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Dietary ω3 Fatty Acids and Cholesterol Modify Intestinal Transport, Enterocyte Membrane Lipid Composition and Enterocyte Lipid Synthesis

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance. A thesis entitled *Dietary ω3 Fatty Acids and Cholesterol Modify Intestinal Transport, Enterocyte Membrane Lipid Composition and Enterocyte Lipid Synthesis* submitted by Monika Maria Keelan in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medical Sciences (Medicine).

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Dedication

This thesis is dedicated to my husband, David, and my two children, Anthony and Brittany. Their love and support gave me the opportunity and the desire to continue with my academic pursuits.

The birth of my children during the early years of my Ph.D. program presented many challenges, but also many gifts. As I finish my graduate studies, my children have grown to the age that they are just beginning their formal education. I wish them as much joy and success as I have experienced with my studies.

Abstract

Nutrient uptake is increased into the intestine of diabetic rats, and this adaptation can be modified further by manipulation of the type of fatty acids in the triglycerides in the diet. Adult Wistar rats were divided into two groups. In half of the animals, diabetes was produced with the injection of streptozotocin, and the other half of the animals were injected with saline and served as non-diabetic controls. Both groups were raised on chow for two weeks and then randomized to one of four semisynthetic diets for two weeks: beef tallow (BT), beef tallow supplemented with cholesterol (BTC), fish oil (FO), or fish oil supplemented with cholesterol (FOC). The enhanced glucose uptake into the small intestine of streptozotocin-diabetic rats can be partially corrected by feeding FO. This 'lowering effect' on the uptake of D-glucose observed with feeding FO may be prevented by supplementing the diet with cholesterol. Alterations in D-glucose uptake could not be explained by changes in mucosal surface area. Feeding FOC to diabetic rats prevented the increase in jejunal cholesterol uptake observed with feeding BTC, yet feeding FO increased jejunal cholesterol uptake as compared with feeding BT.

Lipid uptake examined in rabbit jejunal BBMV suggests linoleic acid uptake occurs by a passive process, but does not exclude the possibility of a membrane fatty acid binding protein. Further studies suggest lipid uptake may occur by three mechanisms: 1) partitioning from the bile acid micelle into BBMV by way of the 'collision' model (cholesterol); 2) uptake from the bile acid micelle by both collision and aqueous/dissociation models (stearic acid); 3) uptake may be mediated by both a concentration-dependent and -independent component (stearic acid).

Changes in the composition of the brush border membrane (BBM) lipids provide a possible mechanism for the observed alterations in transport. Diabetes is associated with an increase in BBM total phospholipids. Feeding FO reduced total phospholipid content in BBM of control and diabetic animals, primarily due to a reduction in sphingomyelin (SM).

Diet and diabetes also influence jejunal and ileal enterocyte microsomal membrane (EMM) lipid composition and the activity of two phospholipid metabolizing enzymes, cholinephosphotransferase (CPT) and phosphatidylethanolamine *N*-methyltransferase (PEMT). Feeding FO reduced EMM total phospholipids (phosphatidylcholine (PC),

phosphatidylethanolamine (PE) and phosphatidylinositol), and decreased jejunal CPT activity up to 82% in diabetic rats and 95% in control rats. Diabetes was associated with increased jejunal EMM total phospholipids (SM and PE), without associated changes in CPT or PEMT activity. Dietary cholesterol supplementation did not affect EMM total cholesterol, phospholipids, CPT or PEMT activity in control rats fed BT or FO. In contrast, dietary cholesterol supplementation increased EMM cholesterol in diabetic rats fed BT or FO, decreased total phospholipids (SM, PC and PE) in diabetic rats fed FO, without associated changes in CPT or PEMT activity.

Isocaloric alterations in the type of dietary lipids and diabetes are also associated with alterations in the phospholipid fatty acyl content of the intestinal BBM and EMM, as well as Δ^5-, Δ^6- and Δ^9-desaturase activities. Feeding a high cholesterol diet increased jejunal Δ^5-and Δ^9-desaturase activities in control rats fed FO, increased jejunal Δ^5-desaturase activity in diabetic rats fed FO, and increased the ileal Δ^5- and Δ^6-desaturase activities in control and diabetic animals fed FO. Dietary fatty acids, cholesterol and diabetes, however, did not always produce the changes in the fatty acids in BBM PC or PE expected from the measured alterations in desaturase activities.

A model is proposed by which the BBM nutrient uptake and lipid composition may be altered with diabetes and diet.

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AA	arachidonic acid
AAT	acylCoA acyltransferase
ACAT	acylCoA:cholesterol acyltransferase
αGP	α-glycerol phosphate
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BBM	brush border membrane
BBMV	brush border membrane vesicles
BLM	basolateral membrane
BT	beef tallow low cholesterol diet
BTC	beef tallow high cholesterol diet
cAMP	cyclic adenosine monophosphate
CEH	cholesteryl ester hydrolase
CM	chylomicron
CoA	coenzyme A
CPT	cholinephosphotransferase
CVA	crypt villus axis
CVD	cardiovascular disease
DG	diacylglycerol
DGAT	diacylglycerol acyltransferase
DHA	docosahexanoic acid
EDTA	ethylenediaminetetraacetic acid
EGF	epidermal growth factor
EMM	enterocyte microsomal membrane
EPA	eicosapentaenoic acid
EPT	ethanolaminephosphotransferase
ER	endoplasmic reticulum
FA	fatty acid
$FABP_c$	cytoplasmic fatty acid binding protein
$FABP_{pm}$	plasma membrane fatty acid binding protein
FFA	free fatty acid
FO	fish oil with low cholesterol diet
FOC	fish oil with high cholesterol diet
GL-TP	glycolipid transfer protein
GLUT2	sodium-independent glucose transporter

GLUT5	sodium-independent fructose transporter
GSL	glycosphingolipid
HDL	high density lipoprotein
HEPES	(*N*-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid])
HgA_1C	hemoglobin A_1C
HMGR	3-hydroxy-3-methylglutarylCoA reductase
IDDM	insulin-dependent diabetes mellitus
$\delta\Delta F_{w\rightarrow l}$	incremental change in free energy from an aqueous to a lipid phase
I-$FABP_c$	intestinal type cytoplasmic fatty acid binding protein
IV	intravenous
K_d	diffusion constant
K_i	inhibition constant
K_m	Michaelis constant
LA	linoleic acid
LCAT	lecithin:cholesterol acyltransferase
LDL	low density lipoprotein
L-$FABP_c$	liver type cytoplasmic fatty acid binding protein
LNA	linolenic acid
LPC	lysophosphatidylcholine
LPE	lysophosphatidylethanolamine
LTB_4	leukotriene B_4
LTB_5	leukotriene B_5
LTP	lipid transfer protein
mdr	mouse multidrug resistance gene
MDR	human multidrug resistance gene
MG	monoacylglycerol
MGAT	monoacylglycerol acyltransferase
M/S	monounsaturated fatty acids / saturated fatty acids
MTTP	microsomal triacylglycerol transfer protein
MVM	microvillus membrane
NADH	α-nicotinamide adenine dinucleotide, reduced form
NADPH	α-nicotinamide adenine dinucleotide phosphate, reduced form
NHE_3	sodium/hydrogen exchanger in the brush border membrane
NIDDM	non-insulin-dependent diabetes mellitus
nsL-TP	non-specific lipid transfer protein
OA	oleic acid
P	polyunsaturated fatty acid diet

PC	phosphatidylcholine
PC-TP	phosphatidylcholine transfer protein
P_d	passive permeability coefficient
PE	phosphatidylethanolamine
PEMT	phosphatidylethanolamine N-methyltransferase
PFK_1	6-phosphofructo-1-kinase
PGI_2	prostacyclin
PI	phosphatidylinositol
PI-TP	phosphatidylinositol transfer protein
PL/C	phospholipid/cholesterol
PPAR	peroxisome proliferator-activated receptor
P/S	polyunsaturated fatty acids / saturated fatty acids
PS	phosphatidylserine
PUFA	polyunsaturated fatty acid
S	saturated fatty acid diet
SAM	S-adenosylmethionine
SCD1	stearoylCoA desaturase 1
SCD2	stearoylCoA desaturase 2
SCP-2	sterol carrier protein 2
SFA	saturated fatty acid
SGLT1	sodium-dependent D-glucose cotransporter
SM	sphingomyelin
TC	taurocholic acid
TG	triacylglycerol
TGN	trans-Golgi network
TLC	thin layer chromatography
TXA_2	thromboxane A_2
TXA_3	thromboxane A_3
UWL	unstirred water layer
VLDL	very low density lipoprotein
V_{max}	maximal transport rate
ω	omega

CHAPTER 1

LITERATURE REVIEW

1.1 Overview

The role of diet in the maintenance of health has been well studied, although a continuing controversy exists on what the minimum requirements of nutrient intake should be for the average individual in the general public. In recent years, a great deal of study has also taken place on the role of diet as a nutritional therapy for various illnesses. Despite the increasing amount of interest in the mechanisms by which diet can improve the health status of an individual suffering from a variety of diseases, few studies have examined the effect of diet on the function of the small intestine with aging, or in the presence of injury due to insult (i.e. alcohol, radiation, resection), or disease (i.e. diabetes).

The lipid components of the diet are often the major focus of studies which examine correlations between North American diet and disease. The current average North American diet contains 34% fat (NHANES III, 1993). This high dietary fat is associated with an increased incidence of cardiovascular disease (CVD) and several types of cancer. Not only the amount of dietary fat is a problem, however; it is the type of dietary fat that correlates to an increased risk of disease. An increased incidence of CVD is observed with a high dietary intake of cholesterol and saturated fats, but not with a high dietary intake of monounsaturated fats. A high dietary intake of ω6 polyunsaturated fats may promote tumor growth in some types of cancer. This contrasts with potential health benefits that may be achieved when a diet is enriched in ω3 polyunsaturated fatty acids. The initial evidence originated from studies of the dietary habits of the Greenland Eskimos. Despite the high dietary fat intake of the Greenland Eskimos arising from their staple diet of marine animals, the incidence of CVD, cancer, diabetes, hypertension and chronic diseases associated with abnormalities in immune function and the inflammatory response, is virtually non-existent (Bang and Dyerberg, 1980; Carroll, 1986a). This was attributed to the nature of the type of fat consumed: marine animal fat is enriched in ω3 polyunsaturated fatty acids.

Diabetes is associated with an elevation in blood glucose due to the inability of cells to access blood glucose. This inability results from an absolute insulin deficiency, or a decline in the production and release of insulin from pancreatic β cells to the blood, with or without a peripheral insulin resistance. Diabetes is also associated with a variety of changes in the small intestine:

1) alterations in nutrient absorption: an enhanced intestinal uptake of sugars and lipids (Thomson, 1983a),

2) changes in brush border membrane lipid composition: an increase in the ratio of phospholipid to cholesterol, due to increases in the content of phosphatidylcholine and phosphatidylethanolamine (Keelan et al., 1985a),

3) abnormalities in lipid metabolism: increased cholesterol and phospholipid synthesis (Feingold et al., 1982a; Hoffmann et al., 1981).

With insulin administration, insulin binding to the insulin receptors of insulin-sensitive cells permits glucose to enter cells, thereby reducing blood glucose to normal levels. Insulin administration or islet cell transplantation also decreases intestinal nutrient uptake and lipid synthesis to near normal levels in diabetic rats (Thomson and Rajotte, 1984, 1985; Feingold et al., 1983; Bremer, 1981).

Is it possible to alter intestinal function in diabetics by altering the quality of the dietary fat? Feeding diabetic rats a high fat diet enriched in ω6 polyunsaturated fatty acids is associated with a decline in the intestinal uptake of sugars and an improvement in the clinical control of the diabetes (Rajotte et al., 1988; Thomson et al., 1987a,b, 1988a). In control animals, feeding a diet enriched in ω3 polyunsaturated fatty acids reduces glucose uptake to a greater degree than observed with feeding a diet enriched in ω6 polyunsaturated fatty acids (Thomson et al., 1988b). Feeding diabetic rats a diet enriched in ω3 polyunsaturated fatty acids may be a useful strategy to reduce the enhanced nutrient absorption associated with diabetes.

The impact of either diabetes or diet on the small intestine is significant. The concept that diet may reverse changes in the small intestine observed with diabetes is worthy of investigation. The mechanisms by which diet may mediate changes in membrane structure and function are not well understood. Accordingly, this thesis examines the influence of diet on nutrient transport, digestive enzyme activity, enterocyte membrane lipid composition and the activity of several enzymes involved in lipid metabolism in both control and diabetic animals.

1.2 Composition and Digestion of Dietary Fat

Approximately 81-83 g of fat is consumed each day in the average adult diet in the western world (NHANES III, 1993). The majority of dietary fat is triacylglycerol, and contains predominantly oleic and palmitic acids. The source of the dietary fat dictates the triacylglycerol fatty acid composition. For example, fats derived from animal sources contain primarily saturated fatty acids at the α (*sn*-1) and α_1 (*sn*-3) position, while fats derived from vegetable sources contain primarily unsaturated fatty acids at the β position. The position of the fatty acid on the triacylglycerol molecule influences the digestion and absorption of fat. The digestion and absorption of fat has been recently reviewed (Thomson et al., 1989a, 1993). Lingual and gastric lipases hydrolyze fatty acid from the *sn*-3 position of triacylglycerols to form diacylglycerols and free fatty acid. Pancreatic lipases hydrolyze fatty acid from the *sn*-1 and *sn*-3 position of triacylglycerols to form monoacylglycerols and free fatty acids.

Fatty acids may be saturated (no double bonds), monounsaturated (one double bond), or polyunsaturated (more than one double bond). Each carbon of a saturated fatty acid is bound to its full complement of hydrogen atoms, and is therefore 'saturated' with hydrogen atoms. Unsaturated fatty acids contain double bonds because not all of the carbons are bound to their full complement of hydrogen atoms. When fatty acids are abbreviated numerically, the first number refers to the number of carbon atoms, and is then followed by a colon and a second number which refers to the number of double bonds in the fatty acid molecule. The term ω (omega) or *n*- refers to the position of the first double bond from the methyl terminus of the fatty acid molecule. A double bond has two isomeric forms: *cis*, which introduces a bend in the hydrocarbon chain, and *trans*, which maintains the linear form of the hydrocarbon chain. Animal and vegetable sources of fatty acids are present in the *cis* configuration. The presence of *trans* isomers in the diet arises from the ingestion of shortening and margarine which contain vegetable oils that have been partially hydrogenated during processing. The two most common dietary fatty acids, palmitic acid (saturated fatty acid) and oleic acid (monounsaturated fatty acid), can be synthesized by both animal and vegetable cells. Saturated fatty acids make up 12% of total calorie intake each day (NHANES III, 1993). Two important polyunsaturated fatty acids (PUFA) that are synthesized by vegetable cells cannot be synthesized by animal cells: linoleic acid (18:2ω6) and linolenic acid (18:3ω3). These fatty acids have been termed essential fatty acids because they are required for the synthesis of PUFA precursors for prostaglandin and leukotriene synthesis: arachidonic acid (20:4ω6), eicosapentanoic acid (20:5ω3) and docosahexanoic acid (22:6ω3). 1-2% of linoleic acid and less than 0.5% of linolenic acid are required as essential fatty acids each day. This minimum requirement is easily met since essential fatty acid intake in the Western diet is usually between 5-10% of dietary calories (Kinsella, 1986).

In the western world, only 4-8g of phospholipid are consumed per day in the average adult diet (Noma, 1964; Rizek et al., 1974). Approximately two-thirds of the dietary phospholipid is of animal origin. The primary source of luminal phospholipid is bile and sloughed intestinal epithelium, which together supply 7-22g of phospholipid per day (Borgstrom, 1976; Northfield and Hofmann, 1975; Shiau, 1987). Phosphatidylcholine is the major component of both dietary and endogenous phospholipid sources. Phospholipids are composed of two fatty acid chains esterified to a glycerol backbone by a phosphodiester bond. The α (*sn*-1) position of phosphoglycerides is usually occupied by saturated fatty acids while the β (*sn*-2) position is usually occupied by unsaturated fatty acids. In the presence of colipase, pancreatic phospholipase A_2 hydrolyzes fatty acid from the *sn*-2 position of phosphatidylcholine to form lysophosphatidylcholine and free fatty acid. A calcium-independent phospholipase A_2 and phospholipase B have been described in the brush border membrane (BBM) of villus cells of guinea pig and rat small intestine, and they may also play a role in phospholipid digestion (Diagne, et al., 1987; Pind and Kuksis, 1989, 1991). The intestinal BBM phospholipase A_2 and

phospholipase B have the ability to hydrolyze bile salt solubilized phospholipid (Pind and Kuksis, 1989). Up to 50% of luminal phosphatidylcholine was completely hydrolyzed to free fatty acids and glycerophosphocholine in rats fed a test meal (Le Kim and Betzing, 1976). The BBM also has the ability to hydrolyze glycosylceramides, sphingomyelin and ceramides via the activity of glycosylceramidase, sphingomyelinase, and ceramidase (Leese and Semenza, 1973; Nilsson, 1969).

Dietary cholesterol intake is approximately 0.3-0.5 g per day (NHANES III, 1993). Approximately 50% of the daily cholesterol intake is also derived from bile and sloughed intestinal cells. Cholesterol may be present in either the free or esterified form. Cholesterol ester contains a long-chain fatty acid esterified to the 3β position of the cholesterol molecule. Cholesterol ester is hydrolyzed by cholesterol ester hydrolase to form free cholesterol and free fatty acid.

Modulating the quantity and the quality of dietary fat has been observed to be important for the clinical control and pathogenesis of atherosclerosis and diabetes. The composition of dietary fat has been demonstrated to be important in the regulation of plasma cholesterol levels and the prevention of cardiovascular disease (Kinsella, 1986; Herold and Kinsella, 1986). Increased dietary intake of ω3 fatty acids is associated with a decline in plasma triacylglycerol and lipoprotein levels, reduced platelet thrombogenicity, and alterations in immune cell function and inflammatory response (Leaf and Weber, 1988; von Schacky, 1987). The ability of ω3 fatty acids to reduce plasma triacylglycerol and cholesterol levels, however, depends upon the dietary ratio of linoleic acid to saturated fatty acid and the cholesterol content of the diet (Garg et al., 1988f, 1989). The major dietary source of long-chain ω3 fatty acids is fish oil. The incorporation of ω3 fatty acids into membrane phospholipids may alter membrane permeability and the function of such membrane proteins as the insulin receptor and the glucose transporter (Ginsberg et al., 1982; Thomson et la, 1988b). Omega-3 fatty acids alter the synthesis of leukotrienes and prostaglandins, which, in turn, may influence insulin secretion (Robertson, 1984). Leukotrienes and prostaglandins derived from ω3 fatty acids mediate different functions than those derived from ω6 fatty acids. It remains controversial as to whether long-chain ω3 fatty acids are beneficial in the treatment and control of diabetes (Adler et al., 1994; Axelrod, 1989; Axelrod et al., 1994; Kasim, 1993; Jensen, 1991; Lacky and Noble, 1990; Lefebvre and Scheen, 1992; Malasanos and Stacpoole, 1991; Nosari et al., 1994; Vessby, 1991; Zambon et al., 1992).

1.3 Intestinal Morphology

The intestinal mucosa consists of a layer of epithelial cells supported by the lamina propria, which are seen as finger-like projections called villi (Figure 1.1). Approximately 95% of the villus cells are enterocytes which are columnar in appearance (Figure 1.2). The enterocyte plasma membrane is polarized in composition and function. Tight junctions divide the plasma membrane into two domains: the apical brush border membrane (BBM) and the basolateral

membrane (BLM). The BBM or microvillus membrane lies in direct contact with the luminal contents. Measurements of BBM surface area are inconsistent and vary widely, but may increase the surface area of the villus up to 40-fold (Brown, 1962; Kapadia and Baker, 1976; Penzes and Skala, 1977, Hardin et al., 1993). Mucosal surface area includes measurements of villus height, width and density, and is subject to direct or indirect regulation by food (Johnson and McCormack, 1994). Direct regulation of mucosal surface area occurs via polyamines (spermine, spermidine, putrescine) which are able to bind to nucleic acids, modify growth-regulating genes (c-myc, c-fos, H_2A), regulate membrane-bound enzymes, transporters, second messengers, and stabilize membranes (Seidel et al., 1985; Schuber, 1989; Wang and Johnson, 1992). Indirect modulation of mucosal surface area is achieved by the release of intestinal peptides (gastrin, enteroglucogon, neurotensin), increased pancreato-biliary secretions, and motor stimulation (Schuber, 1989).

The BBM separates the enterocyte from the lumen and acts as a permeability barrier. The BBM is the site of nutrient transport into the cell which is mediated by endocytosis, carrier proteins and permeability properties of the membrane itself. In addition, several enzymes, receptors and antigens are present in the BBM. The BLM is the site of absorbed nutrient exit from the enterocyte into portal blood and into lymph, but it is also the entry site of nutrients, hormones and ions from the bloodstream. The BLM contains enzymes such as the Na^+/K^+-ATPase to maintain the gradient of Na^+ across the cell. Cell shape is maintained by the cytoskeleton; and actin-myosin filaments permit motility of the villi.

The crypts of Lieberkühn are located at the base of the villi, and provide the source of immature undifferentiated cells. Each villus is supplied with cells from several crypts. These cells are more rounded in shape when they leave the crypt, and develop into fully functional absorptive cells as they migrate up the villus. The microvilli that constitute the BBM become narrower and more numerous as the cells mature and migrate up the villus. This migration takes appproximately 2-3 days, at which time the cells are sloughed from the villus tip into the lumen. The crypt constantly produces new cells in order to maintain villus structure and function. Cells from each crypt are derived from a single progenitor cell in mice (Schmidt et al., 1988). A spatial differentiation of the epithelium is maintained along the crypt-villus and duodenal-colonic axis (Leung and Lebenthal, 1988). The ontogenic patterns of digestive enzymes are encoded within each stem cell so that each cell has its own positional address (Rubin et al., 1991, 1992). Alterations in the rate at which cells migrate along the villus may influence the functional surface area by altering the size of the enterocyte transport pool (number of mature, functioning enterocytes).

The uptake of sugars, lipids and amino acids occurs predominantly at the upper third of the villus (Haglund et al., 1973; King et al., 1981; Kinter and Wilson, 1965; Thomson et al., 1994). Approximately 70% of nutrient uptake is handled by the upper 30% of the villus. Nutrients must cross two barriers in series in order to be taken up by the enterocyte: the unstirred water layer and the BBM. The rate of nutrient uptake is determined by the dimensions and properties of

these two barriers (Dietschy et al., 1971; Thomson and Dietschy, 1977; Westergaard and Dietschy, 1974; Wilson and Dietschy, 1975; Winne, 1976), and also by the activity of the protein-mediated components of transport such as the sodium-dependent glucose cotransporter (SGLT1) and the sodium-independent fructose transporter (GLUT5) in the BBM (Burant et al., 1992a; Davidson et al., 1992; Wright, 1993).

1.4 The Unstirred Water Layer and the Acidic Microclimate

The unstirred water layer (UWL) thickness is approximately 100-500 μm in rabbit and rat intestine (Westergaard and Dietschy, 1974; Wilson and Dietschy, 1975; Winne, 1976), and greater than 500 μm in man (Read et al., 1977). The UWL is formed by "a hydrated mucus and a series of water lamellae extending outward from the BBM, each progressively more stirred, until the layers blend imperceptibly with the bulk phase" (Thomson, 1979a). The effective resistance of the UWL must be considered when describing nutrient uptake (Dietschy et al., 1971; Thomson,1984b; Thomson and Dietschy, 1977,1980; Westergaard and Dietschy, 1974). The rate of solute diffusion across the UWL is determined by the thickness of the UWL, the surface area of the UWL, the aqueous diffusion constant of the solute and the concentration gradient of the solute from the bulk phase to the BBM (Wilson et al., 1971). Failure to correct for the effective resistance of the UWL will lead to the overestimation of the value of the Michaelis constant (K_m), an underestimation in the value of the passive permeability coefficient (P_d), and result in the overestimation of the value of the maximal transport rate (V_{max}).

The pH of the UWL is below 6, in contrast with the significantly higher pH of the bulk phase (Matsueda et al., 1989). The pH of the UWL is lowest in the mucus layer immediately adjacent to the BBM, known as the acidic microclimate. Since nutrients must diffuse through the UWL to reach the BBM, an alteration in pH may result in a change in the proportion of ionized and non-ionized solute (Hogerle and Winne, 1983) which, in turn, influences the ability of the solute to be taken up by the BBM. The presence of epidermal growth factor in the intestinal lumen reduces the pH of the acidic microclimate and may influence nutrient uptake (Iwatsubo et al., 1989). A low pH facilitates fatty acid dissociation from mixed micelles and protonation of the free fatty acid for permeation across the BBM (Shiau, 1990). The low pH of the acidic microclimate is maintained by the activity of the Na^+/H^+ exchanger in the BBM and the physical properties of the mucus which retard the diffusion of H^+ to the bulk phase of the lumen (Shiau et al., 1985).

Intestinal mucus is primarily composed of mucin -- a glycoprotein that when linked together forms a gel system which gives it unique properties (Neutra and Forstner, 1987). Mucus not only has a protective function; it may also play a role in nutrient uptake by maintaining the low pH of the microclimate through its ability to retard the diffusion of H^+ from the BBM surface to the lumen (Shiau et al., 1985), thereby altering the proportion of ionized and non-ionized forms of fatty acids. The pK of fatty acids in aqueous solutions has been reported to be approximately 4.2

(Small et al., 1984), which would put the majority of fatty acids in the non-ionized form in the acidic microclimate pH of 6. These protonated fatty acids permeate the BBM more readily than non-protonated fatty acids (Perkins and Cafiso, 1987). Mucus may also influence the distribution of ions at the mucosal surface. Iron absorption has been reported to be modulated by intestinal mucus (Wien and van Campen, 1991). Mucus may also play a role in the absorption of cholesterol since a component of mucus has been reported to have a high affinity for cholesterol and the ability to bind it in a stoichiometric manner (Mayer et al., 1985).

1.5 Brush Border Membrane

A membrane is a lipid bilayer with both intrinsic and extrinsic proteins. The lipids and proteins are asymmetrically distributed across the bilayer (Op den Kamp, 1979). Lipids are able to move in the bilayer, although their movement depends upon their structure, physical state, and the ratio of lipid-to-cholesterol or lipid-to-protein (Vaz et al., 1984; Rubenstein et al., 1979; O'Leary, 1987). Most of the membrane proteins are immobilized in the membrane, likely by attachment to the cytoskeleton (Koppel et al., 1981; Utsumi et al., 1982; Pink, 1985; Axelrod, 1983; Aszalos et al., 1986).

The BBM is composed of carbohydrates (<10%), proteins (30%-40%) and lipids (50%-60%) which are distributed asymmetrically in the membrane (Dudeja et al., 1991a,b). The glycocalyx, located on the external luminal surface of the BBM, is comprised of carbohydrates covalently bound to proteins and lipids. Glycosylation permits asymmetric distribution of the protein or lipid to the luminal surface of the BBM. Sialic residues on the glycocalyx provide a net negative charge to the luminal surface of the enterocyte.

The proteins of mouse duodenal BBM have been analyzed (Billington and Nayudu, 1975). Enzyme and transporter activity associated with the BBM have been described and may be used to differentiate the location of cells along the crypt-villus axis. Disaccharidases, dipeptidases and alkaline phosphatase present in the BBM of villus cells are absent from crypt cells (Hartman et al., 1982; Nordstrom et al., 1968; Nordstrom and Dahlqvist, 1972). Sodium-dependent glucose cotransporter (SGLT1) activity is maximal in the upper villus cells (Dudeja et al., 1990; Freeman et al., 1987; Meddings et al., 1990). Asymmetric distribution of the proteins facilitates the orientation of the protein function to a specific side of the membrane. Seventy percent of the total membrane proteins are integral or intrinsic proteins embedded within the lipid bilayer and examples of these intrinsic BBM proteins are alkaline phosphatase, and transport proteins. The remaining membrane proteins are peripheral or extrinsic proteins which are loosely associated with the surface of the membrane by weak, non-covalent interactions. Digestive enzymes such as lactase, maltase, sucrase, leucine aminopeptidase and γ-gluyamyl transpeptidase are extrinsic BBM proteins. Sucrase, located on the luminal surface of the BBM, is part of the sucrase-

isomaltase complex and has recently been cloned (Hunziker, et al., 1986). Cytoskeletal proteins are located on the cytosolic surface of the BBM.

The lipid composition of the BBM has been reported for several species including rats, mice, hamster, rabbit, and pig (Billington and Nayudu, 1978; Bloj and Zilversmit, 1982; Brasitus et al., 1984; Christiansen and Carlsen, 1981; Dudeja et al., 1991a; Forstner et al., 1968; Hauser et al., 1980; Kawai et al., 1974; Keelan et al., 1985a, b, c, d, 1986, 1990a; Meddings and Thiessen, 1989; Meddings et al., 1990; Millington and Gritchley, 1968). Phospholipids, neutral lipids and glycolipids are the three lipid classes present in the BBM in a ratio of 1:1:2 in the rat (Forstner et al., 1968) and 1:1:1 in the mouse (Kawai et al., 1974). In contrast, the ratio of phospholipids to neutral lipids to glycolipids in the BLM was 2.5:1:0.3 in mouse small intestine.

Lipid domains exist in biological membranes (Op den Kamp, 1979; Schroeder, 1984; Tocanne et al., 1989; Yechiel and Edidin, 1987), including those of the intestinal epithelial cell (Dudeja et al., 1991b; Kawai et al., 1974; Brasitus and Schachter, 1980; Schroeder et al., 1991). The differences in lipid composition of BBM and BLM demonstrate that intracellular domains do exist. For example, more glycosphingolipids are in the BBM than in the BLM (Hauser et al., 1980). The asymmetrical distribution of lipids across the BBM bilayer demonstrate that intramembrane domains exist (Barsukov et al., 1986; Dudeja et al., 1991b; Pelletier et al., 1987). Lateral membrane domains also exist within the bilayer. Fluorescent sterol and [^{3}H]-cholesterol exchange studies demonstrated the presence of two cholesterol pools in the membrane: one readily exchangeable (kinetic) pool and one virtually non-exchangeable (structural) pool (Schroeder et al., 1991). Microlipid domains present around membrane proteins may be a consequence of the protein lattice structure trapping lipids within it, thereby preventing their diffusion (Dupou et al., 1988). Lipid domains may be important in the regulation of BBM transport proteins and enzymes, as well as the regulation of passive permeability.

Phospholipids are amphipathic molecules composed of a charged polar head group (base) and two non-polar aliphatic hydrocarbon chains (fatty acyl chains or fatty acids). The hydrophobic fatty acids are esterified to a glycerol backbone and the phosphate ester to the hydrophilic base. Phospholipids are generally distributed asymmetrically across the membrane lipid bilayer (Devaux, 1991). Membrane lipid asymmetry may be determined by a variety of techniques, including phospholipase treatment, selective quenching of trinitrophenyl groups, steady-state fluorescence polarization and differential polarized phase fluorescence using lipid-soluble fluorophores such as 1,6-diphenyl-1,3,5-hexatriene (Dudeja et al., 1991b). The fluorophores are incubated with membranes under penetrating (37°C) or non-penetrating (4°C) conditions to determine the asymmetrical distribution of the membrane lipids. Phospholipid distribution depends upon species and site. In human BBM, phosphatidylcholine and phosphatidylserine are symmetrically distributed across the bilayer (Dudeja et al., 1991a), while in rabbit BBM both phosphatidylcholine and phosphatidylethanolamine are predominantly located on the cytoplasmic half of the bilayer (Barsukov et al., 1986; Lipka et al., 1991). In rat BBM,

phosphatidylcholine and sphingomyelin are primarily situated on the luminal half of the membrane bilayer, while phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine are chiefly located on the cytoplasmic half of the membrane bilayer (Dudeja et al., 1991b). In trout posterior intestine, phosphatidylserine and phosphatidylethanolamine were both mainly found on the cytoplamic leaflet, compared to the middle intestine where phosphatidylethanolamine is asymmetrically distributed across the bilayer (Pelletier et al., 1987). Lysophosphatidycholine and lysophosphatidylethanolamine may also be present in the BBM as a result of the deacylation of phosphatidylcholine and phosphatidylethanolamine. The fatty acids of the BBM phospholipids are 14-24 carbons in length, although the most abundant fatty acids are the 16 and 18 series. The fatty acids may be saturated (containing their full complement of hydrogen), monounsaturated (containing one double bond), or polyunsaturated (containing more than one double bond). The fatty acid composition of phosphatidylethanolamine and phosphatidylserine is more highly unsaturated than the fatty acid composition of phosphatidylcholine and sphingomyelin (Brasitus et al., 1984; Keelan et al., 1990a; Meddings, 1988). Unlike the other phospholipids, sphingomyelin is highly enriched in saturated fatty acids that are esterified to a ceramide backbone, which gives this molecule a rigid structure that packs tightly in the membrane. These phospholipid and fatty acid differences suggest that the inner cytoplasmic monolayer is more fluid than the outer luminal monolayer of the BBM. Alterations in the fluidity of the two hemi-leaflets of the BBM have been reported (Dudeja et al., 1991a,b; Meddings, 1988; Meddings and Thiessen, 1989) and confirm the suggestion that the luminal hemi-leaflet (BBM_L) is more rigid than the cytoplasmic hemi-leaflet of the BBM (BBM_c).

The neutral lipid composition of the BBM consists primarily of cholesterol and some free fatty acid, although trace amounts of diglyceride, triglyceride and cholesterol ester may also be present. Cholesterol-rich and cholesterol-poor domains are present in the BBM (Schroeder et al., 1991). Cholesterol is located predominantly in the cytoplasmic half of the BBM bilayer (Bloj and Zilversmit, 1982).

Free fatty acids make up 5-15 % of the total lipid content of the BBM (Keelan et al., 1985a,b,c,d, 1986, Pind and Kuksis, 1987; Dudeja et al., 1989). The presence of free fatty acids in the BBM is not considered a result of phospholipid breakdown during membrane purification. It has been postulated that a pool of free fatty acids is generated as a result of the continuous turnover of membrane phospholipid fatty acid groups (Lapetina et al., 1980; Spector and Steinberg, 1967; Spector et al., 1981). The content of this free fatty acid pool may be altered during the absorption of luminal free fatty acid. It has been suggested that membrane free fatty acids mediate functional effects (Spector and Yorek, 1985). A free fatty acid pool within the membrane may be important for the acylation of some membrane proteins by facilitating their attachment to the membrane lipid bilayer, channeling proteins for recycling, or for conformational changes in the protein (Magee and Schlessinger, 1982) and local retailoring of membrane phospholipids (Lynch and Thompson, 1988).

BBM glycolipids are comprised of cerebrosides, ceramide, ceramide monohexoside, ceramide dehexoside, ceramide trihexoside, ceramide tetrahexoside, ceramide sulphatide, and gangliosides (predominantly GM_3). The ceramide monohexoside glucosylceramide is the major component of rat BBM (Forstner and Wherret, 1973). These glycolipids are primarily located in the cytoplasmic leaflet of the BBM bilayer.

The physicochemical properties of the BBM, such as permeability and fluidity, are determined by the lipid composition of the membrane and the temperature of the test conditions. The term "fluidity" describes the motional freedom of lipid molecules in the membrane, and depends on lipid structure and packing in membrane. At physiological temperatures, most membrane lipids are in the liquid-crystalline (fluid) state, which is necessary to maintain membrane functions. The liquid-crystalline state is influenced by:

1) the length and degree of unsaturation of the phospholipid fatty acyl chains: long unsaturated fatty acyl chains (i.e. 20:4) are more fluid than short saturated fatty acyl chains (i.e. 16:0);
2) the phospholipid base: the nature of the phospholipid polar head group influences the mobility of the fatty acyl chains. A large polar head group (i.e. phosphatidylcholine) allows more mobility than a small head group (i.e. phosphatidylethanolamine);
3) the amount of cholesterol: cholesterol is a rigid planar molecule which decreases the motional freedom of nearby fatty acyl chains. Cholesterol increases the electrostatic interaction between the hydroxyl and phosphate groups which help maintain order and rigidity in the bilayer. Cholesterol functions to decrease the fluidity of a membrane to an intermediate state between gel (viscous) and liquid-crystalline (fluid);
4) the temperature: temperature influences the molecular packing and motional freedom of the fatty acyl chains. At low temperatures, the membrane is in the gel state, which describes the close molecular packing of fatty acyl chains in an *all-trans* state. With an increase in temperature, fatty acyl chains change to a fluid liquid-crystalline *cis-trans* state which allows for increased molecular motion and decreased molecular packing, resulting in a decreased membrane width. The *cis* double bonds of the unsaturated fatty acyl chains provide an angular conformation which disorders the bilayer lattice structure, and decreases the hydrophobic interactions of the ester bond as well as the terminal methyl groups. The membrane becomes more disordered by the formation of a *trans-gauche* conformations in the fatty acyl chains which contain double bonds. The unsaturated fatty acyl chains possess more motional freedom than the straight saturated fatty acyl chains;
5) the uptake and incorporation of unsaturated fatty acids and acylation in the β-position of glycerophospholipids (Hegner, 1980) which increase the fluidity of the membrane.

Membrane lipid physicochemical properties play an important role in modulating the function of intrinsic membrane proteins, such as transport proteins and enzymes. The boundary

or annular lipids which immediately surround the membrane protein influence protein function by interacting with the protein through charge, packing and motion. The composition and properties of the boundary lipids may be quite different from those of the bulk lipids.

Fluidity is a property of the BBM that describes the motional freedom of lipid molecules in the membrane, and may be used as a tool to provide an overall assessment of membrane lipid composition (Meddings et al., 1990). Lipid molecules have three types of motion in membranes: rotational, lateral, and translocation from one half of the bilayer to the other half of the bilayer. Fluidity is determined by the membrane lipid composition and how tightly the membrane lipids are packed together. The fluidity of the BBM has been investigated in rats (Brasitus and Schachter, 1980; Brasitus et al., 1979,1980,1984; Dudeja et al., 1991b; Meddings, 1988, 1989; Schachter and Shinitzky, 1977; Schwarz et al., 1985) and rabbits (Meddings et al., 1990; Mütsch et al., 1983; Schwarz et al., 1984). The presence of proteins in the membrane do not affect the fluidity of the BBM at physiological temperature (Brasitus and Schachter, 1980; Brasitus et al., 1979; Mütsch et al., 1983). Figure 1.3 illustrates intestinal membrane fluidity gradients. Enterocyte BBM fluidity is greater in proximal, compared with distal intestine (Meddings, 1988; Schachter and Shinitsky, 1977). BBM fluidity decreases as cells migrate up the crypt-villus axis (Brasitus and Dudeja, 1985; Meddings et al., 1990). In fact, BBM are less fluid than many other biological membranes, including the BLM, due to the unusually high molar ratio of cholesterol to phospholipid (approximately 1:1). The low fluidity of the BBM has been suggested to be functionally important for efficient enterocyte nutrient and electrolyte transport (Papahadjopoulos et al., 1973; Schachter and Shinitzky, 1977), and optimal conformation of surface receptors and enzymes (Shinitzky and Inbar, 1976). Differences in fluidity between the inner and outer halves of the membrane bilayer may also influence the activity of BBM enzymes and the uptake of nutrients across the BBM. When the fluidity of the cytoplasmic leaflet is less than the luminal leaflet, leucine aminopeptidase activity decreases, while D-glucose transport increases (Dudeja et al., 1991a; Meddings, 1990) The jejunal BBM is exposed to the majority of dietary lipids and correlates with an increase in the fluidity of the outer third of the luminal bilayer (Meddings, 1988). This contrasts with the decrease in the fluidity of the outer third of the ileal BBM, which is exposed to far less dietary lipid. The passive movement of lipid across the two bilayers of the BBM depends upon the composition and properties of the two membrane bilayers.

Passive and carrier-mediated transport processes depend on the nature of the fluidity of the BBM and its lipid composition: fatty acyl chains, the charge of the phospholipid head group, the interaction between phospholipid and cholesterol, as well as protein-lipid interactions. Any changes in the BBM lipid composition may alter membrane fluidity, and this reflects an adaptive change by which nutrient transport may be altered.

1.6 Transport Mechanisms

Nutrient transport across the intestinal epithelium occurs by one of two mechanisms: passive permeation or carrier-mediated uptake. Passive permeation includes transport through tight junctions (paracellular pathway) and through the enterocyte (transcellular pathway). Carrier-mediated uptake (also known as protein-mediated uptake) may or may not require energy, and it is referred to as active transport or facilitated transport respectively.

Paracellular nutrient transport through tight junctions occurs with changes in the permeability of the tight junctions to fluid. Nutrients move with the fluid into the subepithelial space by a process of solvent drag. Although sugars may be taken up by the paracellular pathway (Pappenheimer, 1987, 1990; Pappenheimer and Volpp, 1992; Pappenheimer and Reiss, 1987), this route may not be physiologically significant (Ferraris et al., 1990). Only during periods of high intraluminal glucose concentration does the paracellular pathway comprise up to 30% of the total glucose absorbed (Pappenheimer and Reiss, 1987). Intracellular modulators (Ca^{2+}, calmodulin, cAMP, G proteins, protein kinase C, inositol triphosphate) may regulate the movement of solutes through the paracellular pathway (Madara, 1983). Nutrients also influence paracellular transport (Madara and Carlson, 1991; Atisook and Madara, 1991). Glucose plays an important role in the modulation of the paracellular pathway. Glucose-mediated physiological regulation of absorptive tight junctions is Na^+-dependent and inhibited by phorizin. This suggests that the activation of SGLT1 triggers this response (Atisook et al., 1990), which possibly arises from increases in intracellular Ca^{2+} (Madara and Carlson, 1991; Atisook and Madara, 1991).

Passive permeation through the enterocyte is characterized by:

1) the linear uptake of solute with concentration;
2) the absence of competition for uptake by other solutes;
3) the predictable increase in the membrane permeability coefficient with solute hydrophobicity (i.e., plot the natural logarithm of uptake divided by the diffusion coefficient versus a homologous series of fatty acids, increasing in the number of CH_2 groups from 2 to 12); and
4) solute uptake into liposomes which do not contain any protein.

Carrier-mediated uptake may or may not require energy, and is characterized by:

1) the saturable uptake of solute with increasing concentration;
2) inhibition of uptake by proteases, or chemical modification of proteins;
3) dependence on an ion gradient across the membrane for cotransport (i.e. Na^+);
4) competitive inhibition for uptake by other solutes;
5) inhibition of solute uptake by antibodies to carrier-protein; and
6) a high Q_{10} effect, i.e.,the change in transport observed with a 10 degree change in temperature.

D-glucose uptake and amino acid uptake are transcellular carrier-mediated processes (Karasov and Diamond, 1983a). Recent discoveries of membrane lipid binding proteins (Stremmel et al., 1985 ; Thurnhofer and Hauser, 1990a,b; Thurnhofer et al., 1991) and reports of sodium dependence (Stremmel, 1988; Ling et al., 1989; Schoeller et al., 1995a,b; Thomson, 1995) suggest that a proportion of lipid uptake may also be carrier-mediated, although transcellular lipid uptake generally has been accepted to occur by passive diffusion due to the hydrophobic properties of lipids (Borgstrom et al., 1957; Borgstrom, 1977; Sallee et al. 1972; Westergaard, 1987). The different lipid composition of the BBM and BLM confers distinct hydrophobic properties to each membrane which may influence the passive transport of lipids via the transcellular route (Meddings, 1989). Alterations in membrane lipid composition may also influence the activity of membrane bound nutrient transporters (Meddings et al, 1990).

Carrier-mediated uptake is saturable and may be characterized by two kinetic constants: the maximal transport rate (V_{max}) and the Michaelis-Menton constant (K_m) which describes solute binding affinity and is represented by the concentration of solute at one-half V_{max}. A change in transport at low solute concentration represents an alteration in the value of the K_m, while a variation in transport at high solute concentration (at least 4 times the K_m) represents a change in V_{max} and/or the permeability coefficient (P_d). Protein-mediated transport is predominantly altered by changes in V_{max} (Karasov and Diamond, 1983b).

Changes in the value of the V_{max} reflect alterations in the quantity or activity of transporter, which may occur when:

1) the mucosal surface area is enhanced, thereby increasing the number of transporting enterocytes, i.e., following intestinal resection (Keelan et al., 1985d);
2) the number of transporting enterocytes increases without any change in the total number of enterocytes, i.e. recruitment of enterocytes along the villus in the ileum of chronically diabetic rats (Fedorak et al., 1989);
3) the number of transporters per enterocyte increases, i.e., following prolonged feeding of a single nutrient in high concentration (Ferraris et al., 1988) or in chronic diabetes (Fedorak et al., 1989);
4) there are alterations in the sodium (Na^+) gradient across the BBM due to changes in the intracellular concentration of Na^+ or variations in the activity of the Na^+K^+-ATPase (Hopfer, 1975; Luppa et al., 1978; Murer and Hildmann, 1984);
5) there are alterations in the membrane potential; and
6) an increase in carrier activity per unit protein occurs, without any change in the total number of transporters present.

Changes in the value of the K_m may be influenced by alterations in the unstirred water layer resistance. A change in the BBM lipid environment may alter the conformation of a transporter, thereby modifying the K_m, and result in a change in transporter activity. The Na^+-

dependent D-glucose cotransporter activity of the more fluid crypt cell BBM has a lower V_{max} and twice the K_m, compared to the less fluid villus tip cell BBM (Meddings et al., 1990).

The value of the passive permeability coefficient (P_d) may be altered with changes in the:

1) membrane surface area;
2) membrane pore size;
3) number of pores in the membrane;
4) unstirred water layer resistance;
5) incremental changes in free energy from an aqueous to a lipid phase ($\delta\Delta F_{w \rightarrow l}$);
6) membrane lipid composition; and
7) membrane fluidity.

The magnitude of the kinetic parameters calculated for the transporter vary depending upon the various mathematical methods used to estimate them (Meddings and Westergaard, 1989); and they must include correction for the effective resistance of the unstirred water layer and the contribution of the passive component which may proceed concurrently with a carrier-mediated component (Thomson and Dietschy, 1980). Simple linear transformations of the Michaelis-Menton equation, such as the Lineweaver-Burke or Eadie-Hofstee plot, will not necessarily provide accurate estimates of V_{max} and K_m, especially if the UWL and P_d have not been included in the calculation (Thomson and Dietschy, 1977; Thomson, 1979a,b,1981c). Another method suggests that using a substrate concentration several times above the value of the K_m may be used to estimate V_{max} after correction for the passive permation of the solute (Karasov and Diamond, 1983b). Non-linear methods of analysis generally provide the best approach to estimating kinetic parameters, since they incorporate variable weighting of the data, although the transport model must have been defined previously (Meddings and Westergaard, 1989; Fingerote et al., 1994).

Studies in animal models demonstrate that although alterations in the characteristics of the unstirred water layer do occur, these changes insufficiently explain the direction and magnitude of the observed transport changes (Thomson, 1984b). Alterations in mucosal surface area do not consistently agree with changes in transport. Although Hardin and coworkers (1993) demonstrated that epidermal growth factor (EGF)-enhanced brush border surface area could be correlated to EGF-stimulated increases in nutrient absorption, other studies could not establish a consistent relationship between transport and mucosal surface area (Keelan et al., 1985a,b,c,d,1986; Thomson, 1986b; Thomson and Keelan, 1986; Thomson et al., 1986). Alterations in the incremental change in free energy relate to the physicochemical properties of the membrane, which, in turn, are determined by the lipid composition of the membrane. Changes in BBM phospholipid content and composition are associated with altered nutrient transport in several models of intestinal adaptation including aging, diabetes, chronic alcohol feeding, intestinal resection and following external abdominal irradiation (Table 1.1; Keelan et al., 1985a,b,c,d,1986).

1.6.a. Enterocyte Hexose Absorption

The three major dietary hexoses -- glucose, galactose, and fructose -- are absorbed across the intestinal epithelium by intrinsic membrane transport proteins (Pessin and Bell, 1992; Thorens, 1993; Wright, 1993). Entry to the enterocyte from the lumen, is achieved by transport across the BBM by way of the sodium-dependent glucose cotransporter (SGLT1) for glucose and galactose (Semenza et al., 1984; Stevens et al., 1984; Wright, 1993), and by the sodium-independent facilitative transporter (GLUT5) for fructose (Burant et al., 1992a,b; Crouzoulon and Korieh, 1991; Davidson et al., 1992; Mahraoui et al., 1992; Rand et al., 1993a,b; Schultz and Strecker, 1970; Sigrist-Nelson and Hopfer, 1974). GLUT5 may also transport glucose across the BBM of rat enterocytes (Burant et al., 1992b; Rand et al., 1993). Exit from the enterocyte across the BLM into the interstitial space and blood vessels may be the same for all three hexoses by way of GLUT2, a facilitative transporter (Thorens et al., 1990; Burant and Bell, 1992; Cheeseman, 1993; Colville et al., 1993; Maenz and Cheeseman, 1987; Thorens et al., 1989,1990). In contrast to SGLT1, GLUT2 has a low affinity and high capacity for glucose that adapts to the concentration of carbohydrate in the diet (Burant and Bell, 1992; Cheeseman, 1992). The kinetics of GLUT2 are not rate-limited and therefore glucose uptake increases with concentration (Mueckler, 1990). When glucose is actively transported across the BBM by SGLT1, the kinetics of GLUT2 at the BLM allows efficient, uni-directional transfer of glucose out of the enterocyte to the blood or interstitial space. A recent report suggests GLUT5 is also localized at the BLM of human jejunum to facilitate the transfer of fructose out of the enterocyte (Blakemore et al., 1995).

The uptake of D-glucose across the BBM occurs via sodium-dependent, carrier-mediated transport (Crane, 1962; Hopfer et al., 1976; Semenza et al., 1984; Stevens et al., 1984; Hediger et al., 1987; Wright, 1993). D-glucose transport is driven by sodium movement down its electrochemical gradient (Kaunitz et al., 1982; Murer and Hopfer, 1974; Semenza et al., 1984; Stevens et al., 1984). Sodium and glucose bind to the transporter in a 1:1 molar ratio. Sodium must first bind to its active site on the transporter in order to facilitate a conformational change that exposes the D-glucose binding site. D-glucose then, can bind and be transported across the membrane (Peerce and Wright, 1984).

SGLT1 is classified as a tightly coupled secondary active transport mechanism. Sodium enters the cell down its own gradient, accompanied by D-glucose, and D-glucose is accumulated at the expense of the dissipation of the sodium gradient (Semenza et al., 1984). Evidence for a carrier-mediated glucose transporter in the BBM is supported by :

1) saturable uptake with increasing concentration observed with *in vitro* BBM vesicles (BBMV), intestinal sheets and *in vivo* perfusion (Hopfer et al., 1973; Thomson, 1981a; Hotke et al., 1985);
2) inhibition of uptake following protease treatment of BBMV (Semenza et al., 1984);

3) Na^+-dependence for transport across the membrane, demonstrated by the Na^+-dependent overshoot observed with time in BBMV, and the enhanced uptake observed in the presence of luminal sodium (Hopfer et al., 1973);
4) competitive inhibition of uptake with phloridzin, galactose, 3-O-methyl-glucose or α-methyl-glucose (Hopfer et al., 1973);
5) inhibition of uptake with monoclonal antibodies prepared against the renal BBM Na^+-dependent glucose transporter (Haase et al., 1990); and
6) expression cloning and cDNA sequencing of the intestinal BBM Na^+-dependent glucose transporter (Hediger et al., 1987; Ikeda et al., 1989).

Sodium-dependent glucose transport has been examined in BBMV of rat intestine (Dudeja et al., 1990; Freeman et al., 1987; Hopfer et al., 1973; Maenz and Cheeseman, 1986), rabbit intestine (Keelan et al., 1992, Meddings et al., 1990), bovine intestine (Kaunitz and Wright, 1984) and human intestine (Harig et al., 1989; Berteloot et al., 1991; Malo and Berteloot, 1991). Thomson et al. (1987d) discussed the possibility of more than one sodium-dependent transporter based on theoretical considerations, but it remains controversial. Studies in BBMV have provided evidence for two transporters: a high capacity, low affinity transporter; and a second low capacity, high affinity transporter were described in rat, bovine and human intestine (Kaunitz and Wright, 1984; Freeman and Quamme, 1986; Harig et al., 1989). Brot-Laroche et al. (1986) described two distinct sodium-dependent D-glucose transporters in guinea pig that could be differentiated by their sensitivity to temperature and substrate specificity. Another study suggests that two transporters are present in fetal, but not adult human intestine (Malo, 1990). However, studies done with BBMV isolated from normal adult human jejunum using a fast sampling, rapid filtration apparatus support the existence of a single sodium-dependent glucose cotransporter (Malo and Berteloot, 1991). Only one sodium-dependent glucose cotransporter has been isolated, cloned and characterized from rabbit intestine (Hediger et al., 1987; Ikeda et al., 1989). The kinetics of the sodium-dependent glucose cotransporter are different in rabbit crypt versus villus tips cells. Although the difference in kinetics could suggest the existence of two different transporters, it may also be explained by the variation in the lipid environment of the transporter in the crypt and villus tip cells (Meddings et al., 1990), or the changes in transporter density within the BBM along the crypt-villus axis (Haase et al., 1990). The crypt cell transporter had a low V_{max} and twice the K_m compared to villus cells. Fluidization of the villus tip cell BBM resulted in a decline in V_{max} and K_m to that observed in crypt cells (Meddings et al., 1990). A careful reconsideration of the kinetic evidence suggests the presence of only one sodium-dependent glucose cotransporter (Chenu and Berteloot, 1993). A six-state kinetic model predicts differences in sodium and sugar binding for human, rabbit and rat SGLT1 which are likely due to differences in the primary amino acid sequences (Parent et al., 1992; Loo et al., 1993; Hirayama et al., 1996).

Glucose uptake is highest in the mid to upper villus tip cells of the small intestine, as shown by autoradiography (Kinter and Wilson, 1965; King et al., 1981; Fedorak et al., 1989),

uptake by isolated epithelial cells (Gall et al., 1974, 1977; Gall and Chapman, 1976), and BBMV from cells fractionated from along the crypt villus axis (Dudeja et al., 1990; Freeman et al., 1987; Meddings et al., 1990). Greater uptake at the villus tip cells may be due to the increase in BBM surface area as the cells migrate from the crypt to the villus tip (Brown, 1962). Studies using monoclonal antibodies to the SGLT1 demonstrate that transporter density is similar along the rat crypt-vilus axis, which suggests that the concentration of the transporter remains constant (Haase et al., 1990). This contrasts with a recent study that demonstrated SGLT1 was localized at the apical plasma membrane of differentiated absorptive epithelial cells and virtually absent from crypt cells (Yoshida et al., 1995).

Differences in transport may arise from the finding that there are varying surface areas of the transporter-containing-BBM per unit intestinal length, as well as different densities of the transporter within the BBM (Haase et al., 1990). Glucose uptake is greater in the proximal than in the distal intestine (Hopfer et al., 1976; Thomson 1981a,1983a) due to differences in the glucose transport capacity per mg protein for both BBM and BLM (Maenz and Cheeseman, 1986). In addition, SGLT1 may be associated with a regulatory protein (RS1) which could modulate Na^+/D-glucose cotransport (Veyhl et al., 1993; Koepsell and Spangengerg, 1994). The hypothesis suggests that Na^+/D-glucose cotransport is composed of two SGLT1 subunits in the membrane with one or two RS1 proteins located extracellularly, but anchored to the BBM at the C-terminus. The RS1 regulatory protein(s) may play a role in the modulation of glucose uptake and provide an explanation for the biphasic response observed by some researchers.

The molecular genetics of intestinal glucose transport has been reviewed (Wright et al., 1991; Philpott et al., 1992). Rabbit, rat and human SGLT1 have been cloned and sequenced (Hediger et al., 1987, 1989a; Lee et al., 1994). The protein contains 663 residues, of which 87% are identical in rabbit, rat and human amino acid sequences, while another 10% are conservative substitutions. Clones of the sodium-dependent glucose cotransporter in rabbit and pig kidney share 84% homology with the intestinal transporter (Coady et al., 1990). This high degree of homology suggests similar secondary and tertiary structures. The differences in the primary amino acid sequences of human, rabbit and rat SGLT1 are localized to the NH_2 terminus and two hydrophilic regions, one in the center and one near the COOH terminus (Hirayama et al., 1996).

SGLT1 is a 73 kD integral membrane protein (Semenza et al., 1984) that has 12 transmembrane spanning domains, with the amino and carboxyl terminals on the cytoplasmic face of the membrane. The primary amino acid sequence targets SGLT1 to the BBM (Kong et al., 1993). Glycosylation of the protein at Asn^{248} affects its function little (Hediger et al., 1991). Differences in substrate specificity are likely due the amino acid sequences near the COOH terminus (Panayotova-Heiermann et al., 1996; Hirayama et al., 1996). The human SGLT1 gene resides on chromosome 22 at the q11.2→qter region and is thought to be larger than 80 kb (Hediger et al., 1989b). Glucose-galactose malabsorption is an autosomal recessive disease resulting from a mutation on the SGLT1 gene. A single base mutation from guanine to adenine at

position 92 has been described that results in a change in amino acid 28 from an aspartate to asparagine (Turk et al., 1991). This single base mutation is sufficient to cause a severe, chronic diarrhea that can only be controlled by the removal of lactose, glucose and galactose from the diet.

SGLT1 may be regulated post-transcriptionally in lamb intestine. The decline in SGLT1 mRNA with weaning does not coincide with a reduction in activity or amount of SGLT1 protein. Age-related decline in Na^+/D-glucose transport in sheep is due to a decrease in glucose and galactose reaching the small intestine after the development of the rumen (Shirazi-Beechey et al., 1991). This study suggests that glucose promotes the maintenance and up-regulation of BBM transport protein.

The amount of SGLT1 protein does not correlate with the amount of SGLT1 mRNA in the ovine intestine (Lescale-Matys et al., 1993). In ovine intestine, SGLT1 mRNA is detectable just below the crypt-villus junction, peaks rapidly at 150 μ, and decreases toward the villus tip (Freeman et al., 1993a), similar to the pattern observed in rabbit (Freeman et al., 1993b). *In situ* hybridization studies in rabbit small intestine demonstrate SGLT1 mRNA is present in villus but not crypt cells, and increases six-fold from the villus base to the tip (Hwang et al., 1991). Another study in rabbit suggests that mRNA levels are constant from the crypt-villus junction to the villus tip (Smith et al., 1992). Differences in mRNA levels and SGLT1 expression may be due to transcriptional and post-transcriptional mechanisms (Wright, 1993).

There is no structural or functional homology between SGLT1 and the facilitated hexose transporters, including GLUT2 and GLUT5, which are expressed in the small intestine. SGLT1 is exclusive to the BBM of the small intestine. GLUT2 has been cloned and found to be present in the BLM of the small intestine, but is found also in liver, pancreas and kidney (Thorens et al., 1988). GLUT2 is present along the villus, but absent from the crypts.

GLUT5 is present in the BBM and BLM of the human small intestine, but also in brain and insulin-responsive tissues (kidney, heart, skeletal muscle, adipocytes) (Shepherd et al., 1992; Blakemore et al., 1995). GLUT5, a 47 kD protein, shares 40% homology with GLUT2, a 60 kD protein (Kayano et al., 1990). GLUT5 is maximally expressed in the mid-villus and is absent at the villus tip (Rand et al., 1993). GLUT5 is a high affinity fructose transporter in the human BBM (Burant et al., 1992a; Davidson et al., 1992; Kayano et al., 1990; Miyamoto et al., 1994) that has been cloned and sequenced in the rat (Inukai et al., 1993; Rand et al., 1993). Unlike the human GLUT5, rat GLUT5 is only expressed in intestine, kidney and brain. Rat GLUT5 shares 81.5% identity with human GLUT5, but has an affinity for fructose and glucose (Rand et al., 1993). GLUT5 mRNA levels are higher in rat duodenum and proximal jejunum than in distal jejunum and ileum (Castello et al., 1995). The levels of GLUT5 mRNA are low in fetal life, but progressively increase during the suckling period to almost adult levels after weaning. GLUT5 mRNA is also subject to circadian rhythm -- a 12 fold increase at the beginning of the dark cycle is correlated with increased GLUT5 protein at the BBM. Human GLUT5 may be regulated by cAMP to

increase transcription (Mahraoui et al., 1994). GLUT5 protein levels are regulated by fructose itself, by fructose interacting with the BBM to stabilize the protein (Burant and Saxena, 1994). During food deprivation, GLUT5 mRNA levels are elevated , suggesting insulin may be a negative regulator of GLUT5 expression (Castello et al., 1995).

1.6.b Enterocyte Lipid Absorption

The majority of lipids are absorbed in the proximal intestine and at the villus tips (Borgstrom et al., 1957; Haglund et al., 1973; Ladman et al., 1963; Fingerote et al., 1994). To be absorbed, lipids must diffuse across the UWL and permeate the BBM in order to be taken up by the cytoplasmic fatty acid binding proteins. The topic of lipid uptake has been reviewed carefully (Shiau, 1987; Thomson et al., 1989a, 1993; Davidson, 1994; Tso et al., 1991,1994). The products of lipid digestion (free fatty acids, monoacylglycerols, lysophosphatidylcholine and free cholesterol) are solubilized in mixed micelles composed of bile acids, cholesterol and phospholipids which dissociate prior to absorption. The bile acids are usually taurine or glycine conjugates of cholic and chenodeoxycholic acids and the phospholipid is predominantly phosphatidylcholine. Dietary cholesterol is solubilized in dietary triacylglycerol, and may not be absorbed as easily as biliary cholesterol (Dulery and Reisser, 1982; Samuel and McNamara, 1983).

The bile salt concentration of hepatic bile is approximately 35 mM. Subsequent dilution of bile with the luminal contents of the proximal to mid-intestine results in a concentration of 10-20 mM in fed animals, and this diminishes to 2-3 mM in the terminal ileum. Bile acid micelles greatly enhance the uptake of fatty acids and cholesterol (Westergaard and Dietschy, 1976). Cholesterol and phosphatidylcholine transfer to BBMV occurs at a faster rate from taurocholate mixed micelles than from phospholipid mixed micelles or small, unilamellar phospholipid vesicles (Thurnhofer et al., 1991; Nichols, 1988a,b). BBMV incubation with phospholipid vesicles is associated with a 1:1 lipid exchange that comes to equilibrium. All phospholipid classes, cholesterol and glycolipids, are exchanged (Lipka et al., 1991). Bile salts increase the transfer rate of lipid from the micelles when uptake across the BBM is not protein-mediated. The type of bile salt used to solubilize cholesterol influences the efficiency of cholesterol absorption, taurocholate being the most efficient solubilizer. Cholesterol absorption is reported to be increased when solubilized in taurocholate, a trihydroxy bile acid; and it is unaffected or reduced when the cholesterol is solubilized in dihydroxy bile acids (Ponz de Leon et al., 1979). Cholesterol absorption from taurocholate mixed micelles, however, is not accompanied by taurocholate absorption (Thurnhofer et al., 1991).

Passive Lipid Uptake

Lipid uptake has been described generally as an energy-independent, passive diffusion process down a concentration gradient, from the intestinal lumen, across the BBM, to the cytosol of the enterocyte. Passage across the BBM is the rate-limiting step for the uptake of short- and medium-chain fatty acids, while the passive uptake of long-chain fatty acids is rate-limited by

passage through the unstirred water layer (Proulx et al., 1984a; Westergaard and Dietschy, 1974). Fatty acid binding to BBM increases with fatty acid chain length and saturation (Proulx et al. 1984a,b,c). These fatty acids cannot be removed with the addition of fatty acid-free albumin, which suggests that these lipids intercalate within the hydrophobic hydrocarbon core of the BBM. The fatty acid partition coefficient increases with chain length by a factor corresponding to a decrease in the incremental change in free energy moving from an aqueous to a lipid phase ($\delta\Delta F_{w \rightarrow l}$) equal to -820 cal/mol. The partition coefficient decreases with increases in fatty acid unsaturation by a factor corresponding to an increase in the value of $\delta\Delta F_{w \rightarrow l}$ equal to +1178 cal/mol per double bond.

Passive lipid uptake has been proposed to occur by three models (Thomson and Dietschy, 1981):

1) Uptake of the entire intact mixed micelle across the BBM. This model is not supported by any experimental evidence (Wilson and Dietschy, 1972).
2) Collision of the mixed micelle with the BBM allows lipid to be taken up directly from the micelle to the BBM. This "collision" model has been proposed for cholesterol uptake, since cholesterol uptake has been demonstrated to increase linearly with increasing bile acid concentration (Proulx et al., 1984c; Burdick et al., 1994).
3) Lipids must first dissociate from the micelle into the aqueous compartment of the UWL before being taken up by the BBM. Westergaard and Dietschy (1976) provided support for this "aqueous" or "dissociation" model by demonstrating that fatty acid uptake decreased in the presence of an increasing number of bile acid micelles where fatty acid concentration was kept constant. Cholesterol monomer concentration has been observed to increase linearly in proportion to cholesterol concentration and cholesterol uptake (Chijiiwa and Linscheer, 1987).

A modification of the third model suggests the dissociation of lipids from the mixed micelles is thought to be mediated via the low pH of the acidic microclimate adjacent to the BBM (Shiau and Levine, 1980; Shiau, 1990). A low pH increases the critical micellar concentration of fatty acids and cholesterol. Fatty acids become protonated in the presence of an acidic microclimate, thereby increasing their hydrophobic and lipophilic properties so that they may partition into the outer bilayer of the BBM. Protonated fatty acids partition quickly or flip from the outer bilayer to the inner bilayer of the BBM (Small et al., 1984; Higgins, 1994). Exposure to the neutral pH of the enterocyte cytoplasm results in the deprotonation of the fatty acids and subsequent release of the ionized (charged) fatty acid and the proton into the cytosol. Since fatty acid uptake across the BBM is likely coupled with a positive charge, as observed with arachidonic acid uptake into the mucosa of the distal colon (Calderaro et al., 1991), the electrical potential across the BBM may play a role in the uptake of fatty acids (Perkins and Cafiso, 1987). Whether the collision or the

aqueous-dissociation model prevails depends upon the lipid itself, and the composition of the micelle (Burdick et al., 1994; Thomson and Cleland, 1981).

The rate-limiting step in fatty acid permeation across the BBM may be the outer third of the BBM bilayer (Meddings, 1988). The jejunal BBM is exposed to the majority of dietary lipids, and correlates with an increase in the fluidity of the outer third of the luminal bilayer (Meddings, 1988). This contrasts with the decrease in the fluidity of the outer third of the ileal BBM, which is exposed to far less dietary lipid. It has also been suggested that the rate-limiting step for lipid uptake is the translocation of lipid from the outer bilayer to the inner bilayer (Devaux, 1991). This transmembrane movement depends upon the composition and properties of the two membrane bilayers, as well as on the properties and composition of the lipid to be absorbed (Meddings, 1988; Meddings and Thiessen, 1989). Uncharged lipids, such as protonated fatty acids, are transported more rapidly across the bilayer than charged lipids, like unprotonated fatty acids and lysophospholipids (Perkins and Cafiso, 1987). Extrinsic factors, therefore, influence the rate of lipid absorption. A low pH environment allows protonation of the fatty acids which increases their rate of permeation across the membrane (Higgins, 1994). The type of other lipids present in the intestinal lumen also influences the absorption of cholesterol and fatty acids by altering the saturation of the bile acid micelles serving as lipid carriers in the luminal fluid. In addition, cytosolic fatty acid binding proteins may provide a 'sink' (intracellular enzymes that metabolize fatty acids) which promotes fatty acid absorption by changing the equilibrium of fatty acids across the membrane (Higgins, 1994).

Polyunsaturated fatty acids (18:1. 18:2, 18:3, 20:4) and phosphatidylcholine (but not lysophosphatidylcholine) may inhibit cholesterol absorption (Hollander and Morgan, 1980) due to:

1) changes in cholesterol solubility in the micelle which shifts the partition coefficient of cholesterol away from the cell membrane to the micelle; therefore an increase in micellar solubility results in a decline in monomer concentration; and
2) fatty acids and phospholipids which increase micelle size, and therefore the micelle diffuses more slowly than in the smaller micelles containing only cholesterol; the larger fatty acid- and phospholipid-containing micelles have an increased resistance in the UWL that is compounded by the increase in negative charge at the surface at the micelle.

In the jejunum, bile acids are absorbed passively. Bile acid absorption increases by protonation, fewer hydroxyl groups and by deconjugation, but decreases when bile acids form mixed versus simple micelles (Thomson et al., 1993).

Protein-Mediated Lipid Uptake

Lipid uptake may also be protein-mediated. Evidence for protein-mediated fatty acid uptake into the intestine is supported by the following studies:

1) a curvilinear relationship between uptake and very low concentrations of fatty acid (Chow and Hollander, 1979; Stremmel, 1988; Schoeller et al., 1995a);

2) inhibition of fatty acid uptake and loss of the saturable component of fatty acid uptake by trypsin, chymotrypsin, pronase or heat treatment (Potter et al., 1989; Schoeller et al., 1995a; Stremmel, 1988);
3) Na^+- and pH-dependent fatty acid uptake (Stremmel, 1988; Ling et al., 1989; Schoeller et al., 1995a);
4) identification of a plasma membrane fatty acid binding protein ($FABP_{pm}$) in the BBM of rat intestine (Stremmel et al., 1985); and
5) inhibition of fatty acid uptake by a polyclonal antibody to the $FABP_{pm}$ (Stremmel, 1988) and monoclonal antibody to the $FABP_{pm}$ (Schoeller et al., 1995b).

Recently, Schaffer and Lodish (1994) used an expression cloning strategy and a cDNA library from 3T3-L1 adipocytes to identify a cDNA that when expressed, increases the rate of long chain fatty acid uptake. This cDNA encodes a novel 646 amino acid, 71 kDa hydrophobic fatty acid transport protein (FATP) with six membrane-spanning segments. FATP was expressed in mouse skeletal muscle, heart, fat, and to a lower degree in brain, kidney, lung and liver, but was not expressed at all in spleen or jejunal mucosa under the hybridization conditions used and awaits further study.

At high concentrations, fatty acid uptake is linear with increasing fatty acid concentration (Chow and Hollander, 1979; Keelan et al., 1992; Ling et al., 1989; Thomson and Dietschy, 1984). Reducing luminal sodium concentration was associated with a decline in the *in vitro* uptake of short-, medium- and long-chain fatty acids into intestinal sheets (Thomson, 1982). Stremmel (1988) was able to demonstrate Na^+-dependent fatty acid uptake at low concentrations of fatty acid with isolated rat jejunal enterocytes, and with *in vivo* rat jejunal perfusion, by inhibiting Na^+/K^+-ATPase or by replacement of Na^+ with K^+ in the pH 6.5 bath solutions. He was unable to demonstrate Na^+-dependent fatty acid uptake into isolated brush border membrane vesicles. Ling et al. (1989) reported the presence of a Na^+-dependent overshoot when studying the uptake of linoleic acid with time into rabbit jejunal BBMV. Under the same experimental conditions, this Na^+- dependent overshoot could not be confirmed by Keelan et al. (1992). A time-course of oleic acid uptake into rabbit jejunal BBMV was similar in the presence of only an inwardly-directed Na^+ gradient, only an outwardly-directed H^+ gradient, or the absence of either a Na^+ or H^+ gradient, but it increased markedly in the presence of opposing Na^+ and H^+ gradients (Schoeller et al., 1995a). Following treatment of the BBMV with trypsin and chymotrypsin, the time course of oleic acid uptake in the presence of opposing Na^+ and H^+ gradients was reduced to the level observed in the absence of Na^+ and H^+ gradients. A curvilinear relationship between oleic acid uptake and increasing concentration was observed only in the presence of opposing Na^+ and H^+ gradients. This curvilinear relationship was not observed following trypsin and chymotrypsin treatment of the BBMV. These results suggest that fatty acid uptake is indirectly dependent on Na^+, and requires the double exchange of Na^+ and H^+ that is facilitated by NHE_3, the Na^+/H^+-antiporter in the BBM (Schoeller et al., 1995a). Oleic acid binding to trypsin-treated rat BBMV is maximal at pH 4, and

can be reduced by 80% at pH 8 (Stremmel et al., 1985). Stremmel (1988) observed an inhibition in the uptake of oleic acid into isolated rat jejunal enterocytes and *in vivo* rat jejunal perfusion in the presence of rabbit anti-rat liver $FABP_{pm}$ antibody. The antibody to the $FABP_{pm}$ reduces oleic acid uptake into rabbit jejunal BBMV only in the absence of opposing Na^+ and H^+ gradients (Schoeller et al., 1995b). These studies suggest that:

1) oleic acid binds passively in an uncharged, protonated form to the BBM lipids under the conditions of low luminal pH, typical of the acidic microclimate (Stremmel et al., 1985; Schoeller et al., 1995a); and
2) the $FABP_{pm}$ plays an important role in fatty acid uptake only in the absence of sodium or at a low luminal pH (Stremmel, 1988; Schoeller, 1995b), as observed with some conditions of malabsorption (Lucas and Mathan, 1989; Lucas et al., 1976,1978).

The intestinal BBM $FABP_{pm}$ is a 40 kDa extrinsic membrane protein that has been localized to the BBM in the villi and crypts of rat jejunum and ileum (Stremmel et al., 1985). No carbohydrates are associated with the $FABP_{pm}$, which has a pI of approximately 9. $FABP_{pm}$ is conspicuously absent from the *m*ucosa of the esophagus and colon; and this supports the suggestion that $FABP_{pm}$ is involved in the transport of fatty acid into the small intestine (Stremmel et al., 1985). The small intestinal $FABP_{pm}$ is immunologically and functionally similar to $FABP_{pm}$ found in hepatocytes, adipocytes and cardiac myocytes (Potter et al., 1987; Sorrentino et al., 1988), but it is distinct from the family of cytosolic fatty acid binding proteins (Veerkamp et al., 1991). The $FABP_{pm}$ has a high affinity for long chain fatty acids when they are present in low concentrations. It can also transport other lipids including monoacylglycerol, lysophosphatidylcholine and cholesterol, but not bile acids (Stremmel, 1988). The kinetic constants for fatty acid uptake have been reported for rat jejunal enterocyte $FABP_{pm}$: a Vmax of 2.1 nmol/min, and a Km of 93 nM (Stremmel, 1988).

Other workers have also demonstrated that cholesterol and phosphatidylcholine uptake is protein-mediated (Thurnhofer and Hauser, 1990a,b). The BBMV absorption of cholesterol from either mixed micelles or small unilamellar phospholipid vesicles is a second-order reaction consistent with the collision model of lipid transfer (Mütsch et al., 1986; Thurnhofer and Hauser, 1990a,b). Proteolytic treatment of the BBMV changes cholesterol absorption from a second-order to a first-order reaction, which is consistent with the monomeric diffusion model of lipid transfer through an aqueous phase and passive permeation through the BBM (Thurnhofer and Hauser, 1990b; Thurnhofer et al., 1991). Phospholipid exchange between BBMV and small unilamellar phospholipid vesicles is abolished when BBMV are treated with proteinases (Lipka et al., 1991; Thurnhofer and Hauser, 1990a). The proteins released into the supernatant following proteolytic treatment of BBMV by either intrinsic or extrinsic proteinases exhibited cholesterol and phospholipid exchange activity between two populations of small unilamellar phospholipid vesicles (Thurnhofer and Hauser, 1990a,b). A cholesterol-transfer protein has been partially purified and

characterized in the BBM of rabbit small intestine (Thurnhofer et al., 1991). Cholesterol-transfer protein is an integral membrane protein with an apparent molecular weight between 12000 and 14000. The pI of the purified cholesterol-transfer protein is between 9.1 and 9.4.

Protein-mediated transfer of slightly water soluble lysophospholipid and short-chain diacyl phospholipid has been described across rabbit intestinal BBM using fluorescent labelled phospholipid (Zhang and Nichols, 1994). Lysophospholipid and phospholipid transport occurred without any redistribution of highly water insoluble long-chain diacyl phospholipid between the outer and inner leaflets of the BBM. Phospholipid transport across membranes may be an active process requiring ATP (Devaux, 1991). Expression of a mouse liver multidrug resistance gene 2 (mdr2) in yeast revealed the gene product to be a phospholipid transporter (Ruetz and Gros, 1994). A null mutation at the mdr2 gene in mouse bile canalicular membrane resulted in a 50% decline in bile phosphatidylcholine of heterozygotes and absolutely no phosphatidylcholine in bile from homozygotes (Smit et al., 1993). It is not known if mdr2 is expressed in the intestine, or whether its gene product will function as a phospholipid transporter as it does in liver. The human multidrug resistance gene 2 (MDR2) has 90% homology with multidrug resistance gene 1 (MDR1), which encodes a P-glycoprotein capable of pumping hydrophobic cytotoxic drugs out of the cell.

Bile acid absorption has been recently reviewed (Aldini et al, 1995). The major site of bile acid absorption is the terminal ileum (Lewis and Root, 1990). The BBM bile acid transporter is sodium-dependent and possesses a higher affinity for unconjugated than for conjugated bile acids (Lin et al., 1990; Marcus et al., 1991). The Na+/bile acid cotransporter has been cloned from rat, hamster and human ileum (Schneider et al., 1995; Wong et al., 1994, 1995), and is subject to both translational and post-translational regulation (Minami et al., 1993). Dihydroxy bile acids are the most potent inhibitors of uptake of the other bile acids. The active site of the bile acid transporter consists of a steroid recognition site, a positive charge for coulombic interaction with the anionic bile salts, and a negative charge near the positively charged site that interacts with Na^+ (Thomson et al., 1993). An anion exchanger may transport bile acids across the BLM (Weinberg et al., 1986), although recent studies suggest a kinetically and structurally distinct transporter exists in enterocyte BLM (Lin et al., 1993; Simon et al., 1990).

Intracellular Transport of Absorbed Lipids

In the cytosol of the enterocyte, fatty acids are bound and transported by two cytosolic fatty acid binding proteins ($FABP_c$) to their sites of metabolism: the intestinal type (I-$FABP_c$) which is present exclusively in the intestine, and the liver type (L-$FABP_c$) which is present in both the liver and intestine. These $FABP_c$ are 14-15 kDa proteins that belong to an ancient gene family of fatty acid binding proteins (Kaikaus et al., 1990). Studies examining the crystal structure of rat I-$FABP_c$ suggest that the carboxylate group of a bound fatty acid forms a quintet of electrostatic interaction with Arg_{106}, Glu_{115}, and two solvent molecules, with several possible entry and exit locations within the I-$FABP_c$ (Sacchettini et al., 1988, 1989a). The fatty acid-protein interactions

have been further studied and suggest that side chains of the residues of the carboxyterminus end of I-$FABP_c$ are in contact with the bound fatty acid (Sacchettini et al., 1989b, 1990).

I-$FABP_c$ is localized to the mature villus tip cells and is not usually present in the crypt cells, while L-$FABP_c$ is confined to the crypt-villus junction and not present in villus tip cells (Iseki and Kondo, 1990a,b; Iseki et al., 1990; Sweetser et al., 1988a,b). L-$FABP_c$ appears in the crypt cells only under the conditions of fasting, likely in order to obtain fatty acids from the blood (Iseki and Kondo, 1990a,b). Both L-$FABP_c$ and I-$FABP_c$ exist in greater concentration in the proximal, compared with the distal intestine by a factor of 2 and 1.3 fold respectively (Bass, 1985; Sweetser et al., 1987,1988a,b).

The FABP concentration in the cytosol is approximately 0.2-0.4 mM (Ockner et al., 1982). L-$FABP_c$ and I-$FABP_c$ bind free fatty acid that has been desorbed from the BBM, but there is no direct interaction with the BBM (McCormack and Brecher, 1987). The lipid binding characteristics of the two $FABP_c$ are quite different (Lowe et al., 1987; Cistola et al., 1989). The stoichiometry of moles of fatty acid, bound to moles of binding protein, is 1:1 for I-$FABP_c$ and 1-3:1 for L-$FABP_c$. Earlier reports stated I-$FABP_c$ binds palmitic acid, oleic acid and arachidonic acid with equal affinity, while L-$FABP_c$ binds both saturated and unsaturated fatty acids, monoacylglycerols, lysophospholipids and bile salts, but not cholesterol (Cistola et al., 1989,1990; Kaikus et al., 1990; Peeters et al., 1989). In addition, low molecular weight microsomal and cytosolic bile acid binding proteins may be involved in the intracellular transport of bile acids (Lin et al., 1990). However, I-$FABP_c$ binds saturated fatty acids with greater affinity than L-$FABP_c$, while L-$FABP_c$ binds polyunsaturated fatty acids with greater affinity than I-$FABP_c$. A more recent study used fluorescently labelled Lys_{27} of I-$FABP_c$ to measure the K_d for a series of fatty acids (Richieri et al., 1994). Lys_{27} undergoes a change in orientation upon fatty acid binding to I-$FABP_c$, permitting a direct measure of fatty acid bound to protein. The K_d ranged from 2-1000 nM and varied as much as 80 fold, with the K_d for saturated fatty acids (palmitate, stearate) much less than for polyunsaturated fatty acids (linoleate, linolenate, arachidonate). L-$FABP_c$ also binds growth factors, prostaglandins and leukotrienes, which suggests that L-$FABP_c$ may have a role in the regulation of enterocyte growth and differentiation (Ockner, 1990). Unlike the pH sensitive L-$FABP_c$, I-$FABP_c$ has the unique property of pH insensitivity that allows it to bind fatty acids at a pH as low as 4.

The reason for the presence of two different $FABP_c$ is not known. Two $FABP_c$ may be required in the enterocyte to control the movement of fatty acids from different enterocyte cytosolic compartments: I-$FABP_c$ binds luminally derived fatty acids absorbed across the BBM, while L-$FABP_c$ binds fatty acids from the bloodstream (Gangl and Ockner, 1975). The ability of I-$FABP_c$ to bind fatty acids at low pH suggests that the I-$FABP_c$ binds the protonated fatty acids, while L-$FABP_c$ binds only the unprotonated fatty acids (Cistola et al., 1989, 1990). Different binding specificities and properties may aid in the targetting of lipids to their sites of metabolism.

Only a few long chain fatty acids can bind to I-FABP$_c$, while L-FABP$_c$ binds non-specifically to many lipids (Cistola et al., 1989, 1990; Kaikus et al., 1990; Peeters et al., 1989).

The rat intestinal L-FABP$_c$ and I-FABP$_c$ genes and human I-FABP$_c$ have been cloned in E. coli (Gordon and Lowe, 1985; Gordon et al., 1985; Lowe et al., 1987; Sweetser et al., 1986,1987). The rat I-FABP$_c$ gene has been partially defined and contains 3400-4000 nucleotides, four exons and three introns (Sweetser et al., 1987). The mouse I-FABP$_c$ gene has been sequenced (Green et al., 1992). Comparison with the rat and human I-FABP$_c$ gene shows three conserved domains of the 5' nontranscribed sequences. One 14-base pair (bp) element may establish and maintain region-specific expression along the duodenal-colonic axis. In mice, this 14-bp element binds to two members of the steroid hormone receptor superfamily of transcription factors produced in enterocytes and CaCo-2 cells: hepatic nuclear factor 4(HNF-4) and apolipoprotein regulatory protein-1 (ARP-1). These factors interact with the 14-bp element to activate I-FABP$_c$ -103 to +28/hGH+3. This activation is affected by elements between nucleotides -277 and -104 and other transcription factors (Rottman and Gordon, 1993).

Of the total mRNA present in the rat intestine, approximately 2.1 % is L-FABP$_c$ mRNA and approximately 1% is I-FABP$_c$ mRNA. L-FABP$_c$ mRNA makes up 0.7% of rat liver (Bass, 1985). FABP$_c$ concentrations and mRNA levels are similar in male and female rats (Bass et al., 1985). However, I-FABP$_c$ mRNA levels are species-dependent. I-FABP$_c$ mRNA is present in both the small and large intestine of humans, rats and monkeys (Gordon and Lowe, 1985; Sweetser et al., 1987). But in the mouse, I-FABP$_c$ mRNA exists largely in the small intestine, with very little present in the large intestine (Sweetser et al., 1988a).

Starvation, diet, and peroxisomal proliferators influence the quantity of FABP$_c$ in rat intestine, and suggest a differential nutritional regulation of I- and L-FABP$_c$ gene expression. Fasting is associated with increases in the concentrations of L-FABP$_c$ and I-FABP$_c$ concentration in the villus cells of the proximal intestine and the appearance of L-FABP$_c$ in the crypts (Bass, 1985; Iseki et al., 1989; Ockner and Manning, 1974). Feeding a low fat diet results in a reduction of both L-FABP$_c$ and I-FABP$_c$ concentration in the proximal intestine, while feeding a high fat diet increases the concentration of ileal L-FABP$_c$ and ileal I-FABP$_c$. In addition, a high fat diet decreases jejunal L-FABP$_c$, but not jejunal I-FABP$_c$ (Bass, 1985; Clark and Armstrong, 1989; Besnard et al., 1991). Peroxisomal proliferators, such as acetylsalicylic acid, clofibrate and tiadenol, increase FABP$_c$ in liver and intestine (Wilkinson and Wilton, 1986; Kawashima et al., 1985) which correspond to increases in mRNA content (Bass et al., 1985).

1.7 Metabolism of Dietary Fat in the Enterocyte

Once in the enterocyte cytosol, lipids diffuse to the endoplasmic reticulum (ER) for further metabolism within the enterocyte, including lipoprotein assembly for exit via the lymphatic pathway, or direct passage through the enterocyte into the portal vein. Fatty acid chain-length and

unsaturation influences how fatty acids are transported or metabolized (Gangl and Ockner, 1975; Shepherd et al., 1978, 1980). Short- and medium-chain fatty acids remain unesterified and pass directly into the portal vein (Bloom et al., 1951; Carlier and Bezard, 1975). Long-chain fatty acids may also pass into the portal blood, although this pathway is saturable at a level that varies with each fatty acid (McDonald et al., 1980). Studies that infuse linoleate and linolenate reported that more than 50% of these unsaturated fatty acids bypass the lymphatic pathway and enter the portal route (McDonald et al., 1980). The portal route of long-chain fatty acid transport may play an important role in conditions such as pancreatic insufficiency: lipase deficiency results in reduced luminal concentrations of long-chain fatty acids and no chylomicron synthesis. Yet 50% of the dietary fat is absorbed (Shimoda et al., 1974). With abetalipoproteinemia, patients are able to absorb up to 80% of their dietary fat despite the fact that the enterocytes are unable to form apolipoprotein particles. As a result there is accumulation of lipid within the absorptive cells (Ways et al., 1967).

Metabolism may direct fatty acids for re-synthesis of triacylglycerols via reacylation of mono- and diacylglycerols, esterification of cholesterol, synthesis of phospholipids via reacylation of lysophospholipids or *de novo* pathways. A study of radio-labelled palmitic and oleic acid demonstrated their diffusion throughout the cell after absorption (Haglund et al., 1973). These re-synthesized and newly synthesized lipids may be directed to membrane synthesis or lipoprotein assembly. Cholesterol and most of the saturated fatty acids are used for the assembly of chylomicrons and very low density lipoproteins (VLDL). The lipoprotein particles then pass into the lymphatic circulation via exit across the basolateral membrane (Gangl and Ockner, 1975).

Biliary phospholipid (primarily phosphatidylcholine) is absorbed from the lumen as lysophosphatidylcholine (LPC), re-acylated to phosphatidylcholine (PC) and then used for the formation of chylomicrons (Nilsson, 1968a; Scow et al., 1967; Rodgers et al., 1975). Luminal PC is the primary source of chylomicron PC (Mansbach, 1977). Dietary choline is used for *de novo* PC synthesis at the smooth ER for membrane synthesis (O'Doherty et al., 1973). Free cholesterol is absorbed and esterified at the ER for the formation of lipoproteins and chylomicrons, which then move toward the Golgi. Golgi vesicles fuse with the basolateral membrane to release chylomicrons into the intracellular space and then to the lymphatic circulation.

1.7.a. Fatty Acid Synthesis

Fatty acids which are absorbed from the intestinal lumen are converted to acyl-CoA by acyl-CoA synthetase for use in the synthesis of polyunsaturated fatty acids, diacylglycerols, triacylglycerols, phospholipids and cholesterol esters (Figure 1.4) (Brindley and Hubscher, 1966; Ohkubo et al., 1991). Acyl-CoA synthetase is distributed throughout the villus and crypts (Mansbach et al., 1975). Long-chain saturated fatty acids are activated faster than short-chain fatty acids or polyunsaturated fatty acids (Brindley and Hubscher, 1966).

Polyunsaturated fatty acids (PUFA) can be classified into 3 families: ω9, ω6, ω3 (Figure 1.5). These families cannot be interconverted, and thus the availability of fatty acids for metabolism depends upon the diet. Linoleic and linolenic acids are essential fatty acids obtained only from the diet, and cannot be synthesized *de novo*. Linoleic acid is the principle essential fatty acid in mammalian tissues and the precursor of ω6 fatty acids like arachidonic acid. Arachidonic acid is important for the synthesis of prostaglandins, thromboxanes and leukotrienes (Holman and Johnson, 1981). Although linolenic acid is the precursor of the ω3 fatty acids, the function and importance of these fatty acids remains unknown (Holman and Johnson, 1981). It has been speculated that ω3 fatty acids may be important in the regulation of blood cholesterol levels. Free fatty acids are alternately desaturated and elongated in the ER to form long-chain PUFA (Strittmatter and Rodgers, 1975; Holloway and Holloway, 1975, 1977). The ω6 family and the ω3 family of fatty acids use the same desaturase and elongase enzymes in their pathways of fatty acid synthesis (Figure 1.5). The conversion of linoleic acid (18:2ω6) to γ-linolenic acid (18:3ω6), as well as the conversion of α-linoleic acid (18:3ω3) to 18:4ω3 by Δ^6-desaturase is the rate-limiting step in arachidonic acid (20:4ω6) and eicosapentanoic acid (20:5ω3) synthesis respectively. Elongation is much slower than desaturation, and depends upon the presence of albumin to mediate the initial and terminal reactions (Sprecher, 1981).

Desaturases are intrinsic membrane proteins of the endoplasmic reticulum (Rogers and Strittmatter, 1973, 1974) that catalyze the stereospecific removal of two hydrogen atoms from a pre-formed fatty acyl-CoA derivative of exogenous or endogenous origin, to form a *cis* double bond (Jeffcoat, 1979). Only partial purification of desaturases has been reported due to the difficulty in overcoming their requirement of microsomal lipids for their activity (Holloway, 1971; Shimakata et al., 1972). Desaturases require a short electron transport chain comprised of NADH-cytochrome *c* reductase and cytochrome b_5 , an electron donor (NADH or NADPH) and molecular oxygen (Holloway and Holloway, 1975, 1977). Desaturases are classified according to the position on the fatty acid from which the hydrogen atoms are removed (Δ^9, Δ^6, Δ^5-desaturases). There is no direct evidence for the existance of a Δ^4-desaturase. The proposed existence of Δ^4-desaturase to convert 22:5ω3 to 22:6ω3 has been disproved. A study in rat liver by Voss and coworkers (1991) demonstrated that 22:5ω3 is not converted directly to 22:6ω3. Instead, 22:5ω3 is initially elongated to 24:5ω3, desaturated by a Δ^6-desaturase to 24:6ω3, and converted to 22:6ω3 by one round of β oxidation (Figure 1.5).

Microsomal membrane lipid composition as well as hormonal and nutritional factors influence hepatic desaturase activity (Brenner, 1981; Table 1.2). Desaturase activity is increased by insulin and reduced by glucagon, epinephrine, glucocorticoids and thyroxine. ω6 and ω3 PUFAs inhibit the activity of Δ^9, Δ^6 and Δ^5 desaturases (Mahfour et al., 1980; Cook, 1981; Shimp et al., 1982; Pugh and Kates, 1984). Linoleate regulates Δ^6 and Δ^5 desaturase activities, but

evokes an antagonistic effect to avoid accumulation of 20:3ω6, thereby coordinating the synthesis of PUFA (Brenner, 1981). The ω3 fatty acids inhibit ω6 fatty acid synthesis, and ω6 fatty acids weakly inhibit ω3 fatty acid synthesis. But both ω6 and ω3 fatty acids inhibit ω9 fatty acid synthesis (Holman and Johnson, 1981). The dietary ω3 fatty acids contained in linseed oil (18:3ω3) and fish oil (20:5ω3, 22:6ω3) reduce rat liver microsomal Δ^9, Δ^6 and Δ^5 desaturase activities (Garg et al., 1988c,d,e; Muriana et al., 1992). Dietary ω3 fatty acids do not cause any changes in Δ^6 desaturase activity in small intestinal microsomes (Garg et al., 1990). Dietary cholesterol stimulates Δ^9 desaturase activity, but it inhibits or reduces Δ^6 and Δ^5 desaturase activities in rat liver microsomes (Garg et al., 1986; Muriana et al., 1992). When cholesterol is supplemented to a diet enriched in ω3 fatty acids, the cholesterol-associated increase in Δ^9 desaturase activity is prevented (Garg et al., 1988c). Fasting reduces the activity of Δ^9 and Δ^5 desaturases, while feeding a fat-free diet increases Δ^9 desaturase activity in liver microsomes (Brenner 1981; Oshino and Sato, 1972). In the small intestine, Δ^6 desaturase activity increases with fasting, while Δ^9 desaturase activity reduces (Garg et al., 1990, 1992). Refeeding is associated with increases in hepatic desaturase activity which may be blocked in the presence of cycloheximide. This suggests that the enzyme can be induced (Oshino and Sato, 1972). Liver microsomal Δ^9 desaturase activity increases when animals are refed with a high carbohydrate diet, while Δ^6 desaturase activity increases when animals are refed a high protein diet (Brenner 1981; Oshino and Sato, 1972). Many pathological conditions also may be associated with alterations in desaturase activity, including diabetes mellitus, chronic alcoholism, inflammatory bowel disease, cystic fibrosis, hepatomas and certain neurological disorders (Mercuri et al., 1974; Garg et al., 1990). Diabetes mellitus is correlated with a decline in desaturase activity that may be restored to normal levels by insulin treatment (Gelhorn and Benjamin, 1966; Garg et al., 1990).

Although desaturases are present in the microsomes of liver, brain, testes and kidney microsomes (Brenner, 1971; Clandinin et al., 1985a,b; Brenner et al., 1986; Kawata et al., 1987), desaturases are not evenly distributed in all organs; therefore, some organs may depend upon the liver for their synthesis and supply of arachidonic acid (Brenner, 1971). Although the presence of Δ^9 desaturase activity in the small intestine was briefly reported (Ehrlich et al., 1985), it was not until 1988 that the presence of Δ^9 and Δ^6 desaturase activity was described in the homogenates of jejunal and ileal mucosa (Garg et al., 1988a). Further studies localized enterocyte Δ^6 desaturase activity to microsomal membranes with no activity detected in BBM or BLM (Garg et al., 1990). Desaturation and elongation of 18:3ω3 is highest in villus cells, but acylation of 18:3ω3 into phospholipids in crypts is two-fold greater than in villus cells (Chen and Nilsson, 1994). Since the enterocyte is capable of *de novo* synthesis of arachidonic acid, the small intestine does not depend on the liver for supply of this fatty acid. The differences in Δ^6 desaturase activity reported in jejunal versus ileal mucosal homogenates and microsomes (Garg et al., 1988a,b) suggest that

desaturase activity may influence the differences in fatty acid composition reported in jejunal and ileal EMM (Garg et al., 1990) and BBM (Keelan et al., 1990a).

1.7.b. Triacylglycerol Synthesis

In the enterocyte, triacylglyerol is synthesized by reacylation of absorbed mono- and diacylglycerols in the monoacylglycerol (MG) pathway, or *de novo* synthesis by the α-glycerophosphate (αGP) pathway (Figure 1.6). Both pathways are located on the outside cisternae of the Golgi (Mansbach, 1976, 1977). Re-esterification of 2-monoacylglycerol is catalyzed by a triacylglycerol synthetase complex comprised of monoacylglycerol acyltransferase (MGAT), diacylglycerol acyltransferase (DGAT), acyl CoA ligase (fatty acid activating enzyme) and acyl CoA acyltransferase (AAT) (Lehner and Kuksis, 1995). AAT must bind an acyl group before triacylglycerol synthesis begins. The MG pathway is rate-limited by diacylglycerol acyltransferase (Rodgers et al., 1967). Both pathways produce diacylglycerols for triacylglycerol synthesis, but it was previously thought that only the αGP pathway produces diacylglycerol for phospholipid synthesis. This would result in the presence of two diacylglycerol pools within the enterocyte. More recent studies suggest only one pool of diacylglycerol exists in the enterocyte (Lehner and Kuksis, 1992). This finding is in agreement with the existence of only one diacylglycerol acyltransferase (Grigor and Bell, 1982). Diacylglycerol is preferentially acylated to triacylglycerol (Johnston et al., 1970; Mansbach, 1973). DGAT does not exhibit any fatty acid specificity. MGAT is not influenced by diet (Rao and Abraham, 1974). 2-Monoacylglycerol inhibits the αGP pathway; therefore, when 2-monoacylglycerol levels are low, the αGP pathway becomes the major pathway of TG synthesis (Johnston, 1976; Kuksis and Manganaro, 1986).

Acyltransferases used for re-esterification of mono- and diacylglycerols are more active in the proximal than the distal small intestine, corresponding to a greater triacylglycerol synthesis in the proximal small intestine (Rodgers and Bochenak, 1970; Hoving and Valkema, 1969). The activity of these enzymes is also much greater in the enterocytes of the villus tips than in those of the intestinal crypt (Hoffman and Kuksis, 1982). This corresponds to the physiological and morphological site of mono- and diacylglycerol absorption. Acyltransferases are located in microsomes (Dallner, 1963; Rodgers and Singh, 1972; Mansbach, 1976) and dietary lipid is found predominantly in the smooth ER. Newly synthesized triacylglycerol is used for lipoprotein synthesis.

1.7.c. Phospholipid Synthesis

Membrane PC is synthesized by *de novo* pathways (Rodgers et al., 1973; Holub and Kuksis, 1978; Mansbach, 1976, 1977; McMurray and Magee, 1972). The intestine is a major source of *de novo* PC (Mansbach and Arnold, 1986). PC is the largest phospholipid constituent of many membranes, including intestinal BBM. The primary pathway of PC synthesis is the CDP-choline pathway (Figure 1.7) which is present in the small intestine (Weiss et al., 1958; Gurr et al., 1965; Mansbach, 1973). The enzymes of *de novo* PC synthesis are located in the microsomes,

mainly the rough ER (Mansbach, 1976), and are likely arranged in an organized fashion to facilitate channelling of the water-soluble substrates (Vance and Vance, 1990). Choline kinase converts endogenous or exogenous choline to phosphocholine. CTP:phosphocholine cytidylyltransferase converts phosphocholine to CDP-choline (Pelech et al., 1983a) and is the rate-limiting enzyme in this pathway (O'Doherty, 1980; Mansbach and Arnold, 1986). In the final step, CDP-choline: 1,2-diacylglycerol cholinephosphotransferase (CPT), in the presence of 1,2-diacylglycerol, converts CDP-choline to PC. *De novo* PE synthesis occurs by a similar pathway. Phospholipid synthesis may also be influenced by the availability of diacylglyerol and its fatty acid composition (Radominska-Pyrek et al., 1976; O'Doherty, 1980). In rat hepatocytes, cAMP-induced inhibition of PC biosynthesis is associated with decreased levels of diacylglycerol (Jamil et al., 1992). In HeLa cells, increased binding of cytidylyltransferase to diacylglycerol-enriched membranes results in increased PC biosynthesis (Utal et al., 1991). In the intestine, the CDP-choline pathway is the most active in the crypts, where membrane lipid must be rapidly synthesized for the newly generated enterocytes (Brindley, 1977).

In rat hepatocytes and liver microsomes, CPT prefers 1-palmitoyl over 1-stearoyl species of diacylglycerol in the *sn*-1 position (Holub, 1978; Tijburg et al., 1991). As a result, more PC have palmitate in the *sn*-1 position. Remodelling of the *sn*-1 and *sn*-2 positions is required to produce different species of PC. One possibility is the deacylation of palmitate from PC by phospholipase A_1 and reacylation of LPC by acyl CoA:2-acylglycerophosphocholine acyltransferase. *In vivo* rat studies demonstrate preferential reacylation of 2-linoeoyl-*sn*-glycerophosphocholine with stearate (Akesson et al., 1976). Another possibility is through the transacylase sytem which transfers an acyl residue from another phospholipid as described for rat liver microsomes (Sugoira et al., 1988). Remodelling the *sn*-2 position requires a phospholipase A_2, which is located in the mitochondria. Rat liver microsomes do not have any phospholipase A_2 (Waite, 1987). This suggests that PC must be transported to the mitochondria for remodelling the *sn*-2 position (Tijburg et al., 1991). The similar composition of microsomal and mitochondrial membranes would permit reversible transport of PC between the membranes.

A cytosolic form of CTP:phosphocholine cytidylyltransferase has been reported in rat liver (Choy et al., 1977), which may act as reservoir for the microsomal enzyme (Sleight and Kent, 1983; Tercé et al., 1988). PC synthesis is regulated by the distribution of the cytidylyltransferase enzyme between the cytosol and the microsomes. The enterocyte cytidylytransferase activity may be modulated by covalent phosphorylation/dephosphorylation, as it is in the liver where it is activated by a protein phosphate phosphatase and inactivated by a cAMP-dependent protein kinase (Pelech and Vance, 1982, 1984). When stimulated, the cytosolic enzyme is more active in the intestine than in any other organ, except the Chinese hamster ovary cell (Mansbach and Arnold, 1986). This enzyme is sometimes fully active under non-stimulated *in vitro* conditions, suggesting a rapid turnover of the microsomal enzyme, and necessitating replacement with cytosolic enzyme to return activity to normal. A continuous influx of phospholipid into the

enterocyte under resting conditions, for example, lysophospholipid from bile, could explain this nearly fully active state, except that LPC did not stimulate activity *in vitro*. If only a small amount of a specific phospholipid is required to partially activate the cytosolic enzyme, the digestion of sloughed cells may provide enough luminal phospholipid to activate this enzyme. Stimulation of the cytosolic enzyme with phospholipid is minimal (two-fold) when compared with other tissues. It is unknown why the intestinal cytidylyltransferase responds differently from other systems. It has been suggested that intestinal cytidylyltransferase connects closely to a positively charged lipid stimulator that increases activity submaximally so that additional lipid stimulators are effective (Mansbach and Arnold, 1986). Fatty acids inhibit intestinal cytidylyltransferase activity (Mansbach and Arnold, 1986), in contrast with the stimulated activity observed in lung (Weinhold et al., 1984) and liver (Pelech et al., 1983b). Human CTP:phosphocholine cytidylyltransferase has been cloned from a human erythroleukemic K562 cell library, and has close homology with cytidylyltransferase from rat liver, mouse testes, mouse B6SutA hemopoietic cells and Chinese hamster ovary (Kalmar et al., 1994). 100% homology was observed with the rat liver catalytic domain, but the most change was observed near the putative phosphorylation domain close to the C-terminus, where 7 changes occurred within 34 residues.

PC can also be formed by the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway (Figure 1.7). PEMT catalyzes the conversion of phosphatidylethanolamine (PE) to PC by base exchange using S-adenosylmethionine (SAM) as the methyl donor (Bremer and Greenberg, 1961). In rat liver, PEMT is regulated by reversible phosphorylation (Pelech et al., 1986), and contributes to 20-40% of synthesized PC (Vance and Vance, 1990). PEMT activity has been described in the BBM of the colon (Dudeja et al., 1986) and of the small intestine (Dudeja and Brasitus, 1987). PEMT has been cloned from rat liver (Cui et al., 1993). It has been shown that methylation of PE is not required to maintain cellular PE and PC composition in rat hepatocytes (Samborksi et al., 1993). In addition, methylation of PE to PC cannot substitute for an impaired CDP-choline pathway in mutant Chinese hamster ovary cells (Houweling et al., 1995).

A fourth source of PC synthesis within the enterocyte exists from sphingomyelin. Sphingomyelinase breaks down luminally absorbed sphingomyelin to phosphocholine and ceramide (Nilsson, 1968b, 1969). The liberated phosphocholine may be utilized for PC synthesis in the CDP-choline pathway (Figure 1.7). In rat hepatocytes, sphingomyelinase is regulated by an unidentified protein kinase and by either protein phosphatase 1 and/or 2A activity (Hatch and Vance, 1992).

Phosphatidylcholine (PC) is required for the synthesis of lipoproteins which transport triacylglycerols out of the enterocyte across BLM and into lymph. Luminal PC is absorbed as lysophosphatidylcholine (LPC) following its hydrolysis by phospholipases and LPC is then reacylated to PC by LPC acyltransferase in the presence of acylCoA (Figure 1.7). This pool of PC is specifically directed to lipoprotein synthesis, although the fatty acids are subject to modification from diet-derived fatty acids (Mansbach, 1972, 1973, 1975, 1977).

An alternative pathway has been proposed for PC synthesis from 2-monoacylglycerol resulting from inhibition of the αGP pathway by 2-monoacylglycerol (Lehner and Kuksis, 1992). When 2-monoacylglycerol levels are high, inhibition of the αGP pathway results in the inhibition of diacylglycerol production for phospholipid synthesis. However, the increased levels of diacylglycerol from the 2-monoacylglcyerol pathway may be utilized in the CDP-choline pathway for the synthesis of PC. This is possible if the diacylglycerol pool is the same for both triacylglycerol and phospholipid synthesis.

PC synthesis is influenced by diet. A high fat diet stimulates lysophosphatidylcholine (LPC) acyltransferase and cholinephosphotransferase (CPT) activity (Mansbach, 1975). LPC acyltransferase is stimulated to a greater extent, since LPC sources of PC are required for triacylglycerol transport. CTP:phosphocholine cytidylyltransferase activity is stimulated by LPC (O'Doherty et al., 1978), although a previous study did not observe any stimulation by LPC and, in fact, described the inhibition of cytidylyltransferase activity by fatty acids (Mansbach and Arnold, 1986).

De novo PE synthesis is similar to *de novo* PC synthesis. In rat liver, the enzymes for *de novo* PC and PE sythesis are located in the ER and Golgi (Jelsema and Morre, 1978). In the developing rat liver, CPT activity increases with cytidylyltransferase activity (Weinhold et al., 1973). Although the phosphatidylserine decarboxylase enzyme exists only in the mitochondria (Dennis and Kennedy, 1972), decarboxylation of phosphatidylserine (PS) provides another source of PE which is used for the assembly of VLDL in the ER and Golgi. PS synthesis occurs via PS synthase activity which catalyzes phospholipid base exchange of PE or PC with serine (Kuge et al., 1986). PC is the major substrate of PS synthase in the synthesis of PS (Kuge et al., 1986). PS synthase is located primarily in the ER, but also in the Golgi and the plasma membrane (Jelsema and Morre, 1978; Voelker, 1985; Vance and Vance, 1988). Phosphatidylinositol (PI) synthase is present in ER and Golgi (Jelsema and Morre, 1978). The phospholipid enzymes are present on the cytosolic surface of the ER and Golgi (Ballas and Bell, 1981; Coleman and Bell, 1978). The synthesized phospholipids must translocate to the luminal half of the bilayer in order for lipoprotein assembly to occur (Vance and Vance, 1990).

Studies in Wistar rats demonstrate that the enzymes which synthesize PC, PE, SM, PS and PI exist in enterocytes along the entire crypt-villus axis (O'Doherty, 1978). A gradient in enzyme activity was observed for several enzymes: LPC and lysophosphatidylethanolamine (LPE) acyltransferase activities, and enzymes involved in the synthesis of CDP-diacylglycerol, SM, PS and PI, were greater in villus tip cells compared with crypt cells. The greater activity of LPC acyltransferase in the villus tip cells is important for the synthesis of PC for chylomicron assembly, but this localization may also suggest that local retailoring of membrane phospholipids is necessary for mature enterocyte membrane function. LPC acyltransferase has been demonstrated in the intestine of the rat and hamster (Subbiah et al., 1969, 1970; Mansbach,

1972). LPC acyltransferase is located in the microsomes and is more active in cells of the villus tip than those of the crypt (Mansbach, 1975, 1976). The activity of this enzyme is also much greater in the proximal than the distal intestine (Rodgers and Bochenek, 1970; Hoving and Valkema, 1969). Choline- and ethanolaminephosphotransferase activities and phosphatidylserine decarboxylase activity were similar along the crypt-villus axis (O'Doherty, 1978).

1.7.d. Cholesterol Synthesis

Rapid cell turnover in the small intestine and lipoprotein synthesis demand a continuous supply of cholesterol. There are four sources of cholesterol for the enterocyte: diet, synthesis from biliary precursors, *de novo* synthesis from acetate, and cholesterol from LDL taken up by receptors at the BLM. A large number of low density lipoprotein (LDL) receptors are present on the BLM in the small intestine, second in amount only to liver (Spady et al., 1986). The small intestine is a major site of cholesterol synthesis except in the rat (Spady and Dietschy, 1983). For example, in man, only 19% of cholesterol is synthesized by the liver (Turley and Dietschy, 1982). *De novo* cholesterol synthesis from acetate is rate-limited by the enzyme 3-hydroxy-3-methylglutarylCoA reductase (HMGR), which catalyzes the conversion of 3-hydroxy-3-methylglutarylCoA to mevalonic acid. HMGR activity is located in the ER, and is much greater in distal than proximal intestine (Stange and Dietschy, 1983a). Some authors report HMGR to be similar in the villus and crypt cells (Sugano et al., 1977; Merchant and Heller, 1977: Muroya et al., 1977), while others report a greater HMGR activity in crypt than in villus tip cells (Stange and Dietschy, 1983a). Sterol synthesis in mature villus cells depends upon chylomicron synthesis (Gebhard and Prigge, 1981), while in crypt cells depends upon the rate of cell division (Stange et al., 1981a). When sterol synthesis is examined in isolated cells, ileal synthesis is 3-4 times greater than in the jejunum (Stange and Dietschy, 1983a). In mature villus cells, synthesis of sterol is inhibited by dietary biliary cholesterol absorbed in the jejunum. In crypt cells, sterol synthesis is uniform along the intestine.

Brasitus and Schachter (1982) have suggested that cholesterol synthesis in the enterocyte modulates the BBM cholesterol content. Cholesterol synthesis is regulated by the amount of cholesterol absorbed, as well as by bile acids, lipoproteins, insulin, and dietary protein (Dietschy and Siperstein, 1965; Westergaard and Dietschy, 1976; Stange et al., 1980; Oku et al., 1986; Feingold, 1989). Long-term regulation of HMGR actvity results from end-product inhibition of enzyme synthesis, repression of transcription (Osborne et al., 1988), and regulation at the level of translation (Nakanishi et al., 1988; Auwerx et al., 1989). Short-term regulation of HMGR involves reversible phosphorylation of the enzyme (Beg and Brewer, 1981; Oku et al., 1984; Gibson and Parker, 1987). Cholesterol synthesis from bile acid precursors bypasses HMGR in *de novo* synthesis, possibly due to the need to package fatty acids for transport out of the enterocyte (Strandberg, 1983).

Exogenous dietary and biliary cholesterol are absorbed in the upper two-thirds of the small intestine (Dulery and Reisser, 1982), likely due to the absence of a micellar phase in the

distal one-third of the small intestine, which is required for the uptake of cholesterol. Cholic acid, a trihydroxy bile acid, is a powerful stimulator and cofactor of cholesteryl ester hydrolase (CEH) (Treadwell and Vahouny, 1968). Absorbed free cholesterol is esterified by CEH or by acylCoA:cholesterol acyltransferase (ACAT) (Figure 1.8). Oleic acid is the preferred fatty acid for esterification of cholesterol. CEH is located in the cytosol, while ACAT is situated in the microsomal membrane (Heider et al., 1983; Johnson et al., 1983). CEH esterifies cholesterol prior to its lymphatic transport in the form of chylomicrons. Therefore, CEH activity is important under normal conditions (Gallo et al., 1978), whereas ACAT activity may be important under the conditions of cholesterol load, thereby regulating cholesterol absorption. Heider and coworkers (1983) demonstrated that if ACAT activity is blocked with an inhibitor (57-118) without affecting CEH, secretion of cholesterol into the lymph reduces.

Serum LDL levels regulate cholesterol synthesis in the small intestine (Stange et al., 1980). Gebhard and Prigge (1981) perfomed studies using the Thiry Vella loop technique to provide three pieces of evidence to support the regulation of cholesterol synthesis by serum LDL:

1) an increase in cholesterol synthesis in the loop which subsequently fell, suggested that cholesterol was obtained from a non-luminal source;
2) loop HMGR activity was inversely proportional to serum cholesterol levels during cholesterol and cholestyramine feeding, suggesting that the loop utilized LDL cholesterol; and
3) LDL inhibited HMGR activity in cultured mucosa from the loop, but not from intact ileum.

Another study suggests that an increase in lipoprotein cholesterol delivery to the intestine results in increased cholesterol synthesis in both crypt and villus tip cells (Panini et al., 1979). Studies by Stange and Dietschy (1983b) suggest that cholesterol synthesis and LDL uptake are regulated independently, while ACAT actvity may be regulated by LDL.

Diet-induced changes in hepatic and intestinal membrane fatty acid unsaturation may result in regulation of HMGR and ACAT activities -- the metabolic enzymes which monitor the amount of unesterified cholesterol within cells (Bochenek and Rodgers, 1977, 1978; Field et al., 1987). HMGR and ACAT regulate the amount of unesterified cholesterol required to process dietary fatty acids, and respond to diet-associated changes in membrane lipid composition in liver and intestine (Field and Salome, 1982). Stimulation of HMGR activity is similar by all C18 fatty acids (Gebhard and Prigge, 1981) and ω3 fatty acids influence ACAT expression (Drevon et al., 1979). Intestinal ACAT activity increases in rabbits fed safflower oil (Johnson et al., 1983) and in rats fed salmon oil, but not in those fed corn oil (Chauton et al., 1989). Cholesterol feeding reduces cholesterol synthesis (Feingold et al., 1983), but it may be influenced as well by the fatty acid composition of the high cholesterol diet. Dietary unsaturated fat may be a regulator of ACAT activity, but not HMGR activity. Dietary cholesterol supplementation increased ACAT activity in rats fed corn oil, but it did not increase activity any further when rats were fed salmon oil (Chauton

et al., 1989). When animals were fed menhaden oil, compared with those fed cocoa butter, ACAT activity increased, but could not be increased further with cholesterol supplementation (Field et al., 1987). HMGR activity was similar in animals fed either menhaden oil or cocoa butter, and reduced similarly with cholesterol supplementation on either lipid diet (Field et al., 1987). Cholesterol feeding decreases hepatic and intestinal HMGR activity, but increases the activity of ACAT (Purdy and Field, 1984). Prolonged cholesterol feeding completely inhibits hepatic HMGR activity, but does not completely inhibit intestinal HMGR activity; this suggests that cholesterol synthesis may shift to the intestine (Stange et al., 1981b). This contrasts with a study where liver ACAT activity decreased while HMGR activity increased in liver microsomes from rats fed either 10% olive oil or fish oil diets supplemented with 1 % cholesterol (Muriana et al., 1992). Intestinal cholesterol synthesis also depends upon the type of dietary protein. An increase in HMGR activity and cholesterol synthesis was observed in animals fed a soybean diet, compared to those fed a casein diet (Nagata et al., 1982; Tanaka et al., 1983).

1.7.e Lipoprotein Synthesis and Secretion

Intestinal lipoprotein synthesis, secretion and transport have been reviewed extensively (Green and Glickman, 1981; Bisgaier and Glickman, 1983; Thomson et al., 1989a, 1993; Vance and Vance, 1990; Davidson, 1994; Shepherd, 1994; Levy et al., 1995). The intestine synthesizes triacylglycerol-rich very low density lipoproteins (VLDL) and chylomicrons (CM), and some cholesterol-rich high density lipoproteins (HDL). VLDL are the major lipoproteins secreted by the intestine during fasting, while CM become the major lipoproteins secreted by the intestine following fat feeding. The formation and secretion of CM and VLDL by the intestine is not well understood. Calcium, copper and zinc are required for the assembly and release of CM (Strauss, 1977; Koo and Williams, 1981). CM and VLDL are synthesized as pre-CM and pre-VLDL. The pre-CM have a different phospholipid composition (less PC, more PE) than CM, and contain more free fatty acid, cholesterol and protein. Following assembly in the smooth ER, free pre-CM or pre-VLDL lipid droplets bud off from the smooth ER tubules to merge with the Golgi complex. Pre-CM and pre-VLDL are transported and released in groups, not as single particles (Sabesin and Frase, 1977). Vesicles containing pre-CM and pre-VLDL pinch off from the Golgi to become secretory vesicles containing coated pits which later incorporate into the BLM (Pearse 1978). In the *trans* Golgi secretory vesicles, the apoproteins of the pre-CM and pre-VLDL are glycosylated before exocytosis and release as CM and VLDL, across BLM, into the lymphatic circulation (Swift et al., 1984). Release of CM and VLDL occurs as a result of the merger of Golgi vesicles with the BLM; the Golgi vesicles are not exocytosed (Ottosen et al., 1980). Microtubules may also play a role in CM secretion by directing secretory vesicles to cell membrane recognition sites (Glickman et al., 1976; Stein et al., 1976).

Lipoproteins are composed of a hydrophobic core of triacylgycerol and cholesterol ester that is surrounded by a hydrophilic coat of phospholipid and free cholesterol interspersed with apoprotein. Unlike the fatty acid composition of CM phospholipids and cholesterol esters, the fatty

acid composition of CM triacylglycerols reflects the fatty acid composition of the diet (Feldman et al., 1983a,b). Biliary PC is preferentially incorporated into CM (Mansbach, 1977) and luminal PC is essential for maintaining lipoprotein secretion during high fat intake (Tso and Simmonds, 1977). During fasting, fatty acids derived from biliary lipids are used for CM and VLDL formation. Increases in the amount of free and esterified cholesterol in VLDL reflect increases in the dietary cholesterol content (Klein and Rudel, 1983; Swift et al., 1988; Vance and Vance, 1990). Several pieces of evidence suggest that CM and VLDL are assembled via different pathways:

1) The triacylglycerol fatty acid composition is different for CM and VLDL (Feldman et al., 1983a,b): CM TG reflects the fatty acid composition of the diet while VLDL TG reflects an endogenous origin;
2) CM formation is preferentially increased with increasing fatty acid absorption from triacylglycerides, while VLDL formation is preferentially enhanced with increasing cholesterol absorption (Feldman et al., 1983a,b; Tso and Simmonds, 1984);
3) Triacylglycerol infusion stimulates CM synthesis, while PC infusion stimulates VLDL synthesis (Tso and Simmonds, 1984);
4) Golgi vesicles contain either pre-CM or pre-VLDL, but not both (Mahley et al., 1971); and
5) CM formation and transport is inhibited by the hydrophobic surfactant Pluronic L-81, while VLDL is not (Feldman et al., 1983a; Tso and Simmonds, 1984; Nutting et al., 1989).

At low infusion rates, luminal fatty acids are incorporated into both VLDL and CM (Shiau et al., 1985). Exogenous PC is assembled with VLDL, while endogenous PC is more likely used for HDL assembly (Patton et al., 1984). HDL are primarily composed of free cholesterol which is synthesized in two forms: one with apoB, the other with apoA-I and apoA-IV.

The small intestine synthesizes approximately 20% of plasma apoproteins (Green and Glickman, 1981). The small intestine synthesizes all of the plasma CM and apoA-IV, approximately 50% of plasma apoA-I, 10% of VLDL with apoB-48, as well as small amounts of apoB-100, apoC-II, apoA-V and apoA-II (Wu and Windmueller, 1979; Fidge, 1980; Fidge and MCullagh, 1981; Gordon et al., 1983; Davidson et al., 1987). ApoA-I plays a role in the modulation of lecithin cholesterol acyltransferase (LCAT) activity which is required for the esterification of HDL cholesterol (Glickman, 1980). ApoA-I is the predominant apoprotein associated with HDL, although it also makes up 30% of CM protein. CM metabolism, therefore, provides a source of HDL (Glickman, 1985).

ApoA-IV is the major apoprotein synthesized by the intestine in the fasting or lipid-fed state (Bisgaier and Glickman, 1983; Sherman and Weinberg, 1988). After the administration of a high saturated fatty acid and high cholesterol diet, apoA-IV increases (Apfelbaum et al., 1987). However, synthesis of apoA-IV is stimulated by events involved in the packaging and secretion of

CM, not the level of luminal fat (Hayashi et al., 1990b). ApoA-IV may play a role as a centrally-acting satiety factor, reducing the size of meals (Fujimoto et al., 1992,1993a,b; Tso, 1994).

The small intestine synthesizes apoB-48 exclusively. ApoB-100 is synthesized by both the liver and small intestine in rats, but only by liver in humans (Levy et al., 1990). ApoB-100 consists of a single polypeptide chain with 4536 amino acids. ApoB-48 consists of only the N-terminal portion of apoB-100. ApoB-48 is synthesized in the rough ER during fasting, and then tranfers to the smooth ER after fat feeding for assembly into pre-CM (Christensen et al., 1983). ApoB-48 is essential for the secretion of lipoproteins, but may not be the rate-limiting step in their formation (Hayashi et al., 1990a).

ApoA-I synthesis and secretion continues in the absence of CM formation following biliary diversion (Bearnot et al., 1982), as well as during fatty acid absorption. This suggests that apoA-I synthesis may be regulated by factors other than triacylglycerol resynthesis. Most of the apoB in the enterocyte is associated with triacylglycerol-rich lipoproteins, while most of the apoA-I synthesized is not associated with any lipoproteins (Christensen et al., 1983). The synthesis of apoA-I and apoB-48 is unaffected by the amount or composition of dietary triacylglyerol (Davidson and Glickman, 1985), although an increased binding of apoA-I and apoB to lipoproteins is observed with feeding lipid (Davidson et al., 1987). The presence of apoC-II isoforms in the lymph are primarily the result of exchange with HDL after secretion, and are important for later metabolism by lipoprotein lipase (Koster and Holasek, 1972). The phospholipid, cholesterol ester and apoprotein composition of intestinal HDL differs from liver HDL (Green et al., 1978).

The molecular biology of the human apolipoprotein genes has been reviewed recently (Zannis et al., 1992), including current knowledge of the transcription of the human apoB gene (Kardassis et al., 1990a,b, 1992). Lipid transport from the intestine may be regulated by a process which edits apoB mRNA (Scott et al., 1989). Post-translational editing of apoB mRNA has been described in baboon enterocytes (Driscoll and Casanova, 1990) and in differentiating human enterocytes (Jiao et al., 1990; Teng et al., 1990). ApoB mRNA editing is a site-specific cytidine deamination reaction mediated by a protein complex which includes REPR, the catalytic component. REPR mRNA is distributed all along the crypt-villus axis, but apoB mRNA does not extend to crypts (Funahashi et al., 1995).

Dietary fatty acids and cholesterol have different effects on transcriptional and post-transcriptional mechanisms of apoprotein production (Srivastava et al., 1992). Dietary fiber is involved in the regulation of apoprotein gene expression as observed with the reduction in ileal apo AI and apo AIV mRNA levels (Mazur et al., 1991; Felgines et al., 1993, 1994; Sonoyama et al., 1995). Bile may also have a pretranslational role in apoprotein gene expression, as demonstrated by the decline in ileal apo AI and apo AIV mRNA following biliary diversion (Sonoyama et al., 1994).

Lipid transfer protein (LTP) is a 74,000 molecular weight glycoprotein composed of a high percentage (45%) of non-polar amino acid residues (Morton, 1990); these make the molecule

structurally different and more hydrophobic than other intracellular transfer proteins such as lipoproteins (Drayna et al., 1987). LTP has two molecular weight forms: LTP-I and LTP-II (Morton and Zilversmit, 1982). LTP-I specificity is species-dependent (Zilversmit et al., 1982), and facilitates the transfer of cholesterol esters (preferentially monounsaturated species) and triacylglycerols between lipoproteins, but not free cholesterol (Morton, 1986). LTP-I mRNA has been detected in the small intestine, which suggests that it has a role in cholesterol metabolism or membrane synthesis (Drayna et al., 1987). LTP-II is the plasma phospholipid transfer protein (Tall et al., 1983). LTP can mediate either a lipid exchange or a net lipid transfer between lipoproteins, and likely plays an important role in LDL and HDL metabolism.

Microsomal triacylglycerol transfer protein (MTTP) is required for the assembly and secretion of lipoproteins containing apoB VLDL by liver and CM by intestine (Sharp et al., 1993). MTTP is found in the lumen of liver and intestinal mitochondria, and catalyzes the transport of triacylglycerol, cholesterol ester and phosphatidyl choline between phospholipid surfaces using a ping pong bi-bi kinetics, shuttling triacylglycerol and cholesterol between membranes (Atzel and Wetterau, 1993). Non-polar lipids (triacylglycerol, diacylglycerol, cholesterol ester) bind to MTTP and are more readily transported than polar lipids (cholesterol, PC) (Jamil et al., 1995). A mutation in the gene for the large subunit of MTTP results in abetalipoproteinemia, a recessive genetic disease which results in the absence of MTTP in enterocytes, which is characterized by the virtual absence of apoB and apoB-containing lipoproteins in plasma (Gregg and Wetterau, 1994). The MTTP gene subunit structure has been described (Sharp et al., 1994).

Previous studies were unable to detect LDL receptors and mRNA in the small intestine. Recently, the small intestine has been found to be one of the most abundant sites of LDL receptor expression in a normal animal (Fong et al., 1995). LDL receptor and mRNA abundance are much greater in villus enterocytes from ileum than from jejunum, and are two-fold higher than LDL receptor protein and mRNA levels in unstimulated liver. Cholesterol feeding may down-regulate the LDL receptors and mRNA at the level of transcription. LDL receptor levels are not regulated by serum cholesterol. LDL receptor degradation is likely regulated by lipoprotein synthesis and luminal factors. Biliary cholesterol and lipids are absorbed in the jejunum, and may explain the down-regulation of LDL receptors in the jejunum as compared with the ileum. Another possibility may be the absence of cholesterol that reaches the ileum, or perhaps the ileal reabsorption of bile salts may up-regulate the LDL receptors.

1.8 Membrane Lipid Composition: Synthesis, Traffic and Tailoring

The lipid composition of plasma membranes differs from that of other organelles (Voelker, 1990). For example, SM and cholesterol are enriched in plasma membranes while mitochondrial

membranes are the exclusive site of cardiolipin (bisphosphatidylglycerol) and do not contain any SM or cholesterol (Colbeau et al., 1970). Differences in lipid composition also distinguish ER from Golgi and plasma membranes. ER does not contain any glycosphingolipids, and contains less cholesterol, SM, and PS than plasma membranes. A lipid compositional gradient also exists along the Golgi stack, with the cis-Golgi being similar to ER, while the trans-Golgi resembles plasma membranes (Zambrano et al., 1975). Epithelial cell apical plasma membranes differ in lipid composition compared with their basolateral membranes (van Meer et al., 1992). Even the two plasma membranes of the enterocyte differ in their lipid composition: the BBM is enriched in cholesterol and glycosphingolipids when compared with the basolateral membrane (Brasitus and Schachter, 1980; Hauser et al., 1980).

Villus enterocytes have a very rapid cell turnover rate. For example, in humans the rate is 48-72 hours (Croft and Cotton, 1973). Such a rapid cell turnover requires a constant supply of new lipids to be used for membrane synthesis. Where does this lipid come from? Enterocytes are capable of *de novo* synthesis of cholesterol, phospholipid and saturated fatty acids. Polyunsaturated fatty acids used for phospholipid synthesis can be synthesized only from exogenous sources of the essential fatty acids, linoleic and linolenic acids. Cholesterol, phospholipids and fatty acids are available from the diet and from bile.

The majority of lipid synthesis occurs at the microsomal membranes of mammalian cells (Friedman and Nylund, 1980). Microsomal membranes represent the combined membranes of the cell organelles that have a capacity for lipid synthesis, and include the smooth and rough ER and the Golgi. Although some are in the plasma membranes, most enzymes involved in lipid synthesis are localized to the smooth and rough ER, and the Golgi. Synthesized lipids must then be sorted and moved to their targeted destination within the cell. Little is known about the mechanisms which control lipid traffic and membrane lipid composition.

The current knowledge of intracellular lipid traffic in animal cells has been reviewed (Bishop and Bell, 1988; van Meer, 1989, 1993; van Meer and van Genderen, 1994). Pagano and Longmuir (1983) presented evidence for the synthesis, transport and assembly of lipids into intracellular membranes of cultured mammalian cells. Since lipids are water-insoluble, they must be carried from one membrane site to another with the aid of lipid transfer proteins (Pagano and Sleight, 1985). It is likely that fine tuning of the lipid composition is done at the destination membrane through the activity of deacylation-reacylation enzymes which may alter the fatty acid composition of membrane phospolipids. Or, the fine-tuning may also be accomplished by enzymes which alter the head group of a phospholipid to convert the molelcule to another phospholipid. The possibility of phospholipid interconversion was confirmed with the study by Dudeja and Brasitus (1987) which identified and characterized the presence of phosphatidylethanolamine methyltransferase (PEMT) activity in the BBM of rat small intestine. Phospholipase A_2 and acyltransferase activities are present in the BBM, enterocyte microsomal

membranes (EMM) and mitochondria (Tagesson et al., 1987; Pind and Kuksis, 1987,1988; Clark and Hubscher, 1960).

De novo lipid synthesis occurs primarily in the ER, but it may also occur in Golgi and nuclear membranes (Bishop and Bell, 1988; Jelsema and Moore, 1978; Van Golde et al., 1974). Plasma membranes (i.e., BBM, BLM), mitochondrial membranes and lysosomal membranes are not capable of synthesizing either some or all of their lipids, and therefore must acquire their lipids by some means of interorganelle transport.

Lipid translocation from the ER and Golgi to the plasma membrane occurs by two processes:

1) a rapid, ATP-independent process which is coupled to biosynthesis that likely uses a carrier such as the phospholipid exchange protein, like that observed for PC and PE (Wirtz and Gadella, 1990); and
2) a slower ATP-dependent process that uses transport vesicles which cycle among the plasma membrane, ER and Golgi, similarly observed for PC, SM, cholesterol and glycolipids (Sleight and Pagano, 1984; Lipsky and Pagano, 1985; Kaplan and Simoni, 1985b; Sasaki, 1990).

Many aspects of membrane lipid synthesis and assembly are not well understood. Lipid transfer proteins, fatty acid binding proteins and translocases may be involved in membrane synthesis, structural maintenance or membrane recycling. It is not known if lipid transfer vesicles exist; and if they do exist, then how they might be sorted and targetted to various membranes within the cell. It is possible that cellular processes may influence lipid transport within the cell. The mechanisms by which lipid transport can influence membrane lipid composition and subsequently modulate the activity of membrane-bound proteins are not clear, and must be elucidated in future studies.

1.8.a Generation of Membrane Lipid Asymmetry

Cholesterol, PC, SM, PE, PS, PI, and glycolipids are distributed heterogeneously among different membranes, and asymmetrically across the two halves of any membrane bilayer (Op den Kamp, 1979; Zachowski, 1993). There is a gradual enrichment of cholesterol and sphingomyelin in membranes as one moves along the path of the ER, to the *cis* Golgi, to the *trans* Golgi, and then to the plasma membrane (van Meer, 1989; Voelker, 1990). The lipid composition of the exoplasmic leaflet adapts to the needs of the external environent, compared to the stable and uniform lipid composition of the cytoplasmic leaflet that is required by cells (van Meer et al., 1992). Asymmetry in intestinal BBM lipid composition and fluidity has been reported (see Section 1.4; Barsukov et al., 1986; Pelletier et al., 1987; Meddings et al., 1990; Dudeja et al., 1991a).

The process by which membrane bilayer lipid asymmetry is achieved and maintained within the cell is not well understood. The ER itself contains little lipid asymmetry; therefore it is unlikely that plasma membrane lipid asymmetry occurs during synthesis at the ER (Hermann, et al., 1990). The ER synthesizes PC, PE, PS and PI on the cytoplasmic ER surface. This location

permits the synthetic enzymes access to their water-soluble substrates in the cytosol, and the incorporation of their phospholipid products into the cytoplasmic leaflet of the ER. Transmembrane phospholipid movement must occur to allow expansion of the luminal leaflet of the ER membrane (Hermann, et al., 1990).

Membrane lipid asymmetry may be generated and maintained by:

1) spontaneous diffusion or flip-flop across the bilayer;
2) facilitated diffusion by a phospholipid flippase;
3) active transport by aminophospholipid translocase;
4) asymmetric lipid synthesis;
5) asymmetric charge distribution may stabilize lipid asymmetry; and
6) interaction of lipids with the membrane cytoskeleton.

Cholesterol makes up to 50% of plasma membrane lipids, and can diffuse rapidly (in seconds) across membranes, despite the polar hydroxyl group on the 3 position of the sterol ring (Brasaemble et al., 1988; Zachowski and Devaux, 1990). The asymmetical distribution of cholesterol in the membrane alters with the incorporation of polyunsaturated fatty acids (Sweet and Schroeder 1988; Schroeder et al., 1990), chronic exposure to ethanol (Wood et al., 1990) or by the expression of cytosolic sterol carrier proteins (Jefferson et al., 1991).

Diacylglycerol does not exist permanently in membranes, but only as an intermediate following hydrolysis of phosphatidylinositol by phospholipase C (Berridge, 1987). Like cholesterol, the absence of charge allows diacylglycerol to diffuse rapidly across the membrane (Allan et al., 1978). The presence of diacylglycerols in the membrane bilayer may catalyze the movement of lipids across the membrane, by facilitating the occurance of hexagonal H_{II} structures that destabilize the membrane and permit rapid diffusion of other lipids (Siegel, 1989).

Depending on the pH, fatty acids may be neutral in the non-ionized form or charged in the ionized form. Neutral fatty acids diffuse quickly across the membrane bilayer (Broring et al., 1989). The carboxyl group provides a single negative charge on the ionized form of fatty acids, which reduces the ability of the fatty acid to diffuse across the membrane. Translocation of fatty acids across the membrane is very rapid, and represents the combined diffusion time of the ionized and non-ionized forms. A pH gradient across an artificial phospholipid membrane produces an asymmetrical distribution of fatty acids on each side of the bilayer (Hope and Cullis, 1987). Some phospholipids also classify as weak acids due to the single negative charge of the phosphate group: PI, phosphatidic acid, phosphatiylglycerol and diphosphatidylglcycerol, and only the neutral dehydrated phospholipid can cross the bilayer. PI and diphosphatidylglcycerol move very slowly across the membrane, due to the presence of hydroxyl groups on both the glycerol and the inositol moieties (Zachowski and Devaux, 1990).

PC, SM and PE (the zwitterionic phospholipids), as well as PS and glycolipids (which have a net negative charge on the polar head group) are charged molecules at any pH, and therefore may diffuse only across membranes through defects in the lipid bilayer, such as

hexagonal H_{II} phases, or destabilization in the bilayer at protein boundaries. Phospholipid diffusion is faster in erythrocyte ghosts than in liposomes from the lipid extract (Zachowski et al., 1985). Phospholipid diffusion is slow (hours), and it varies depending upon the lipid composition of the membrane, while glycolipid diffusion may not occur at all due to the bulky head group.

Facilitated transfer of lipid across the bilayer may occur via energy-independent protein activity (Devaux, 1992). Flippases are proteins proposed to be involved in the formation of non-bilayer structures in rat liver microsomes that might allow equilibration of phospholipid between the two monolayers (Van Duijn et al., 1987). Transmembrane movement of phospholipids may occur via flippase proteins during membrane synthesis (Rothman and Kennedy, 1977; Hermann et al., 1990). The presence of an ATP-independent PC-flippase has been proposed to explain the observed rapid translocation of PC to the luminal ER surface (Bishop and Bell, 1988), and thereby permit the synthesis of a lipid bilayer (Bell et al., 1981). The ability to translocate PC in the ER is lost in the trans-Golgi onward through the endocytic pathway. PC in the trans-Golgi, endosomal and plasma membranes is present, therefore, in two pools which do not rapidly equilibrate. PC-flippase has a low lipid specificity which also permits PE, PS and PI to equilibrate rapidly across the ER bilayer during synthesis. This results in virtually no membrane asymmetry across the bilayer (Hermann et al., 1990). Enzymes involved in lipid synthesis are distributed asymmetrically in microsomal membranes; therefore, rapid equilibration of lipids would prevent the accumulation of products in one monolayer, and permits intermediates synthesized on one monolayer to reach the next site of synthesis on the other monolayer (Zachowski and Devaux, 1990). In rat liver ER, PC flippase facilitated the rapid distribution of PC during membrane assembly (Bishop and Bell, 1985). Also, LPC was translocated in liver ER by the same flippase activity (Kawashima and Bell, 1987). PEMT activity present in the BBM results in the methylation of PE to PC (Dudeja and Brasitus, 1987), and likely coincides with translocation of the phospholipid similarly observed with PEMT activity in erythrocytes (Hirata and Axelrod, 1978).

The transmembrane movement of phospholipids may also occur via energy-dependent translocase proteins: the lipid is moved from one monolayer where it is less concentrated to another monolayer where the lipid is more concentrated. ATP provides the energy required to overcome the lipid concentration and/or electrical gradient across the bilayer. The presence of aminophospholipid translocases, which facilitate the transmembrane movement of PS and PE, have been described in erythrocyte membrane (Seigneuret and Devaux, 1984; Tilley et al., 1986), the plasma membranes of lymphocytes, platelets, cultured hamster fibroblasts, and in synaptosomes form the Torpedo electric organ (Zachowski and Devaux, 1990). The affinity for PS is ten times greater than for PE. Translocation of the phospholipids across the membrane is an ATP-dependent process that prefers phospholipids containing at least one unsaturated fatty acid (Devaux, 1988, 1991). Following phospholipid translocation, asymmetry may be maintained by interactions between the aminophospholipids and the cytoskeleton (Middlekoop et al., 1988).

SM is synthesized on the luminal surface of the Golgi (Pagano, 1988). Glycosphingolipids (GSL) are derived from ceramide synthesis at the ER, followed by glycosylation at the luminal surface of the Golgi (Lipsky and Pagano, 1985). Translocation of GSL and SM from the luminal to the cytoplasmic surface of the Golgi has not been described. This is consistent with the exclusive location of GSL and SM on the outer leaflet of the apical plasma membrane (i.e., BBM).

Cholesterol synthesis occurs on the ER (Reinhart et al., 1987) and in the peroxisomal lumen (Appelkvist, 1987). Cholesterol enrichment takes place along the cisternae of the Golgi: the cis-Golgi face is cholesterol-poor, while the trans-Golgi face is cholesterol-rich (Orci et al., 1981a,b). Cholesterol enrichment may be achieved by phospholipid retrieval from the Golgi via phospholipid transfer proteins (Wieland et al., 1987).

1.8.b Lipid Sorting

The trafficking of lipids to the BBM, BLM and other membranes is not well understood. Signals which sort and target cholesterol and phospholipid, and maintain membrane bilayer asymmetry, occur via lipid synthetic pathways or protein microdomains which sort lipids (van Meer, 1993). Lipid sorting may occur through molecular filters during vesicle generation prior to transport from the Golgi to other intracellular membranes (Bretscher, 1976). The trans-Golgi network has been determined to be the site of exocytic lipid sorting, which may occur through lipid association with proteins or via a sorting protein (Simons and van Meer, 1988). Studies in intestinal Caco-2 cells suggest that lipid sorting occurs in the trans-Golgi to direct lipid movement to the BBM or BLM (van't Hof et al., 1992). In addition, phospholipids may be incorporated into nascent lipoproteins during transist through the ER to Golgi and secretory vesicles. The major site of phospholipid packaging into lipoproteins occurs in the Golgi from specific biosynthetic pools (Vance and Vance, 1986a,b; Higgins and Fieldsend, 1987).

Vesicular transport between cis-Golgi and ER appears to be bi-directional (Pelham, 1989a,b). This suggests that ER proteins entering the cis-Golgi would have to be sorted into the return pathway to the ER. SM is not present in ER, and therefore may be excluded from the return pathway by lipid sorting at the cis-Golgi (van Meer, 1989). Although uni-directional sorting has been proposed, there is no evidence to suggest that vesicles are not moving bi-directionally between the trans-Golgi and the Golgi stack. Lipid sorting also occurs in the luminal leaflet of the endosomes, which determines whether lipids recycle to the endosomes or exit to the lysosomes. The sorting mechanism is not defined, but likely involves the lateral segregation of lipids. The mechanism of lipid sorting and recycling from the endosomes to the plasma membrane is unknown. In the transcytosis pathway, lipid sorting may occur in the endosome in a similar fashion to the exocytic pathway (van Meer, 1989).

GSL have the ability to self-associate by hydrogen bonding, and then interact with apical proteins directly or indirectly, via a possible sorting protein to form a microdomain at the site of apical vesicle budding on the luminal surface of the trans-Golgi (Simons and van Meer, 1988). A

sorting protein likely would define the destination of the vesicle at the cytoplasmic surface of the plasma membrane.

Phospholipid asymmetry is generated during membrane synthesis in the Golgi (see Section 1.8). Since many membrane proteins have a requirement for specific phospholipids for optimal activity, synthesis of the membrane protein at the EMM may dictate the type of phospholipid which surrounds the protein, and consequently the membrane protein itself may direct it to the correct membrane location within the cell. Specific phospholipid sorting mechanisms have not been defined in the Golgi, but may involve the lateral segregation of lipids.

Cholesterol has a differential affinity for phospholipids, but it interacts preferentially with SM. This suggests that cholesterol may be incorporated into a SM microdomain so that cholesterol may be sorted as a complex with SM in the Golgi. This would result in the bulk of cholesterol situated in the luminal leaflet of the Golgi and endosomal membranes, so that cholesterol would be asymmetrically located on the luminal surface of the plasma membrane. Cholesterol translocation from the luminal to the cytoplasmic leaflet has not been described for Golgi, endosomal or plasma membranes, but it may be present in lysosomal membranes (Liscum and Faust, 1987).

1.8.c Transport of Lipids from the Microsomal Membranes to the Intracellular Membranes

The t½ of transport for newly synthesized PC and cholesterol from the ER to the plasma membrane is approximately 1 min., and 10 min. to 2 hours, respectively (Kaplan and Simoni, 1985a,b; Lange and Matthies, 1984). PC and cholesterol transport to the plasma membrane is insensitive to colchicine (an inhibitor of microtubule assembly) or to monensin (an inhibitor of exocytosis), while cholesterol transport depends on cellular ATP. Newly synthesized membrane lipids may be delivered to their destination membrane by one of three mechanisms (Bishop and Bell, 1988):

1) the vesicular pathway -- an energy-dependent movement of vesicles between membranes or organelles;
2) the transfer of lipid monomers by spontaneous diffusion or protein-facilitated transport; or
3) lateral diffusion of lipids between membranes at regions of direct intermembrane contact.

It is also possible that microtubule assembly plays a role in lipid trafficking within the enterocyte, since colchicine treatment results in alterations in the morphology of subcellular organelle membranes and the BLM (Pavelka and Gangl, 1983).

The Vesicular Pathway

The primary mechanism of intermembrane lipid transfer occurs via the vesicular pathway: this is a slow ATP-dependent process using transport vesicles that cycle among the plasma membranes, ER and Golgi. Lipids are transferred to their destination via membrane vesicles

through either the endocytic or exoctyic pathways. In Chinese hamster ovary cells, cholesterol transport from the ER to the plasma membrane is mediated by a vesicular pathway (Kaplan and Simoni, 1985b). Mitochondria and peroxisomes are not connected by either vesicular pathways, although a study in baby hamster kidney cells suugests that vesicles mediate the transfer of phosphatidylserine from microsomes to mitochondria (Voelker, 1985).

In the exocytic route, vesicles first recycle between the ER and the *cis*-Golgi. It is not known if the intra-Golgi movement is uni-directional. Lipids are sorted in the *cis*- and *trans*-Golgi (see Section 1.8.b). Vesicles are then targetted to deliver their contents to the plasma membranes. From there, vesicles enter the endocytic pathway. Endosomes (vesicles) remove membrane lipid from the plasma membrane and recycle to the *trans*-Golgi, the plasma membrane, or the lysosomes. Additional lipid sorting may occur in the luminal leaflet of the endosomes in order to distinguish lipids recycling to endosomes from lipids destined for the lysosomal compartment. A short route connects the *trans*-Golgi with a pre-lysosomal compartment to the lysosomes. Vesicles are not recycled from the lysosomal compartment (Kornfeld and Mellman, 1989).

Monomer Diffusion or Protein-Mediated Facilitated Transport

Lipid monomers may diffuse through the cytosol by spontaneous diffusion or by protein-facilitated transport (Bishop and Bell, 1988). Monomeric phospholipid diffusion may be important for the movement of PE from the outer mitochondrial membrane to ER, and for intracellular equilibration of PE of rat hepatocytes (Blok et al., 1971; Yaffe and Kennedy, 1983). Monomeric cholesterol transport occurs to the inner mitochondrial membrane for the first step in hormone production, and also occurs from LDL in lysosomes to the ER for cholesterol ester synthesis (van Meer, 1989). Monomeric phospholipid and cholesterol transport has not been described in the enterocyte.

Phospholipid transfer proteins were initially described as phospholipid exchange proteins (Wirtz and Zilversmit, 1968). Since then, phospholipid transfer proteins have been studied extensively in bovine brain, heart and liver, as well as in rat liver (Wirtz and Gadella, 1990; Wirtz, 1991). Lipid movement by phospholipid transfer protein is a rapid, ATP-dependent and often phospholipid-specific process (Wirtz and Gadella, 1990) which has not been well characterized in the enterocyte.

PC-transfer protein (PC-TP) is highly specific for PC and may be very important for the transfer of PC to mitochondria, since they are incapable of synthesizing their own PC. PI transfer protein (PI-TP) is specific for PI and PC, but has a 16-fold higher affinity for PI than PC (Van Paridon et al., 1987a). PI-TP is required for the rapid equilibration of PI between ER and mitochondria. The dual specificity of PI-TP may be useful for replenishing PI in the plasma membranes used for signal transduction, by transferring PI from the ER in exchange for PC at the plasma membrane. This ensures a continuous supply of PI to the plasma membrane, and a continuous return of PC to the ER. Non-specific lipid transfer protein (nsL-TP), also known as

sterol carrier protein 2 (SCP-2), transfers cholesterol as well as a variety of phospholipids and glycolipids. nsL-TP or SCP2 is involved in cholesterol synthesis and steroidgenesis. SCP-2 has a non-enzymatic stimulatory effect on cholesterol metabolism (Trzaskos and Gaylor, 1983). SCP-2 is involved in the transport of cholesterol to the cytoplasmic outer mitochondrial membrane, and from the outer to the inner membrane (Scallen et al., 1985). SCP-2 is primarily located in peroxisomes, and may have a role in peroxisomal cholesterol synthesis (Amerongen et al., 1989; Keller et al., 1989). Glycolipid transfer protein (GL-TP) transfers all sphingolipids and some glycodiacylglycerols, but it does not transfer phospholipids, cholesterol or cholesterol esters (Sasaki, 1990).

SCP-2 is found in high concentrations in the liver (0.78 ug/mg tissue) and intestine (0.46 ug/mg tissue), as well as in other tissues involved in steroidgenesis. But in other cells, it is present in only low concentrations (0.1 ug/mg tissue) (Teerlink et al., 1984). The concentrations of PC-TP, PI-TPand GL-TP in the small intestine have not been reported.

Bovine liver PC-TP, rat brain PI-TP, and bovine liver nsL-TP are each a single polypeptide chain consisting of 213, 271 and 121 amino acids respectively, and have corresponding molecular weights of 28 kDa, 32 kDa and 14.5 kDa (Akeroyd et al., 1981; Dickeson, 1989; Westerman and Wirtz, 1985). The pI of PC-TP is 5.8, while nsL-TP has a pI of 9.55. PC-TP, PI-TP, nsL-TP and GL-TP do not have any significant sequence homologies. PI-TP PC-TP contains very hydrophobic peptide segments, while PI-TP does not (as determined from amino acid sequences deduced from the cDNA clone). The lipid binding site of PC-TP involves the peptide Val^{171}-Phe^{176}. Isolated PC-TP and PI-TP contain phospholipids bound non-covalently in a 1:1 molar ratio (Demel et al., 1973: Van Paridon et al., 1987b). Isolated nsL-TP does not have any bound lipid, but cholesterol has been observed to bind in a 1:1 molar ratio (Scallen et al., 1985). Phospholipids, cholesterol and glycoplipids may also be transfered by nsL-TP. Glycolipid binds to GL-TP in a 1:1 molar ratio, but it is the only lipid transfer protein to exist with or without bound lipid (Sasaki and Demel, 1985). PC-TP and PI-TP have two different hydrophobic binding sites for *sn*-1 and *sn*-2 fatty acyl chains (Berkhout et al., 1984; Van Paridon et al., 1988). The *sn*-2 chains of PC and PI share a common binding site (Van Paridon et al., 1988).

The mechanism by which lipid transfer proteins exchange or transfer lipids, to or from membranes, is not clear. PC-TP and PI-TP have been proposed to act as freely diffusible carriers in an aqueous phase that compete with membranes for the exchange of phospholipid (Wirtz and Gadella, 1990). GL-TP also facilitates net transfer of glycolipid between the GL-TP and the membrane (Sasaki, 1990). nsL-TP does not act as a freely diffusible carrier of lipids, but it mediates either net transfer or exchange of lipid by one of three possible models:

1) by lowering the energy barrier to lipid monomer-interface dissociation (Nichols and Pagano, 1983);
2) by collision with the membrane (Nichols, 1988b); or

3) by facilitating the juxtaposition of two membrane interfaces by forming dimers (Van Amerongen et al., 1989).

The rate of bulk flow from the ER to the Golgi to the plasma membrane equals the rate of cholesterol movement to the plasma membrane (Kaplan and Simoni, 1985b), but does not agree with the rapid movement of phospholipid to the cell surface (Kaplan and Simoni, 1985a). Approximately 50% of the ER phospholipid is transferred out of the Golgi every 10 minutes (Wieland et al., 1987), suggesting that this massive loss is recovered by recycling from Golgi and the plasma membrane via phospholipid transfer proteins.

Intermembrane Lateral Diffusion

Lipids may also sort between membranes by lateral diffusion at regions of direct intermembrane contact, even though these associations may be transient or stable (Bishop and Bell, 1988). Tight junctions block lipid diffusion between the outer luminal leaflets, but not between the inner cytoplasmic leaflets of the BBM and the BLM (van Meer and Simons, 1986; van Meer et al., 1987; Simons and van Meer, 1988). Lipids in the cytoplasmic leaflets are free to diffuse between the BBM and BLM, but it is unclear to what extent this occurs.

1.8.d Post-transport Membrane Lipid Modification

It is unlikely that the lipid asymmetry derived from membrane synthesis and lipid sorting at the ER/Golgi is in itself sufficient to achieve the desired lipid asymmetry which characterizes each intracellular membrane. There are a number of ways that the lipid asymmetry of membranes may be altered following membrane synthesis and transport within the cell:

1) Phospholipid headgroups may be interconverted at the site of the plasma membrane. The BBM contains enzymes which may modulate its phospholipid composition. PEMT activity has been described in the BBM, which has the ability to convert and translocate PE to PC and vice versa (Dudeja and Brasitus, 1987).
2) Phospholipids may be deacylated and reacylated with a different fatty acid (Lynch and Thompson, 1988). Phospholipases exist at the BBM which may have the ability to alter the fatty acid composition of a membrane phospholipid. A Ca^{2+}-independent phospholipase A_2 with a high substrate specificity for PE has been isolated from rat BBM (Pind and Kuksis, 1987). The physiological significance of this enzyme is not known, but it may play a role in local fine tuning of the BBM lipid composition. Acyltransferases are commonly found in plasma membranes and were reported to exist in the BBM (Clark and Hubscher, 1960). But a later study suggested that the previous report on the presence of acyltransferases may have been due to contamination with EMM during the BBM isolation procedure (Robins et al., 1971). It is now possible to obtain highly purified BBM that would be suitable for the assay of acyltransferase activity (Lands and Crawford, 1976).
3) Phospholipid fatty acids may be desaturated directly on the phospholipid molecule (Pugh and Kates, 1977, 1979). This type of glycerolipid retailoring provides for

additional capabilities of fine tuning membrane lipid composition locally at the membrane. These membrane-bound phospholipid desaturases have not yet been described in the BBM, but they are present in other plasma membranes (Pugh and Kates, 1977, 1979).

4) Phospholipids may be 'flipped' from one hemi-leaflet of the membrane bilayer to the other. The existence of ATP-translocase or flippase has not yet been defined in the enterocyte, but it does exist in the plasma membranes of other cells (Zachowski and Devaux, 1990).

In addition to dietary fatty acid (FA), luminal phospholipases which act on biliary PC may also influence the type of free fatty acid (FFA) presented to the BBM. The amount of FFA derived from luminal phospholipases represents at least 5-10% of the total FFA presented to the BBM.

Dietary and luminally derived FFA may significantly alter the composition of the BBM FFA pool -- the potential source of FFA for reacylation reactions or permeation across the BBM bilayer into the enterocyte. The transported FFA would be available for subsequent incorporation into phospholipids, and then targetted for lipoprotein or membrane synthesis. Changes in membrane lipid composition may influence the permeability properties of the BBM and the activity of intrinsic BBM or EMM proteins. As a result, dietary FA influence the composition and fluidity of the BBM, and possibly the activity of BBM proteins such as SGLT1.

1.9 Diabetes Alters Lipid Metabolism

Lipid uptake significantly increases in diabetic animals (Nervi et al., 1974; Thomson, 1980c, 1983a,b) and this increased absorption cannot be attributed to changes in the animal's food intake or intestinal morphology. How is this increased concentration of lipid handled within the enterocyte? Intestinal *de novo* cholesterol synthesis is increased by up to three times in diabetic animals (Feingold et al., 1982a), due to an increase in HMGR activity (Young et al., 1983). This increase appears shortly after the onset of diabetes, and persists for at least five weeks (Feingold, 1989). Intestinal HMGR and ACAT activity are both increased, while liver ACAT is reduced in genetically obese rats with non-insulin dependent diabetes (Jiao et al., 1991). Insulin administration returns cholesterol synthesis to near normal levels (Feingold et al., 1983). The diabetes-associated increase in cholesterol synthesis is specifically localized to the intestinal epithelium and the muscularis, but not to any other part of the body (Feingold et al., 1982a). In addition, the increase is greater in distal than in proximal small intestine (Feingold and Moser, 1984). In the distal small intestine, this increase in cholesterol synthesis is greatest in the villus tip cells, while in the proximal small intestine, it is greatest in the crypt cells (Feingold and Moser, 1987). Intestinal HMGR protein and mRNA are increased 2.5-fold and 2.1-fold respectively in streptozotocin-diabetic rats, while liver HMGR mRNA is unaffected (Feingold et al., 1994). LDL receptor mRNA levels are enhanced 43% in the small intestine of diabetic rats which may

adversely affect lipoprotein metabolism and increase the risk of atherosclerosis. Hyperphagia may be responsible for the increase in HMGR observed in diabetic animals (Young et al., 1982). Insulin deficiency in alloxan-diabetic rabbits may influence apoE gene expression and tissue cholesterol homeostasis (Lenich et al., 1991). When food intake is reduced or the dietary cholesterol increased, cholesterol synthesis returns to normal (Young et al., 1983; Feingold and Moser, 1984). The hypercholesterolemia observed with diabetes may be due to hyperphagia or to changes in the routes of cholesterol flux (Young et al., 1983). An increase, for instance, in cholesterol flux from the intestine to the blood may result in decreased cholesterol synthesis by the rest of the body. But with the decrease in body cholesterol synthesis, diabetic rats stop growing. This decline in cholesterol synthesis is balanced by an increase in liver bile acid synthesis, which results in an increased bile acid pool. Fecal sterol excretion and total utilization are normal. Therefore the increase in plasma cholesterol occurs because of an increased influx from the intestine, which, when combined with a decline in body cholesterol synthesis, results in a larger portion of the total body cholesterol passing through the blood. An increase in cholesterol secretion via the thoracic duct has been reported (Nervi et al., 1974). Cholesterol feeding markedly inhibited hepatic and intestinal cholesterol synthesis in diabetic rats. This virtually obliterated the three-fold increase in cholesterol synthesis associated with diabetes (Feingold et al., 1983).

In diabetes, fatty acid metabolism alters due to a decrease in Δ^6 desaturase activity, which can be reversed with insulin administration (Brenner, 1981; Horrobin, 1993). Depressed Δ^6 desaturase activity results in the impaired conversion of linoleic to arachidonic acid. The resulting increase in the ratio of linoleic acid/arachidonic acid is observed in phospholipids of many tissues including sciatic nerve, renal cortex, platelets and liver microsomes (Chattopadhyay et al., 1992; Jones et al., 1986; Ramsammy et al., 1993; Venkatraman et al., 1991; Zhu and Eichberg, 1993). In serum phospholipids and hepatic smooth ER, the total PUFA increases due to a rise in ω6 but not ω3 fatty acids. The amount of linoleic acid increases, while arachidonic acid decreases. Many metabolites of linoleic acid are present despite the reduction in Δ^6 desaturase from the increased activity of Δ^5 and Δ^4 desaturases. Based on membrane phospholipid fatty acid composition, diabetes was proposed to be associated with a decline in Δ^9 and Δ^6 desaturase activities in rat liver microsomes (Faas and Carter, 1981, Mercuri et al., 1974). Intestinal desaturase activity has not previously been studied in diabetic animals.

BBM PC and PE content have been reported to increase with diabetes (Keelan et al., 1985a). Total phospholipid content of rat liver microsomes increased with diabetes due to an increase in PE content (Faas and Carter, 1981). PEMT activity was reduced in liver microsomes of alloxan diabetic rats (Hoffman et al., 1981). There are no reports in the literature on the effects of diabetes on intestinal PC metabolism. Insulin has been observed to increase sphingomyelinase activity in rat liver microsomes (Petkova et al., 1990) This suggests that a

diabetes-associated decline in insulin may be associated with a decline in the activity of sphingomyelinase that may, in turn, result in increased levels of sphingomyelin, compared to non-diabetic controls.

1.10 Therapeutic Potential of ω3 Fatty Acid Enriched Diets

Despite the fact that the Greenland Eskimos consume a high fat diet, they have a low incidence of cardiovascular disease, cancer, diabetes, hypertension and chronic diseases associated with abnormalities in immune function and the inflammatory response (Bang and Dyerberg, 1980; Carroll, 1986b; Leaf and Weber, 1988; Schmidt and Dyerberg, 1994). Since the Eskimo diet is enriched with ω3 fatty acids derived from marine animals (approximately 400 g fish/day), the potential usefulness of fish oil supplements has drawn a large interest in nutritional strategies that may attenuate or prevent the development of the above mentioned pathological conditions (Dyerberg, 1994). This idea was supported with the observation that the incidence of cardiovascular disease was also significantly lower in the Japanese population, which also consumes a diet high in fish, up to 200 g fish/day (Dyerberg, 1986; Kagawa et al., 1982). Diets enriched in ω3 fatty acids such as eicosapentanoic acid (EPA, 20:5ω3) and docosahexanoic acid (DHA, 22:6ω3), the major unique fatty acids in fish oils, reduce arachidonic acid synthesis, which has significant consequences for the production of eicosanoids (Weaver and Holub, 1988). In addition, ω3 fatty acids are readily absorbed and acylated preferentially into tissue phospholipids such as PE and PC, although incorporation of EPA and DHA into tissue phospholipids may vary (Bruckner et al., 1984; Croft et al., 1984). Rat platelet phospholipids exclude DHA, but not EPA; yet rat aorta incorporates both EPA and DHA into its phospholipids (Bruckner et al., 1984). Incorporation of ω3 fatty acids into tissue phospholipids occurs at the expense of arachidonic acid, which results in important biological consequences.

Arachidonic acid (20:4ω6) is a precursor for the synthesis of prostaglandins and leukotrienes. EPA competitively inhibits the cyclooxygenase and lipoxygenase enzymes, and thereby reduces the products of these pathways (Lee et al., 1985; Needleman et al., 1979). EPA competes with 20:4ω6 for eicosanoid synthesis, and as a result may modify eicosanoid production (Kinsella, 1986). Eicosanoids derived from 20:5ω3 have significantly different biological activites compared with those derived from 20:4ω6. Thromboxane A_2 (TXA_2) is a potent stimulator of blood vessel constriction, platelet aggregation and thrombi formation. Prostacyclin (PGI_2) stimulates blood vessel relaxation, but it prevents platelet aggregation as well. PGI_2 is therefore considered antithrombotic (Bunting et al., 1983). Under normal conditions the TXA_2/PGI_2 ratio is controlled to permit clotting only following blood vessel injury (Bunting et al., 1983). Thromboxane A_3 (TXA_3) is a poor stimulator of platelet aggregation, but PGI_3 functions exactly as PGI_2. Thus dietary ω3 fatty acid supplementation results in a decline in clotting ability (Dyerberg, 1981). The

decline in TXA_2 synthesis may decrease the risk of thrombosis, which may block arteries enough to result in a heart attack or stroke (Dyerberg, 1986; Herold and Kinsella, 1986). In a study where a DHA enriched diet preferentially reduced TXA_2 synthesis, while an EPA enriched diet decreased the synthesis of both TXA_2 and PGI_2, Bruckner and coworkers (1984) suggested DHA may be more anithrombotic than EPA.

Kromhout et al. (1985) did an epidemiological study which suggests that fish consumption is inversely correlated with mortality from coronary heart disease. In that study, the consumption of 30 g fish/day reduced the incidence of mortality from ischemic heart disease in a population of adult Dutch males. Plasma VLDL, LDL, triacylglycerol and cholesterol levels declined while HDL levels increased following dietary ω3 fatty acid supplementation in animals and humans (Harris et al., 1983; Herold and Kinsella, 1986). This may prove beneficial in the prevention of cardiovascular disease (Leaf and Weber, 1988; von Schacky, 1987). When hyperlipidemic human subjects were fed a fish oil supplement at 30% of calories/day (equal to 30 g of ω3 fatty acid per day), serum lipid levels were also significantly reduced (Phillipson et al., 1985). The decline in plasma lipid levels may be due to:

1) decreases in VLDL and triacylgycerol synthesis (Dyerberg, 1986; Nestel et al., 1984);
2) decreased fatty acid synthesis (Yang and Williams, 1978); and/or
3) enhanced VLDL removal from the circulation and increased biliary excretion of cholesterol (Harris et al., 1983).

In studies on mammary, pancreatic, intestinal and prostatic tumors, high amounts of dietary ω3 fatty acids do not stimulate tumor growth, and in fact, it may actually inhibit it (Braden and Carroll, 1986; O'Connor et al., 1985; Karmali, 1986a,b). The reason for this protective effect is not clear. It may be due to:

1) low concentrations of ω6 fatty acids in fish oils. Omega-6 fatty acids are precursors to PGE_2 which is present in increased levels in tumor tissues and blood from patients with tumors (Carroll, 1986b).
2) oxidation products from ω3 fatty acids may inhibit tumor growth. Omega-3 fatty acids are more susceptible to oxidation than ω6 fatty acids (Carroll, 1986a,b).
3) incorporation of ω3 fatty acids into tissues. Prostaglandins and leukotrienes synthesized from ω3 fatty acids have a different biological activity than those synthesized from ω6 fatty acids, and may prevent tumor growth (Tashjian et al., 1984; Karmali et al., 1984).

Several chronic conditions such as hypertension (Lands, 1986), systemic lupus (Robinson et al., 1985; Kelley et al., 1985), multiple sclerosis (Mertin, 1984; French, 1984), rheumatoid arthritis (Kremer et al., 1987), and inflammatory bowel disease (Sharon and Stenson, 1984) are associated with alterations in the inflammatory response and immune function, possibly as a

result of abnormalities in eicosanoid synthesis (Carroll, 1986a). Leukotriene B_4, (LTB_4) is elevated in the colonic mucosa of patients with ulcerative colitis: LTB_4 induces neutrophil aggregation, and increases microvascular permeability (Sharon and Stenson, 1984). Leukotriene B_5, produced from 20:5ω3, is an inflammatory mediator with 30 times less biological activity than 20:4ω6-derived LTB_4 (Terano et al., 1984). A decrease in the production of inflammatory mediators with dietary ω3 fatty acid supplementation correlates with a clinical benefit in patients with rheumatoid arthritis and ulcerative colitis, but not Crohn's disease (Kremer, et al., 1987; McCall et al., 1989; Lorenz et al., 1989). However, in some situations, dietary ω3 fatty acid supplementation may exacerbate rather than ameliorate disease symptoms; and the reason for this apparently paradoxical effect is unknown (Carroll, 1986a). Eicosanoid synthesis is usually low in normal tissues, but significantly increases in pathological or stimulated tissues, and therefore the function of eicosanoids may be important only in pathological states (Lands, 1985).

The usefulness of dietary ω3 fatty acid supplementation in the control of diabetes is controversial and may have detrimental metabolic effects (Adler et al., 1994; Axelrod, 1989; Axelrod et al., 1994; Clandinin et al, 1993; Dines et al., 1993; Glauber et al., 1988; Jensen, 1991; Kasim, 1993; Lackey and Noble, 1990; Lefebvre and Scheen, 1992; Malasanos and Stacpoole, 1991; Nosari et al., 1994; Vessby, 1991; Zambon et al., 1992). Prostaglandins and leukotrienes synthesized from 20:5ω3 have different metabolic activities than those synthesized from 20:4ω6, and may influence insulin secretion from pancreatic beta cells (Robertson, 1984). In rats, dietary fish oil supplementation (3 g ω3 fatty acids per day) may adversely affect glycemic control in non-insulin-dependent diabetes (NIDDM), without any significant benefit on plasma lipids (Borkman et al., 1989). Insulin sensitivity increases with dietary ω3 fatty acid supplementation (3 g/day for eight weeks) in NIDDM and results in enhanced transport of glucose into peripheral cells (Lardinois, 1987; Popp-Snijders et al., 1987). Postprandial insulin concentrations were decreased in NIDDM men receiving a dietary fish oil supplement containing 8 g of ω3 fatty acids per day for for weeks (Zambon et al., 1992). In addition, VLDL cholesterol was decreased while LDL cholesterol was increased, although total cholesterol and triglyceride concentration were unchanged with fish oil supplementation. Administration of a 3.1 g ω3 fatty acid supplement for 3 weeks resulted in no adverse effects on glucose homeostasis in mildly obese, normotriglyceridemic men with NIDDM treated with oral hypoglycemic agents (Pelikanova et al., 1993). In Alaska Natives, consumption of seal oil at least 5 times per week or daily salmon intake was required to reduce the risk of glucose intolerance and NIDDM (Adler et al., 1994). The data suggests that large doses of ω3 fatty acid supplements of greater than 6 g per day may be detrimental for patients with NIDDM without overt hyperlipidemia (Friday et al., 1989; Kasim et al., 1988; Schectman et al., 1988).

A dietary intake of 5.4 g EPA and 2.3 g DHA per day for four weeks results in a decline in triglycerides, platelets aggregation and thromboxane production in insulin-dependent diabetes (IDDM) (Landgraf-Leurs et al., 1990). Dietary supplementation with ω3 fatty acids at 6 g/day for

three months reduces triglyceride and HDL levels without adversely affecting overall diabetes management in IDDM (Bagdade et al., 1990). Although hypertriglyceridemia may improve, other lipoprotein indices may worsen in diabetic patients given varying doses of ω3 fatty acid supplements over a period of six months (Stacpoole et al., 1989). In addition, insulin requirements increase in diabetics given fish oil supplements for two years. The data suggest that ω3 fatty acid supplementation may be beneficial for reducing some CVD risk factors in IDDM.

An anti-absorptive effect of dietary fish oil supplementation has been observed in the small intestine following short-term feeding in non-diabetic animals (Thomson et al., 1988b). Variations in dietary triacylgycerol saturation alter BBM lipid composition and fluidity (Brasitus et al, 1985), therefore the incorporation of ω3 fatty acids into membrane phospholipids will likely also change membrane fluidity and permeability. Diabetes is associated with an abnormal fluidity in erythrocytes that reverses following dietary ω3 fatty acid supplementation (Kamada et al., 1986).

The effects of dietary ω3 fatty acids, summarized by Kinsella (1986) explain that ω3 fatty acids:

1) displace 20:4ω6 from tissue phospholipids;
2) reduce the amount of 20:4ω6 available for PG synthetase;
3) decrease PG synthetase activity because ω3 fatty acids are poor hydroperoxide substrates, compared with 20:4ω6;
4) competitively inhibit eicosanoid production from 20:4ω6 (K_i <2 μM); and
5) produce eicosanoids of different biological activity than those derived from 20:4ω6.

In rat liver microsomes, feeding fish oil is associated with a decline in Δ^6 and Δ^5-desaturase activities, possibly due to feedback inhibition by 20:5ω3 and 22:6ω3, or a decline in enzyme synthesis (Garg et al., 1988c 1988d; Choi et al., 1989; Christiansen et al., 1991). Δ^9-desaturase is also decreased with feeding fish oil. Fish oil feeding maintains a moderate level of 20:4ω6 and 20:5ω3 to ensure a balance of eicosanoids. The role of 22:6ω3 is not clear, except that the hydroxylated products of 22:6ω3 are potent regulators of 20:4ω6 metabolism (Norum et al., 1989).

The ability of ω3 fatty acids to exert their effects depends upon the total fat content of the diet, as well its fatty acid composition. The optimum amount of dietary ω3 fatty acid supplementation or substitution depends upon dietary habits. A low fat diet enriched with ω3 fatty acids and low in ω6 fatty acids will produce the most noticeable effects. This is supported with studies which demonstrate that the plasma lipid lowering effects of dietary ω3 fatty acids depends upon the saturation of the remaining dietary fatty acid constituents (Garg et al., 1988d,e). Diets enriched with saturated fatty acids and supplemented with ω3 fatty acids result in lower plasma lipid levels than diets enriched with ω6 fatty acids and supplemented with ω3 fatty acids. The effects of dietary cholesterol were attenuated in the presence of dietary ω3 fatty acids (Garg et al.,

1988d,e). The anti-absorptive effect of dietary ω3 fatty acids has been reported for the small intestine (Thomson et al., 1988b).

The source of ω3 fatty acids may influence the biological effects of dietary ω3 fatty acids. Linseed oil is enriched with 18:3ω3 while fish oils are enriched with 20:5ω3 and 22:6ω3. Although 18:3ω3 is the precursor of 20:5ω3 and 22:6ω3, the intake of 18:3ω3-enriched linseed oil may not result in the same level of increased incorporation of 20:5ω3 and 22:6ω3 into tissue lipids as observed following the intake of fish oils. The potential benefits have not yet been elucidated for EPA vs DHA supplementation, the varying EPA:DHA ratios and their dosage. The EPA and DHA content and the EPA:DHA ratio of fish oils varies depending upon the type of fish (Kinsella, 1986). The EPA:DHA ratio is 1:1 in oil derived from Mexican anchovy, 1:2 in oil derived from Atlantic cod; 1:3 in oil derived from American shark; 1:4 in oil derived from Bluefin tuna; 1:6 in rainbow trout; 3:1 in oil derived from Peruvian anchovy, Japanese cod or menhaden.

It has been suggested that acute doses of fish oils may be beneficial in a therapeutic strategy, in quantities such as 30-50 g/day (Harris et al., 1983), although doses as low as 5 g/day and 2 g/day have been shown to decrease serum triacylglycerols (Sanders, 1983; Schmidt et al., 1992). In patients at risk from CVD, a low dose of fish oil (1-6 g/day) may be beneficial in conjunction with a prudent diet (Gibson, 1988). It is not clear whether the benefits of dietary ω3 fatty acids can be obtained simply by increasing fish consumption, or whether refined, concentrated supplements are required. The types of fish most commonly consumed in Western diets contain only 1-2% fat, therefore a large increase in dietary fish consumption may be required in order to realize any benefits. A recent U.S. prospective cohort study of 21,185 male physicians did not find any reduction in the risk of developing CVD with moderate fish consumption, even when fish intake was greater than 5 fish meals per week (Morris et al., 1995). Over the 4 year followup period, there was no relationship between dietary fish intake and myocardial infarction, stroke, or cardiovascular death, even when adjusted for history of hypertension, hypercholesterolemia, diabetes mellius, angina pectoris, parental history of myocardial infarction before age 60, obesity, exercise, smoking, alcohol use, saturated fat inake and use of vitamin supplements.

The metabolism of ω3 fatty acids has not been well-defined in human tissues, and this metabolism cannot be assumed to be comparable to animals studies: differences in ω3 fatty acid metabolism have been described among species, as well as among tissues within a species (Lokesh et al., 1984; Morita et al., 1983).

1.11 Nutrients Alter Gene Expression

An increasing amount of evidence suggests that nutrients play a significant role in the regulation of gene expression (Clarke and Abraham, 1992; Clarke and Jump, 1993, 1994, 1996;

McGrane and Hansen, 1992; Jump et al., 1994, 1995; Sanders and Kline, 1995). Four events control the process of gene expression:

1) gene transcription (regulated by *trans*-acting nuclear proteins and *cis*-acting elements [nucleotide sequences in the 5' or 3'-untranslated regions]),
2) mRNA processing (capping, polyadenylation, splicing and sequence editing),
3)) mRNA stability (regulated by cytosolic proteins),
4) mRNA translation (rates).

The development of transgenic mice has opened the field of nutrition research tremendously. Transgenic mice are used to express exogenous genes, express genes in a tissue-specific manner, or not express endogenous genes (Knapp and Kopchick, 1994). Transgenic mice may be used to study the effect of nutrition on the regulation of gene expression, or conversely, the effect of gene expression on nutrient utilization or metabolism. As a result, studies of the interaction of nutrition in transgenic animal models of human pathologies will provide a greater insight into the underlying mechanisms of diet and disease.

Dietary lipids, carbohydrates, vitamins and minerals are capable of modulating gene expression. Most studies examining the nutrient control of gene expression have been carried out in the liver, adipose tissue, or cultured hepatocytes and islet cells (Clarke and Abraham, 1992). Little is known about the nutrient control of gene expression in the intestine. The type of control mechanism(s) exerted by nutrients depends upon the gene and the tissue where the gene is expressed.

Intact glucose itself plays a role in the post-transcriptional control of SGLT1 gene expression in lamb intestine (Shirazi-Beechey et al., 1991). After the development of the rumen, less glucose reaches the intestine resulting in a decline in SGLT1 mRNA and protein. Weaning from a milk diet to a grass diet is associated with a decline in SGLT1 mRNA without any change in the amount or activity of SGLT1 protein. The decrease in SGLT1 mRNA can be prevented if the animals are maintained on a milk replacer diet beyond the weaning period. Glucose infusion into adult sheep results in the re-expression of SGLT1. These studies suggest that glucose is required to maintain and up-regulate SGLT1 protein. Glucose may also play a role in the regulation of transcription of GLUT2 (Waeber et al., 1994).

Feeding a fructose-enriched diet induces the expression of GLUT5 in proximal jejunum (Burant and Saxena, 1994; Castello et al., 1995; Inukai et al., 1993) possibly through a mechanism that increases cAMP (Mahraoui et al., 1994). Weaning rats onto a high fat diet partially prevents the induction of GLUT5 gene expression associated with weaning (Castello et al., 1995).

Enzymes of hepatic and renal gluconeogenesis (i.e. phosphoenolpyruvate carboxykinase) are subject to nutritional and hormonal regulation in both liver and kidney (Gurney et al., 1994). Dietary glucose induces the hepatic gene transcription of several genes that code for glycolytic enzymes (phosphfructokinase, pyruvate kinase) (Rongnoparut et al., 1991; Munnich et al., 1987),

lipogenic enzymes (acetyl-CoA carboxylase, fatty acid synthase) (Katsurada et al., 1990a, b; Clarke et al., 1990), as well as apo E, (Strobl et al., 1989) and is mediated by a product of carbohydrate metabolism, possibly a novel adenine-containing nucleotide; (Mariash, 1989). Hepatic apoB mRNA editing is altered with a high sucrose diet: cytosine is converted to uracil resulting in the creation of a premature stop codon which converts apoB-100 to apoB-48 (Baum et al., 1990). Increases in dietary carbohydrate increases the hepatic expression of malic enzyme by increasing mRNA stability (Dozin et al., 1986). The *trans*-acting proteins have not been identified, but the *cis*-acting carbohydrate response elements of pyruvate kinase and insulin have been cloned (Thompson and Towle, 1991).

Feeding rats a high glucose diet + 10% triolein enhanced the hepatic mRNAs encoding for several enzymes in the glycolytic pathway (Jump et al., 1994). When the triolein was replaced with menhaden oil, mRNAs for enzymes in the lipogenic pathway were equally suppressed. The data suggest that polyunsaturated fatty acids (PUFA) can coordinately regulate both lipogenic and glycolytic enyzmes.

Liver and adipose tissue levels of lipogenic enzyme mRNA are influenced by diet and hormones (Girard et al., 1994). In liver and adipose tissue, endogenous fatty acid synthesis may be inhibited by PUFA in the diet (Clarke et al, 1976). Exogenous fatty acids from the diet may belong to a different metabolic pool than endogenous fatty acids, and thereby may produce different physiological effects.

Long chain fatty acids are components of complex lipids, including membrane lipids, that function as important metabolic substrates and also play a role in signal transduction (Glatz et al., 1995; Dennis et al., 1991). Arachidonic acid is a precursor for eicosanoids (prostaglandins, leukotrienes, thromboxanes) and a second messenger of some G-protein mediated cellular receptors, while palmitic and oleic acids play a role in the regulation of gene transcription (Clarke and Jump, 1993; Distel et al., 1992). Long chain fatty acids are able to mediate direct effects without undergoing metabolism, and act as signals in the induction of L-FABP, peroxisomal B-oxidation and cytochrome P450 4A1 (Amri, et al., 1994; Grimaldi et al., 1992; Jump et al., 1994; Kaikus et al., 1993a,b). Fatty acid modulation of gene expression may be mediated by ligand-activated transcription factors (Isseman et al., 1990; Gottlicher et al., 1992).

PUFA play a direct role in the hepatic regulation of the gene expression of enzymes involved in lipid and LDL receptor synthesis (Jump et al., 1995). Dietary PUFA induce the expression of the LDL receptor gene when compared with feeding dietary saturated fatty acids (Spady and Dietschy, 1985). In contrast, a continuous diet enriched in PUFA rapidly inhibits the gene transcription of fatty acid synthase (Clarke, 1993), while saturated and monounsaturated fatty acids do not have any effect. A greater fatty acid chain length and degree of unsaturation increases the potency of inhibition, with the fatty acids in fish oil (20:5ω3, 22:6ω3) having the most potency (Clarke et al., 1990). Unknown intracellular metabolites of 18:2ω6 or 18:3ω3 are

responsible for the inhibitory effects. Δ^6-desaturation is required for inhibition and provides support for the increased potency observed with 20 carbon vs 18 carbon fatty acids, although Δ^5-desaturation is not essential in the regulation of the fatty acid synthase gene. The fatty acid synthase gene, its promoter and 2.5 kb 5'-flanking region have been cloned (MacDougald et al., 1992). The 5'-flanking region contains tissue-specific *cis*-acting elements which are hormonally responsive. Weaning animals onto a high carbohydrate diet increases plasma glucose and insulin levels. An increase in glucose metabolism is required to express fatty acid synthase and acetyl CoA carboxylase. Expression of the fatty acid synthase gene in adipose tissue, lung, kidney or small intestine is not inhibited by dietary PUFA (Clarke, 1993). A reduction in plasma glucagon inhibits the accumulation of lipogenic enzyme mRNA in liver and white adipose tissue (Girard et al., 1994). A PUFA response element has not yet been defined but shares similarity with the element responsive to the peroximsome proliferator-activated receptor (PPAR) (Girard et al., 1994). A recent review suggests that PPAR is not the PUFA response factor, and presents evidence to suggest that PPAR and PUFA regulation of gene expression involves separate and independent mechanisms (Clarke and Jump, 1996).

Stearoyl-CoA desaturase 1 (SCD1) and SCD2 genes have been used to study cell differentiation, tissue-specific gene expression and dietary regulation of gene expression in mouse liver (Ntambi, 1995). Dietary carbohydrates and PUFA or their metabolites can directly or indirectly regulate the expression of the mouse hepatic SCD1 gene (Ntambi, 1992). Linoleic acid and arachidonic acid down-regulate the transcription of SCD1 (Landschultz et al., 1994). Fructose or insulin administration to fasted diabetic mice increases hepatic SCD1 mRNA 23-fold and SCD1 transcription 10-fold within 24 hours (Waters and Ntambi, 1994).

Cholesterol also plays a regulatory role in gene expression. The binding of an oxidation product of cholesterol to a nuclear sterol binding protein inhibits the transcription of the HMGR gene in liver and ovaries, and may also inhibit hepatic HMGR mRNA translation (Takagi et al, 1989; Su et al, 1990; Rennert et al, 1990). Rat liver, intestinal and renal mitochondrial HMG CoA synthase mRNA increases slowly during fetal life, increases markely after birth, and remains elevated during suckling or weaning onto a high fat diet (Thumelin et al., 1993). HMG CoA synthase mRNA is reduced by half in liver, and totally absent from intestine when animals are fed a low fat diet.

PUFA are important mediators of gene expression because they affect the activity of transcription factors which target key cis-linked elements associated with specific genes (Clarke and Jump, 1994, 1996). This targeting process is not well understood. The nuclear actions of PUFA require further elucidation in order to provide insight into the potential therapeutic role of PUFA in disease.

1.12 Intestinal Adaptation

Intestinal adaptation is the ability of the intestine to change functionally in response to differences in environmental conditions. The patterns, mechanisms, signals and time course of intestinal adaptation have been reviewed (Karasov and Diamond, 1983a; Diamond and Karasov, 1987; Thomson et al., 1990; Thomson, 1995). Patterns of adaptation arise in response to factors such as diet, metabolic requirements, age or reproductive status. Mechanisms of adaptation describe the manner in which function may be altered, such as changes in transport kinetics, UWL, membrane permeability,or changes in cell maturity. Signals of adaptation may relate to hormone levels, ATP levels or changes in solute concentration. The time course of adaptation addresses its reversibility and whether the adaptation is an acute response from cells already situated along the villus, or whether the adaptation is a long-term response from cells in the crypt. Adaptation may occur via different processes in the jejunum and ileum.

The process of intestinal adaptation has been examined in a variety of models. Glucose transport increases with pregnancy (Musacchia and Hartner, 1970), lactation (Cripps and Williams, 1975), high carbohydrate intake (Diamond and Karasov, 1984), hyperglycemia (Csaky and Fisher, 1981; Fischer and Lauterbach, 1984; Maenz and Cheeseman, 1986) and following bowel resection (Gleeson et al., 1972; Robinson et al., 1982; Thomson, 1986b). Carrier-mediated hexose transport increased, while passive lipid transport decreased with aging (Bowman and Rosenberg, 1983; Hollander and Morgan, 1979a, b; Holt and Dominguez, 1981; Jakab and Penzes, 1981; Klimas, 1968; Pelz et al., 1968; Thomson, 1979c, 1980a,b, 1981b; Toloza and Diamond, 1992). Both carrier-mediated and passive transport were increased with diabetes (Fedorak et al., 1987; Olsen and Rogers, 1970; Thomson, 1980c, 1981a, 1983a,b; Thomson and Rajotte, 1983a,b). Carrier-mediated and passive transport were both decreased with external abdominal irradiation (Thomson et al., 1983, 1984a,b) and chronic ethanol consumption (Thomson, 1984a). In order to study the mechanism of this adaptation, BBM enzyme activity, villus morphology and BBM composition were examined in a variety of animal models and involved the effects of aging, diabetes mellitus, chronic ethanol consumption, short bowel syndrome and external abdominal irradiation (Keelan et al., 1985a,b,c,d; 1986). The changes observed in active and passive nutrient transport also did not correlate with changes in BBM enzyme activity, or static measurements of morphology, but they were associated with alterations in BBM phospholipid content and composition. The ratio of alkaline phosphatase/sucrase was reduced with aging, chronic alcohol consumption and following external abdominal irradiation, but was unchanged with diabetes. Jejunal mucosal surface area reduced with aging and following external abdominal irradiation, yet it remained unchanged with diabetes and chronic alcohol consumption. BBM total phospholipid and phospholipid/cholesterol (PL/C) increased with aging, diabetes and following external abdominal irradiation, but reduced with chronic alcohol consumption. BBM cholesterol content remained constant in the various animal models studied,

and it has been suggested that BBM cholesterol is tightly regulated (Brasitus and Schachter, 1982). Even a high cholesterol diet will not significantly alter the cholesterol content of the BBM of normal animals (Keelan et al., 1987). Aging and chronic alcohol consumption were associated with a decline in phosphatidylcholine (PC) content, while diabetes and external abdominal irradiation were correlated with an increase in PC and phosphatidylethanolamine (PE) content. BBM phospholipid fatty acid composition was not determined in these studies. Changes in BBM phospholipid content and composition then, may have a relationship with alterations in nutrient transport, and may be regulated in an adaptive response to animal model manipulation. These findings directed investigations into the mechanisms and signals by which changes in membrane lipid composition could modulate intestinal function.

Membrane cholesterol content, phospholipid and fatty acid composition can be modified in mammalian cells (Spector and Yorek, 1985). Changes in membrane lipid composition may alter the physical properties of the membrane, which, in turn, may result in alterations in the activity of membrane-bound proteins. Changes in membrane composition may produce alterations in cell function, like enzyme activity, carrrier-mediated and passive nutrient transport. BBM lipid composition has been reported for rat intestine (Brasitus and Schachter, 1984; Brasitus et al., 1984; Forstner et al., 1968; Keelan et al., 1985a,1986; Meddings and Thiessen, 1989; Millington and Gritchely, 1968), mouse intestine (Kawai et al., 1974; Billington and Nayudu, 1978), rabbit intestine (Bloj and Zilversmit, 1982; Hauser et al., 1980; Keelan et al., 1985b,c,d; Meddings et al., 1990), pig intestine (Christiansen and Carlsen, 1981) and human intestine (Dudeja et al., 1991a).

Fluidity is a physical property of the lipid composition of the membrane. Changes in membrane fluidity may be associated with alterations in membrane lipid composition and function (Schachter, 1984; Stubbs and Smith, 1984). Studies of BBM fluidity have been performed in rat intestine (Brasitus and Schachter, 1980,1982; Brasitus et al., 1979,1980,1984; Dudeja et al., 1991b; Meddings, 1988; Meddings and Thiesen, 1989; Schachter and Shinitzky, 1977; Schwarz et al., 1985) and rabbit intestine (Meddings et al., 1990; Mütsch et al., 1983; Schwarz et al., 1984). Intestinal BBM fluidity is low compared to other tissue membranes, due to a low ratio of phospholipid to cholesterol (Brasitus et al., 1980, Nassar et al., 1981; Gourley et al., 1983). BBM fluidity decreases with age (Brasitus et al., 1984; Omodeo-Sale et al., 1991). Passive lipid permeability and carrier-mediated D-glucose uptake are influenced by changes in BBM fluidity (Brasitus et al., 1989; Meddings, 1988, 1990; Meddings and Thiesen, 1989; Meddings et al., 1990). When young animals are weaned from the high fat breast milk diet to a high carbohydrate diet, lipid permeability decreases. BBM fluidity also decreases and is associated with changes in BBM lipid composition (Meddings and Thiesen, 1989).

Dietary constituents may provide a signal for intestinal adaptation (Thomson and Keelan, 1986). Enterocytes lining the small intestine are continually subjected to changes in the quality and quantity of nutrients in the diet. As a result, the intestine must quickly adapt to the constant

variations in dietary load and composition (Diamond, 1991). The response of the small intestine in the mature animal to changes in dietary content and composition is influenced by the type of diets to which the animal is exposed in early life: the ability of the intestine to absorb various nutrients depends on the type of diet fed to the animals at a young age, and has led to the concept of "critical period programming" of the small intestine (Karasov and Diamond, 1983a; Karasov et al., 1985; Keelan et al., 1990c; Thomson et al., 1989b). Critical period programming has been defined as "a biological mechanism which is turned irreversibly on or off only once during an individual's lifetime in response to conditions prevailing at some critical stage" (Karasov et al., 1985). The ability of the enterocyte to adapt to this dynamic environment and to maintain its physiological function is not well understood. Changes in the dietary macronutrient content (i.e. protein, carbohydrate, cholesterol or essential fatty acid content) result in alterations in villus height, BBM enzyme activity, BBM lipid composition and nutrient transport (Thomson and Rajotte, 1983a,b; Keelan et al., 1987, 1990a). Alterations in dietary fatty acid composition are also correlated with changes in intestinal nutrient transport (Thomson et al., 1987c,1988b). Animals fed diets enriched with linoleic acid are associated with a decline in glucose uptake, compared with animals fed a saturated fatty acid diet (Thomson, et al., 1987c), while the lowest glucose uptake was observed with animals fed a diet enriched with ω3 fatty acids (Thomson et al., 1988b).

Dietary fat unsaturation influences the lipid composition and function of membranes isolated from kidney, brain, heart, liver and intestine (Clark et al., 1983; Foot et al., 1982; Forstner et al., 1968; Innis and Clandinin, 1981; Neelands and Clandinin, 1983; Thomson et al., 1986). The functional changes observed in these studies were related to alterations in the membrane phospholipid fatty acid composition. Alterations in dietary fat unsaturation have been reported to specifically affect intestinal active and passive nutrient transport, dependent upon the nutrient absorbed and the site of absorption (Thomson et al., 1986). The BBM cholesterol and phospholipid composition were not significantly influenced by changes in dietary fat unsaturation. Analysis of the BBM fatty acid composition, however, did reveal changes associated with the unsaturation of the dietary fatty acids (Keelan et al., 1990a). An essential fatty acid deficient diet results in changes in villus and crypt sizes, as well as epithelial cell ultrastructure, yet does not alter rat BBM cholesterol and phospholipid content or phospholipid composition. BBM phospholipid fatty acid composition, however, is markedly influences by feeding an essential fatty acid diet (Christon et al., 1991). It is unknown whether BBM fatty acids and phospholipids respond to the same signal or adapt concurrently.

Glucose perfusion in the ileum produces an increase in jejunal glucose absorption that is inhibited by cycloheximide, and this suggests an induction of glucose transporter synthesis (Debnam, 1985). Short-term hyperglycemia results in an induction in transcellular glucose transport (Csaky and Fisher, 1981; Fischer and Lauterbach, 1984), due to a specific induction of glucose transporter in the jejunal BLM (Csaky and Fisher, 1981; Maenz and Cheeseman, 1986). Feeding adult rats a glucose-enriched diet results in the up-regulation of both BBM and BLM

glucose transport (Cheeseman and Maenz, 1989; Cheeseman and Harley, 1991; Ferraris et al., 1992). In mice fed a high carbohydrate diet, an increase in BBM glucose uptake may be partially explained by an increase in SGLT1 expression in enterocytes emerging from the crypt (Ferraris and Diamond, 1992). Feeding a fructose-enriched diet enhanced GLUT5 protein up to eight-fold at seven days, with only a transient increase in GLUT5 mRNA at day one that was normalized by day seven (Burant and Saxena, 1994).

Systemic administration of epidermal growth factor results in an up-regulation of ileal glucose transport associated with changes in BBM phospholipid composition and a decline in cholesterol content, resulting in a more fluid membrane (Oplete-Madsen et al., 1991). Orogastric administration of epidermal growth factor was also associated with increased glucose transport, but this functional adaptation was secondary to mucosal hyperplasia.

Glucose absorption increases in alloxan-, streptozotocin-, or genetically diabetic animals (Crane, 1961; Csaky and Fisher, 1981; Leese and Mansford, 1970; Olson and Rosenberg, 1970; Ramaswamy et al., 1980; Schedl and Wilson, 1971; Thomson, 1981a). An increase in tight junction permeability is observed with diabetes (Phipott etl al., 1992). Both BBM and BLM glucose absorption increases with diabetes (Debnam et al., 1990; Fedorak et al., 1[illegible], Philpott et al., 1992). Diabetes is associated with an increase in carrier-mediated and passive intestinal nutrient uptake (Thomson, 1980c,1981a,1983a,b) which can further be modified by insulin (Westergaard, 1989) and islet cell transplantation (Thomson and Rajotte, 1984, 1985), alterations in dietary protein, carbohydrate, cholesterol and essential fatty acid content (Thomson and Rajotte, 1983a,b), or alterations in dietary fatty acid composition (Thomson et al., 1987a,b, 1988a). Glucose uptake is altered to different degrees in the jejunum and ileum of diabetic animals (Fedorak et al., 1987) and occurs independently of hyperphagia (Fedorak et al., 1990). The diabetes-associated increase in intestinal glucose uptake may be mediated by a decline in glucose-independent Na^+ leakage across the BBM and not an increase in the number of glucose transporters (Hopfer, 1975). Na^+/K^+-ATPase activity increases in experimentally induced diabetes (Luppa et al., 1978; Wild et al., 1996a), which may, in turn, increase the Na^+ gradient across the BBM and enhance glucose uptake. The increased Na^+ gradient would also explain the increase in carrier-mediated uptake of amino acids and bile salts that is observed with diabetes (Caspary, 1973; Olson and Rosenberg, 1970; Thomson, 1983b). The most marked diabetes-associated increases in Na^+/K^+-ATPase activity, protein expression and mRNA abundance are present in the rat ileum (Wild et al., 1996a). Proximal to distal gradients were observed for Na^+/K^+-ATPase activity and protein expression, but not mRNA abundance. This suggests that alterations in transcriptional and post-transcriptional events are involved in the adaptive response to diabetes.

In addition to an increase in glucose transport, diabetes is associated with an increase in glucose utilization arising from the decreased activity of 6-phosphofructo-1-kinase (PFK_1) (Jamal and Kellet, 1983). Stimulation of the glycolytic pathway by vanadate is also correlated with a

decline in the function and expression of sodium-dependent glucose transport in LLC-PK1 cells (Madsen et al., 1994). Both a down-regulation of glucose transport and an increase in glucose utilizaton are required to achieve an overall reduction in glucose absorption. Oral vanadate supplementation down-regulates the sodium-dependent glucose carrier in both control and streptozotocin-diabetic rats, although Na^+/K^+-ATPase activty was affected only in diabetic rats (Madsen et al., 1993). Vanadate restores the decreased activity of PFK_1 associated with diabetes, normalizes blood glucose levels and glucose uptake (Madsen et al., 1995).

Another mechanism of increased ileal glucose uptake, observed with diabetes, may be a recruitment of enterocytes down the villus of the ileum (Fedorak et al., 1989). An increase in SGLT1 transporters in the lower villus and crypt region, as determined by [^{3}H]-phlorizin binding to the SGLT1, was associated with an increase in transport activity to a greater extent than the villus tip region (Fedorak et al., 1987). Altered expression of the glucose carrier occurred in the ileum of both acute and chronic diabetic rats, but only with chronic diabetes in the jejunum (Fedorak et al., 1991).

Both SGLT1 and GLUT2 mRNA were increased in the mucosal scrapings of chronically diabetic rats probed with human glucose transporter cDNA probes (Miyamoto et al., 1991). Using *in situ* hybridization, Burant et al. (1994) reported 4-to 8-fold increases in glucose transporter mRNA (SGLT1 and GLUT2 > GLUT5), with 1.5- to 6-fold increases in SGLT1, GLUT2 and GLUT5 (BBM fructose transporter) protein in both the jejunum and ileum of diabetic rats. Wild et al. (1996b,c) confirmed these results for GLUT2 and GLUT5, as well as for SGLT1. Castello and coworkers (1995) reported an 80% increase in GLUT5 mRNA in the mucosa from proximal jejunum. Insulin administration reversed the diabetes-associated increases in transporter expression and mRNA (Burant et al., 1994). The data suggests that the increase in hexose transport observed with diabetes is due to a premature expression of hexose transporters in enterocytes along the crypt-villus axis, resulting in a cumulative increase in transporter protein during enterocyte maturation (Burant et al., 1994). The mechanism by which diabetes increases glucose transporter expression is not clear. It is not due simply to a lack of insulin because a high carbohydrate diet increases intestinal glucose transport at the BLM (Cheeseman and Maenz, 1989; Debnam and Chowrimootoo, 1992) and BBM (Ferraris et al., 1992), while glucose infusion also increases BLM glucose transport (Cheeseman and Maenz, 1989). It is the increased loads of glucose in either the blood or intestine (secondary to hyperphagia with diabetes) that appear to induce precocious expression of the glucose transporter genes in the crypt cells (Burant et al., 1994).

Using immunogold cytochemistry, longer villi observed in diabetic rats supported the concept of larger number of enterocytes in jejunum (Debnam et al., 1995). Normal distal ileal villi had no detectable glucose transport or SGLT1 protein until the rats were treated with streptozotocin. Protein and transport increased from the crypt to the villus tip. The close

correlation of positional expression of SGLT1 and transporter function suggest that transport density is important for the up-regulation of transport in diabetes.

BBM lipid composition and minor morphological changes have been reported for 5-day diabetic rats (Gourley et al., 1983) and for 14-day diabetic rats (Keelan et al., 1985b,1990b). The total phospholipid content of BBM increased with diabetes due to increases in PC and PE content. Diabetes is also correlated with changes in the membrane lipid composition of other rat tissues (Chandramouli and Carter 1975, Clark et al., 1983, Faas and Carter, 1981, 1983; Holman et al., 1983; Lemieux et al., 1984). Although phospholipid content and composition were not altered in the renal and hepatic tissues of diabetic rats, the arachadonic acid content in PC and PE was reduced while linoleic acid content increased. Similar changes in phospholipid fatty acid composition were observed in other rat tissues. Insulin returned the fatty acid composition to normal. Diet may influence the fatty acid composition of diabetic rat tissue phospholipids since mammalian cells require exogenous sources of linoleic acid in order to synthesize long-chain polyunsaturated fatty acids (PUFA) (Holman et al. 1982). The mechanisms by which diet and diabetes may influence intestinal lipid traffic and metabolism have not yet been elucidated.

Since diets may alter intestinal function, diets possibly may be designed as a therapeutic nutritional strategy to alter nutrient absorption in the desired direction. For example, feeding a saturated fatty acid diet prevented the decrease in intestinal function observed in animals exposed to external abdominal irradiation or chronic ethanol consumption, compared to those fed a polyunsaturated fatty acid diet (Thomson et al., 1989b, 1991). On the other hand, feeding a polyunsaturated fatty acid diet prevented the increase in nutrient uptake observed with diabetes in animals fed a saturated fatty acid diet (Thomson et al., 1987a,b, 1988a).

1.13 Aims and Scope of Research

The manner in which dietary lipids modify passive and carrier-mediated nutrient absorption is postulated to occur by alterations in membrane lipid synthesis/turnover, which thereby dictate membrane lipid composition, and subsequently influence membrane function. Diabetes and alterations in dietary fatty acid composition are associated with changes in nutrient transport and BBM phospholipid fatty acid composition. Since very little is known about the regulation and control of lipid metabolism within the enterocyte, future studies must define the regulatory and control mechanisms for lipid synthesis, as well as the subcellular traffic of lipids for membrane synthesis.

The intestinal mucosa is a site of rapid cell turnover (every 48-72 hours) and therefore it is a site of rapid membrane synthesis. Since diet has an influence on BBM composition and function, it is highly probable that diet affects lipid and membrane synthesis by influencing the subcellular traffic of lipids. There is no direct information on whether the type of dietary fatty acids

will influence the pathways of enterocyte phospholipid synthesis, and ultimately, membrane composition.

Many enterocyte functions depend upon the lipid composition of the membrane. For example, intestinal alkaline phosphatase activity is influenced by the content of PC in the membrane (Seetharum et al., 1985). The permeability properties of the BBM are a result of the membrane lipid composition, thereby influencing the passive and protein-mediated uptake of nutrients (Meddings, 1990). Changes in BBM fluidity and lipid composition alter lipid permeability (Meddings, 1988; Meddings and Thiesen, 1989; Meddings, 1990). Glucose uptake is also influenced by the fluidity and lipid composition of the BBM (Brasitus et al., 1989; Dudeja et al., 1990, 1991a; Meddings et al., 1990).

Diabetes mellitus alters BBM composition and function in animal models (Keelan et al., 1985a; Thomson, 1980c, 1981a, Thomson and Rajotte, 1983a,b). Alterations in dietary fatty acid composition are associated with changes in nutrient uptake in the diabetic animal (Thomson et al., 1987a,b, 1988a). An understanding of the mechanisms which regulate membrane composition and how the membrane responds to changes in nutrient absorption must be defined. The information acquired from these studies would allow investigation into nutritional strategies which may normalize membrane function in diseases such as diabetes.

The objectives of this research proposal are:

1) to determine whether dietary lipids are modulators of intestinal BBM and EMM composition and function; and
2) to identify the mechanism by which these compositional and functional changes are mediated.

These aims will be achieved by testing the following hypotheses:

Hypothesis 1: Modifications in dietary fat unsaturation produce changes in the *in vitro* and *in vivo* intestinal permeability. Chapter 2 establishes the BBMV preparation as a technique suitable for the study of *in vitro* glucose and lipid uptake in normal chow-fed animals. Chapter 3 examines the possible mechanisms of lipid uptake across the BBM in normal chow-fed animals.

Hypothesis 2: Alterations in dietary fat unsaturation normalize the altered intestinal transport function observed with diabetes. Chapter 4 describes the effect of modifications in dietary fat on the *in vitro* uptake of glucose into BBMV isolated from control and diabetic rat jejunum. Chapter 5 investigates the effect of modifications in dietary fat on the *in vitro* sheet intestinal uptake of glucose and lipids in diabetic rats. Chapter 6 examines the effect of modifications in dietary fat on the *in vivo* intestinal uptake of glucose in control and diabetic rats.

Hypothesis 3: Alterations in dietary fat unsaturation and enterocyte lipid metabolism are associated with changes in BBM and EMM composition. Chapter 7 describes the effect of feeding animals diets enriched with different fatty acids on BBM phospholipid fatty acid composition. Chapter 8 investigates the effect of feeding animals diets enriched with different

fatty acids on EMM phospholipid fatty acid composition, Δ^9, Δ^6 and Δ^5 desaturase activities, as well as CPT and PEMT activity.

Hypothesis 4: Alterations in dietary fat unsaturation normalizes the altered intestinal membrane composition and function observed with diabetes due to changes in enterocyte lipid metabolism. Chapter 7 describes the alterations in BBM lipid composition in control and diabetic rats fed either a saturated or a polyunsaturated fatty acid diet. Chapter 8 investigates the alterations in EMM lipid composition, CPT and PEMT activity in control and diabetic rats fed either a saturated or a polyunsaturated fatty acid diet. Chapter 9 examines the alterations in BBM and EMM phospholipid fatty acid composition, as well as EMM desaturase activities in control and diabetic animals.

As a result of the testing of these hypotheses, and accomplishing the specific objectives, a model is proposed in the general discussion (Chapter 10) to attempt to explain the diet- and diabetes-associated alterations in nutrient transport.

Table 1.1 Transport and BBM lipid changes in 5 models of intestinal adaptation.

Model	Hexose Uptake	Lipid Uptake	Phospholipid/ Cholesterol	Phosphatidylcholine
Aging	increased	decreased	increased	increased
Diabetes	increased	increased	increased	increased
Chronic Alcohol Feeding	decreased	decreased	decreased	decreased
External Abdominal Irradiation	decreased	decreased	increased	increased
Distal Resection	increased	increased	increased	increased

Table 1.2 Influence of hormonal factors and nutritional factors on liver microsomal desaturase activity.

	Δ^9 Desaturase Activity	Δ^6 Desaturase Activity	Δ^5 Desaturase Activity
Hormonal Factors			
Insulin	increased	increased	increased
Glucagon	decreased	decreased	decreased
Epinephrine	decreased	decreased	decreased
Glucocorticoids	decreased	decreased	decreased
Thyroxine	decreased	decreased	decreased
Nutritional Factors			
ω6 and ω3 PUFA*	decreased	decreased	decreased
Cholesterol	increased	decreased	decreased
Cholesterol plus ω3 PUFA	unchanged	decreased	decreased
Fasting**	decreased	unchanged	decreased
Refeeding with a high carbohydrate diet	increased	unchanged	unchanged
Refeeding with a high protein diet	unchanged	increased	unchanged

* Small intestinal microsomes are unaffected by feeding ω3 PUFA.

** Small intestinal microsomal Δ^9-desaturase activity is reduced with fasting while Δ^6-desaturase activity is increased.

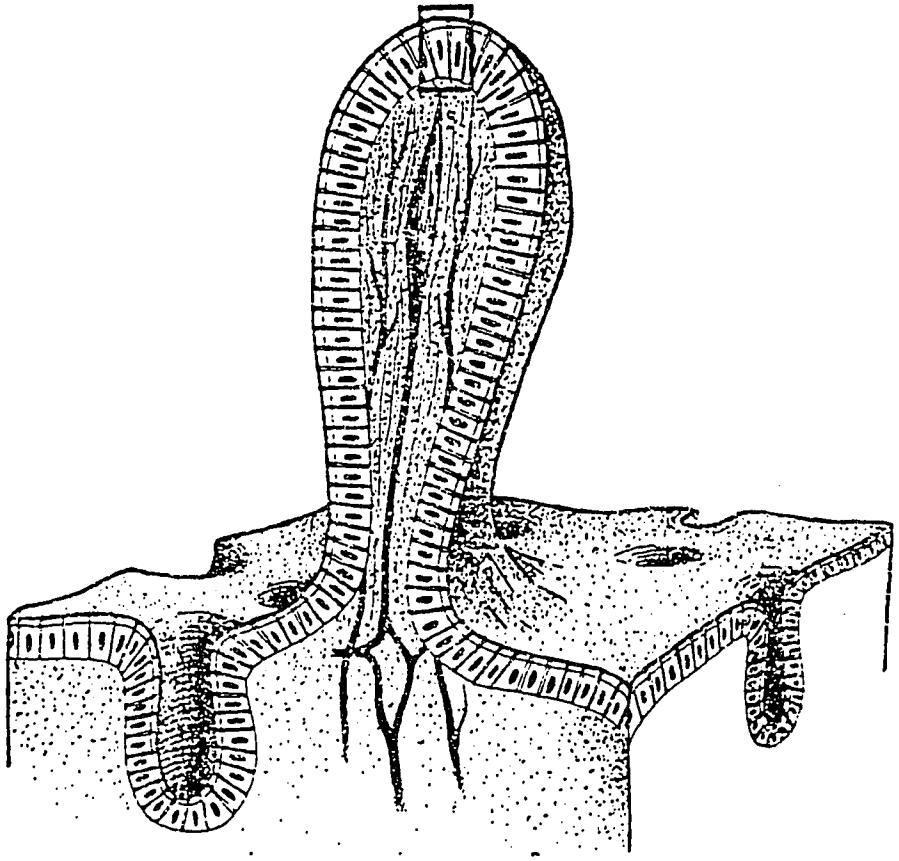

Figure 1.1. Villus structure. The box highlights the enterocyte.

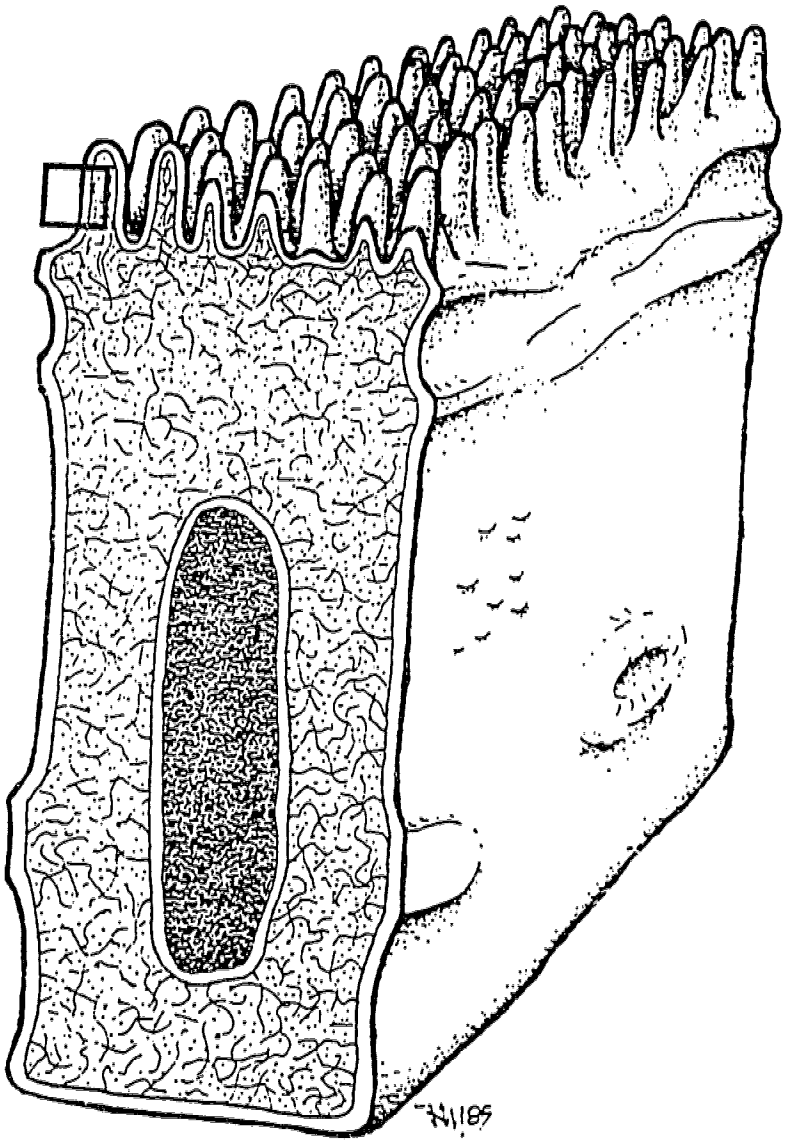

Figure 1.2. Enterocyte. The box highlights the brush border membrane.

Jejunum > Ileum

Crypt > Villus tip

BLM > BBM

BBM_C > BBM_I

Figure 1.3. Intestinal membrane fluidity.

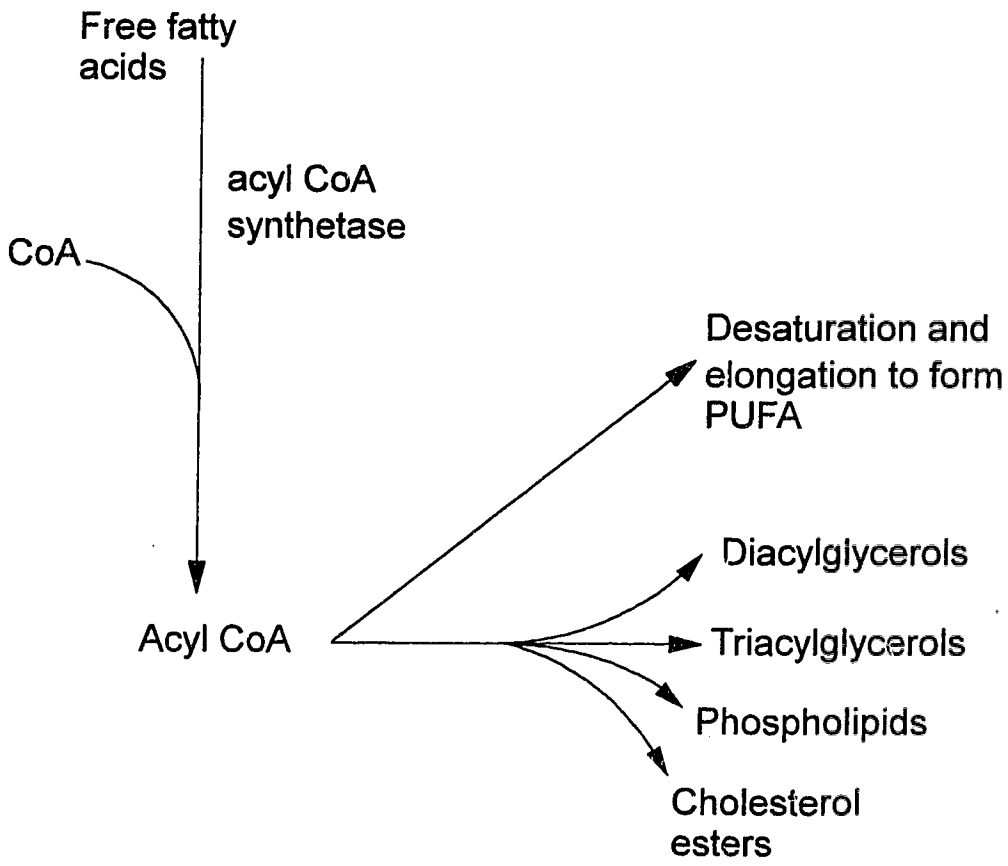

Figure 1.4. Fatty acid metabolism.

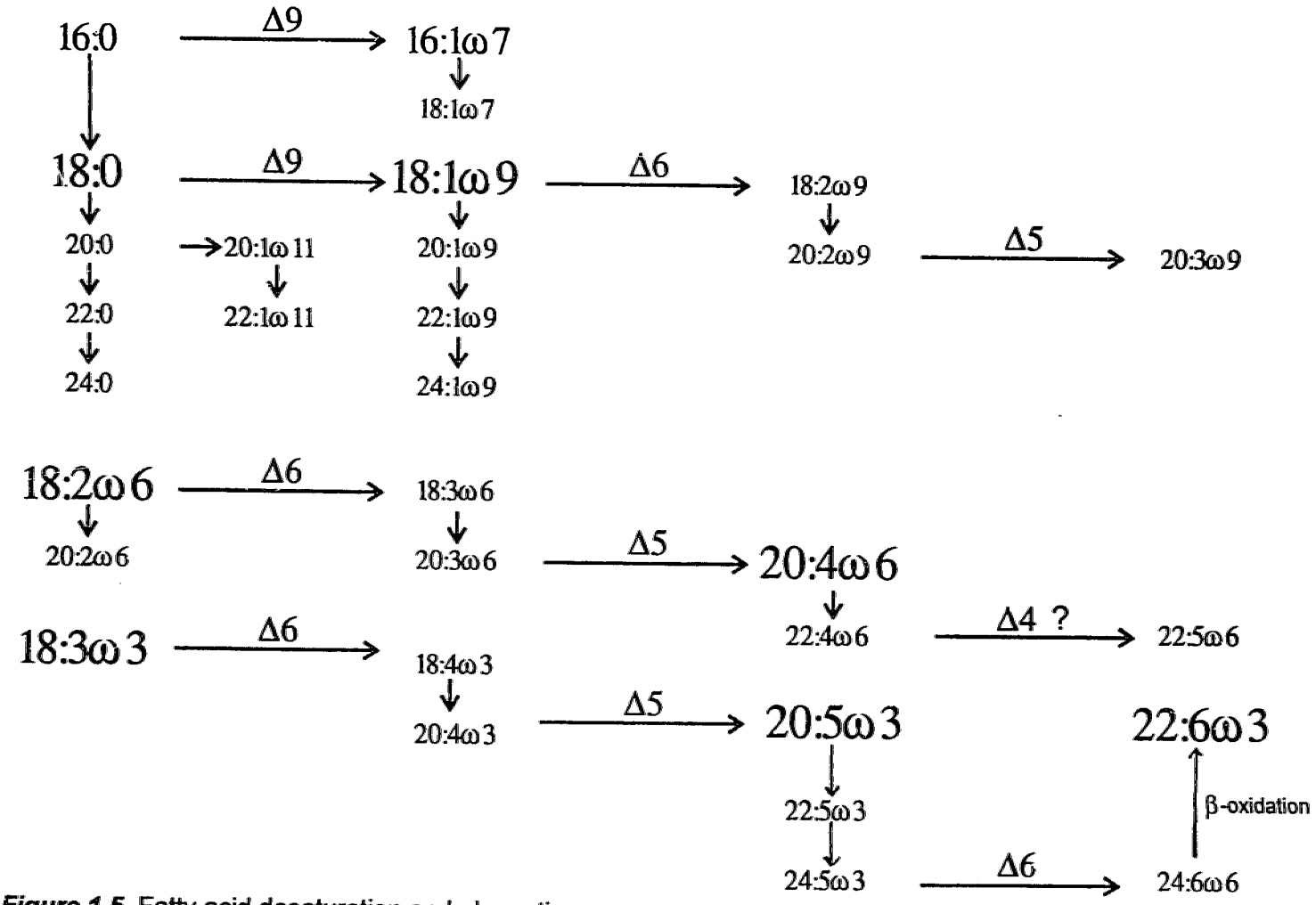

Figure 1.5 Fatty acid desaturation and elongation..

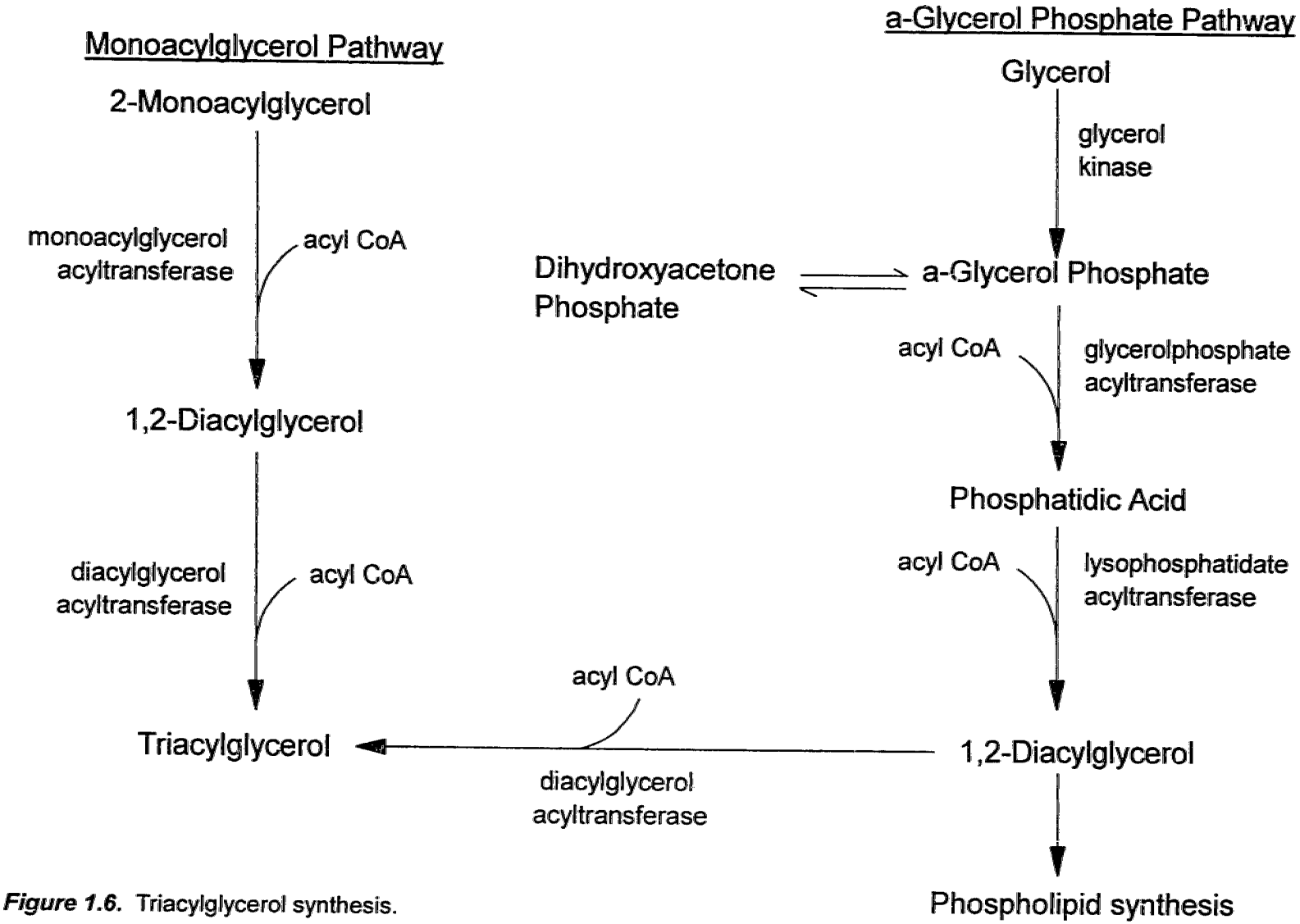

Figure 1.6. Triacylglycerol synthesis.

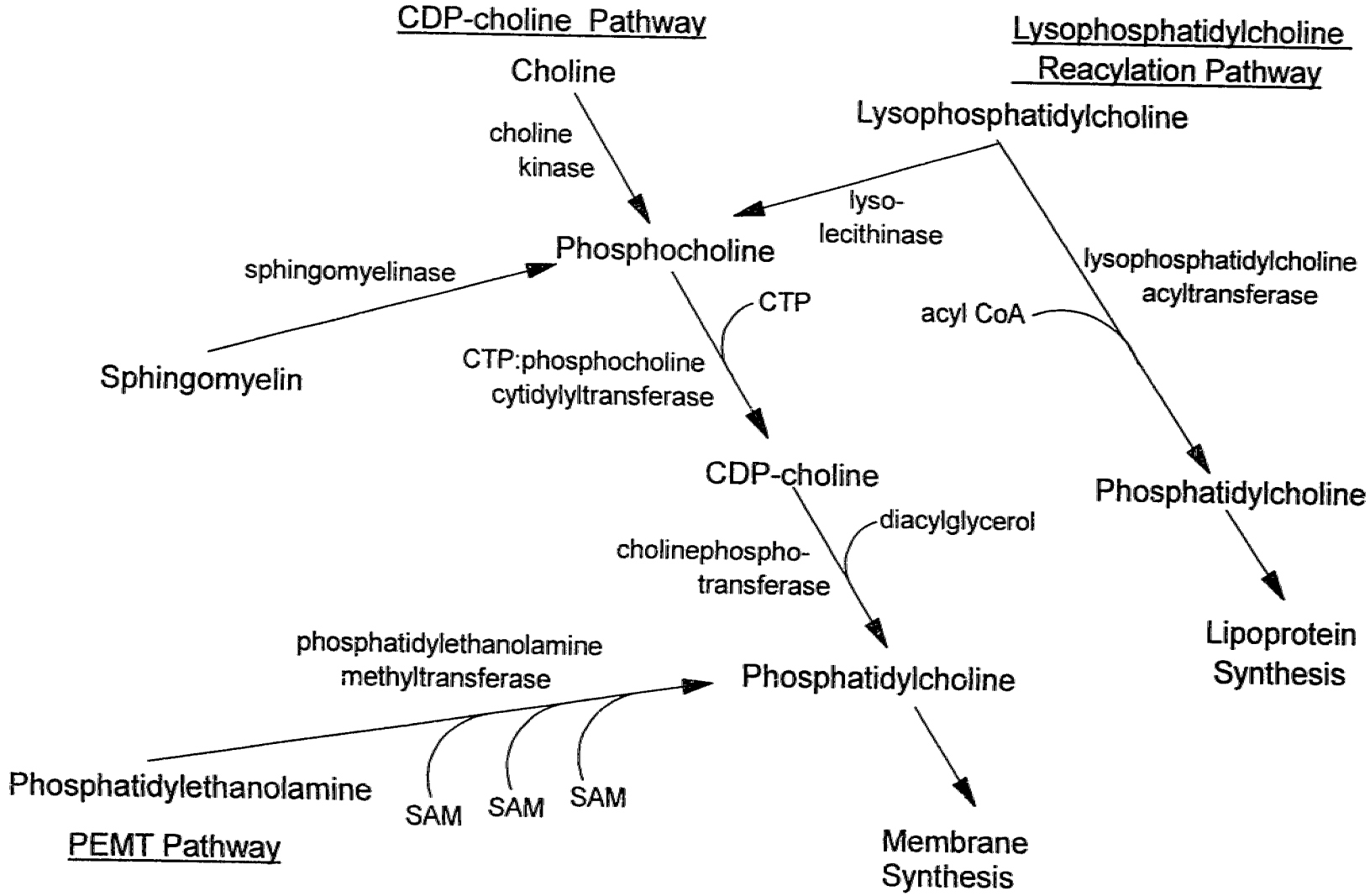

Figure 1.7. Enterocyte phosphatidylcholine metabolism.

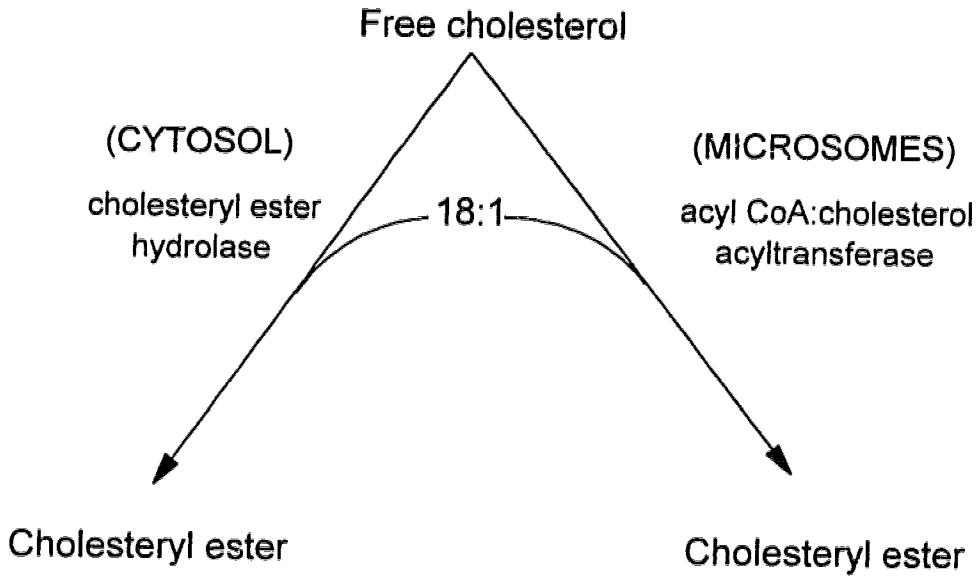

Figure 1.8. Esterification of cholesterol within the enterocyte.

1.13 Bilbiography

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CHAPTER 2

CHARACTERIZATION OF LIPID UPTAKE INTO RABBIT JEJUNAL BRUSH BORDER MEMBRANE VESICLES[1]

2.1 Introduction

A variety of techniques have been used to assess the mechanism of the intestinal uptake of lipids. The general consensus is one of the passive diffusion of lipids across the intestinal unstirred water layer followed by partitioning from the micelle into an aqueous phase and then into the lipophilic phase of the brush border membrane (Thomson et al., 1989). This process may be influenced by fatty acid binding proteins in the cytosol (Shields et al., 1986), and by a membrane-binding protein (Stremmel et al., 1985; Stremmel, 1988). Recently, intestinal brush border membrane vesicles (BBMV) have been used to study the uptake of linoleic acid (LA) (Ling et al., 1989; Proulx et al., 1982). For example, in rabbit BBMV there is an inhibitory effect of phloretin on the uptake of LA and there is an overshoot phenomenon which was interpreted to represent the ability of the BBMV to accumulate LA in the intravesicular space in the presence of a sodium gradient.

We wished to use the BBMV technique to examine the permeability properties of the intestine under a variety of experimental conditions, and initially we set out to attempt to confirm this earlier study.

2.2 Methods and Materials

2.2.a Chemicals

[^{14}C]D-glucose (55 Ci/mol) and [^{14}C]-linoleic acid (55.6 Ci/mol) were purchased from Amersham. All other chemicals were purchased from Sigma, Fisher Scientific or BDH and were of the highest quality available.

2.2.b Vesicle Preparation

Female New Zealand white rabbits weighing 2.0-2.5 kg were sacrificed by an anaesthetic overdose (sodium pentobarbital 800 mg/kg body weight). Brush border membrane vesicles (BBMV) were always prepared fresh on the day of the uptake experiments. BBMV were prepared at 4°C by a modification of the technique of Schmitz et al. (1973) and of Malathi et al. (1979), using the buffers described by Ling et al. (1989). Approximately 80 cm of proximal intestine was

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[1] A version of this chapter has been published in Can. J. Physiol. Pharmacol. 70: 1128-1133, 1992.

removed and flushed three times each with 60 ml of ice-cold saline. The intestine was placed on a pre-chilled glass plate moistened with saline, and the mesenteric border was removed. The mucosa was exposed by cutting along the mesenteric border and then gently blotting it with lint-free tissue to remove excess moisture. The mucosa was removed by scraping with two glass slides, and then was placed into pre-weighed vials containing 5.0 ml isolation buffer (300 mM D-mannitol - 10 mM Tris-HCl pH 7.0) to determine the wet weight of the mucosal scrapings. All steps were done at 4°C. Mucosal scrapings were homogenized in 115 ml of isolation buffer using a Brinkman Polytron® (Brinkman Instruments, Westbury, NY) at a setting of "9" for 30 s. The homogenate was centrifuged at 500 x g for 15 min (Beckman J2-21 high speed centrifuge with JA-14 rotor, Beckman Instruments, Palo Alto, CA). Sufficient 1 M $CaCl_2$ was added to the supernatant to yield a final concentration of 10 mM $CaCl_2$ and the solution was then stirred on ice for 10 min. The solution was then centrifuged at 7500 x g for 20 min (JA-14 rotor). The resulting supernatant was centrifuged at 28000 x g for 20 min (JA-20 rotor). The pellet was resuspended in Vesicle Resuspension Buffer (300 mM mannitol - 10 mM HEPES-Tris, pH 7.4) and was homogenized with the Polytron® at a setting of "8" for 30 s. The homogenate was centrifuged at 30000 x g for 20 min. The final pellet was resuspended in Vesicle Resuspension Buffer and was homogenized with the Polytron® at a setting of "6" for 15 s. Vesicles were equilibrated in Vesicle Resuspension Buffer for 90 min at 4°C prior to uptake studies.

The protein concentration of the vesicle preparation was 3-5 mg/ml as determined by the Hartree modification (1972) of the method of Lowry (1951), using bovine serum albumin as a standard. Sucrase and alkaline phosphatase activities (Dahlqvist, 1964; Bowers et al., 1967) of the final BBMV preparation were enriched 12-15-fold over their activities in the initial homogenates. The BBMV preparation was free of other subcellular membranes (data not shown) as assessed by the lack of activity of Na^+K^+-ATPase (Scharschmidt et al., 1979) glucose-6-phosphatase (Freedland and Harper, 1957), ß-glucuronidase (Glaser et al., 1973), and by DNA (Burton, 1956; Giles & Myers, 1965).

2.2.c Probe Preparation

[^{14}C]D-glucose was dried down under nitrogen and was reconstituted in Glucose Transport Buffer (100 mM D-mannitol - 1 mM $MgCl_2$ - 2 mM $CaCl_2$ - 10 mM HEPES-Tris - 100 mM NaSCN, pH 7.4), giving a final specific activity of 55 Ci/mole. For the LA uptake studies, a 600 µm cold LA stock solution was prepared by weighing out the appropriate amount of LA and dissolving it in 25 ml chloroform. Aliquots were pipetted into tubes, dried down under nitrogen and stored at -80°C until required. The appropriate amount of unlabelled LA (previously reconstituted in chloroform) was added to the [^{14}C]-labelled LA and then dried down together under nitrogen before reconstitution in Lipid Transport Buffer (100 mM mannitol - 1 mM $MgCl_2$ - 2 mM $CaCl_2$ - 10

mM HEPES-Tris - 2 mM sodium taurocholate - 100 mM NaSCN, pH 7.4). The solution was then sonicated until clear. The final specific activity was 7 Ci/mole.

2.2.d Transport Studies

BBMV uptake of substrate was performed at room temperature (20°C) using the rapid filtration technique described by Hopfer et al., (1973) and by Maenz and Cheeseman (1986), using the buffers described by Ling et al., (1989). For incubation times of less than 1 minute, 20 µl probe and 20 µl BBMV preparation (60 µg) were pipetted as separate drops in a 16 x 100 mm polystyrene test tube, ensuring that the two drops did not mix. To start the uptake incubation, the tube was placed on a vortex and mixed for the duration of the incubation time. The incubation was stopped by the addition of 1.25 ml ice-cold Stop Buffer (100 mM NaSCN - 50 mM KCl - 2 mM HEPES-Tris, pH 7.4 with 0.6 mM phloridzin for the glucose uptake studies (Hopfer et al., 1973); and 0.2 mM phloretin for the lipid uptake studies (Abumrad et al., 1984).

For incubation times of greater than 1 min, 50 µl BBMV preparation (150 µg) was added to a 16 x 100 mm polystyrene tube containing 50 µl probe. The mixture was vortexed for 10 seconds and allowed to stand for the duration of the incubation. To stop the reaction, 40 µl of the incubation mixture was added to 1.25 ml ice-cold Stop Buffer. Then, 1.0 ml of the stopped incubation mixture was rapidly filtered through pre-washed 0.45 µm cellulose acetate filters (Millipore [Bedford, MA] Micron Sep Fisher E04WP02500, Cincinnati, OH) under vacuum on an Amicon Filtration Manifold VFMI (Danvers, MA). (The filters were pre-washed twice with 2 ml of 1 mM unlabelled D-glucose in Glucose Transport Buffer or 500 µM unlabelled LA in Lipid Transport Buffer for glucose and lipid experiments, respectively. Pre-wash was necessary to reduce non-specific binding of labelled substrate to the filters.) The filters were then rinsed twice with 2 ml of ice-cold stop buffer and was transferred to scintillation vials.

After drying the filters for 10 minutes at 55°C, HP ReadySolv Scintillant® (Beckman) was added, the vials were vortexed and counted on a Beckman LS 9800 liquid scintillation counter (Beckman Instruments Inc., Palo Alto, CA 94304).

Blanks were prepared in the same way as samples except that the BBMV preparation was substituted by Vesicle Resuspension Buffer. Osmolarity studies were done in the presence of varying concentrations of sucrose in the final vesicle suspension medium.

2.2.e Data Analysis

The data represents the mean ± standard error of the mean of at least four experimental days for each graph. On each experimental day each time point was performed in triplicate for D-glucose and in quadruplicate for LA uptake. Uptake was the net radioactivity after subtraction of non-specific radioactivity bound to filters in the absence of vesicles (blank). This blank value was equivalent to the radioactivity obtained in the presence of BBMV at time 0 (vesicle ± probe ± immediate quenching and filtering). Uptake was expressed as pmol per mg BBMV protein for D-

glucose and nmol per mg BBMV protein for linoleic acid. The time curve (rate of uptake versus time) was used to determine the linearity range of the initial rate of uptake and the time required to reach equilibrium. The initial rate of uptake represents the one-way movement of substrate across the BBMV. Beyond this linear portion of the time-uptake course, substrate movement represents 'net transfer' into the BBMV.

The concentration curve for D-glucose allowed estimations to be made of the maximal transport rate (V_{max}) and the Michaelis constant (K_m). The concentration curve of LA uptake allowed estimation of the diffusion constant (K_d). The commercial computer-generated curve-fitting program, Systat® (SYSTAT Inc., Evanston, Illinois) was used for the estimation of the kinetic constants of the saturable or non-saturable processes.

2.3 RESULTS

2.3.a D-Glucose Uptake

When D-glucose uptake was studied as a function of time, brush border membrane vesicles (BBMV) demonstrated an 'overshoot' phenomenon (Figure 2.IA). This represents the ability of the BBMV to accumulate D-glucose in the intravesicular space in the presence of a sodium gradient. When the scale of the initial portion of this figure was expanded, it was apparent that the glucose uptake was linear to 15 seconds (Figure 2.IB). Accordingly, all subsequent studies at initial rate conditions were done at 5 seconds. Equilibrium uptake was achieved within 10-20 min. The intravesicular space was 2.1 µl/mg protein based on the accumulation of 214 pmol D-glucose/mg protein after a 30 min incubation.

The D-glucose "overshoot" was abolished when glucose uptake was performed in the presence of 0.6 mM phloridzin but not with 0.2 mM phloretin (Figure 2.2A), in the absence of sodium (Figure 2.2B),or in the presence of sodium but in the absence of a sodium gradient (Figure 2.2C). Under these experimental conditions the BBMV uptake of D-glucose resembled the uptake of L-glucose (Figure 2.2D). Since the D-glucose overshoot was unaffected by the presence of phloretin in the incubation medium, these results support the conclusion that we were examining BBMV D-glucose uptake and not basolateral membrane D-glucose uptake (which is inhibited by phloretin but not phloridzin, and is not a sodium-dependent process). Glucose uptake was not affected by the addition of 2 mM taurodeoxycholic acid (data not shown).

In order to establish whether substrate uptake was due to intravesicular accumulation or due to binding to the brush border membrane, osmolarity studies were performed under equilibrium conditions, whereby substrate uptake is examined in the presence of varying concentrations of sucrose in the final vesicle resuspension medium. Sucrose is impermeable to the BBMV and osmolarity is proportional to the sucrose concentration. When glucose uptake is

plotted versus 1/osmolarity, the extrapolated y-intercept represents an infinite osmolarity, which corresponds to the point at which the vesicle intravesicular space is negligible. Accordingly, the amount of substrate associated with the vesicles at infinite osmolarity represents the amount of substrate bound to the membrane. A linear relationship was noted between 1/osmolarity and glucose uptake (Figure 2.3). Negligible amounts of D-glucose were associated with the BBMV (29 pmol/mg protein), suggesting that at least 90% of the glucose uptake represents glucose that is present in the intravesicular space, not bound to the brush border membrane. This result demonstrates that the BBMV preparation was composed of sealed and osmotically reactive vesicles, and is in agreement with other reports in the literature (Hopfer et al., 1973; Maenz and Cheeseman, 1986).

The initial rate of uptake of varying concentrations of D-glucose was determined with 5 second incubation of BBMV. A curvilinear relationship was noted between glucose concentration and uptake (Figure 2.4), which was unaffected by the presence of 2 mM taurocholic acid. The maximal transport rate, V_{max}, was 916 pmol/mg/sec and the K_m was 0.4 mM, as estimated by non-linear regression.

2.3.b Linoleic Acid Uptake

When linoleic acid (LA) uptake was studied as a function of time, no overshoot was demonstrated in the presence of a sodium gradient, and equilibrium was achieved within 2 min (Figure 2.5). The magnitude of LA uptake was 10-fold greater at 1 min and 56-fold greater at 30 min than that observed for D-glucose uptake (Figures 2.1A and 2.5). LA uptake was inhibited to approximately 88% of control values by the addition of 0.6 mM phloridzin, and to approximately 58% of control values by 0.2 mM phloretin (Figure 2.6), but was unaffected by the absence of sodium or by the presence of a sodium gradient (data not shown). This latter data does not agree with the results of Ling et al., (1989) where a spike overshoot phenomenon was observed with LA uptake. This spike was defined by only a single point at 15 seconds. The uptake of LA was not significantly altered by the presence of glucose, nor was the uptake of glucose affected by LA (data not shown).

Changes in the osmolarity of the buffer had no effect on the uptake of LA (Figure 2.7). This was not surprising, since there was no fatty acid binding protein present in the intravesicular space. Without a carrier of some type, lipid would not easily move out of the hydrophobic environment of the BBMV into the aqueous intravesicular space. Lysis of BBMV incubated with LA by the addition of ice-cold deionized water (Boumendil-Podevin et al., 1985) did not alter the amount of LA associated with the BBMV (data not shown). This provides further evidence that no intravesicular accumulation of LA had occurred. This data also differed from the results of Ling et al., (1989) where 40% of the accumulated LA was released by lysis of the BBMV. The LA concentration curve was linear to 160 μM, when carried out under initial rate conditions (Figure

2.8). At higher concentrations, there was considerable variability in the data, possibly due to solubility limitations of higher concentrations of LA with 2 mM taurocholic acid.

2.4 Discussion

Long-chain fatty acids and cholesterol are solubilized by bile acids in the intestinal lumen, diffuse across the intestinal unstirred water layer and then partition from the micelles into an aqueous phase and from there into the brush border membrane (Thomson and Dietschy, 1984; Thomson et al., 1989). This is thought to be a passive process, although several observations have raised the possibility that this consideration must be re-examined: the curvilinear relationship between uptake and low concentrations of lipids (Chow and Hollander, 1979a,b); the presence of fatty acid binding proteins in the cytosol (Shields et al., 1986) and in the brush border membrane (Stremmel, 1988); and recently, by the demonstration of an inhibitory effect of phloretin on the vesicular uptake of linoleic acid (LA) and also by the overshoot phenomenon observed with short durations of incubation of LA (Ling et al., 1989).

Our first step in this series of studies was to confirm that the uptake of D-glucose into BBMV in our hand was comparable to that reported in the literature. We were able to demonstrate an overshoot phenomenon (Figure 2.IA), an inhibitory effect of phloridzin (Figure 2.2A) but not of phloretin (Figure 2.2A), and D-glucose uptake in the absence of sodium (Figure 2.2B) similar to that of L-glucose (Figure 2.2D) and the absence of a sodium gradient (Figure 2.2C), a curvilinear relationship between glucose concentration and uptake (Figure 2.4), and confirmation that glucose was being transported into an intravesicular space (Figure 2.3). Thus, we have satisfactorily demonstrated that our BBMV were capable of transporting glucose in an anticipated manner and in quantities similar to that reported in the recent literature (Ling et al., 1989).

Next, we wished to confirm the recent findings (Ling et al., 1989) of the nature of uptake of LA into BBMV. Quantitatively the uptake of LA was much higher than that of glucose (Figures 2.1A and 2.5), and was comparable to that reported by Ling et al. (1989). Similar to this previous report, LA uptake was inhibited by 0.2 mM phloretin (Figure 2.6) and was unaffected by a sodium gradient (data not shown). Also, LA uptake was not affected by variations in osmolality, suggesting that uptake was associated with the BBMV and not into an intravesicular space (Figure 2.7). Furthermore, the possibly altered rate of uptake of LA at concentrations above 160 µm may have been due to solubility limitations of higher concentrations of LA with 2 mM taurocholic acid rather than necessarily being due to an altered uptake process; our findings are in agreement with the work of Ling et al. (1989). However, we were unable to confirm the results of Ling et al. (1989) with regards to the effect of lysis of BBMV: lysis of BBMV incubated with LA by

addition to ice-cold deionized water did not alter the amount of LA associated with the BBMV, whereas Ling et al. (1989) demonstrated that 40% of accumulated LA was released by lysis of the BBMV. Also, in contrast to the results of these workers, we did not find an overshoot phenomenon at short incubation periods, and equilibrium was achieved by 2 min, and not at 30 min (Figure 2.5).

Our data are compatible with the model of the partitioning of lipids into the brush border membrane: 1) the absence of an overshoot phenomenon and constant rate of uptake with incubation for as long as 20 min (Figure 2.5); 2) the lack of effect of a sodium gradient (data not shown); 3) the lack of effect of variations in osmolality (Figure 2.7); and 4) the linear relationship between concentration and uptake of LA (Figure 2.8). Furthermore, the uptake of LA was unaffected by glucose (data not shown). None of these data disprove, however, the possibility that there may be an energy-dependent or protein-associated component to the lipid uptake process. We might have expected that such a process would have been more likely to have been demonstrable at lower concentrations of LA, but this did not occur (Figure 2.8). The spike overshoot phenomenon reported by Ling et al. (1989) was defined by a single point at 15 sec. There were a number of minor differences between our methods and those used by Ling and co-workers which might explain our inability to confirm the presence of an overshoot with LA, and the accomplishment of an earlier equilibrium (Table 2.1).

Finally, from the known rates of uptake of the LA (Figure 2.5), and with the knowledge that uptake occurs into the BBMV but not into an intravesicular space (Figure 2.7), it may be possible, for example, to enrich the vesicles in a predictable manner by varying the concentration of LA (Figure 2.8), and to thereby determine whether the short-term enrichment of the BBMV with a given fatty acid could modify the subsequent vesicular uptake of D-glucose.

Although it is predictable that mixing membranes with an aqueous solution of fatty acid micelles leads to the partitioning of fatty acid into the membrane, the purpose of this study was to characterize this process in BBMV, in order to assess the affinity of lipid for BBM. Lipid uptake studied in vesicles allows the determination of the impact of the membrane in lipid uptake in the absence of the unstirred water layer or cytosolic fatty acid binding protein. Whether the affinity of lipid for BBM is protein-mediated or lipid-mediated or both must be the subject of further studies.

Table 2.1 Summary of differences in methods used in the present and in a previous study (Ling et al., 1989) of linoleic acid uptake into rabbit intestinal BBMV.

	Ling et al., 1989	Present study
BBMV Preparation		
Mucosal scrapings	- frozen	- fresh
Homogenization	- Polytron and glass teflon	- Polytron only
Stir with $CaCl_2$	- 45 minutes	- 10 minutes
BBMV uptake done on	- frozen vesicles	- fresh vesicles which equilibrated 90 min. prior to uptake
Protein concentration	- 10-12 mg/ml	- 3-5 mg/ml
Transport Studies		
Amount of protein/uptake	- 100-120 µg	- 60-100 µg
Filter pre-wash	- no	-yes (2 x 2 ml)
Data point replicates:		
D-glucose	- duplicate	- triplicate
Linoleic acid	- duplicate	- quadruplicate
Probe specific activity:		
D-glucose	- not stated	- 55 Ci/mole
Linoleic acid	- not stated	- 7 Ci/mole
Results		
D-glucose:		
Overshoot	- 90 seconds	- 60 seconds
Equilibrium	- 20 minutes	- 10-15 minutes
Linoleic acid:		
Overshoot	- 15 seconds	- none
Equilibrium	- 30 minutes	- 2 minutes

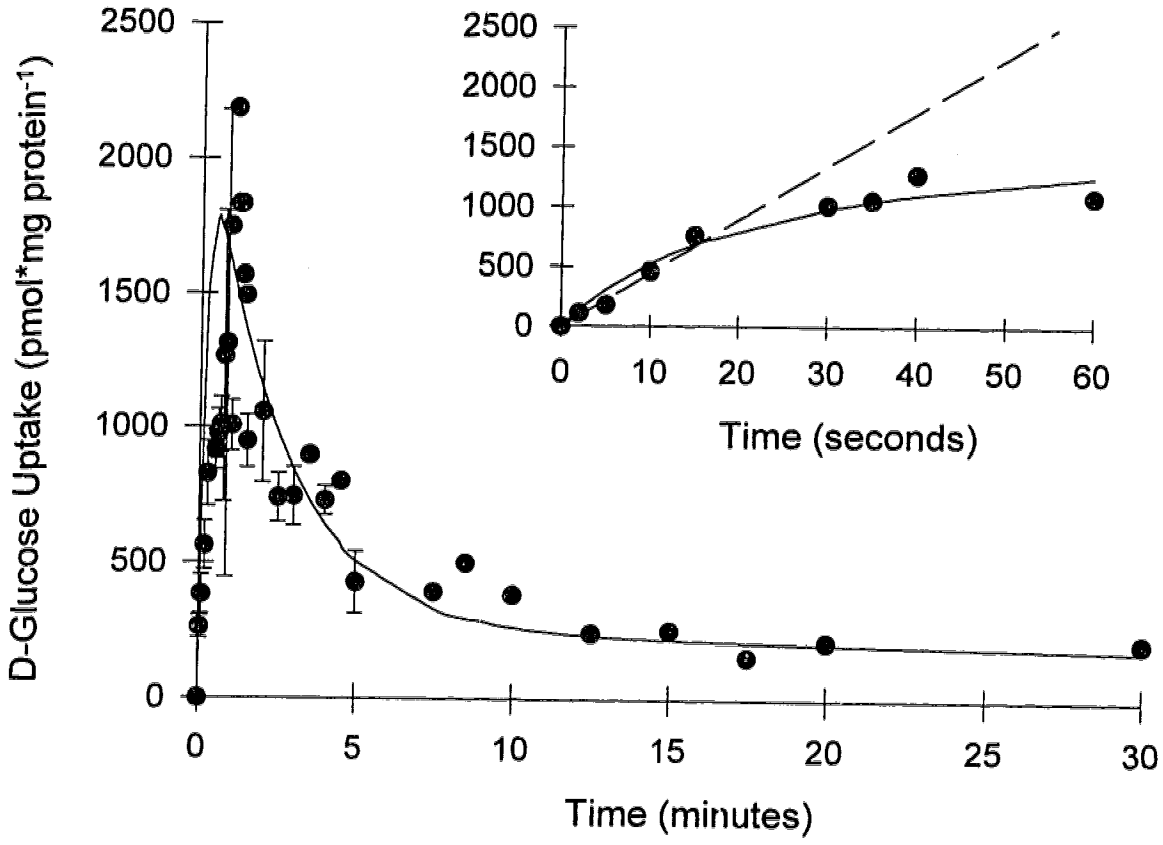

Figure 2.1 D-glucose uptake time course. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Buffer and incubated at room temperature with Glucose Transport Buffer containing 100 µM D-glucose, 100 mM NaSCN (see Methods). The inset represents the initial rate of D-glucose uptake time course.

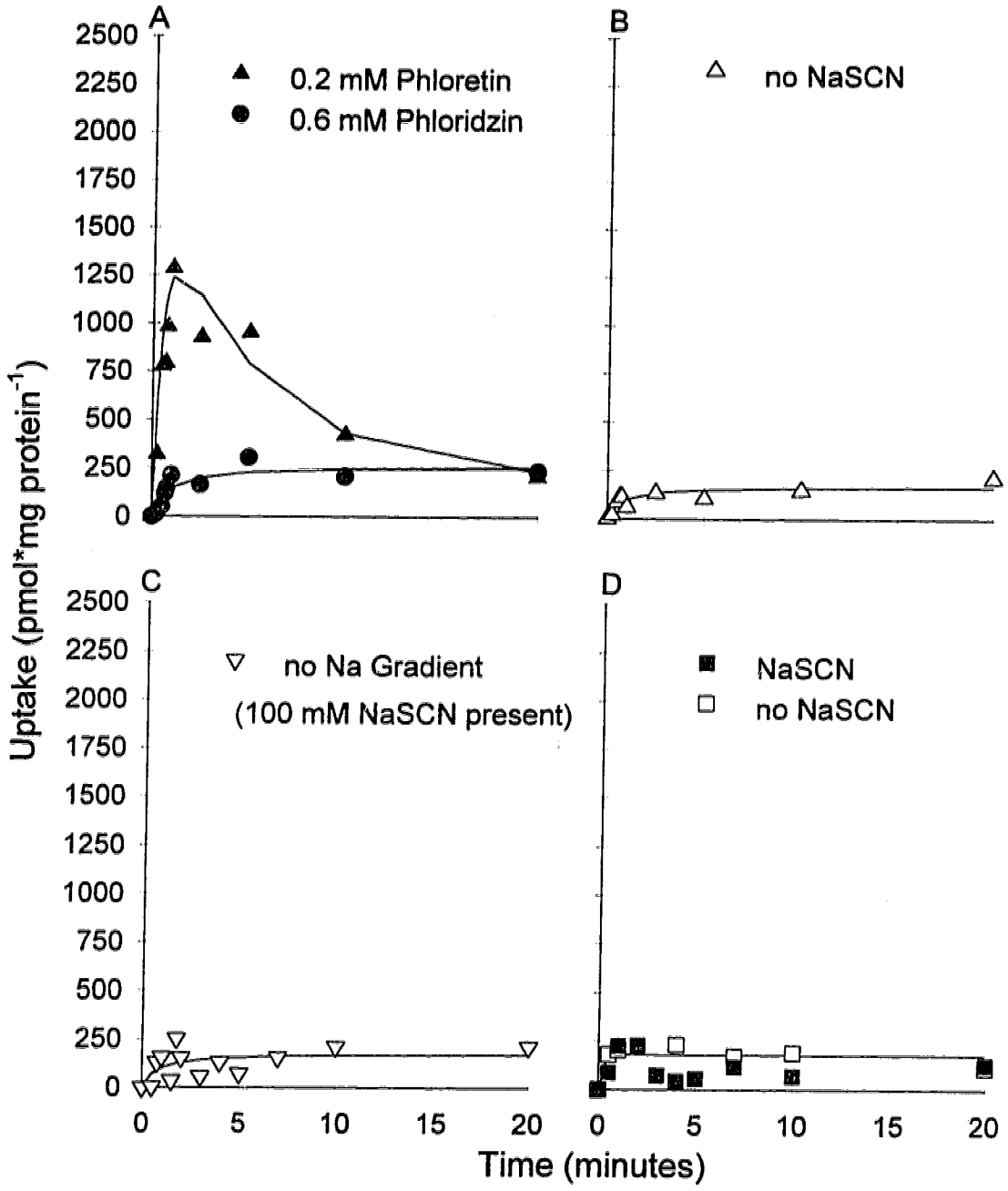

Figure 2.2 ***(A)*** Abolition of D-glucose overshoot by phloridzin but not by phloretin. BBMV were pre-loaded with Vesicle Resuspension Solution (VRS) containing either 0.6 mM phloridzin or 0.2 mM phloretin, and were incubated at room temperature (RT) with Glucose Transport Buffer (GTB) containing 100 µM D-glucose, 100 mM NaSCN, and 0.6 mM phloridzin or 0.2 mM phloretin. ***(B)*** Abolition of D-glucose overshoot in the absence of sodium. BBMV were pre-loaded with VRS and incubated at RT with GTB containing 100 µM D-glucose and no NaSCN. ***(C)*** Abolition of D-glucose overshoot in the presence of sodium but without a sodium gradient. BBMV were pre-loaded with VRS containing 100 mM NaSCN and incubated at RT with GTB containing 100 µM D-glucose and 100 mM NaSCN. ***(D)*** L-glucose uptake time course in the presence or absence of sodium. BBMV were pre-loaded with VRS and incubated at RT with GTB containing 100 µM L-glucose with or without 100 mM NaSCN. (see Methods)

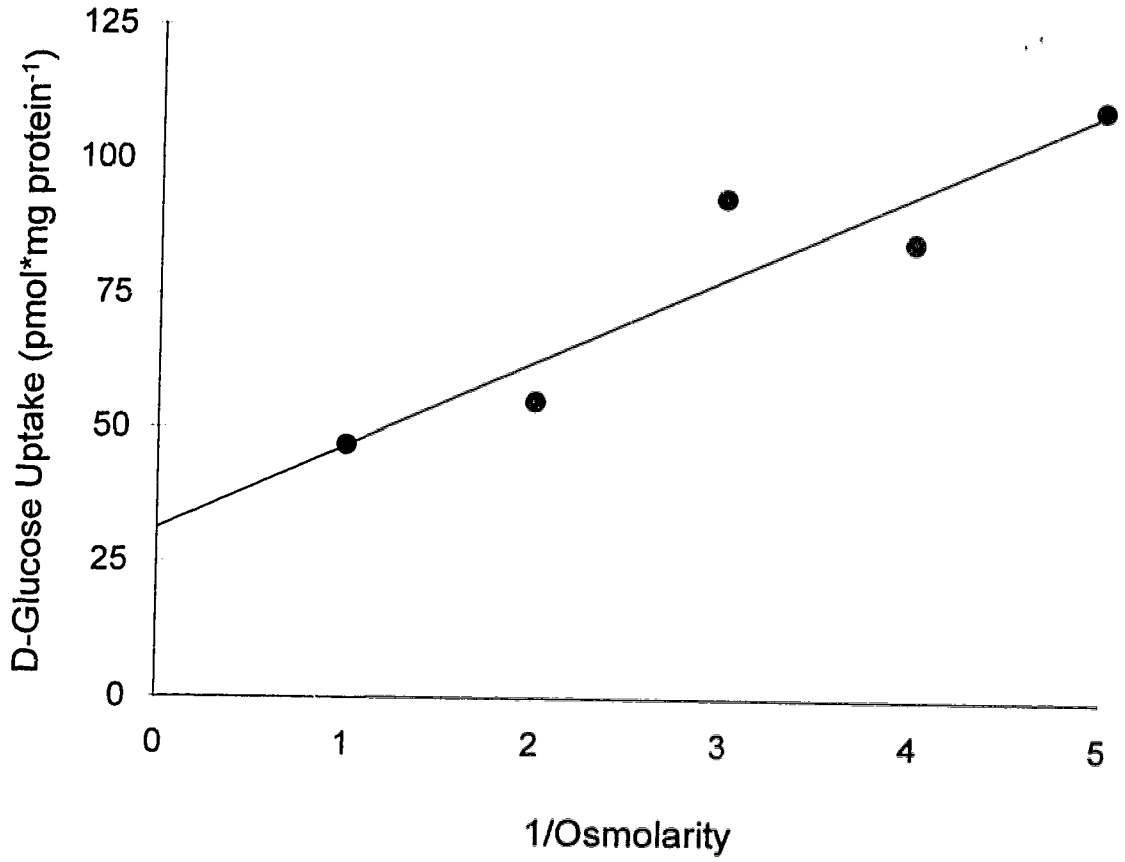

Figure 2.3 Effect of osmolarity on D-glucose uptake. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution containing increasing concentrations of sucrose and incubation at room temperature with Glucose Transport Buffer containing 100 µM D-glucose and 100 mM NaSCN under equilibrium conditions (see Methods).

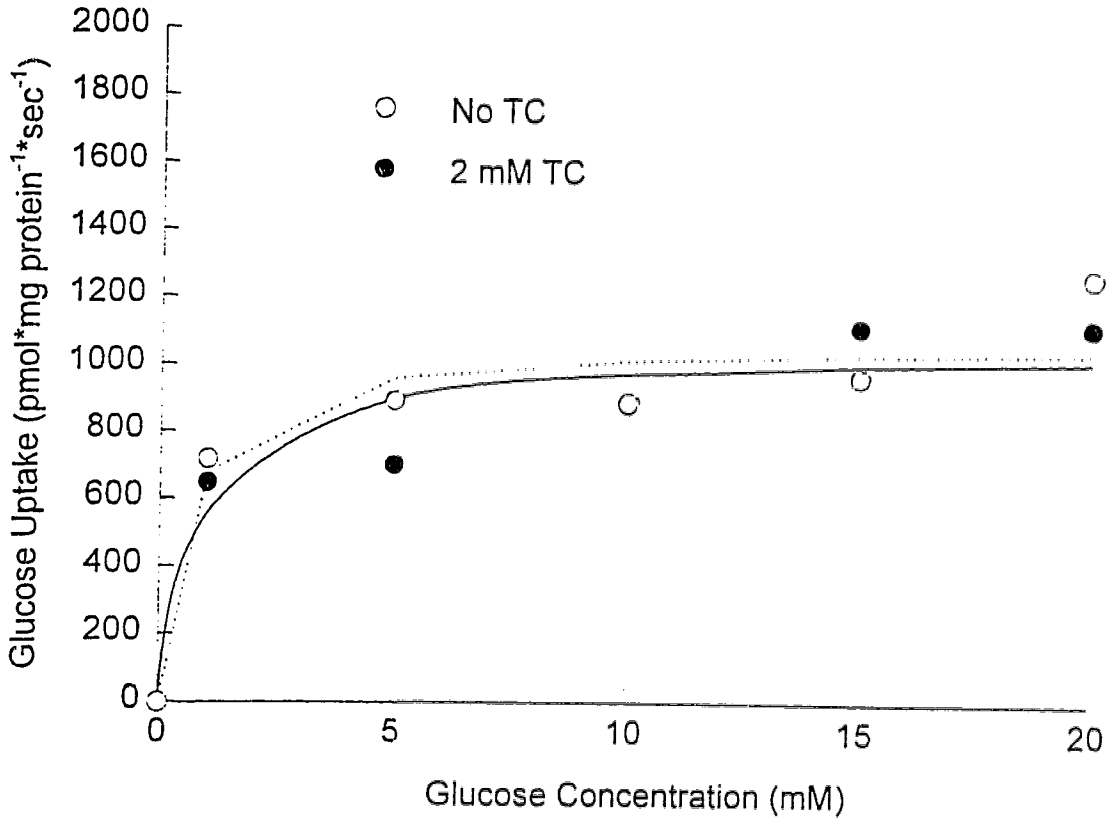

Figure 2.4 D-glucose uptake concentration curve. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution and incubated at room temperature with Glucose Transport Buffer containing 100 mM NaSCN and increasing concentrations of D-glucose (0.1 to 20 mM) in the presence or absence of 2 mM taurocholic acid (TC) (see Methods).

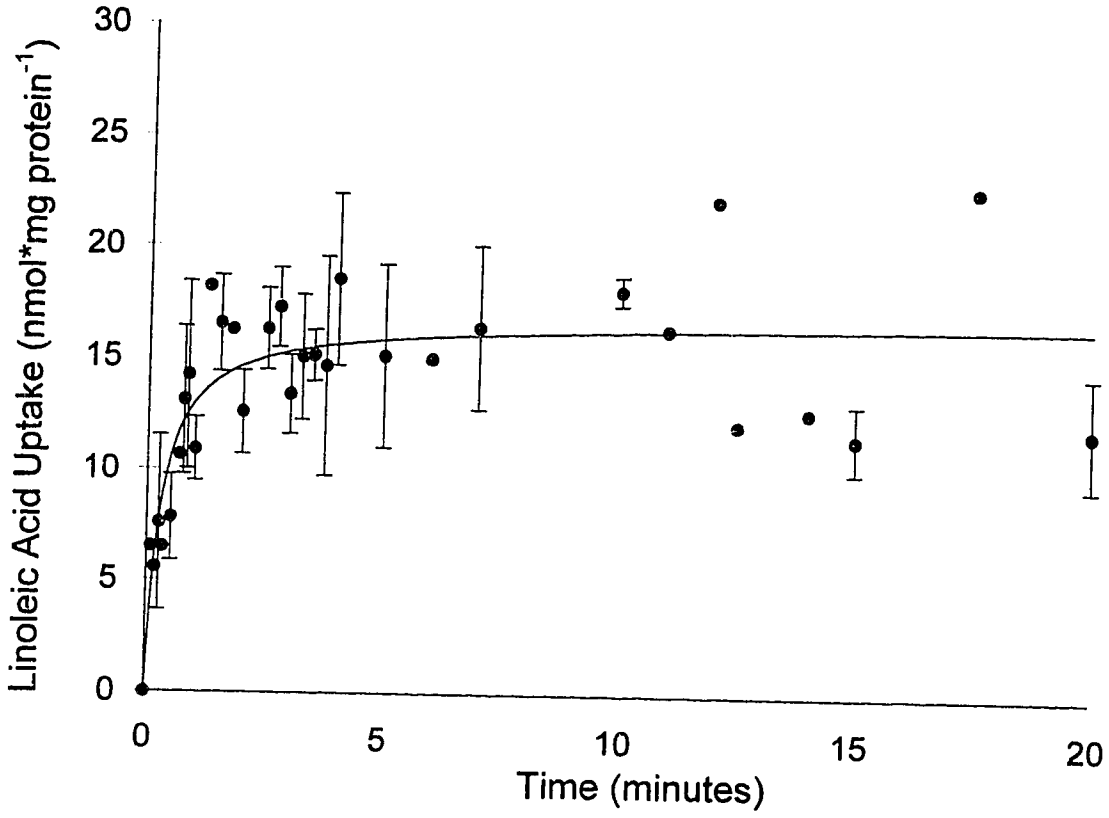

Figure 2.5 Linoleic acid uptake time course in the presence of sodium. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution and incubated at room temperature with Lipid Transport Buffer containing 100 µM linoleic acid and 100 mM NaSCN (see Methods).

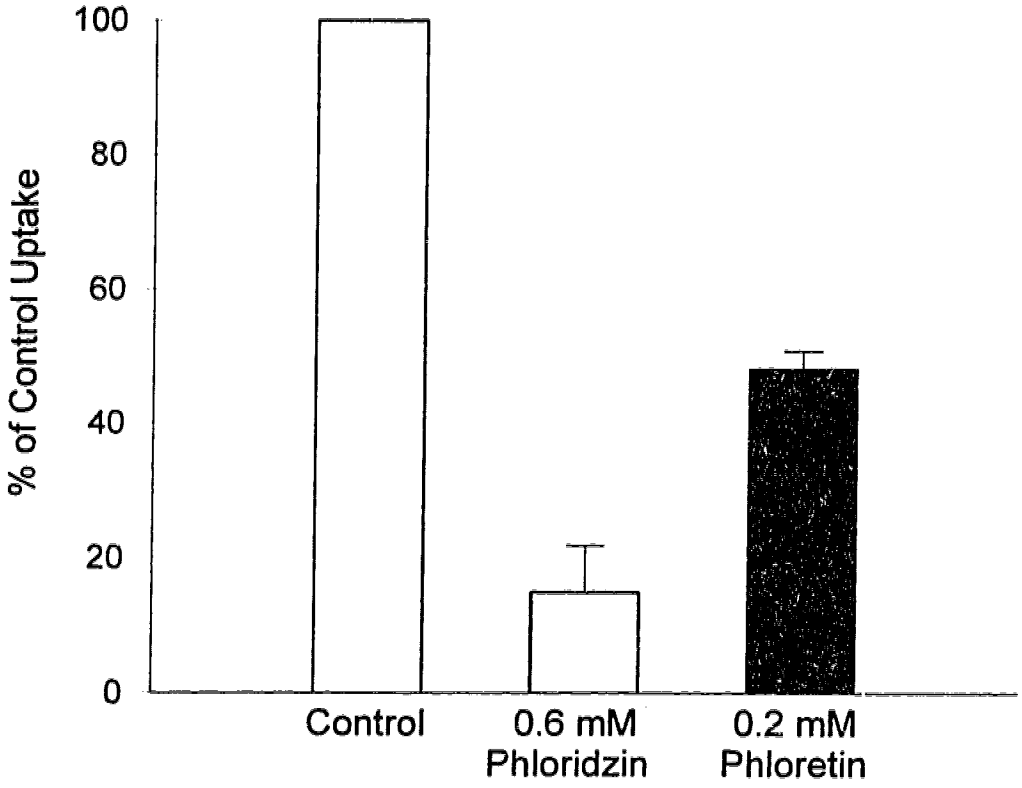

Figure 2.6 Inhibition of linoleic acid uptake in the presence of phloridzin or phloretin. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution (VRS) or VRS containing 0.6 mM phloridzin or 0.2 mM phloretin, and incubated at room temperature with Lipid Transport Buffer containing 100 µM linoleic acid and 100 mM NaSCN (see Methods).

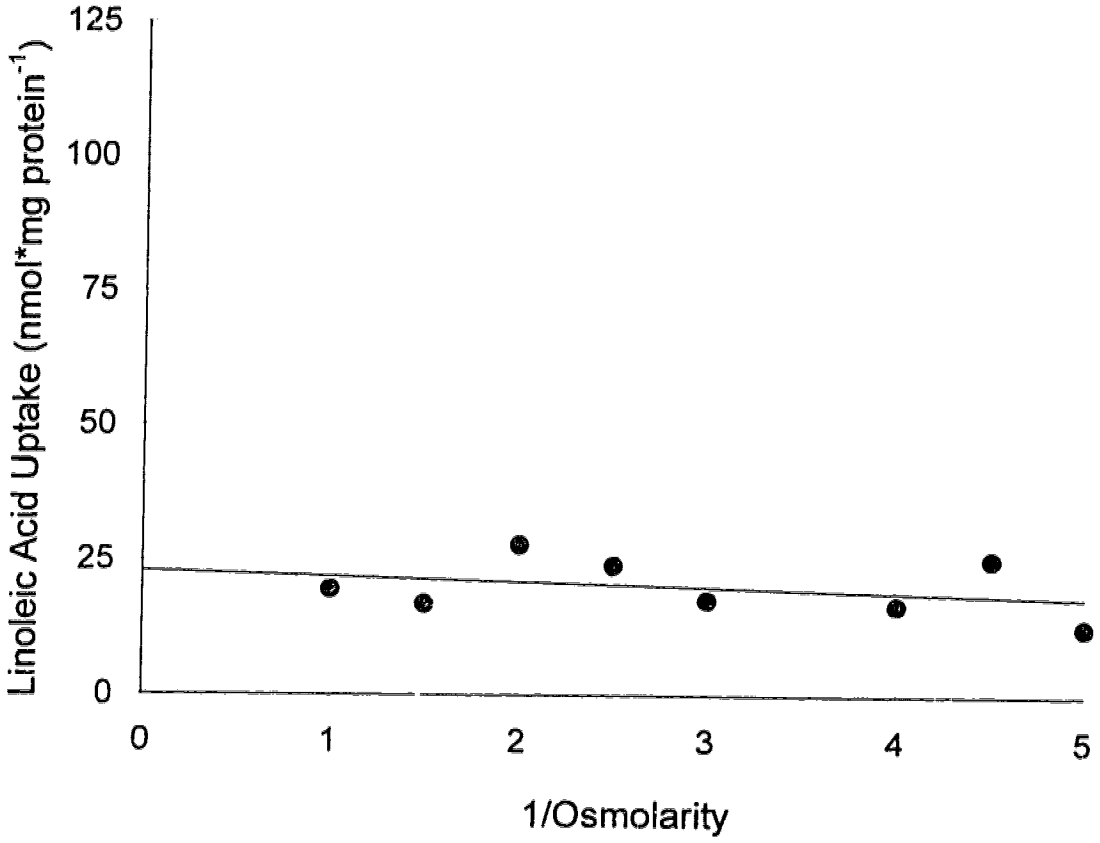

Figure 2.7 Effect of osmolarity on linoleic acid uptake. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution containing increasing concentrations of sucrose and incubated at room temperature with Lipid Transport Buffer containing 100 μM linoleic acid under equilibrium conditions (see Methods).

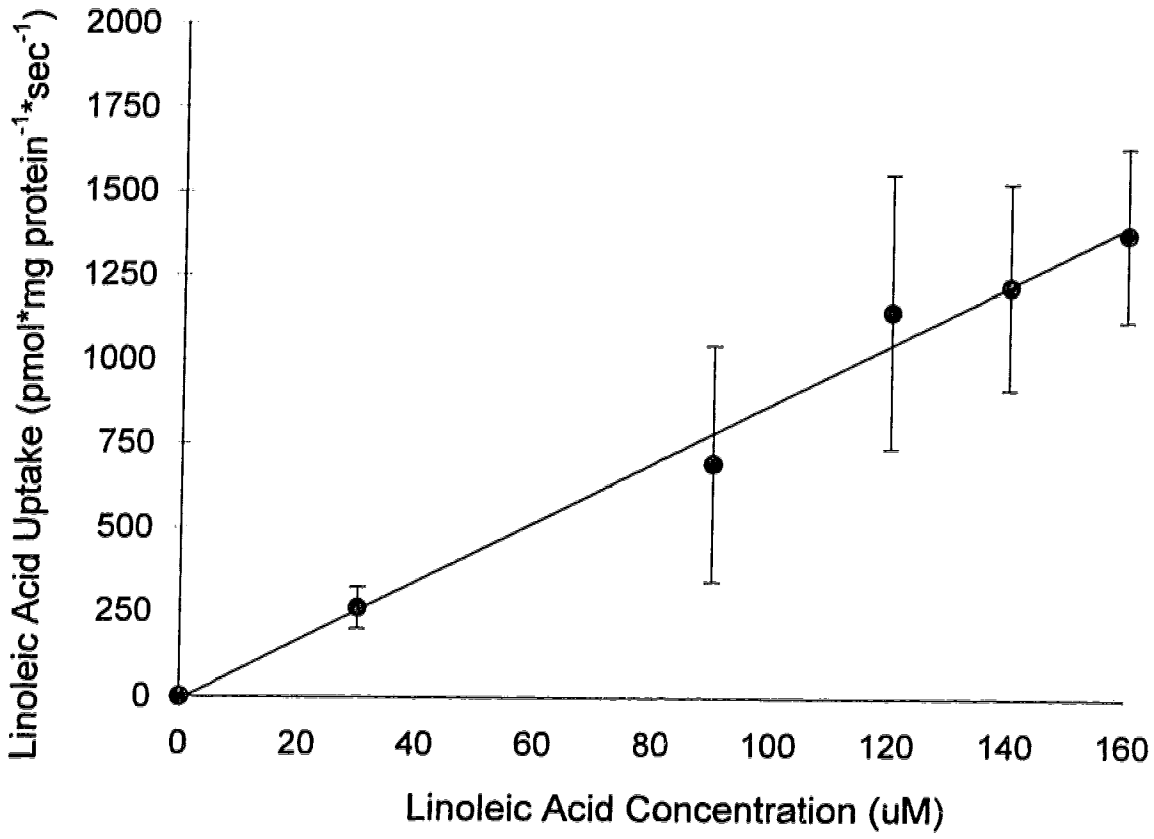

Figure 2.8 Linoleic acid concentration curve. Brush border membrane vesicles were pre-loaded with Vesicle Resuspension Solution and incubated at room temperature with Lipid Transport Buffer and increasing concentrations of linoleic acid (see Methods).

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CHAPTER 3

DIFFERENT MECHANISMS OF UPTAKE OF STEARIC ACID AND CHOLESTEROL INTO RABBIT JEJUNAL BRUSH BORDER MEMBRANE VESICLES[2]

3.1 Introduction

The intestinal uptake of lipids involves a variety of mechanisms in which lipids interact with the brush border membrane (BBM) of the small intestine (Shiau, 1987; Thomson and Dietschy, 1981; Thomson et al., 1989; Tso, 1985). Uptake of lipids is thought to occur passively via an energy-independent diffusion process (Sallee and Dietschy, 1973; Westergaard, 1987). A membrane fatty acid binding protein may also be involved in fatty acid absorption in the intestine (Stremmel et al., 1985), and the uptake of both cholesterol and phosphatidylcholine may also be protein-mediated in rabbit small intestine (Thurnhofer, and Hauser, 1990a,b).

The mechanism by which lipid is transferred from the bile acid micelle to the BBM has been studied using *in vitro* experiments. These results provide evidence for movement of the lipid from the micelle into an aqueous phase, with the lipid then permeating into the BBM (Chijiiwa and Linscheer, 1987; Westergaard and Dietschy, 1976). Studies using intestinal BBM vesicles (BBMV) have suggested that some portion of the uptake of lipids results from collision of micelles with the BBMV (Mutsch et al., 1986; Proulx et al., 1985; Thurnhofer and Hauser, 1990a). BBMV represent a useful method to study the uptake of lipid in the absence of an unstirred water layer and cytosolic fatty acid binding proteins. Accordingly, the aim of this study was to examine the uptake of stearic acid and cholesterol into jejunal BBMV of rabbits under conditions of varying concentrations of bile acid, and varying ratios of bile acid to stearic acid or cholesterol. The results suggest that stearic acid and cholesterol may be taken up from bile acid micelles into intestinal BBMV by different mechanisms.

3.2 Materials and Methods

3.2.a Chemicals

[^{14}C]-stearic acid (55.3 mCi/mmol) and [^{14}C] cholesterol (55 mCi/mmol) were purchased from ICN Radiochemicals (Irvine, CA). All other chemicals were purchased from Sigma, Fisher Scientific or BDH and were of the highest quality available.

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3.2.b *Preparation of Brush Border Membrane Vesicles*

Female New Zealand white rabbits weighing 2.0-2.5 kg were sacrified by an anaesthetic overdose injection of sodium pentobarbital (800 mg/kg) in the marginal ear vein. Brush border membrane vesicles (BBMV) were prepared at 4°C as described in Chapter 2. BBMV were resuspended and equilibrated in Vesicle Resuspension Buffer (300 mM mannitol - 10 mM HEPES -Tris, pH 7.4) for 90 min at 4°C prior to uptake studies. The protein concentrations was determined using Hartree modification (1972) of the Lowry method (1951) using bovine serum albumin as a standard. The purity of the BBMV was examined using sucrase and alkaline phosphatase activities (Dahlqvist, 1964; Bowers et al., 1967). The final BBMV were enriched 10-15-fold over their activities in the initial homogenates.

3.2.c *Preparation of Stearic Acid Probe*

[^{14}C] stearic acid was combined with unlabelled stearic acid and dried down under a stream of nitrogen, and reconstituted in Lipid Transport Buffer (1 mM $MgCl_2$ - 2 mM $CaCl_2$ - 100 mM D-mannitol - 10 mM HEPES-Tris - 20 mM taurocholic acid, pH 7.4) such that the final concentration of 18:0 was 50 µM or 200 µM, and the final specific activities were 8.8 Ci/mol and 9.8 Ci/mol, respectively. The final probes were sonicated for 15 min in a water-bath sonicator (Branson 1200, Branson Ultrasonic, Danbury, CT) at 37°C.

3.2.d *Preparation of Cholesterol Probe*

[^{14}C] cholesterol was combined with unlabelled cholesterol and dried down under a stream of nitrogen and reconstituted in Lipid Transport Buffer, yielding a final concentration of 50 µM and a final specific activity of 20.8 Ci/mol. The probe was sonicated for 15 min in a water-bath sonicator.

3.2.e *Preparation of Filter Prewash*

300 µM stearic acid was solubilized in Stock Stop Buffer (0.15 M KCl - 2 mM HEPES-Tris, pH 7.4) containing 30 mM taurocholic acid (TC). Similarly, for cholesterol, 300 µM was solubilized in Stock Stop Buffer containing 20 mM TC. All filter prewash solutions were stirred at 48°C until the solutions cleared.

3.2.f *Uptake Studies*

Transport studies were performed using a method of rapid filtration, as described in Section 2.2d of Chapter 2.

3.2.g *Experimental Procedures*

Stearic acid (25 or 100 µM) or cholesterol (25 µM) uptake was studied as a function of time. Both lipids were solubilized in 20 mM TC and were incubated with BBMV suspension for 5 s to 16 min. Lipids were solubilized with 20 mM TC, and were studied at initial rate with 5 s incubation periods. Stearic acid was examined at concentrations of 1 to 200 µM, and cholesterol was studied at increasing concentrations of 1 to 50 µM.

Stearic acid (25 or 100 μM) and cholesterol (25 μM) were examined at 5 s incubation periods with TC concentrations ranging from 2-20 mM. Stearic acid uptake with TC at fixed ratios of 12.5:1 or 1.25:1 (μM 18:0:mM TC) and cholesterol (1.25:1) were examined at initial rates of 5 s incubation periods. Stearic acid (25 μM) and cholesterol (25 μM) uptake was studied when the pH of the vesicle resuspension buffer and lipid probe were maintained at pH 7.4; the concentration of TC was 20 mM and the duration of incubation was 5 s.

3.2.h Data Analysis

The data for each point on the subsequent graphs represents the mean ± standard error of the mean of four experimental days in which four replicates were performed. Uptake values for the time-course experiments were expressed as nmol/mg BBMV protein, and represent the total radioactivity of the sample minus the nonspecific binding of labelled lipid to the filter. To determine the best curve fit, Sigmaplot (4.1) graphics program (Jandel Scientific, San Rafael, CA) was used for the time-course experiments. For the concentration curves, lipid/TC ratio (constant ratio of lipid/TC with increasing concentrations of both) and constant concentration of lipid with increasing TC, the uptake is presented as nmol per mg BBMV protein per second. First order regressions and regression coefficients were calculated for experiments done under initial rate conditions (5 s).

3.3 Results

3.3.a Time Course of Stearic Acid and Cholesterol

Studies of 100 μM stearic acid (18:0) uptake as a function of time showed an initial rate of uptake that plateaued within 2 min at a value of approximately 35 nmol per mg BBMV protein (Figure 3.1A). Similarly, 25 μM 18:0 (Figure 3.1B) and 25 μM cholesterol (Figure 3.1C) demonstrated a rapid initial rate of uptake, equilibrating within 2 min at values of 3 and 4 nmol per mg protein, respectively.

3.3.b Lipid Concentration Curve

The uptake of 18:0 was greater from low (2 mM) than from high (20 mM) concentrations of TC (Figures 3.2A and 3.2B). The relationship between the concentration of 18:0 and uptake was linear when the 18:0 was solubilized with 2 mM TC (Figure 3.2A), but a curvilinear relationship was noted at low concentrations of 18:0 when the fatty acid was solubilized with 20 mM TC (Figure 3.2B). For cholesterol, there was a linear relationship between concentration and uptake (Figure 3.2C).

3.3.c Lipid and Taurocholic Acid (TC) Varied to Maintain Constant Ratio

The concentrations of 18:0 and TC were both increased in unison, maintaining a constant ratio of 12.5 μM/1 mM (Figure 3.3A) or 1.25:1 μM/1 mM (Figure 3.3B); there was a linear increase

in 18:0 uptake. Similarly, cholesterol uptake increased at a linear rate as both cholesterol and TC concentrations were increased at a constant ratio of 1.25 μM/1 mM (Figure 3.3C).

3.3.d Constant Lipid Concentration with Increasing TC

The uptake of 100 or 25 μM 18:0 decreased as the concentration of TC was increased from 2 to 20 mM (Figures 3.4A and 3.4B), whereas cholesterol uptake increased as the TC concentration was increased (Figure 3.4C).

3.4 Discussion

Several mechanisms have been postulated to explain the sequence of events occurring when lipid is transferred from the bile salt micelles in the intestinal lumen into the brush border (BBM) membrane. Uptake of the entire micelle is not supported by experimental data (Johnston, 1978). The second model involves a "collision" between the micelle and the BBM whereby the solubilized lipid moves directly from the micelle into the BBM. In the third model, the movement of lipid occurs via transfer from the micelle to an aqueous phase followed by permeation into the BBM. This "monomer" or "aqueous" hypothesis is based on an equilibrium between fatty acids that exist in monomeric form and those in a micellar form (Westergaard and Dietschy, 1976). In solution there is a maximum concentration of monomers and a finite amount of lipid that can be solubilized into the micellar form. When the solubilizing agent has reached the total carrying capacity for the lipid, any increase in the lipid concentration will result in the formation of an emulsion (Mansbach et al., 1975; Thomson and Dietschy 1981). These monomeric and micellar forms are present in the bulk phase in the intestinal lumen and in the unstirred water layer. As the monomeric form of the lipid permeates into the membrane, there is further partitioning of lipid from the micelle into the water phase.

A variation of the aqueous model, the "dissociation" model, suggests that fatty acids in a low pH microclimate just external to the BBM will be protonated, resulting in increased permeation through the lipid membrane (Shiau and Levine, 1980). The presence of an acid microclimate has been demonstrated *in vitro* and *in vivo* (Lucas et al., 1975; Said et al., 1986). Stremmel et al., (1985) showed that binding of oleate to rat jejunal microvillus membranes increased at pH values below 6.8 with a maximum at pH 4.0, and at pH values above 8.0 there was a decrease in binding. Shiau (1990) studied fatty acid uptake in everted rat jejunal sacs and demonstrated increased fatty acid uptake at lower pH values. He interpreted this data to suggest that the pH microclimate provides an environment in which fatty acid preferentially partitions from the micelle. Stearic acid is protonated in a low pH environment and becomes more lipid soluble, whereas cholesterol is not protonated. Thus, the "dissociation" model may be a possibility for the uptake of stearic acid but not for cholesterol.

In addition to the passive permeation of lipids through the BBM, there may be a mediated component of this process. Recent findings with linoleic acid uptake into rabbit jejunal BBM vesicles (BBMV) demonstrate uptake showing a sodium-dependent "overshoot", suggesting that there is a protein-mediated component involved for fatty acid uptake (Ling et al., 1989). The finding of this overshoot has not been confirmed (Keelan et al., 1992, See Chapter 2), but this may relate to technical differences between the two studies. The use of the BBMV overcomes an effect of cytosolic fatty acid binding proteins (Stremmel, 1985) and of unstirred layers, and allows the comparison of the collision versus the aqueous/dissociation models of lipid uptake. Under the conditions of low concentration of 18:0 and 20 mM TC, but not 2 mM TC, a curvilinear relationship was noted between 18:0 concentration and uptake (Figure 3.2B). This suggests that under certain experimental conditions there may be a linear and a nonlinear component describing fatty acid uptake, in contrast to the single component describing cholesterol uptake under the same conditions (Figure 3.2C). Caution must be used in this interpretation, however, since the regression line between 10-25 μM 18:0 intersects with the origin, and since this curvilinear relationship was not obtained at equilibration (4 min or beyond).

The increasing uptake of 18:0 and cholesterol with increasing concentrations of both lipid and solubilizing TC (but with a constant ratio of lipid:TC) (Figure 3.3) is suggestive of partitioning of fatty acid or cholesterol from the micelle directly into the BBMV (Westergaard and Dietschy, 1974), the so-called "collision" model. This same lipid:lipid model is supported by the finding of an increasing uptake of cholesterol with increasing concentrations of TC (Figure 3.4C). In contrast, the reduction in the uptake of 100 or 25 μM 18:0 with increasing concentrations of TC (Figure 3.4A and 3.4B) supports but does not prove the model of partitioning of the fatty acid from the micelle into an aqueous phase, and then into the BBM (Westergaard and Dietschy, 1974), the so-called aqueous/partitioning model. Again, one must be cautious with these interpretations, since the increasing cholesterol uptake with increasing concentrations of TC (Figure 3.4C) may reflect increasing amounts of TC enhancing the solubility of cholesterol in the micelles; the fact that there is no deviation from the linear relationship in the uptake of cholesterol above and below the critical micellar concentration of TC suggests that cholesterol solubility is present over the concentration range tested. On the other hand, the effect of increasing the amount of TC versus 18:0 (Figure 3.4A and 3.4B) might be explained by increasing competition of TC micelles for 18:0 versus the BBMV. We do not have data on the activity or solubility of 18:0 in the different solutions, so that our data supports but does not prove one or the other model. Notwithstanding, there appeared to be qualitative differences in the uptake of stearic acid and cholesterol (Figures 3.3 and 3.4): the partitioning of cholesterol from the bile acid micelle into the BBM appears to be by way of the "collision" of the cholesterol into the membrane. In contrast, the uptake of stearic acid from the micelle into the BBM may be by both the collision and the aqueous/dissociation models, in which

the acid microclimate may play an additional role. Furthermore, the curvilinear relationship between concentration and uptake of low concentrations of 18:0 solubilized in 20 mM TC, but a linear relationship for cholesterol under the same conditions further suggests that stearic acid and cholesterol may be taken up by different mechanisms from bile acid micelles into the intestinal BBM.

The uptake of D-glucose into BBMV is not affected by 2 mM TC, but is reduced by 20 mM TC (Keelan et al., 1992; Ling et al., 1989). We were aware therefore of the possibility that the higher concentrations of TC might open the sealed vesicles and dissipate the sodium gradient. This might be one of the several possible explanations for our not demonstrating an "overshoot" in the uptake of 18:0 or cholesterol (Figure 3.1). However, it was at the condition of 20 mM TC that there was a curvilinear relationship between concentration and uptake of 18:0 (Figure 3.2A), so that a possible leakiness of the vesicles due to TC could not explain the lack of a curvilinear concentration/uptake curve for cholesterol, or 18:0 at a lower concentration of 18:0 (Figure 3.2A, 3.2C). Furthermore, any possible effect of TC on BBMV integrity did not produce a break in the linear relationship between the ratio of lipid/TC and uptake (Figure 3.3), nor could the possibility of leakiness explain the qualitatively different relationship between the uptake of 18:0 or cholesterol when the concentration of TC is increased (Figure 3.4). We recognize, of course, that this data suggesting different mechanisms for the uptake of stearic acid and cholesterol applies only to the special conditions of BBMV, and may not necessarily apply to the intact intestine *in vivo*.

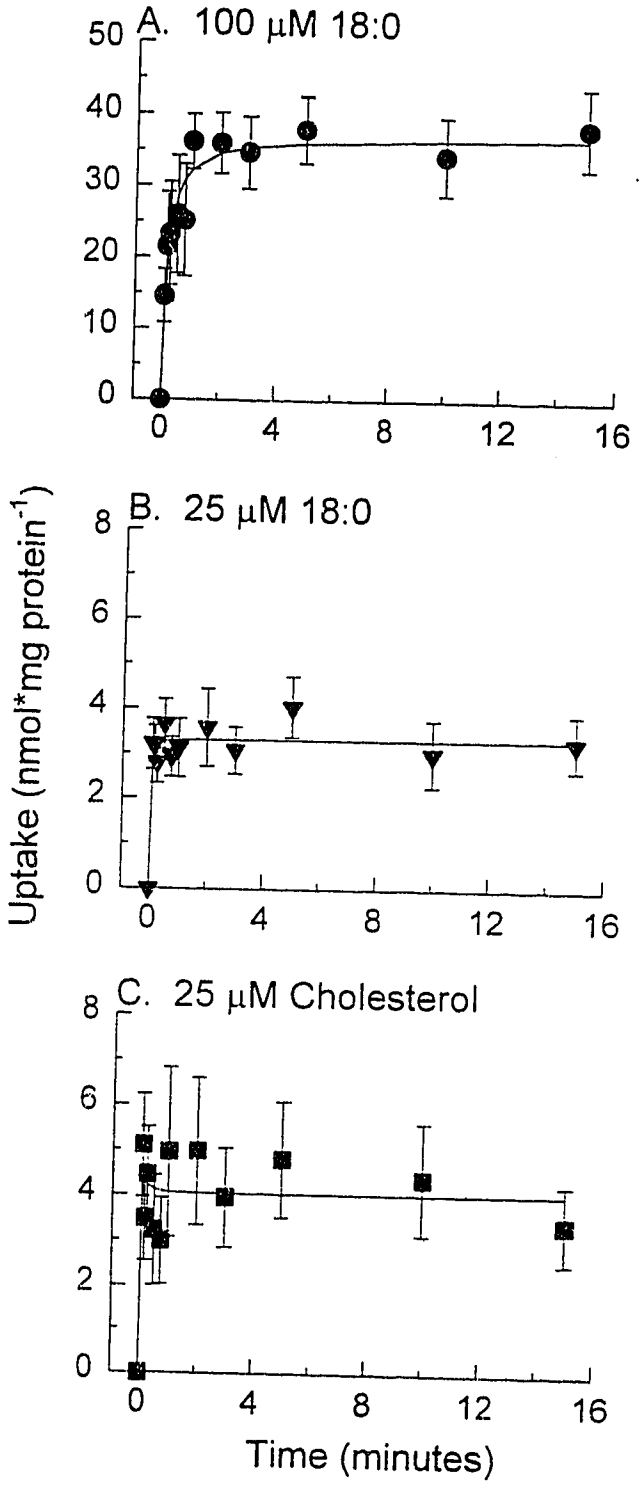

Figure 3.1 Time course of ***(A)*** 100 µM stearic acid (18:0), ***(B)*** 25 µM 18:0, or ***(C)*** 25 µM cholesterol uptake into brush border membrane vesicles (BBMV). BBMV were pre-loaded with Vesicle Resuspension Buffer and incubated at room temperature with Lipid Transport Buffer containing 20 mM taurocholic acid and stearic acid or cholesterol (see Methods).

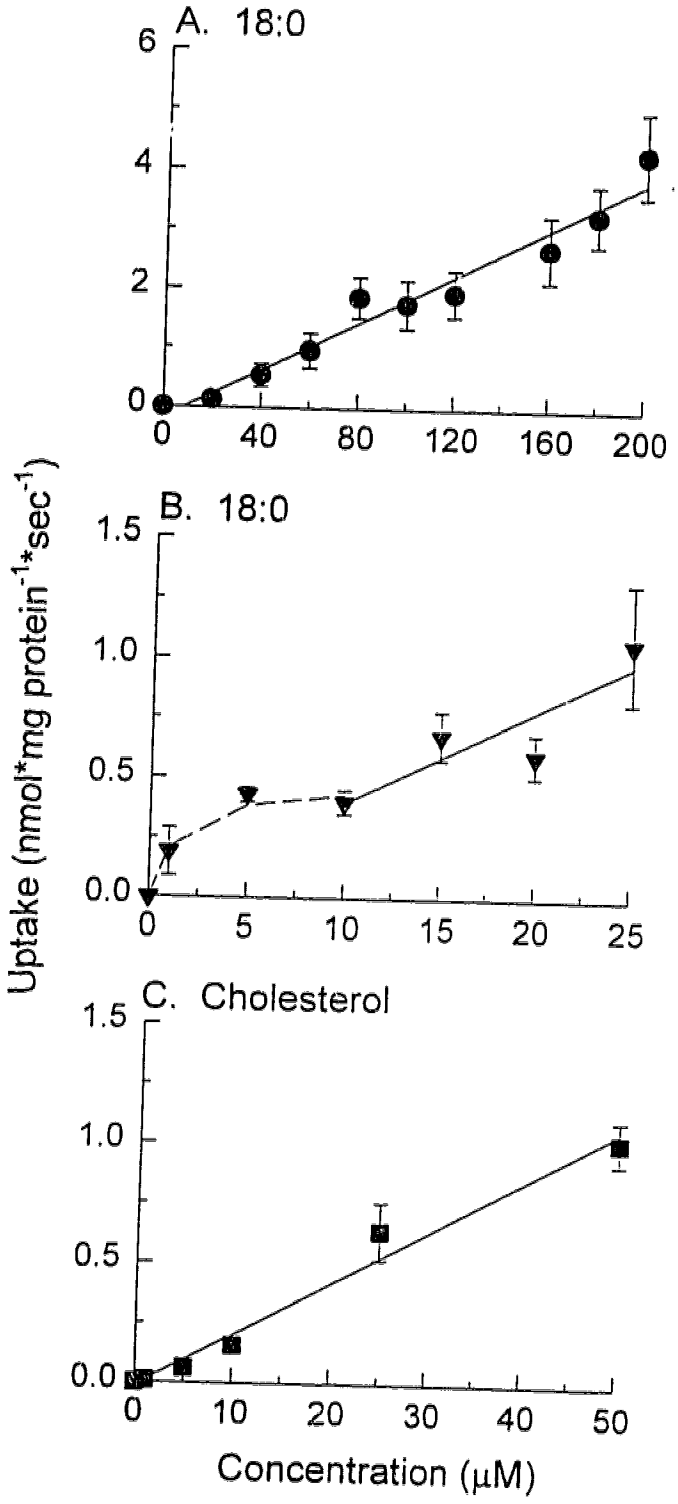

Figure 3.2 Lipid uptake concentration curve. Brush border membrane vesicles (BBMV) were pre-loaded with Vesicle Resuspension Buffer and incubated for 5 sec at room temperature with Lipid Transport Buffer containing ***(A)*** 20-200 μM stearic acid (18:0) and 2 mM taurocholic acid (TC), ***(B)*** 1-25 μM 18:0 and 20 mM TC, or ***(C)*** 1-50 μM cholesterol and 20 mM TC (see Methods).

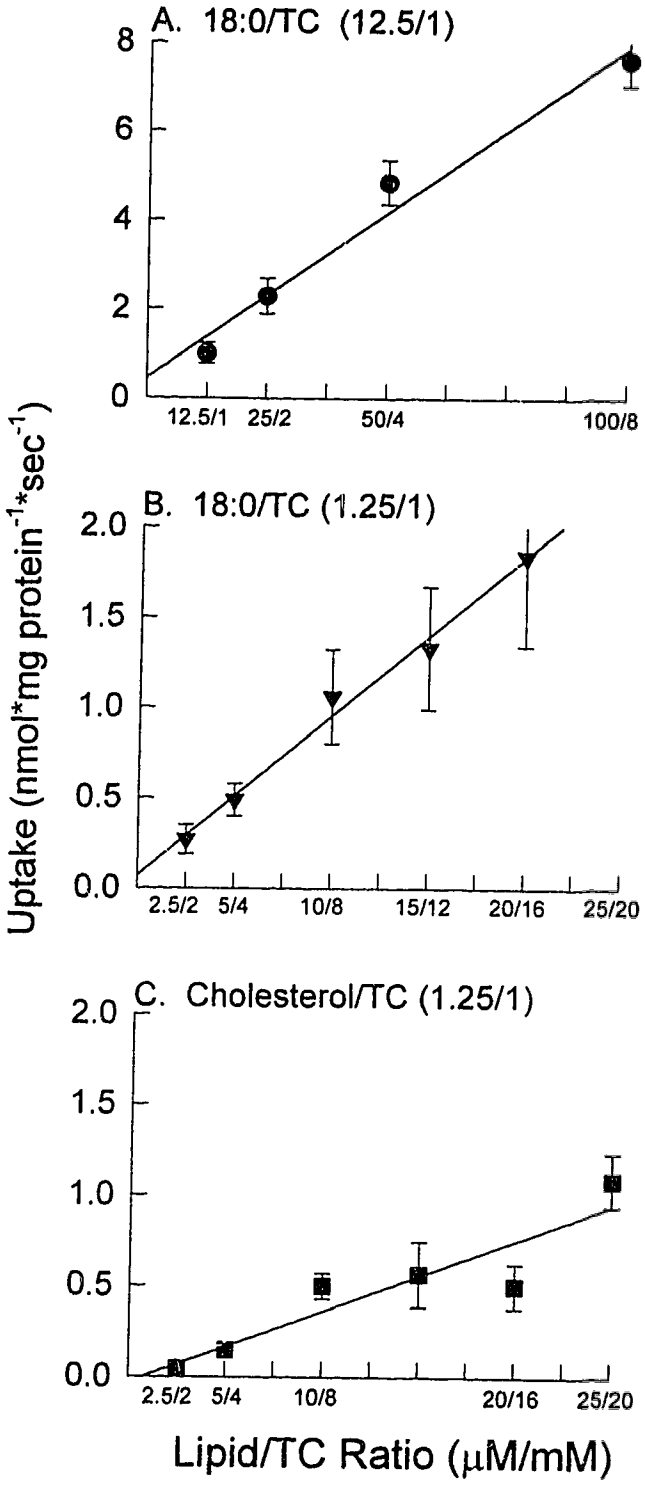

Figure 3.3 Effect of increasing the concentrations of both lipid and taurocholic acid (TC), while maintaining a constant ratio of lipid/TC, on the uptake of stearic acid (18:0) or cholesterol into brush border membrane vesicles (BBMV). BBMV were pre-loaded with Vesicle Resuspension Solution and incubated for 5 sec at room temperature with Lipid Transport Buffer containing increasing concentrations of lipid and TC while maintaining a fixed lipid/TC ratio of ***(A)*** 12.5 μM 18:0/1 mM TC, ***(B)*** 1.25 μM 18:0/1 mM TC, or ***(C)*** 1.25 μM cholesterol/1 mM TC (see Methods).

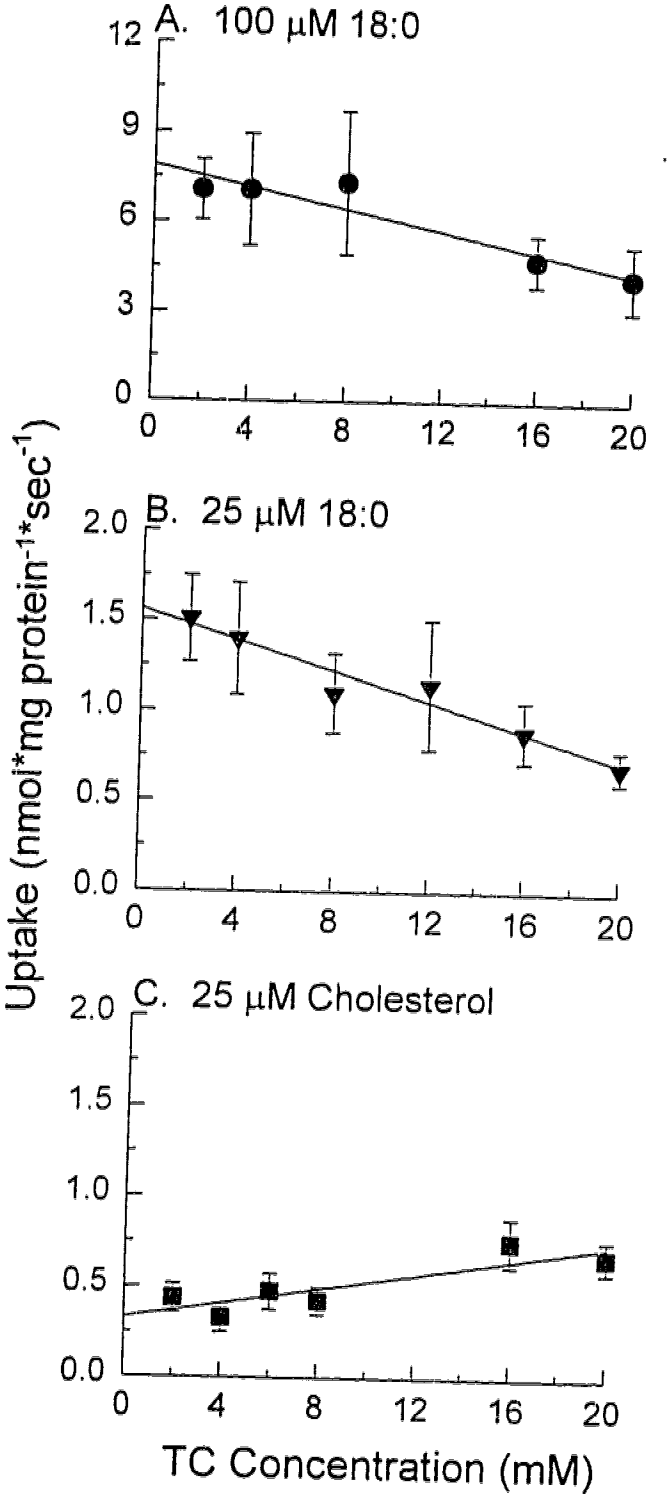

Figure 3.4 Effect of varying the concentration of taurocholic acid (TC), while maintaining a constant concentration of lipid, on the uptake of lipid into brush border membrane vesicles (BBMV). BBMV were pre-loaded with Vesicle Resuspension Solution and incubated for 5 sec at room temperature with Lipid Transport Buffer containing 2-20 mM TC and ***(A)*** 100 µM stearic acid (18:0), ***(B)*** 25 µM 18:0, or ***(C)*** 25 µM cholesterol (see Methods). The slope and correlation coefficient for each panel are -0.018, 0.937 ***(A)***; -0.042, 0.972 ***(B)***; and 0.020, 0.884 ***(C)***.

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CHAPTER 4

FEEDING AN ISOCALORIC OMEGA-3 FATTY ACID DIET REDUCES THE BRUSH BORDER MEMBRANE VESICLE UPTAKE OF GLUCOSE IN STREPTOZOTOCIN-DIABETIC RATS[3]

4.1 Introduction

The *in vivo* and *in vitro* uptake of glucose is increased into the intestine of streptozotocin-diabetic rats (Churnratanakul et al., 1990; Debnam et al., 1988, 1990; Thomson, 1980, 1981, 1983; Thomson et al., 1988a). Feeding an omega-3 or an omega-6 isocaloric diet as compared with a saturated fatty acid diet reduces the enhanced intestinal uptake of glucose in diabetic rats (Keelan et al., 1989, 1990; Rajotte et al., 1988; Thomson et al., 1987c, 1990;). Intestinal brush border membrane vesicles (BBMV) have been used to study the characteristics of intestinal nutrient uptake in the absence of an intestinal unstirred water layer (Hopfer et al., 1973; Hopfer, 1975; Keelan et al., 1992). This study was undertaken to examine the uptake of glucose into BBMV obtained from control and streptozotocin-diabetic rats fed an isocaloric, semisynthetic diet enriched with saturated fatty acids from beef tallow or with polyunsaturated fatty acids from fish oils. The results demonstrate that the enhanced glucose uptake into BBMV of streptozotocin-diabetic rats can be partially corrected by feeding an isocaloric semisynthetic diet enriched with polyunsaturated omega-3 fish oils.

4.2 Methods and Materials

4.2.a Animals and Diets

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Male Wistar rats weighing 300-400 g were split into two groups. The first group was rendered hyperglycemic by intraperitoneal injection with the pancreas β-cell cytotoxic agent streptozotocin (70 mg/kg) dissolved in 0.9% saline. The second group was injected with normal saline and served as non-diabetic controls.

All rats were housed in pairs and allowed *ad libitum* access to standard Purina® rat chow and water, with the amounts of food recorded. On day 7 after injection, tail vein blood was tested

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[3] A version of this chapter has been published in Diabetes Research 25:65-75. 1994.

for hyperglycemia, using the glucose oxidase method. Streptozotocin-injected animals with blood glucose levels of 350 mg/dl were judged to be diabetic and included in the study. Control animals did not exhibit hyperglycemia.

The next two weeks the animals were fed one of two semisynthetic diets, each containing 20% (w/w) fat providing either high amounts of 16:0 and 18:0 (saturated fatty acids) from beef tallow (BT), or high amounts of 20:5ω3 and 22:6ω3 (polyunsaturated fatty acids) from fish oil (FO). Nisshin EP-28 fish oil was obtained from Nissho Iwai Corporation, Tokyo, Japan. The semisynthetic diets were isocaloric and nutritionally adquate, providing for all known essential nutrient requirements. Macronutrient composition, preparation and fatty acid composition of the diets are detailed in Appendix I.

Food intake was monitored every two days. The animals were weighed weekly and tail vein glucose was rechecked at the time of sacrifice.

4.2.b Preparation of Intestinal Brush Border Membrane Vesicles

Animals were anesthetized by intrahepatic injection of Euthanyl® (sodium pentobarbital, 450 mg/kg body weight). Approximately 60 cm of the rat jejunum was removed and irrigated with 60 ml of ice cold saline (0.9% NaCl). Six rats were used for each experiment. The intestine was cut into 7x6 cm segments and was inverted directly onto plastic pegs which were placed directly into 600 ml of chilled (0-4°C) oxygenated 2.5 mM EDTA buffer, and were fitted into a plexiglass pegboard. Intestinal villous cells were released by mechanical vibration of the entire apparatus at 1600 rpm for 30 min (Harrison and Webster, 1964). The suspension was centrifuged 15 min at 15,300 x g. The resultant pellet of cellular components was resuspended in ice-cold 300 mM D-mannitol - 10 mM Tris-HCl buffer (pH 7.4), homogenized using a Polytron® at a setting of 9 for 15 s, and centrifuged for 20 min at 600 x g. Sufficient 1 M $CaCl_2$ was added to the supernatant to yield a concentration of 10 mM and stirred on ice for 10 min to aggregate the intracellular components. The solution was then centrifuged 15 min at 3000 x g. The supernatant was centrifuged 20 min at 29,800 x g, and the resulting pellet was resuspended in Vesicle Resuspension Buffer (300 mM D-mannitol - 10 mM HEPES-Tris, pH 7.4), and centrifuged 20 min at 19,500 x g. The final pellet is was resuspended in an appropriate volume of Vesicle Resuspension Buffer to yield sufficient volume for the experiment. The purity and recovery of the BBMV were determined by measuring marker enzyme activities. Sucrase activity (Dahlqvist, 1964) was measured and final concentration of protein was measured by the method of Lowry (1951).

The time-course studies were performed in the presence of a Na^+ gradient so that the final resuspension buffer was 300 mM D-mannitol - 10 mM HEPES-Tris, pH 7.4. In order to obtain kinetic data from concentration curves that would be independent of a Na^+ gradient, the vesicles were resuspended in 100 mM D-mannitol - 100 mM NaSCN - 10 mM HEPES-Tris, pH

7.4. As such, NaSCN was present on both sides of the vesicle membrane, and changes in vesicle leakiness to Na^+ do not affect transport rates (Maenz and Cheeseman, 1986).

4.2.c Glucose Uptake Studies

Uptake of D-glucose by the rat jejunal BBMV was determined using a rapid filtration technique (Hopfer, 1975), as described in Chapter 2. Uptake measurements were obtained at 5, 10, 20, 30, 40, 50 and 60 s, and at 1.5, 2.0, 2.5, 3.0, 5.0, 10 and 15 min, using a D-glucose concentration of 100 µM. The concentration curves were established over a concentration range of D-glucose 10 µM to 1000 µM, using a 5 s incubation. In both the time-course and the concentration studies, Na^+-independent rates of glucose transport were measured by replacing NaSCN with KSCN.

4.2.d Data Analysis

D-glucose uptake was the net radioactivity after subtraction of non-specific radioactivity bound to filters in the absence of BBMV (blank). This blank value was equivalent to the radioactivity obtained in the presence of BBMV at time 0 (BBMV + probe + immediate quenching and filtering). Uptake was expressed as pmol per mg BBMV protein. The time-course was used to determine the initial rate of uptake and the time required to reach equilibrium. The initial linear rate of rapid uptake represents the one-way movement of substrate across the BBM. Beyond this time, substrate movement represents 'net transfer' into the BBMV.

The concentration curve for D-glucose allowed estimations to be made of the maximal transport rate (V_{max}) and the Michaelis constant (K_m). The kinetics of glucose uptake were determined by the Systat program for best fit curves.

All values represent the mean ± standard error of the mean of four to six vesicle preparations. Each uptake point was performed in triplicate. Analysis of variance and the Student Newman-Keul's multiple range test were used to search for differences between means, with 0.05 as the accepted upper limit of statistical significance.

4.3 Results

Food intake was greater in diabetic than in control rats (Table 4.1). Food intake was higher in diabetic rats fed beef tallow (BT) as compared with fish oil (FO). Weight gain was lower in diabetic than in control animals. In both control and in diabetic rats, weight gain was lower in animals fed FO than BT ($p<0.05$). The fasting blood glucose was higher in diabetic than in control rats, but there was no difference between animals fed BT as compared with FO.

The time-course for the uptake of 100 µM D-glucose demonstrated an overshoot which peaked at approximately 30 s, and then declined to achieve an equilibrium plateau (Figures 4.1A

and 4.1B). In rats fed the isocaloric saturated or polyunsaturated diet, glucose uptake was greater in diabetic as compared with non-diabetic control animals.

With increasing concentrations of glucose, uptake was greater in diabetic than in control rats, particularly at concentrations at or above 5 mM (Figures 4.2A and 4.2B). The maximal transport rate (V_{max}) for glucose uptake was lower in control rats fed FO as compared with BT (difference not statistically significant), and this inhibitory effect of feeding FO on glucose uptake was much more marked in diabetic animals (Table 4.2). In diabetic rats fed BT, the value of the V_{max} was increased approximately 9-fold as compared with non-diabetic rats fed BT ($p<0.05$), whereas the increase was approximately 6-fold in diabetic as compared with control rats fed FO ($0.05<p<0.1$). Thus, the value of the V_{max} was approximately 50% lower in diabetic rats fed FO as compared with those fed BT. No difference was noted in the values of the K_m between control and diabetic rats, and between those animals fed BT versus FO.

4.4 Discussion

The enhanced intestinal uptake of glucose has been demonstrated *in vivo* and *in vitro* in streptozotocin-diabetic rats (Churnratanakul et al., 1990; Debnam et al., 1988, 1990; Fedorak et al., 1987, 1989, 1991; Thomson, 1980, 1981, 1983; Thomson et al., 1988a). This is due to an increase in the value of the maximal transport rate (V_{max}), which results from the recruitment of glucose transporters from the mid-portions of the ileal villus, and from an increase in the activity of the glucose carrier in the enterocytes in the upper third of the jejunal villus (Dudeja et al., 1990; Fedorak et al., 1989, 1991). Isocaloric modifications in the dietary fatty acids modify the *in vitro* intestinal absorption of glucose (Thomson et al., 1987a, b, 1988a, b), with uptake being higher in animals fed a saturated as compared with a polyunsaturated diet (Thomson et al., 1986, 1989). The type of polyunsaturated diet also influences nutrient uptake (Keelan et al., 1987; Thomson et al., 1987a, d, 1988a). Feeding an isocaloric semisynthetic polyunsaturated fatty acid diet reduces the enhanced *in vivo* uptake of glucose demonstrated in diabetic rats fed a saturated diet, and this is associated with modest improvements in the diabetic clinical control (Churnratanakul et al., 1990; Rajotte et al., 1988; Thomson et al., 1987b, 1988a).

Brush border membrane vesicles (BBMV) have been widely used to examine nutrient uptake. This technique generally demonstrates lower values of the Michaelis affinity constant (K_m) than the K_m values demonstrated with *in vitro* intestinal discs or *in vivo* perfusion (Churnratanakul et al., 1990; Thomson, 1979; Thomson et al., 1988b). This is possibly because of lack of effect of an intestinal unstirred water layer (UWL) in BBMV (Thomson and Dietschy, 1984). The presence of a glucose overshoot (Figures 4.1A and 4.1B) confirms that the BBMV were sealed, and these overshoot values are similar to those reported by other workers (Dudeja

et al., 1990; Hopfer et al., 1973; Ling et al., 1989; Meddings et al., 1990; Tsuji et al., 1988). These time-course studies directed the selection of a duration of incubation of 5 s in the D-glucose concentration studies. Glucose uptake was greater in diabetic than in control BBMV in the concentration studies (Figures 4.2A and 4.2B).

Feeding FO reduces glucose uptake in non-diabetic control animals, when assessed *in vitro* and *in vivo* (Churnratanakul et al., 1990, 1991; Rajotte et al., 1988; Thomson et al., 1987b, 1988b, 1990;). This was also found in the present study (although the difference was not statistically significant), and as well it was confirmed that glucose uptake was increased in diabetic as compared with control rats (Table 4.2), with the value of the V_{max} increased by approximately 9-fold in those fed BT and by 6-fold in FO-fed animals. This represents a greater enhancement of glucose uptake compared with the increase demonstrated in diabetic rats, where glucose uptake is examined *in vitro* with intestinal discs, or *in vivo* with intestinal perfusion (Churnratanakul et al., 1990; Thomson, 1981; Thomson et al., 1987b). Also, the inhibitory effect of the FO diet on the enhanced uptake of glucose observed in diabetic rats fed a saturated diet (Thomson et al., 1987b, 1990) was confirmed using BBMV (Table 4.2).

In this study, BBMV were prepared from an homogenate of membranes obtained from enterocytes collected from all positions along the villus. Most glucose transport occurs from enterocytes along the upper third of the villus (Dudeja et al., 1990), but in diabetes enterocytes from the mid- and lower-third of the villus may play a greater role in glucose transport (Meddings et al., 1990), particularly from the ileum (Fedorak et al., 1987, 1989, 1991). Although the kinetic basis is clear for the changes in glucose uptake observed with diabetes or with changes in dietary lipids, it remains to be established what is the mechanism for the change in V_{max}. Enterocyte fractionation studies must now be performed to determine whether the inhibitory effect of feeding fish oil on the enhanced uptake of glucose in diabetic rats is due to an equal effect on all enterocytes along the villus, or whether this inhibitory effect of feeding an omega-3 fatty acid fish oil diet is due to the alteration in the distribution of glucose carriers along the intestinal villus. Furthermore, studies must now be performed with the recently reported antibody to the BBM Na^+-dependent glucose cotransporter (SGLT1) to determine whether the changes in V_{max} represent the presence of more transporter, or further activation of carrier at the same or different position along the crypt-villus axis.

Table 4.1: Effect of diabetes and dietary ω3 fatty acids on animal characteristics

	CONTROL		DIABETIC	
	BT	FO	BT	FO
FOOD INTAKE (g/day)	23.0 ± 0.8	25.4 ± 2.4	34.3 ± 0.9*	30.2 ± 1.3*+
WEIGHT GAIN (g/day)	2.6 ± 0.3	4.8 ± 0.2+	-2.8 ± 0.5*	0.6 ± 0.7*+
BLOOD GLUCOSE (mg/dl)	115 ± 2	116 ± 2	448 ± 2*	445 ± 3*

* $p<0.05$, diabetic versus control

+ $p<0.05$, fish oil versus beef tallow

The diets were: BT, beef tallow; FO, fish oil.

Table 4.2: Effect of diabetes and dietary ω3 fatty acids on the kinetic parameters of rat jejunal brush border membrane vesicle glucose uptake

	CONTROL		DIABETIC	
	BT	FO	BT	FO
V_{max} (pmol/mg protein/5 s)	890 ± 265	604 ± 246	5000 ± 1116*	3634 ± 2939*
K_m (μM)	10 ± 4	22 ± 12	46 ± 13	43 ± 40

* $p<0.05$, diabetic versus control

The diets were: BT, beef tallow; FO, fish oil.

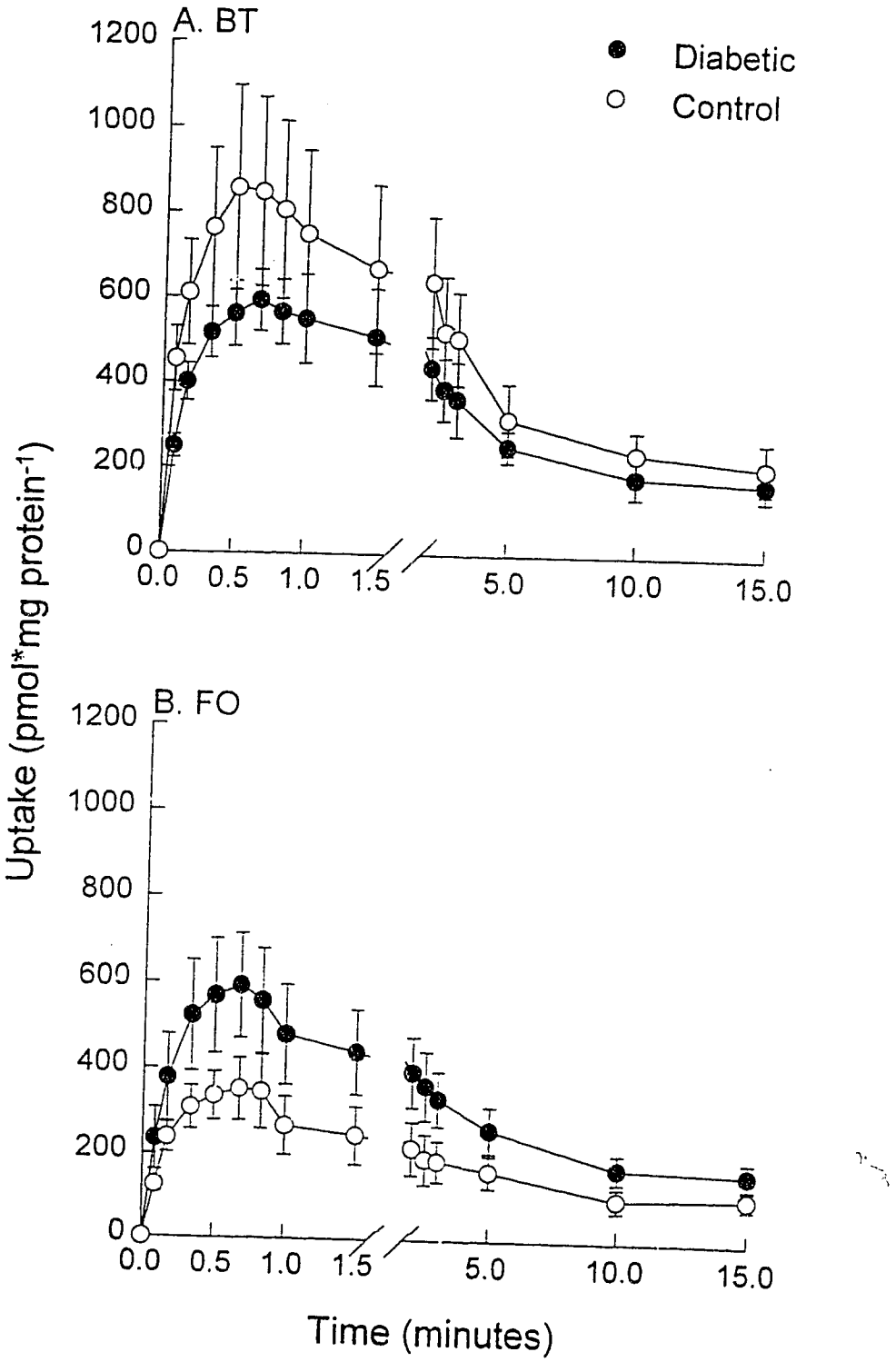

Figure 4.1 Time course of D-glucose uptake into brush border membrane vesicles (BBMV) obtained from diabetic (●) or from non-diabetic control (○) rats fed an isocaloric semisynthetic diet enriched with saturated (beef tallow, BT) or with polyunsaturated (fish oil, FO) fatty acids. BBMV were pre-loaded with Vesicle Resuspension Buffer and incubated at room temperature with Glucose Transport Buffer containing 100 mM D-glucose and 100 mM NaSCN (see Methods).

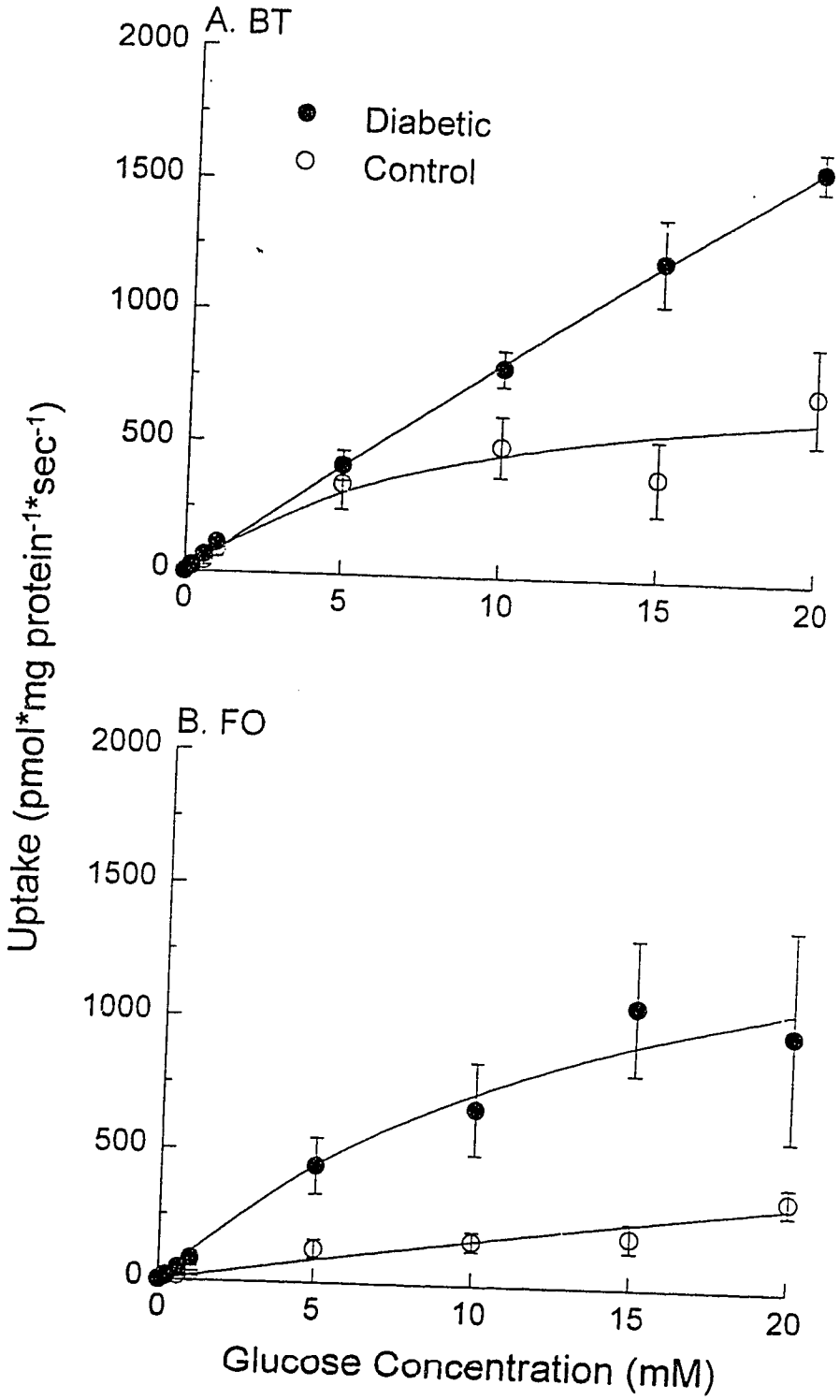

Figure 4.2 Concentration curve for D-glucose uptake into brush border membrane vesicles (BBMV) obtained from diabetic (●) or from non-diabetic control (○) rats fed an isocaloric semisynthetic diet enriched with saturated (beef tallow, BT) or with polyunsaturated (fish oil, FO) fatty acids. BBMV were pre-loaded with 100 mM mannitol - 100 mM NaSCN - 10 mM Tris-Hepes buffer, pH 7.4 and incubated for 5 sec at room temperature with Glucose Transport Buffer containing 100 mM NaSCN and 0.2 - 20 mM D-glucose (see Methods).

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CHAPTER 5

FISH OIL MODIFIES EFFECT OF HIGH CHOLESTEROL INTAKE ON INTESTINAL ABSORPTION IN DIABETIC RATS[4]

5.1 Introduction

The topic of the potential benefit of a fish oil-containing diet in the prevention of atherosclerosis has been reviewed (von Schacky, 1987). The two major omega-3 (ω3) fatty acids found in fish oil are eicosapentaenoic acid (EPA, 20:5ω3) with five double bonds and docosahexaenoic acid (DHA, 22:6ω3) with six double bonds. The ω3 fatty acids have numerous effects on plasma triglyceride and lipoprotein concentrations, platelet thrombogenicity, inflammatory and immune cell function and the development of atherosclerosis (Leaf and Weber, 1988). The abnormal fluidity of cell membranes of diabetics can be reversed with ω3 fatty acid supplementation (Kamada et al., 1986), which may influence the function of membrane-associated proteins such as insulin receptors or glucose transporters (Ginsberg et al., 1981). Omega-3 fatty acids may also influence the insulin secretion of pancreatic beta cells by way of specific prostaglandin and leukotriene mediators; their synthesis and function are influenced by fish oils (Fujimoto and Metz, 1984; Robertson, 1984).

Variations in dietary lipid alters active and passive intestinal transport properties of the intestine of rats (Thomson et al., 1986). Feeding isocaloric diets varying in the type of polyunsaturated fatty acids (such as α-linolenic acid in linseed oil, or EPA and DHA in fish oil) also modifies the form and function of the intestine (Thomson et al., 1988a, b, c). Furthermore, the effects of fish oil dietary supplements on the intestine is influenced by other lipids in the diet. For example, diets supplemented with fish oil have a different effect on the intestine when the main dietary lipid is saturated rather than polyunsaturated ω6 fatty acids (Thomson et al., 1989).

Streptozotocin-induced diabetes in rats is known to alter the active and passive absorption of nutrients (Thomson, 1980, 1981; Hotke et al., 1985). This adaptive process responds to variations in the dietary content of nutrients (Thomson and Rajotte, 1983a, b; Rajotte and Thomson, 1987). These alterations in nutrient transport are associated with improvements in the animals' glucose tolerance, i.e., feeding a polyunsaturated fatty acid diet for 11 weeks reduces significantly the diabetic animal's hemoglobin A_1C concentration, as compared with diabetic rats fed an isocaloric saturated fatty acid diet (Rajotte et al., 1988).

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[4] A version of this chapter has been published in Diabetes Research 22:171-183, 1993.

Accordingly, this study was undertaken to test the hypothesis that the influence of a fish oil diet on nutrient absorption in diabetes is further modified by the presence of cholesterol in the diet.

5.2 Methods and Materials

5.2.a Animals and Diets

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Drug-induced glucose intolerance was produced in female albino Wistar rats weighing 200-250 g by intravenous administration of the pancreas β-cell cytotoxic agent streptozotocin (65 mg/kg). After injection of streptozotocin, the rats were fed chow for three weeks, following which they were switched to one of four semisynthetic diets for two weeks: a diet containing enriched amounts of 16:0 and 18:0 (from beef tallow) with low or high amounts of cholesterol (BT, BTC), or enriched amounts of 20:5ω3 and 22:6ω3 (from fish oil) with low or high amounts of cholesterol (FO, FOC). NISSHIN EP-28 fish oil was obtained from Nissho Iwai Corporation, Tokyo, Japan. The semisynthetic diets were isocaloric and nutritionally adequate, providing for all known essential nutrient requirements. The complete composition of the diets is given in Appendix I.

Food intake was monitored every two days. The animals were weighed at the beginning as well as at the end of the study. Intestinal weights were determined at the time of each uptake study. Rats were allowed *ad libitum* access to food and water until the time of their sacrifice. Induction of glucose intolerance was assessed by the presence of hyperglycemia and glucosuria. Urine glucose was measured using a Beckman glucose analyzer. A few drops of blood were obtained by pricking the tail with a lancet and then placing the blood on a glucose test strip that is read with a Glucoscan® meter (Lifescan Medical Products, Vancouver, B.C).

5.2.b Probe and Marker Compounds

[^{3}H]-Inulin (molecular weight approximately 5,000) was used as supplied by the manufacturer^{+} to measure the adherent mucosal fluid volume. The [^{14}C]-labelled probes included palmitic acid (FA 16:0)$^{++}$, stearic acid (FA 18:0)$^{++}$, oleic acid (FA 18:1)$^{+}$, linolenic acid (*FA 18:2)*, linolenic acid (FA 18:3)$^{+}$, cholesterol*, L-glucose* and D-glucose^{++}. Unlabelled probes were supplied by Sigma Company (St. Louis, No.). [^{3}H] and [^{14}C]-labelled probes were obtained from $^{+}$New England Nuclear (Boston, Massachusetts), $^{++}$ICN Biomedical Inc. (Montreal, Quebec), or *Amersham Canada Ltd. (Oakville, Ontario).

5.2.c Individual Experiments

The method used for preparation of micellar solutions of the fatty acids and cholesterol has been published (Thomson, 1980, 1981; Hotke et al., 1985; Thomson and Rajotte, 1983a, b;

Rajotte and Thomson, 1987; Rajotte et al., 1988; Westergaard and Dietschy, 1976). The long-chain fatty acids and cholesterol were solubilized in 20 mM taurodeoxycholic acid. The concentration of solutes was: cholesterol, 0.05 mM; long-chain saturated and unsaturated fatty acids, 0.1 mM; D-glucose, 1-40 mM; and L-glucose, 1 mM.

5.2.d Tissue Preparation

Animals were sacrificed by intraperitoneal injection of Euthanyl® (sodium pentobarbital, 450 mg/kg body weight). Two 15 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with 50 ml of ice-cold saline, as described in detail elsewhere (Thomson, 1980; Westergaard and Dietschy, 1974, 1976; Lukie et al., 1974). The intestine was opened along its mesenteric border and the mucosal surface was carefully washed with cold saline to remove visible mucus and debris. Pieces of intestine were cut from a segment and tissue was mounted as flat sheets in incubation chambers. Preincubation chambers contained oxygenated Krebs-bicarbonate buffer (pH 7.4) at 37°C; tissue discs were preincubated in this buffer for 10 minutes to allow equilibration at this temperature. After preincubation, the chambers were transferred to other beakers containing [^{3}H]-inulin and various [^{14}C]-probe molecules in oxygenated Krebs-bicarbonate (pH 7.4) at 37°C. The preincubation and incubation solutions were mixed at identical stirring rates with circular magnetic bars and the stirring rates were precisely adjusted by means of a strobe light. Stirring rates were reported as revolutions per minute at which the stirring bar was driven. A stirring rate of 600 rpm was selected to achieve low effective resistance of the intestinal unstirred water layer (Westergaard and Dietschy, 1974), and to thereby allow for demonstration of diet-associated changes in membrane transport properties.

5.2.e Determination of Uptake Rates

After incubation of discs in labelled solutions for 6 min, the experiment was terminated by removing the chamber and quickly rinsing the tissue in cold saline for approximately 5 s. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch and was gently blotted on filter paper. The tissue was dried to a constant weight overnight in an oven at 55 °C. The dry weight of the tissue was determined, the sample was saponified with 0.75N NaOH, scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes.

5.2.f Morphology

Previous reports (Ecknauer et al., 1982; Keelan et al., 1985a, b, c) have described in detail the method used to assess the weight of the scraped mucosa and the remainder of the intestinal wall, and the villus and mucosal surface areas of the intestine.

5.2.g Expression of Results

The uptake rates of the probes were calculated after correcting total tissue [^{14}C]-radioactivity for the mass of the probe molecule present in the adherent mucosal fluid

(Westergaard and Dietschy, 1974). Uptake rates were expressed as nmol of probe molecule taken up into the mucosa per 100 mg dry weight of tissue per minute (nmol*100 mg^{-1}*min^{-1}). Values obtained from different dietary groups are reported as mean ± standared error of the mean of results observed for a minimum of six animals in each group. Analysis of variance and the Student Neuman Keul's multiple range test was used to test the significance of the difference between dietary groups.

5.3 Results

5.3.a Animal Characteristics

Food intake (g/day) was similar in diabetic animals fed beef tallow low cholesterol (BT), beef tallow high cholesterol (BTC), fish oil low cholesterol (FO) and fish oil high cholesterol (FOC) (Table 5.1). Body weight change (g/day) was also similar over the two weeks of feeding BT, BTC, FO and FOC. The dry weight (mg/unit serosal surface area) of jejunum and ileum was higher ($p<0.05$) in BTC than in BT, higher ($p<0.05$) in FO than in BT, and higher ($p<0.05$) in FO than in FOC in the ileum but not in the jejunum.

Cholesterol supplementation increased the percentage of the ileal wall comprised of mucosa when fed with saturated fat (BTC versus BT), whereas the converse was true when cholesterol was fed with fish oil (FOC versus FO). Thus, cholesterol feeding increased the mucosal mass of the jejunum and ileum when fed with BT, but decreased mucosal mass when fed with FO.

After two weeks of feeding semisynthetic diet, the blood glucose was similar in BTC, FO and FOC, but was lower in BT. Furthermore, over the two week interval of feeding the semisynthetic diets, the decline in blood glucose concentration was much greater in BT (240 mg/dL) and FOC (200 mg/dl), than in animals fed BTC (62 mg/dl) and FO (115 mg/dl).

Adding cholesterol to the beef and fish diets had no effect on jejunal mucosal surface area, whereas ileal mucosal surface area was lower ($p<0.05$) when rats were fed FOC as compared with BTC (Tables 5.2 and 5.3).

5.3.b Glucose Uptake

There was no difference in the uptake of 1-40 mM D-glucose into the jejunum of diabetic animals fed the four different semisynthetic diets (Figure 5.1A). Ileal uptake of D-glucose was similar in BT and in BTC, whereas the supplementation of the omega-3 diet with cholesterol (FOC versus FO) was associated with higher ($p<0.05$) uptake of glucose (Figure 5.1B). Ileal uptake of 1 and 40 mM D-glucose was lower ($p<0.05$) in FO than in BT, but this effect was lost when the fish oil diet is supplemented with cholesterol. In contrast, the uptake of 1 mM and 40 mM glucose was significantly lower in the ileum of diabetic rats fed FO as compared with BT of FOC.

5.3.c Lipids

The uptake of palmitic acid (FA 16:0) into the jejunum of diabetic rats was lower in FO than in BT or FOC (Table 5.4). Cholesterol uptake in the jejunum of diabetic rats was higher in BTC than in BT, higher in FO than BT, and higher in BTC than in FOC. The only effect observed with feeding the four semisynthetic diets on ileal uptake was the greater uptake of linoleic acid (FA 18:2) in rats fed FOC as compared with BTC or FO (Table 5.5). Thus, feeding fish oil prevented the increase in jejunal cholesterol uptake observed in diabetic rats fed beef tallow high cholesterol (BTC). And yet, in the presence of low dietary cholesterol, fish oil itself increased the uptake of cholesterol (F0) as compared with animals fed beef tallow low cholesterol (BT).

5.4 Discussion

The intestinal uptake of higher concentrations of glucose is reduced in animals fed isocaloric semisynthetic diets containing fish oil (Thomson et al., 1988b, c). However, this reduction is limited to the ileum and is prevented when the fish oil-containing diet is supplemented with cholesterol (Figure 5.1). We have not determined the concentration of fish oil or cholesterol along the length of the intestinal lumen, but we presume that the amount of these dietary lipids reaching the ileum is less than that to which the jejunum is exposed, because of the absorption of some dietary lipid in the proximal intestine. The response of the ileal but not the jejunal brush border membrane to alter D-glucose transport in response to dietary changes (Figure 5.1) is therefore unlikely to be on the basis of a stronger signal to the ileum than to the jejunum. The lipid composition of the enterocyte microsomal membrane varies between jejunum and ileum (Garg et al., 1988), suggesting the possibility that these organelles are qualitatively different between the two intestinal sites. We postulate that the jejunal microsomes respond less than or differently to the microsomes from the ileum in response to variations in the luminal contents of lipids achieved by varying the source of fatty acid and cholesterol in the diet.

The reduction in the absorption of higher concentrations of glucose into the ileum of animals fed FO did not result in significantly lower mean values for their blood glucose concentration (Table 5.1). It is likely that the contribution of the ileum to the total amount of glucose absorbed is much less than the jejunum, and therefore the alterations in the ileal uptake of glucose might have relatively little influence on the glucose concentration. However, in this study the animals were fed FO for only two weeks, and it is possible that longer durations of feeding would have been associated with lower levels of blood glucose concentration. For example, it has recently been shown that diabetic rats fed a semisynthetic isocaloric diet containing polyunsaturated fatty acids was associated with lower blood glucose and hemoglobin A_1C concentrations after eleven weeks feeding (Rajotte et al., 1988).

In diabetic animals, unlike non-diabetic control animals (Thomson et al., 1988b,c), feeding fish oil did not have a consistent anti-absorptive effect on lipid uptake (Tables 5.4 and 5.5). For example, feeding FO was associated only with reduced jejunal uptake of palmitic acid, which persisted in the presence of added cholesterol (FOC), whereas feeding FO increased the jejunal uptake of cholesterol. Indeed, it was only when cholesterol was added to the fish oil diet (FOC) that the jejunal uptake of cholesterol was reduced. This was in contrast to the enhancing effect of cholesterol supplementation on ileal cholesterol uptake when beef tallow was the major dietary lipid (BTC). Furthermore, jejunal uptake of cholesterol was higher in FO than BT. The blood lipid levels were not measured in this study. Based on results obtained from the jejunal and ileal uptake in diabetic animals, it might be anticipated that FO would not have a major influence on lowering the hyperlipidemia associated with diabetes. However, it must be stressed that most of the hypolipidemic effects of fish oil are exerted after absorption, e.g. inhibition of synthesis and secretion of triglyceride (Nossen et al., 1986), inhibition of cholesterol biosynthesis (Field et al., 1988; Daggy et al., 1987), and an increase in bile flow and excretion (Balasubramaniam et al., 1985).

These effects of feeding fish oil, with or without added cholesterol, on jejunal and ileal active uptake of glucose and passive uptake of lipid could not be explained by difference in the animals' food intake or weight gain (Table 5.1). Although feeding BTC was associated with a heavier jejunum and ileum as compared with animals fed BT, this was not associated with increases in the uptake of glucose or lipid. In addition, the lower ileal weight in animals fed FOC than in those fed FO was associated with higher rather than lower rats of uptake of 1 and 40 mM glucose and palmitic acid (Figure 5.1). Furthermore, the jejunal mucosal surface area was unaffected by the type of lipid or the presence of cholesterol (Table 5.2). Thus, although the ileal mucosal surface area was lower in rats fed FOC as compared with BTC (Table 5.3), the differences in the weight of the intestine or in intestinal morphology was unlikely to be the explanation for the differences in intestinal transport.

What then is the likely mechanism of these dietary changes? The phospholipid composition of the brush border membrane is altered in diabetic rats (Keelan et al., 1985a, 1987) and the lipid composition of the brush border membrane is influenced by the lipid composition of the diet (Keelan et al., 1987, 1990a, b). We anticipate that the brush border membrane lipid composition differs between animals fed FO versus BT and between those fed diets supplemented with cholesterol versus those not supplmemented with cholesterol (FOC and BTC) (see Chapters 7 and 9).

Feeding fish oil may be beneficial for the prophylaxis of atherosclerosis (von Schacky, 1987), and clinical trials are in progress to examine the effects of feeding fish oil on the alternations in glucose metabolism observed in diabetic patients. Glauber and co-workers

(Glauber et al., 1988) fed six men with type 2 (non-insulin-dependent) diabetes mellitus a liquid diet containing 5.5 g of ω3 fatty acids (EPA 3.3 g and DHA 2.2 g) and 108 mg of cholesterol daily. Plasma and erythrocyte content of ω3 fatty acid rose with diet supplementation, associated with which basal hepatic glucose output rose and insulin levels estimated by means or intravenous glucagon fell by 30% and 39%, respectively. After ω3 fatty acid withdrawal, fasting glucose returned to baseline. In these studies, only 21% of calories were obtained from fat, the dietary content of cholesterol was low, there was no fiber in the diet and the amount of ω3 fatty acids in the liquid diet (Sustacal®) was also relatively low. Furthermore, the fatty acid content of this liquid diet differed from that used in this study. Similar deleterious effects have been reported in diabetic patients consuming 8 g per day of ω3 fatty acids for 8 weeks, using a different fish oil concentrate (Friday et al., 1987). These studies in humans differ from the short and longer term benefit of feeding ω3 fatty acids on the intestinal uptake of glucose and lipids in these streptozotocin diabetic rats. Also, the longer term feeding of polyunsaturated fatty acid diets to streptozotocin diabetic rats is associated with improved levels of hemoglobin A_1C concentrations and improved glucose clearance (Rajotte et al., 1988). The differences therefore between the animal and human studies may be species-related, or may be due to the differences in the diets. Diets containing fish oil alter the intestinal transport of nutrients, but the direction and magnitude of these changes depends upon the presence of saturated fatty acids in the diet and the presence of cholesterol. Thus, before these observations of the anti-absorptive effect of fish oil on rat intestine can be applied to the human condition, the mechanism by which fish oil alters intestinal transport must first be elucidated. Secondly, it remains to be established what is the optimal ratio of dietary saturated, polyunsaturated (ω3 and ω6) fatty acids and cholesterol required to normalize the enhanced intestinal transport function observed in diabetic rats.

Table 5.1. Effect of dietary ω3 fatty acids and cholesterol on diabetic rat characteristics

	BT	BTC	FO	FOC
FOOD INTAKE				
g/day	19.6 ± 0.3	20.1 ± 0.5	18.4 ± 1.5	18.5 ± 0.4
WEIGHT CHANGE				
g/day	-1.6 ± 0.7	-0.1 ± 0.5	0.1 ± 0.6	-0.2 ± 0.7
DRY WEIGHT,				
mg/unit serosal				
surface area				
Jejunum	7.6 ± 0.05	10.3 ± 0.6#	8.6 ± 0.3	9.2 ± 0.5
Ileum	5.4 ± 0.5	8.0 ± 0.9#	10.3 ± 0.4+	5.8 ± 1.1#
% OF INTESTINAL				
WALL COMPRISED				
OF MUCOSA				
Jejunum	69.2 ± 1.8	71.1 ± 1.8	70.6 ± 0.9	73.3 ± 1.2
Ileum	57.5 ± 3.7	72.1 ± 3.1#	76.8 ± 1.9+	51.5 ± 7.6#+
BLOOD GLUCOSE				
mg/dL				
before diet	461 ± 22	446 ± 29	423 ± 24	481 ± 14
after diet	221 ± 54	384 ± 48#	308 ± 30	281 ± 43

\+ $p \leq 0.05$, fish oil versus beef tallow

\# $p < 0.05$, high versus low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 5.2. Effect of dietary ω3 fatty acids and cholesterol on morphology of diabetic rat jejunum

MORPHOLOGICAL PARAMETER	BT	BTC	FO	FOC
CRYPT DEPTH, μm	82 ± 5	80 ± 7	68 ± 5	67 ± 6
VILLUS HEIGHT, μm	428 ± 19	415 ± 32	400 ± 10	373 ± 11
VILLUS WIDTH AT 1/2 HEIGHT, μm	110 ± 8	98 ± 8	103 ± 4	98 ± 5
VILLUS BOTTOM WIDTH, μm	122 ± 9	111 ± 8	126 ± 8	139 ± 12
VILLUS THICKNESS, μm	446 ± 27	562 ± 30#	568 ± 37+	566 ± 40
VILLUS SURFACE AREA, μm^2/villus	521 ± 30	602 ± 56	585 ± 16	531 ± 16
NO. OF VILLI/mm SEROSAL LENGTH A	8.5 ± 0.6	9.4 ± 0.7	8.2 ± 0.5	7.7 ± 0.6
NO. OF VILLI/mm SEROSAL LENGTH B	2.3 ± 0.1	1.8 ± 0.1#	1.8 ± 0.1+	1.8 ± 0.1
NO. OF VILLI/mm^2 SEROSA	19.1 ± 1.3	16.8 ± 1.2	14.5 ± 0.9+	13.5 ± 1.1
MUCOSAL SURFACE AREA mm^2/mm^2 serosa	9.9 ± 0.8	10.1 ± 1.2	8.4 ± 0.4	7.3 ± 0.7

\+ $p<0.05$, fish oil versus beef tallow

\# $p<0.05$, high versus low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 5.3. Effect of dietary ω3 fatty acids and cholesterol on morphology of diabetic rat ileum

MORPHOLOGICAL PARAMETER	BT	BTC	FO	FOC
CRYPT DEPTH, μm	81 ± 4	84 ± 6	68 ± 3	56 ± 4#
VILLUS HEIGHT, μm	222 ± 12	302 ± 8#	225 ± 9	183 ± 6#+
VILLUS WIDTH AT 1/2 HEIGHT, μm	89 ± 3	87 ± 3	111 ± 5	84 ± 2#
VILLUS BOTTOM WIDTH, μm	101 ± 2	109 ± 5	130 ± 10+	115 ± 6
VILLUS THICKNESS, μm	434 ± 20	559 ± 46#	598 ± 36+	325 ± 21#+
VILLUS SURFACE AREA, μm^2/villus	266 ± 16	427 ± 13#	376 ± 15+	169 ± 5#+
NO. OF VILLI/mm SEROSAL LENGTH A	10.0 ± 0.2	9.3 ± 0.5	8.1 ± 0.6+	8.9 ± 0.4
NO. OF VILLI/mm SEROSAL LENGTH B	2.4 ± 0.1	2.0 ± 0.2	1.7 ± 0.1+	3.2 ± 0.2#+
NO. OF VILLI/mm^2 SEROSA	23.0 ± 0.5	16.7 ± 0.8#	13.5 ± 1.0+	27.4 ± 1.3#+
MUCOSAL SURFACE AREA mm^2/mm^2 serosa	6.1 ± 0.3	7.1 ± 0.4#	5.1 ± 0.5	4.7 ± 0.3+

\+ $p<0.05$, fish oil versus beef tallow

\# $p<0.05$, high versus low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 5.4. Effect of dietary ω3 fatty acids and cholesterol on lipid uptake into diabetic rat jejunum

SUBSTRATE	BT	BTC	FO	FOC
FA 16:0	1.33 ± 0.23	1.16 ± 0.22	0.43 ± 0.11^{+}	0.81 ± 0.15$^{\#}$
FA 18:0	1.05 ± 0.19	1.38 ± 0.59	0.76 ± 0.15	0.61 ± 0.13
FA 18:1	0.92 ± 0.18	0.53 ± 0.11	0.57 ± 0.10	0.95 ± 0.16
FA 18:2	1.07 ± 0.19	0.92 ± 0.22	0.78 ± 0.16	0.49 ± 0.26
FA 18:3	0.84 ± 0.14	0.96 ± 0.14	1.25 ± 0.23	1.15 ± 0.26
Cholesterol	0.59 ± 0.14	2.47 ± 0.66$^{\#}$	1.68 ± 0.51^{+}	1.07 ± 0.26^{+}

The concentration of the fatty acids was 0.1mM, in 20 mM taurodeoxycholic acid.

The concentration of cholesterol was 0.05mM, in 20 mM taurodeoxycholic acid.

\+ $p<0.05$, fish oil versus beef tallow

\# $p<0.05$, high versus low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 5.5. Effect of dietary ω3 fatty acids and cholesterol on lipid uptake into diabetic rat ileum

SUBSTRATE	BT	BTC	FO	FOC
FA 16:0	1.01 ± 0.24	0.83 ± 0.15	1.19 ± 0.18	1.45 ± 0.22
FA 18:0	0.98 ± 0.20	0.73 ± 0.20	0.53 ± 0.11	0.95 ± 0.19
FA 18:1	0.49 ± 0.14	0.56 ± 0.16	0.45 ± 0.14	0.66 ± 0.10
FA 18:2	0.48 ± 0.11	0.68 ± 0.14	0.68 ± 0.11	1.60 ± 0.26#+
FA 18:3	0.77 ± 0.21	0.64 ± 0.14	0.96 ± 0.25	1.02 ± 1.06
Cholesterol	1.20 ± 0.21	1.34 ± 0.42	0.91 ± 0.24	11.15 ± 0.25

The concentration of the fatty acids was 0.1mM, in 20 mM taurodeoxycholic acid.

The concentration of cholesterol was 0.05mM, in 20 mM taurodeoxycholic acid.

\+ $p<0.05$, fish oil versus beef tallow

\# $p<0.05$, high versus low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

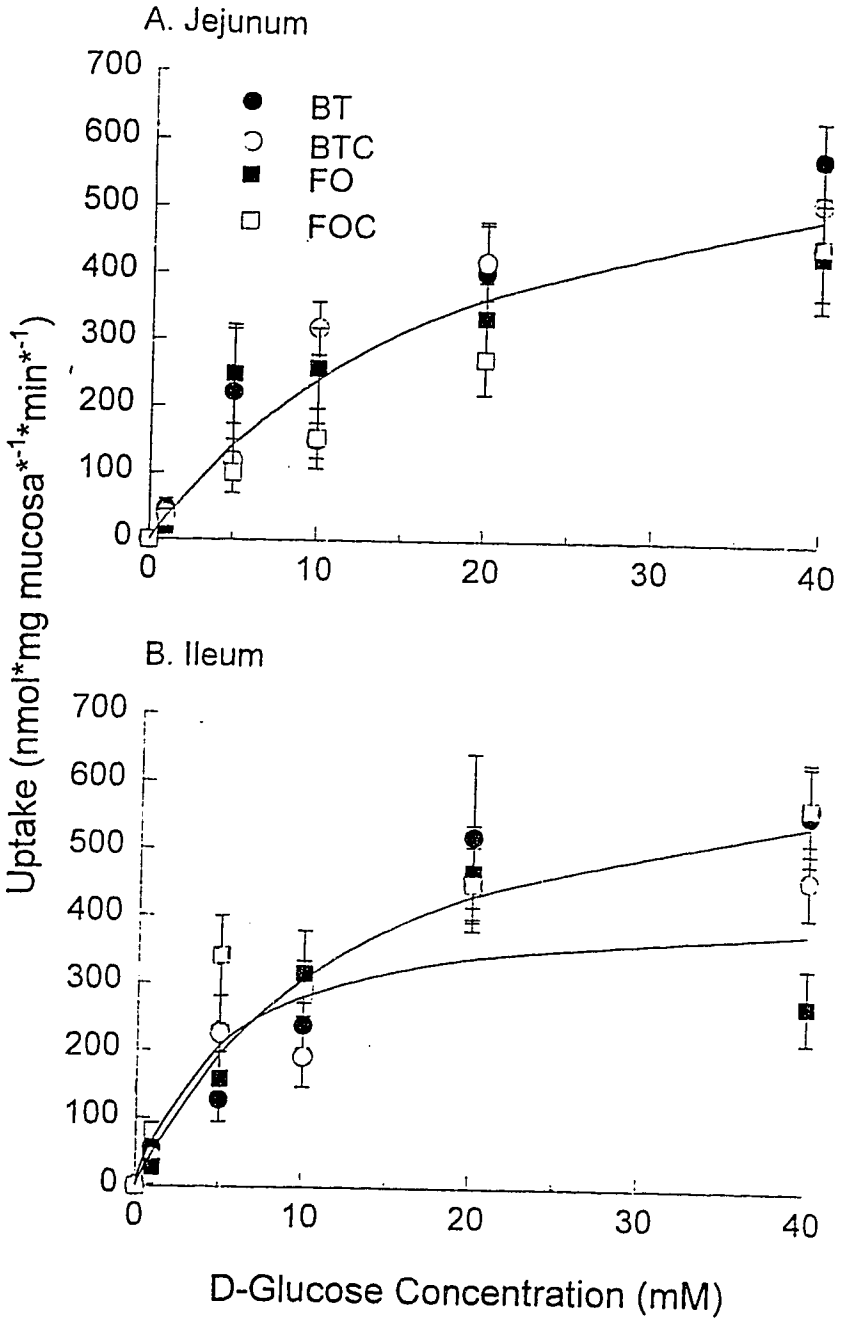

Figure 5.1 Effect of dietary ω3 fatty acids and cholesterol on D-glucose uptake into diabetic rat jejunum (**A**) and ileum (**B**). In panel A there were no significant differences between the mean values of the four diet groups. The values for the means for each group were superimposed on the values for the means of the four groups shown in this figure. For ileal uptake shown in panel B, glucose uptake was lower in rats fed fish oil than in those fed fish oil plus cholesterol or beef tallow with or without added cholesterol. The value for BT, BTC and FOC were combined and for clarity of presentation are shown as a single value for the upper line in this panel. The bulk phase was stirred at 600 rpm to reduce the effective resistance of the intestinal unstirred water layer.

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CHAPTER 6

OMEGA-3 FATTY ACIDS PARTIALLY CORRECT THE ENHANCED IN VIVO UPTAKE OF GLUCOSE IN DIABETIC RATS[5]

6.1 Introduction

Diabetes is associated with changes of the morphology and nutrient uptake into the small intestine (Anderson, 1974; Chang et al., 1986; Fedorak et al., 1987; Keelan et al., 1985; Miller et al., 1977; Schedl and Wilson, 1971; Thomson, 1980, 1981, 1983). *In vitro* studies using streptozotocin-induced diabetic rats have shown increased intestinal absorption of D-glucose and other hexoses, L-amino acids, dipeptides, cholesterol, fatty acids and fatty alcohols (Anderson, 1974; Olsen and Rosenberg, 1970; Schedl and Wilson, 1971; Thomson, 1980, 1981, 1983, 1987). These same results may be confirmed for hexose uptake *in vivo* (Hotke et al., 1985; Westergaard, 1989). Some of the mechanisms responsible for this increased absorption seen in diabetic rats include: a decrease in the effective resistance of the intestinal unstirred water layer (Hotke et al., 1985; Thomson, 1983); an increased maximal transport rate (V_{max}) of the glucose carrier (Fedorak et al., 1987; Hotke et al., 1985; Meddings and Westergaard, 1989; Olsen and Rosenberg, 1970; Thomson, 1981, 1983; Westergaard, 1989); an increase in the apparent passive permeability constant (Thomson, 1980, 1981, 1983); recruitment of additional glucose carriers (Fedorak et al., 1987, 1989; Thomson, 1983); an increase in the height of the villi, and an increase in the number of absorptive cells per villus (Miller et al., 1977; Schedl and Wilson, 1971; Thomson, 1980, 1981, 1983).

The fatty acid content of the diet has been found to alter in *in vitro* intestinal uptake of nutrients (Thomson et al., 1984, 1986). Isocaloric semisynthetic diets with high polyunsaturated/saturated fatty acid (P/S) ratios have been shown to reduce the enhanced *in vitro* uptake of glucose and fatty acids in diabetic rats as compared to animals fed a diet with a low P/S ratio (Thomson et al., 1987a, b, c). Interest in using the omega-3 marine ('fish') oils for the treatment of diabetes has been aroused by studies indicating its ability to lower elevated serum triglyceride and cholesterol levels (Harris et al., 1983, 1988a; Nestel, 1986; Phillipson et al., 1985; Schectman et al., 1989) and to retard the development of atherosclerosis (Holub, 1988; Leaf and Weber, 1988; Weiner et al., 1986). Feeding fish oils may also play a role in the synthesis or prostaglandins involved in the clotting process and is associated with reduced platelet aggregation (Bruckner et al., 1984; Zucker et al., 1988). However, there is considerable literature suggesting,

[5] A version of this chapter has been published in Diabetes Research 15:117-123, 1990.

in humans at any rate, that fish oil may worsen diabetic control, probably decreasing insulin secretion (Axelrod, 1989). The source of omega-3 (vegetable) or fish fatty acids also influences the *in vitro* intestinal uptake of hexoses, with the fish oils showing an antiabsorptive effect on jejunal D-glucose uptake (Thomson et al.,1988). Varying the source of omega-3 is also associated with altered mucosal mass of the intestine, but these morphological changes do not fully account for the variation in nutrient uptake.

The *in vivo* perfusion method is widely applied to the study of nutrient absorption (Harris et al., 1988b; Meddings and Westergaard, 1989; Schectman et al., 1989), but has not yet been applied to the study of the effect of variations in the dietary content of lipids. Accordingly, this study was undertaken to determine the effect of feeding isocaloric semisynthetic diets high in saturated fatty acids from beef tallow (BT) or high in polyunsaturated fatty acids from fish oil (FO), on the *in vivo* intestinal uptake of D-glucose in rats with streptozotocin diabetes.

6.2 Materials and Methods

6.2.a Chemicals

Streptozotocin, unlabelled D- and L-glucose were supplied by Sigma Chemical (St. Louis, MO). Unlabelled lauric acid (FA 12:0) was supplied by Applied Science Laboratories Inc. (State College, PA). Unlabelled glactose was obtained from Fisher Scientific Company (Fair Lawn, NJ). [^{14}C]-labelled D-glucose, lauric acid and galactose were supplied by ICN Pharmaceuticals Ltd. (Irvine, CA), along with methoxy [^{3}H] inulin. L-[1-^{14}C] glucose was supplied by Radiochemical Amersham (Oakville, ON). All labelled chemicals were supplied at greater than 99% purity, while unlabelled chemicals were reagent grade.

6.2.b Animals

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Female Wistar rats weighting 200-250 g were anaesthetized using a mixture of ketamine HCl:xylazine (2:1, providing 70 mg/kg and 7.5 mg/kg body weight, respectively), then divided into control and diabetic groups. Glucose intolerance was induced in the diabetic group by the intravenous injection of the pancreas β-cell cytotoxic agent streptozotocin (65 mg/kg body weight) into the lateral tail vein. The animals were then fed standard rat chow (Purina) *ad libitum* for 7 days. Glucose intolerance was confirmed 7 days post-injection by the presence of hyperglycemia (using the glucose oxidase method). Animals were accepted as being diabetic when their morning blood glucose exceeded 300 mg/dl.

Seven days after injection with streptozotocin, both groups were fed *ad libitum* for two weeks with either a beef tallow (BT) or fish oil (FO) isocaloric semisynthetic diet. Food intake was

monitored by weighing residual food in their feeding dish every two to three days to ensure adequate nutritional intake. Animals were weighed weekly. Blood glucose concentrations were also determined at the time of sacrifice.

On the day of the experiment, animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight) and the abdomen was opened with a midline incision. A jejunal loop of 20 cm was isolated approximately 5 cm dital to the ligament of Treitz, flushed clear with 37°C isotonic saline and cannulated. An ileal loop of 20 cm was isolated approximately 5 cm proximal to the ileocecal valve, flushed and cannulated. Both loops were returned to the abdominal cavity, ensuring that no mesenteric vasculature damage or unnecessary twisting occurred, and that the intestine remained warm and moist. The animals' core body temperature was maintained at 37°C by a heating lamp and pad. The cannulated loops were connected by 0.8 mm Tygon tubing to a SAGE peristaltic pump.

6.2.c Diets

Both beef tallow (BT) and fish oil (FO) diets were composed of 27% protein, 40.7% carbohydrate, 5% fibre, and 20% of the appropriate fat. The beef diet had a polyunsaturated:saturated (P/S) fatty acid ratio of 1:9.33, while the fish oil diet had a P/S ratio of 1:1.65, including 21.8% omega-3 fatty acids. Neither BT nor FO diets contained added cholesterol, and their cholesterol content was similar, 0.06%. These semisynthetic diets were isocaloric and nutritionally adequate, providing for all known essential nutrient requirements. The complete nutrient composition of the diets is been reported in Appendix I.

6.2.d Perfusion

Perfusion was begun in the proximal-to-distal direction at a rate of 1 ml/min. A 10 min equilibration period was allowed before the effluent was collected in a fraction collector at 5 min intervals for the remaining 35 min of the perfusion. Solutions tested were 1, 5, 10, 20, 40, 60, 80 or 100 mM D-glucose, 20 mM L-glucose (for correction of the passive component of glucose uptake), and 0.1 mM lauric acid (for calculation and correction of the effective resistance of the intestinal unstirred water layer (Westergaard et al., 1986). All solutions contained [^{3}H]-inulin as a nonabsorbable volume marker.

Once the perfusion was completed the animal was sacrificed by a lethal injection of sodium pentobarbital (700 mg/kg body weight). The jejunal and ileal loops were removed and rinsed with ice cold saline. The intestine was suspended vertically under a 10 g weight and the *ex vivo* length was obtained. One small segment of intestine was removed, cut open, and mucosal scrapings obtained to determine the dry weight of the scraped mucosa and the remainder of the intestinal wall ('submucosa'). Weights were determined after drying the tissue overnight at 55°C.

The perfusate probe content was measured busing Ready Solv HP/GAA scintillation cocktail (Beckman Instruments) and counted on a Beckman 5801 scintillation counter using

quench correction. The counting efficiencies and the spill-over ratio of [^{14}C] into [^{3}H] channels at variable quenching were used to calculate the disintegration per minute per milliliter of [^{3}H] and [^{14}C] in each sample. The uptake of nutrient was determined from the disappearance of radiolabelled solute from the perfusate.

6.2.e Calculations and Data Analysis

Due to the secretion of absorption of water by the small intestine, the actual effluent concentration (actual C_{out}) needed to be calculated, using Equation 1, which uses inulin as a nonabsorbable marker ($inulin_{in}$ and $inulin_{out}$):

$$\text{Actual } C_{out} = (\text{Apparent } C_{out} \times inulin_{in}) / (inulin_{out}) \qquad \text{Equation 1}$$

Since some of the probe was absorbed and not all of the perfused segment will be exposed to the same concentration of substrate infused (C_{in}), the lumen concentration was also corrected for absorption (Winne and Markgraf, 1979), using Equation 2:

$$\text{Mean } C_{in} = (C_{in} - \text{Actual } C_{out}) / (\log_{n} (C_{in} / \text{Actual } C_{out}) \qquad \text{Equation 2}$$

The values of actual C_{out} and mean C_{in} were used to calculate nutrient uptake and water secretion, as previously described (Westergaard, 1989; Westergaard et al., 1986). The rate of disappearance of the [^{14}C]-probe molecule from the perfusate solution was calculated from a plot of [^{14}C] disintegration per minute (dpm) per milliliter effluent per minute of perfusate. The slope of this line was obtained by linear regression analysis and used as the single value of uptake for that concentration of solute for that individual animal. Because the weight of the mucosa did not vary between control and diabetic rats and between those fed BT versus FO, the uptake results were expressed as $nmol*cm^{-1}*min^{-1}$.

Resistance of the intestinal unstirred water layer (R) was calculated from the rate of uptake of lauric acid (Westergaard et al., 1986). The value of R is solved using Equation 3:

$$R = C_1 D / J_d = d / Sw \qquad \text{Equation 3}$$

C_1 is the concentration of lauric acid (mM/L) in the bulk water phase, D is its free diffusion coefficient ($D=7.59.10^{-6}*cm^{-2}*sec^{*-1}$) (Sallee and Dietschy, 1973), J_d is the uptake observed in $nmol*cm^{-1}*min^{-1}$, d is the thickness of the unstirred water layer (UWL) and Sw is the surface area of the UWL.

The contribution of passive permeation to D-glucose uptake was determined from the rate of uptake of 1 mM L-glucose. The true passive permeability coefficient (Pd) was obtained by

correcting L-glucose uptake for the effective resistaance of the intestinal unstirred water layer. This was done by multiplying the passive permeability coefficient of glucose by the concentration of glucose, corrected for unstirred water layer resistance (C2) (Meddings and Westergaard, 1989).

Transport kinetic parameters V_{max} (maximal transport rate) and K_m^* (apparent Michaelis constant) were determined by using a commercially available statistical program, Systat (SYSTAT, Inc., Illinois), employing an iterative, nonlinear regression method on a microcomputer. As the variance increased with the size of the variable Y (rate of uptake), data points were weighted in proportion to the reciprocal of the within concentration estimates of variance. Nonlinear regression analysis was estimated for each treatment (Meddings and Westergaard, 1989). The interactive analysis employed sought to minimize the residual sum of the squares between the observed and predicted values. Convergence occurred within 20 interactions using the nonlinear regression modules of Systat. To determine whether two sets of transport data differed significantly, we first fit the sets separately, then combined and used the modified F statistic presented by Motulsky and Ransnas (1987) to determine whether the separate fits were better than the combined fit.

Data are shown as mean ± standard error of the mean. Perfusion studies were conducted on seven to eight animals in each group. Differences between groups were determined with the Duncan multiple range test using the SAS statistical program (SAS Institute, Cary, NC) (Khazanie, 1987).

6.3 Results

6.3.a Animal Characteristics

In the first week after induction of diabetes, food consumption was higher in diabetic than in non-diabetic control animals fed either BT or FO, but in the second week, food intake was only higher in diabetic than in control rats fed FO (Table 6.1). In week 2, control rats fed FO ate less than animals fed BT; despite this, their weight gain was higher. Weight gain by the diabetic rats fed BT was lower than control animals fed BT, despite the similar food intakes in week 2. Weight gain in diabetic rats fed FO was lower than in control animals fed FO, despite their higher food intake. In week 2, diabetic rats fed FO gained more weight than those fed BT, despite their similar food intake.

The blood glucose concentration in the diabetic animals was significantly higher than controls while they were initially fed chow after 2 weeks of diet therapy, but no differences were noted between diabetic rats fed BT or FO (Table 6.1).

The jejunal and ileal dry weights (mg/cm length of intestine) were greater in diabetic than in control rats fed either BT or FO, but the percentage of the intestinal wall comprised of mucosa was lower, resulting in a similar mass of mucosa in diabetic as compared with control rats fed BT or FO (Table 6.1).

6.3.b Effective Resistance of the Unstirred Water Layer and Passive Permeability Coefficient

The effective resistance of the intestinal unstirred water layer (UWL) was determined with a diffusion limited probe, lauric acid (Westergaard et al., 1986). The values reported here for the rate of uptake of lauric acid from jejunum perfused at 1 ml/min were close to the values reported in animals fed chow, in which the intestine was perfused at 1.5 ml/min (Westergaard et al., 1986). Jejunal and ileal uptake of lauric acid was higher and therefore the effective resistance of the intestinal unstirred water layer (UWL) was lower in diabetic rats fed FO than control animals fed FO (Table 6.2). UWL was similar in control and diabetic rats fed BT, and UWL was higher in control rats fed FO than BT.

The passive component of glucose uptake was estimated from 20 mM L-glucose. These values for the rate of uptake of L-glucose ($nmol*cm^{-1}*min^{-1}$) corrected for UWL and perfused through jejunum of rats fed FO were within 6% of the value for L-glucose uptake determined in animals fed chow and whose intestine was perfused at 1.5 ml/min (Westergaard et al., 1986).

The uptake into the jejunum and ileum of 20 mM L-glucose was higher ($p<0.05$) in the control rats fed FO than BT, and was higher in diabetic than in control animals only when they were fed BT. When the uptake of L-glucose was corrected for UWL effects, the true passive permeability coefficient (P_d) of the intestine was higher in diabetic than control rats fed BT, whereas the passive uptake of glucose was similar in diabetic and control rats fed FO (Table 6.2). Feeding FO to control rats increased jejunal and ileal P_d as compared with animals fed BT, whereas feeding FO to diabetic rats lowered jejunal P_d as compared with diabetic animals fed BT.

In control rats, feeding FO reduced the value of the maximal transport rate (V_{max}) and the Michaelis constant (K_m) for jejunal and for ileal uptake of D-glucose (Table 6.3). The value for the V_{max} for jejunal uptake of D-glucose in rats fed BT was within 5% of the value of the V_{max} obtained in rats fed chow (Westergaard, 1989). Jejunal and ileal V_{max} and K_m were lower in control than in diabetic rats fed FO. Ileal V_{max} was greater in diabetic than in control rats fed BT, yet the jejunal V_{max} and K_m for D-glucose uptake was numerically lower in diabetic than in control animals fed BT.

6.4 Discussion

The topic of fish oil and diabetes has been reviewed recently (Axelrod, 1989). *In vitro* studies have demonstrated reduced glucose uptake in animals fed fish oil (FO) as compared with those fed beef tallow (BT) (Thomson et al., 1988). This was confirmed *in vivo* with lower jejunal and ileal V_{max} of glucose in control rats fed FO as compared with BT (Table 6.3). The 'antiabsorptive' effect of FO was also due in part to a higher effective resistance of the intestinal unstirred water layer (Table 6.2). However, the lower V_{max} in control animals fed FO was partly compensated for by a greater contribution of passive permeation (Table 6.2) which would be of particular importance at higher glucose concentrations, and by a lower value of the K_m for uptake which would be of importance at lower luminal glucose concentrations. Thus, the effect of FO on glucose uptake in non-diabetic control rats is achieved by an influence on several of the kinetic components of glucose uptake, V_{max}, K_m, unstirred water layer resistance and passive permeation, with the net effect being one of reduced glucose absorption.

This interacting effect of diabetes and diet on glucose uptake was also complex, with lower effective resistance of the unstirred water layer in diabetic as compared with control rates fed FO, higher passive permeability (P_d) of glucose uptake in diabetic than in control rats fed BT yet lower values of P_d in those fed FO (Table 6.2), lower jejunal V_{max} in diabetic than in control rats fed BT yet higher values of ileal V_{max} in diabetic than in control rates fed either BT or FO, and higher values of jejunal V_{max} in diabetic than in control rats fed FO. Although feeding FO had numerous effects on these several parameters of glucose uptake, the net effect was also one of reduced glucose uptake, as comparing diabetic animals fed FO versus BT. However, it must be stressed that whereas diabetes was associated with a higher jejunal and ileal uptake of glucose, the V_{max} for jejunal uptake of glucose was actually lower in diabetic than in control rats fed BT (Table 6.3).

The question of "which diet is best?" depends upon the starting perspective: when starting with a control animal fed BT (with a V_{max} already greater than in rats fed FO), diabetes does not cause a further increase in V_{max}. Yet, feeding BT rather than FO to diabetic rats results in greater glucose uptake because of the enhanced passive permeation and greater V_{max}. When starting from the perspective of a control rat fed FO, diabetes was associated with increased jejunal and ileal V_{max} for glucose. The ideal perspective, however, is to control the enhanced glucose absorption in diabetic animals: under these circumstances FO was superior to BT, with a lower V_{max}, lower passive contribution to glucose uptake, and unchanged Michaelis constant (Tables 6.2 and 6.3).

The weight-versus-length ratios of intestinal tissue in diabetes (mg/cm) indicate that the diabetic animals have a higher mass per unit length in both the jejunum and ileum (Table 6.1), and

that diabetes therefore has a trophic effect on the gut. However, the percentage of the jejunal and ileal intestinal wall comprised of mucosa in the diabetic animals is lower than in the controls. Thus, the apparent functional absorptive tissue of the intestine is not increased in diabetes, and at least under the conditions of this study the alterations in intestinal mass probably does not account for the difference in uptake. However, this study did not specifically address the issue of the proportion of the mucosal mass comprised of transporting enterocytes: only a small portion of the enterocytes at the upper third of the villus are capable of transporting glucose, and the size of the enterocyte transport pool may be increased in diabetes (Fedorak et al., 1989). Thus, it is possible that the differences in V_{max} between diabetic and control animals were due to variations in the functional enterocyte pool not reflected by measurements of the total weight of the mucosa.

In the short-term two week feeding performed in this study, the blood glucose concentrations were similar in diabetic rats fed BT and FO (Table 2). However, with longer periods of feeding FO, there may be modest improvement in the diabetic control, as reflected by lowered levels of HgA_1C and the K value obtained from the intravenous glucose tolerance test (Rajotte et al., 1988). The role of fish oil diets in the treatment of humans with diabetes mellitus type II remains unproven (Glauber et al., 1988), and controversial for type I diabetes (Axelrod, 1989). Indeed, we were surprised that the mean blood glucose concentration in control rats was actually higher in those fed FO than BT (Table 6.1); the mechanism of this effect is unknown, but in diabetic humans fish oil may worsen diabetic control, probably decreasing insulin secretion (Axelrod, 1989). Thus, these rsults cannot be directly extrapolated to the human condition, but they do raise the interesting speculation that the dietary content of lipids may be varied to modify the intestinal response to diabetes mellitus.

Table 6.1. Effect of diabetes and dietary ω3 fatty acids on animal characteristics

	CONTROL		DIABETIC	
	BT	FO	BT	FO
FOOD CONSUMPTION (g/day)	18.4±0.4	14.9±0.3+	18.0±0.3	17.9±0.4*
WEIGHT GAIN (g/day)	0.9±0.1	1.5±0.2+	-0.2±0.2*	0.5±0.3*
TOTAL INTESTINAL WEIGHT (mg dry weight/cm length)				
Jejunum	12.0±0.6	12.0±0.2	15.1±0.4*	15.6±0.4*
Ileum	10.1±0.4	8.2±0.3+	13.3±0.5*	13.1±0.4*
PERCENTAGE OF INTESTINAL WALL COMPRISED OF MUCOSA				
Jejunum	40.3±1.3	41.9±1.6	34.9±2.4*	36.0±1.9*
Ileum	32.5±3.8	34.1±2.2	24.3±2.8*	21.5±1.2*
MUCOSAL MASS (mg dry weight/cm length)				
Jejunum	4.8±0.2	5.0±0.1	5.3±0.2	5.6±0.2
Ileum	3.3±0.1	2.8±0.1	3.2±0.1	2.8±0.1
FINAL BLOOD GLUCOSE (mg/dl)	65±11	134±17+	315±22*	356±19*

* $p<0.05$, diabetic versus control

\+ $p<0.05$, fish oil versus beef tallow

The diets were: BT, beef tallow; FO, fish oil

Table 6.2 Effect of diabetes and dietary ω3 fatty acids on the calculation of unstirred water layer resistance and passive permeation in rat intestine

CHARACTERISTIC	CONTROL		DIABETIC	
	BT	FO	BT	FO
LAURIC ACID UPTAKE ($nmol*cm^{*-1}*min^{-1}*mM^{-1}$)				
Jejunum	14±1	11±1+	12±2	16±1*
Ileum	12± 1	8±1+	11±1	13±1*
UNSTIRRED WATER LAYER RESISTANCE				
Jejunum	0.033±0.003	0.041±0.002	0.038±0.006	0.028±0.001*
Ileum	0.038±0.005	0.056±0.003+	0.041±0.007	0.035±0.002*
L-GLUCOSE UPTAKE ($nmol*cm^{*-1}*min^{-1}$)				
Jejunum	21.9±2.3	35.0±2.9+	46.4±6.3*	32.4±3.9+
Ileum	11.4±1.2	26.8±3.1+	26.3±3.3*	27.5±1.6
PASSIVE PERMEABILITY COEFFICIENT δ (corrected for unstirred layer resistance) ($nmol*cm^{-1}*min^{*-1}*mM^{-1}$)				
Jejunum	1.2±0.1	2.2±0.2+	3.2±0.6*	1.9±0.3+
Ileum	0.6±0.1	1.7±0.2+	1.6±0.3*	1.6±0.1

* $p<0.05$, diabetic versus control

\+ $p<0.05$, fish oil versus beef tallow

The diets were: BT, beef tallow; FO, fish oil

δ The apparent passive permeability coefficient for L-glucose was corrected for the effective resistance of the intestinal unstirred water layer, giving the true permeability coefficient.

Table 6.3 Effect of diabetes and dietary ω3 fatty acids on kinetic parameters of D-glucose uptake into rat small intestine

	CONTROL		DIABETIC	
	BT	FO	BT	FO
V_{max} (nmol*cm^{-1}*min^{*-1})				
Jejunum	196.8±1.4	29.3±0.1+	116.2±0.6*	87.4±0.1+
Ileum	20.5±0.2	6.4±0.1+	41.7±0.4*	23.9±0.1+
K_m (mM)				
Jejunum	25.2±0.3	1.2±0.1+	6.6±0.1*	5.3±0.1*
Ileum	1.3±0.1	0.1±0.1+	1.1±0.1	1.4±0.1*

* $p<0.05$, diabetic versus control

\+ $p<0.05$, fish oil versus beef tallow

The diets were: BT, beef tallow; FO, fish oil

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CHAPTER 7

ISOCALORIC MODIFICATION OF DIETARY LIPIDS INFLUENCES INTESTINAL BRUSH BORDER MEMBRANE COMPOSITION IN DIABETIC RATS[6]

7.1 Introduction

The dietary content of fatty acids influences intestinal transport and brush border membrane (BBM) composition. For example, feeding an isocaloric, semisynthetic, polyunsaturated fatty acid diet reduces the enhanced intestinal uptake of nutrients observed in rats with streptozotocin diabetes mellitus (Thomson et al., 1987a, b, 1988a). These same diets alter BBM phospholipids and fatty acyl constituents (Keelan et al., 1990a, b). The dietary content of cholesterol and the type of polyunsaturated fat in the diet also influence intestinal absorption of nutrients (Thomson et al., 1989). Furthermore, with the feeding of omega-3 (ω3) fatty acids there is generally a diminution in the intestinal uptake of actively and passively transported nutrients (Thomson et al., 1988b). The present study was undertaken to determine the influence of variations in the type of dietary lipid on BBM enzyme activity and lipid composition. The diets studied included: a saturated fatty acid diet containing beef tallow versus a polyunsaturated fatty acid diet containing 'fish oil' with and without cholesterol supplementation (beef tallow, low cholesterol [BT]; beef tallow, high cholesterol [BTC]; fish oil, low cholesterol [FO]; and fish oil, high cholesterol [FOC]). The results show that feeding an ω3 polyunsaturated fatty acid diet (fish oil) reduced total phospholipid content in BBM of control and diabetic rats, primarily due to a reduction in sphingomyelin; and that feeding an ω3 polyunsaturated fatty acid diet or cholesterol supplementation alters the activity of BBM enzymes.

7.2 Methods and Materials

7.2.a Animals and Diets

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Female Wistar rats weighing 200-250 g were split into two groups. The first group was rendered hyperglycemic by intravenous injection of the pancreas β-cell cytotoxic agent streptozotocin (65 mg/kg body weight). The second group was injected with saline and served as non-diabetic controls. Both groups were fed a standard rat chow diet for two weeks to allow stabilization of the

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diabetes. Then the control and diabetic groups were further subdivided into four groups of 20 rats each, and fed one of four semi-synthetic diets for two weeks: beef tallow with low cholesterol (BT), beef tallow with high cholesterol (BTC), fish oil with low cholesterol (FO) and fish oil with high cholesterol (FOC). The semisynthetic diets were nutritionally adequate, providing for all known essential nutrient requirements. The complete composition of the diets is given in Appendix I.

Food intake was monitored every two to three days. Animals were weighed weekly. Blood glucose concentrations were determined at one week post-injection and at the time of sacrifice. Animals were allowed *ad libitum* access to food and water until the time of sacrifice.

7.2.b Brush Border Membrane Preparation and Marker Enzyme Analysis

Animals were sacrificed by intraperitoneal injection of Euthanyl® (sodium pentobarbial, 450 mg/kg). Two 40 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with ice cold saline. The intestine was then opened along its mesenteric border and the mucosal surface carefully washed with cold saline to remove mucus and debris. The mucosal surface was blotted with lint-free tissue to remove excess moisture. The mucosal surface was removed by gently scraping with a microscope slide and then snap frozen in liquid nitrogen and stored at -80°C for later BBM preparation.

Intestinal brush border membranes (BBM) were prepared from mucosal scrapings by homogenization, $CaCl_2$ precipitation, differential centrifugation and density gradient centrifugation. The details of this methodology have been published (Yakymyshyn et al., 1982; Keelan et al., 1985). Mucosal scrapings were homogenized in 50 mM mannitol-2mM tris buffer, pH 7.1, using a Polytron® at setting "8" for 15 s. The homogenate was filtered through gauze, a metal sieve and a 40 μM Millipore filter to remove mucus and debris. A 1 M $CaCl_2$ solution was added to the homogenate to obtain a final concentration of 10 mM $CaCl_2$, and stirred gently on ice for 10 min to precipitate the subcellular components. The homogenate was centrifuged at 3000 x g for 15 min at 4°C to pellet the subcellular components; the pellet was discarded. The supernatant was centrifuged at 43700 x g for 20 min at 4°C. The resulting pellet was resuspended in 50 mM mannitol-2mM tris-5mM EGTA buffer, ph 7.1, and centrifuged again at 43700 x g at 4°C. The pellet was resuspended in in buffer and sonicated before layering onto 25 ml of 40% Percoll and centrifuged at 43700 x g for 20 min at 4°C. The upper fraction (1.019-1.055 g/ml) contained the BBM. Contaminating material remained in the lower fraction. Percoll was removed from the BBM by centrifuging at 115000 x g for 45 min at 4°C. The final BBM preparation was resuspended in the supernatant and aliquotted for immediate lipid extraction. Additional aliquots were taken for protein and marker enzymes.

Protein was determined according to the Hartree (1972) modification of the method of Lowry et al. (1951). The BBM markers that were assayed included sucrase (Dahlqvist, 1964) and alkaline phosphatase (Bowers et al., 1967) and were enriched 11 to 17 fold over homogenates. In

addition, Na^+K^+-ATPase (Scharschmidt et al., 1979), glucose-6-phosphatase (Aronson and Touster, 1974), β-glucuronidase (Glaser and Sly, 1973) and DNA (Burton, 1956; Giles and Myers, 1965) were assayed as markers of basolateral membranes, microsomes, lysosomal membranes, and nuclear material, respectively. The activities of these various enzymes are linear with respect to the amount of membrane protein added to the assay system. All assays were performed at substrate concentrations that yield maximal activity rate values.

7.2.c. Lipid Extraction and Analysis

Lipid extraction of the isolated membranes were carried out immediately after preparation with the sequential addition of methanol and chloroform (1:2, v/v) containing 0.005% (w/v) butylated hydroxytoluene, followed by acidification of the water wash using 0.01 M sulfuric acid according to a modification of the method of Bowyer and King (1977) and of Folch et al., (1957). Aliquots of the chloroform layer dried down under nitrogen at 60°C and stored at -80°C until the time of analysis.

The determination of free and esterified cholesterol was done on dried lipid extract reconstituted in isopropanol (Allain et al., 1974, Morin, 1976). Cholesteryl esterase breaks down any cholesteryl esters that may be present to free cholesterol. Cholesterol oxidase converts free cholesterol to cholest-4-en-3-one, which then is able to react with the 4-aminophenazone and produce a colour that can be measured at 500 nm.

Total phospholipid is determined on dried lipid exracts which have been digested with 2.5 M sulfuric acid and 30% peroxide (Sunderman and Sunderman, 1960). The liberated phosphorus is quantitated by the classic molybdenum reaction. Determinations of phospholipid composition were achieved by first separating the phospholipids by HPTLC in a solvent sytem containing chloroform: methanol: isopropanol: triethylamine: 0.25% KCl (15: 4.5: 12.5: 6.5: 3.5). (Vitiello and Zanetta, 1978; Touchstone et al., 1979). When the plates air-dry, they were immersed in 3% cupric aceteate in 8% phosphoric acid and charred at -80°C. Semiquantitative determination of phospholipid densitometry was obtained by scanning densitometry. Quantitative determinations of phospholipid were performed using the determination of phosphorus as described by Sunderman and Sundernman (1960).

7.2.d Data Analysis

All data are expressed as mean±standard error of the mean. Each of the eight groups contained 20 rats and each sample represented material pooled from two animals, so that each group contained 10 samples. The results were statisticcally tested by analysis of variance and by the Student Neuman Keul multiple range test.

7.3 Results

7.3.a *Animal Characteristics*

For the first two weeks after injection of streptozotocin (diabetes) or saline (control) the animals were on a chow diet. During this period the diabetic animals consumed more food but gained less weight than did their non-diabetic controls (Table 7.1). When the control and diabetic animals were switched to semisynthetic diets, the diabetic rats continued to consume more food and gain less weight. Food intake was similar among control animals, while weight gain was significantly increased in the control rats fed fish oil (FO versus BT, FOC versus BTC). Control animals fed FO gained the most weight, significantly more than FOC, BT and BTC.

Food intake was similar among diabetic animals, yet weight gain was quite different: the animals fed fish oil gained weight while those fed beef tallow lost weight. Diabetic animals fed FOC gained the most weight, as compared with the diabetic rats for BT, BTC or FO.

Blood glucose levels were elevated in diabetic animals as compared with control animals, regardless of diet. Following two weeks of feeding semisynthetic diets, blood glucose levels in the diabetic rats were less elevated than observed one week post-injection, but there were no differences between the four diet groups in the diabetic rats.

7.3.b *BBM Enzymes*

There was reduced jejunal BBM sucrase activity and an increased ratio of alkaline phosphatase/sucrase in control animals fed fish oil (FOC), as compared with those fed beef tallow (BTC) (Table 7.2). Cholesterol supplementation was associated with increased BBM sucrase activity when fed with beef tallow (BTC versus BT), but was associated with reduced sucrase activity and an increased ratio of alkaline phosphatase/sucrase when fed with fish oil (FOC versus FO).

In diabetic rats, supplementing the fish oil diet with cholesterol (FOC versus FO) was also associated with an increased jejunal BBM ratio of alkaline phosphatase/sucrase, due to an elevation of the BBM alkaline phosphatase activity. In animals fed the high cholesterol diet, fish oil feeding was also associated with an increased alkaline phosphatase and ratio of alkaline phosphatase/sucrase (FOC versus BTC). Diabetes interacted with these dietary effects so that there was higher BBM sucrase in diabetic as compared with control rats fed BT, FO and FOC. There was increased BBM alkaline phosphatase activity in diabetic as compared with control animals fed FOC but not with the other diet groups.

In the ileum of control animals, alkaline phosphatase activity was lower in animals fed FO as compared with BT (Table 7.3). When the fish oil diet was supplemented with cholesterol (FOC versus FO), there was an increased ratio of alkaline phosphatase/sucrase due to an elevation in the alkaline phosphatase activity levels in control and diabetic animals. In the ileal BBM of

diabetic rats fed FOC as compared with BTC, there was an increased ratio of alkaline phosphatase/sucrase. This increased ratio of alkaline phosphatase/sucrase was due to an enhanced activity of BBM alkaline phosphatase and reduced activity of sucrase. The reduced ileal BBM alkaline phosphatase/sucrase ratio in diabetic compared with control animals fed beef tallow was due to increased activity of sucrase. In animals fed FO both sucrase and alkaline phosphatase were increased in diabetic as compared with control animals, so that the ratio of alkaline phosphatase/sucrase was unchanged. The ileal BBM sucrase and alkaline phosphatase was higher in diabetic as compared with control animals fed FOC (although the increase in the ileal BBM alkaline phosphatase level failed to achieve statistical significance), and therefore the ratio of alkaline phosphatase/sucrase remained unchanged.

7.3.c BBM Lipids

Because of the decrease in total phospholipids, the jejunal BBM ratio of phospholipid/cholesterol was lower in control animals fed fish oil than in those fed beef tallow, even in the presence of cholesterol supplementation (FO versus BT and FOC versus BTC, Table 7.4). The jejunal BBM content of total phospholipid was higher ($p<0.05$) in diabetic than in control rats fed BT, BTC and FOC. Thus, in the presence of a low cholesterol diet, feeding fish oil prevented the increase in BBM phospholipids observed in diabetic rats. However, the magnitude of the changes was such that the jejunal BBM ratio of phospholipid/cholesterol was greater in diabetic than in control rats fed BTC, FO and FOC. Fish oil feeding and cholesterol supplementation had no effect on jejunal lipid composition of diabetic animals.

In the ileum of control and diabetic rats, there was lower BBM total phospholipid in animals fed fish oil as compared with beef tallow, resulting in a lower ratio of phospholipid/cholesterol (FO versus BT, and FOC versus BTC) (Table 7.5). Cholesterol supplementation had no influence on the lipid composition of control and diabetic rats fed either beef tallow or fish oil (FOC versus FO and BTC versus BT).

Diet did not alter the BBM phospholipid composition in the jejunum of control animals (Table 7.6). Similarly, in diabetic animals there were no differences in the jejunal BBM phospholipids due to diet, except for a decrease in sphingomyelin in animals fed fish oil (FO versus BT, FOC versus BTC). However, in animals fed BT, diabetes was associated with an increased level of sphingomyelin (nmol/mg protein), while in animals fed BTC diabetes was associated with an increase in phosphatidylethanolamine. Diabetes did not influence the phospholipid composition of animals fed FO. When animals were fed FOC, diabetes was associated with an increase in phosphatidylethanolamine. Cholesterol supplementation did not affect the phospholipid composition of diabetic rat jejunum.

In the ileal BBM of control animals, diet did not alter phospholipid composition except for a reduction in sphingomyelin observed with fish oil (FOC versus BTC) (Table 7.7). In the diabetic

rats, fish oil feeding was associated with a reduction in sphingomyelin, regardless of the amount of cholesterol in the diet (FO versus BT, FOC versus BTC). Cholesterol supplementation did not influence the phospholipid composition in the ileal BBM of control or diabetic rats. In animals fed beef tallow, diabetes was associated with an increase in phosphatidylethanolamine, a concomitant reduction in sphingomyelin, and a lower ratio of phosphatidylcholine/phosphatidylethanolamine. When rats were fed FO, diabetes was associated with a higher phosphatidylethanolamine, while in animals fed FOC, diabetes was associated with a higher phosphatidylcholine.

7.4 Discussion

Feeding fish oil, dietary cholesterol supplementation and streptozotocin diabetes each had a distinct effect on the intestinal brush border membrane (BBM) enzyme activity and lipid composition. Variations in the cholesterol content of the diet influence intestinal transport (Thomson et al., 1989), and short-term *in vitro* enrichment of BBM vesicles with cholesterol reduced the activity of alkaline phosphatase (Brasitus et al., 1988). However, feeding a high cholesterol diet for a week does not necessarily result in an increased BBM cholesterol level (Keelan et al., 1987). Furthermore, the major source of the dietary lipid is an important determinant of the influence of dietary cholesterol: feeding a high cholesterol diet increased jejunal BBM sucrase activity in control animals fed BTC as compared with BT and increased ileal BBM alkaline phosphatase activity in those fed FOC versus FO. In diabetic rats, jejunal BBM alkaline phosphatase was higher in FOC than in FO. Thus, feeding a high cholesterol diet, rather than enriching BBM cholesterol levels as seen with short-term incubation of BBM vesicles, does not result in a reduction in the activity of alkaline phosphatase. Finally, feeding a high cholesterol diet, whether with beef tallow or fish oil, did not result in alterations in jejunal or ileal BBM total phospholipid, cholesterol, sphingomyelin or phosphatidylethanolamine. Therefore, the results of short-term *in vitro* lipid enrichment studies do not necessarily translate into the same effects achieved by longer term dietary manipulations.

It is of interest that most of the cholesterol-associated changes in intestinal BBM enzyme activity occur in control or diabetic animals fed fish oil. For example, the increased alkaline phosphatase/sucrase ratios in jejunal and ileal BBM were observed in control and in diabetic animals fed FOC as compared with FO, and not in animals fed BTC versus BT. Thus, the limited changes observed as a result of cholesterol feeding were dependent upon the presence of the polyunsaturated fatty acid in the diet. It is speculated that the lack of similar enzyme changes in animals fed beef tallow may have been the result of the lack of further responsiveness of the BBM enzyme activity in animals fed a saturated fatty acid diet.

The fish oil diet altered the BBM enzyme activities and lipid composition. For example, feeding fish oil reduced jejunal BBM total phospholipid in control animals (FOC versus BTC) and also reduced ileal BBM total phospholipids in control and in diabetic rats. Feeding fish oil also resulted in an increased ratio of alkaline phosphatase/sucrase activity in jejunal BBM of control and diabetic animals (FOC versus BTC). These fish oil-related changes were not dependent upon the presence of cholesterol, since ileal BBM total phospholipids were reduced in both control and diabetic animals fed FO as compared with BT. Thus, feeding fish oil reduces BBM total phospholipids and/or the ratio of phospholipid/cholesterol.

In this study we did not measure the enzymes involved in the synthesis of phospholipids. Phosphatidylcholine may be synthesized *de novo* via the CDP-choline pathway. This pathway may be monitored by assessing the activity of cholinephosphotransferase or the activity of the rate-limiting enzyme CTP-choline cytidylyltransferase. Phosphatidylcholine may also be formed from phosphatidylethanolamine by way of phosphatidylethanolamine methyltransferase (the PEMT pathway). It is of note that the reduced ileal BBM phosphatidylethanolamine, associated with a reduced total phospholipid level in animals fed FOC, raises the possibility that feeding fish oil may influence the activity of the PEMT pathway. Further evidence supporting the suggestion of an alteration in the PEMT activity as a result of these dietary manipulations is derived from the observation of increased phosphatidylethanolamine in jejunal BBM of diabetic rats fed BTC and FOC as well as in ileal BBM of diabetic rats fed BTC and FO. These speculations await direct experimental scrutiny (See Chapter 8).

In addition to the membrane changes resulting from feeding cholesterol or fish oil, there were distinct differences observed in diabetic animals. Diabetes was associated with increased jejunal BBM sucrase activity, as compared with non-diabetic controls, in animals fed BT and FO; similar changes were seen for the ileum. Diabetes was also associated with alterations in the BBM total phospholipid or the ratio of phospholipid/ cholesterol, as compared with control animals: for example, there was increased total phospholipids, phospholipid/cholesterol and phosphatidylethanolamine in diabetic as compared with control animals fed BTC. Thus, the influence of dietary cholesterol and polyunsaturated fatty acids is different in diabetic as compared with non-diabetic control animals. The enzymatic basis for this differential effect remains to be established.

It has recently been reported that these same diets were also associated with alterations in intestinal transport function (Thomson et al., 1989). In general, feeding a beef tallow diet increases glucose and lipid uptake, whereas feeding a fish oil diet has an antiabsorptive effect in both control and diabetic animals. Clearly, however, the interaction between intestinal transport and lipid composition is not explained simply by alterations in the BBM phospholipids or cholesterol. Variations in the dietary content of fatty acid also influence the fatty acyl composition

of the BBM phospholipids, as well as the activity of enterocyte microsomal desaturases (See Chapter 9). Until the effect of these diets on the phospholipid, cholesterol and fatty acid metabolizing enzymes in the enterocyte are elucidated, and until the BBM and basolateral membrane fatty acyl constituents have been described, the results observed in the present study must be considered to be only descriptive. However, this data does provide the first step towards our searching for a possible mechanism of the effect of dietary manipulations on intestinal transport. The alterations in BBM enzyme activities suggest the possibility of changes in the maturity of the enterocytes lining the villus, and the alterations in the BBM phospholipids are suggestive of the possible change in the activity of those enterocyte microsomal and brush border membrane enzymes responsible for phospholipid metabolism.

Table 7.1 Effect of dietary ω3 fatty acids and cholesterol on animal characteristics

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
BLOOD GLUCOSE, mg/dL								
Chow					399 ± 15*	431 ± 16*	442 ± 15*	462 ± 14*
Diet	144 ± 4	151 ± 5	164 ± 10	158 ± 11	288 ± 35*^	238 ± 34*^	271 ± 30*^	291 ± 34*^
WEIGHT GAIN, g/day								
Chow	0.81 ± 0.10	0.80 ± 0.08	0.70 ± 0.11	0.74 ± 0.12	-0.02 ± 0.26*	0.05 ± 0.18*	0.00 ± 0.20*	0.02 ± 0.23*
Diet	1.23 ± 0.15^	1.01 ± 0.10	2.64 ± 0.12+^	1.81 ± 0.25#+^	-0.65 ± 0.33*	-0.85 ± 0.45*	0.84 ± 0.39+*	1.68 ± 0.28+^
FOOD CONSUMPTION, g/day								
Chow	14.6 ± 1.1	14.9 ± 0.7	16.1 ± 0.3	16.4 ± 1.0	17.8 ± 1.0*	17.8 ± 1.2	18.2 ± 0.9*	20.3 ± 1.5
Diet	16.4 ± 0.3	15.4 ± 0.3	16.8 ± 0.3	15.7 ± 0.4	18.0 ± 0.8	18.8 ± 0.8*	19.1 ± 0.8*	20.3 ± 0.9*

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
* $p < 0.05$, diabetic versus control
^ $p < 0.05$, diet versus chow

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.2 Effect of dietary ω3 fatty acids and cholesterol on jejunal brush border membrane markers in control and diabetic rats

MARKER	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
MUCOSAL SCRAPINGS mg/cm length	36 ± 3	37 ± 5	33 ± 1	35 ± 1	35 ± 3	41 ± 5	36 ± 3	40 ± 2
PROTEIN mg/g wet weight	1.05 ± 0.10	0.95 ± 0.05	0.68 ± 0.13+	0.73 ± 0.11	0.93 ± 0.11	1.04 ± 0.08	0.73 ± 0.07	1.08 ± 0.39
ALKALINE PHOSPHATASE U/g protein	2631 ± 294	2807 ± 416	2982 ± 528	3218 ± 414	2703 ± 273	3487 ± 497	3670 ± 589	5316 ± 300+#*
SUCRASE U/g protein	952 ± 40	1084 ± 47#	936 ± 49	632 ± 57+#	1084 ± 45*	1149 ± 83	1148 ± 78*	1119 ± 33
ALKALINE PHOSPHATASE/ SUCRASE	2.68 ± 0.29	2.67 ± 0.44	3.15 ± 0.47	4.65 ± 0.52+#	2.52 ± 0.26	3.26 ± 0.58	3.14 ± 0.40	4.84 ± 0.40+#

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol.
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.3 Effect of dietary ω3 fatty acids and cholesterol on ileal brush border membrane markers in control and diabetic rats

MARKER	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
MUCOSAL SCRAPINGS mg/cm length	36 ± 3	35 ± 2	23 ± 2+	25 ± 2+	34 ± 2	38 ± 2	27 ± 2+	32 ± 2+*
PROTEIN mg/g wet weight	0.68 ± 0.05	0.75 ± 0.05	0.56 ± 0.08	0.68 ± 0.12	0.81 ± 0.09	0.88 ± 0.08	0.87 ± 0.08*	0.84 ± 0.08
ALKALINE PHOSPHATASE U/g protein	272 ± 26	302 ± 44	168 ± 27+	391 ± 84#	302 ± 36	257 ± 51	319 ± 60*	561 ± 129+
SUCRASE U/g protein	324 ± 46	282 ± 24	248 ± 42	233 ± 32	702 ± 79*	559 ± 78*	500 ± 59*	362 ± 45+*
ALKALINE PHOSPHATASE/ SUCRASE	1.02 ± 0.15	1.10 ± 0.16	0.73 ± 0.16	1.63 ± 0.26#	0.55 ± 0.12*	0.56 ± 0.13*	0.62 ± 0.08	1.68 ± 0.45+#

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.4 Effect of dietary ω3 fatty acids and cholesterol on jejunal brush border membrane lipid composition in control and diabetic rats

LIPID	CONTROL				DIABETIC			
(nmol/mg protein)	BT	BTC	FO	FOC	BT	BTC	FO	FOC
TOTAL PHOSPHOLIPIDS	345 ± 20	350 ± 20	296 ± 26	275 ± 20+	450 ± 34*	459 ± 32*	348 ± 18	389 ± 47*
CHOLESTEROL								
TOTAL	304 ± 15	321 ± 18	320 ± 25	329 ± 11	346 ± 27	329 ± 25	305 ± 20	323 ± 21
FREE	280 ± 15	304 ± 15	302 ± 26	306 ± 11	336 ± 25	316 ± 23	288 ± 17	298 ± 19
ESTERS	12 ± 5	11± 4	17 ± 6	16 ± 7	10 ± 5	12 ± 5	17 ± 5	25 ± 10
PHOSPHOLIPID/ CHOLESTEROL	1.2 ± 0.1	1.1 ± 0.1	0.9 ± 0.1+	0.8 ± 0.1+	1.3 ± 0.1	1.4 ± 0.1*	1.2 ± 0.1*	1.2 ± 0.1*

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.5 Effect of dietary ω3 fatty acids and cholesterol on ileal brush border membrane lipid composition in control and diabetic rats

LIPID	CONTROL				DIABETIC			
(nmol/mg protein)	BT	BTC	FO	FOC	BT	BTC	FO	FOC
TOTAL PHOSPHOLIPIDS	268 ± 20	313 ± 19	186 ± 20+	224 ± 32+	389 ± 40*	376 ± 30	266 ± 20+*	234 ± 22+
CHOLESTEROL								
TOTAL	329 ± 27	410 ± 32	368 ± 38	428 ± 17	400 ± 27	348 ± 28	357 ± 8	404 ± 20
FREE	312 ± 27	390 ± 31	342 ± 35	400 ± 19	377 ± 27	335 ± 26	334 ± 11	356 ± 19
ESTERS	8 ± 4	5 ± 3	23 ± 7	29 ± 8+	14 ± 6	13 ± 5	23 ± 6	29 ± 7
PHOSPHOLIPID/ CHOLESTEROL	0.9 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	0.6 ± 0.1+

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.6 Effect of dietary ω3 fatty acids and cholesterol on jejunal phospholipid composition in control and diabetic rats

PHOSPHOLIPID		CONTROL				DIABETIC			
		BT	BTC	FO	FOC	BT	BTC	FO	FOC
SPHINGOMYELIN	%	19.3 ± 2.9	23.6 ± 3.2	18.2 ± 2.8	18.5 ± 2.2	23.3 ± 8.8	20.4 ± 1.6	14.5 ± 2.6	15.0 ± 1.5
	nmol/mg protein	65 ± 10	86 ± 16	50 ± 10	51 ± 7	111 ± 16*	92 ± 32	52 ± 11+	59 ± 15
PHOSPHATIDYLCHOLINE (PC)	%	43.2 ± 2.4	44.8 ± 3.7	42.3 ± 2.2	46.3 ± 2.4	41.3 ± 3.1	42.0 ± 3.5	42.9 ± 2.6	44.5 ± 2.2
	nmol/mg protein	148 ± 14	154 ± 10	129 ± 14	129 ± 12	167 ± 14	205 ± 27	149 ± 9	172 ± 22
PHOSPHATIDYLSERINE	%	0.08 ± 0.4	1.1 ± 0.6	2.1 ± 0.8	1.3 ± 0.7	0.6 ± 0.3	0.4 ± 0.2	1.6 ± 0.6	1.6 ± 1.0
	nmol/mg protein	2.0 ± 1	4 ± 2	5 ± 2	3 ± 2	2 ± 1	2 ± 1	6.0 ± 2.0	3.0 ± 2.0
PHOSPHATIDYL-ETHANOLAMINE (PE)	%	31.6 ± 1.1	24.0 ± 2.8	30.0 ± 1.6	26.3 ± 2.3	27.3 ± 2.0	28.2 ± 3.6	32.4 ± 2.6	30.8 ± 1.4
	nmol/mg protein	109 ± 7	90 ± 13	82 ± 10	72 ± 9	123 ± 14	142 ± 18*	112 ± 12	108 ± 13*
PHOSPHATIDIC ACID	%	1.4 ± 0.6	1.4 ± 0.6	2.2 ± 0.8	2.6 ± 1.1	0.5 ± 0.2	1.0 ± 0.5	0.7 ± 0.3	2.0 ± 0.6
	nmol/mg protein	5 ± 2	4 ± 2	8 ± 3	7 ± 3	2 ± 1	5 ± 2	2 ± 1	4 ± 1
PHOSPHATIDYLINOSITOL	%	3.2 ± 0.8	4.8 ± 1.9	2.8 ± 1.0	2.0 ± 0.6	2.9 ± 0.7	2.6 ± 0.6	4.0 ± 0.7	3.0 ± 0.7
	nmol/mg protein	12 ± 3	12 ± 6	9 ± 4	5 ± 2	12 ± 3	12 ± 3	14 ± 3	10 ± 4
PC/PE		1.4 ± 0.1	1.9 ± 0.4	1.4 ± 0.1	1.9 ± 0.2	1.7 ± 0.3	1.6 ± 0.2	1.4 ± 0.2	1.5 ± 0.1

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
ND, not detected

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 7.7 Effect of dietary ω3 fatty acids and cholesterol on jejunal phospholipid composition in control and diabetic rats

PHOSPHOLIPID		CONTROL				DIABETIC			
		BT	BTC	FO	FOC	BT	BTC	FO	FOC
SPHINGOMYELIN	%	34.0 ± 4.0	36.8 ± 1.7	28.6 ± 5.4	22.7 ± 2.8+	28.8 ± 1.7	27.3 ± 3.2*	22.5 ± 3.5	18.8 ± 1.7
	nmol/mg protein	95 ± 16	114 ± 8	81 ± 30	41 ± 12+	125 ± 18	119 ± 18	61 ± 13+	46 ± 5+
PHOSPHATIDYLCHOLINE (PC)	%	36.3 ± 2.7	34.2 ± 1.7	29.4 ± 2.4	35.0 ± 5.2	32.1 ± 2.9	37.6 ± 2.5	37.4 ± 4.8	38.1 ± 2.7
	nmol/mg protein	95 ± 6	109 ± 9	123 ± 59	61 ± 13	145 ± 29	157 ± 28	97 ± 10	98 ± 9*
PHOSPHATIDYLSERINE	%	0.6 ± 0.3	2.6 ± 1.7	2.4 ± 2.4	3.2 ± 0.9	1.8 ± 0.6	0.9 ± 0.4	2.3 ± 1.3	2.8 ± 1.0
	nmol/mg protein	2 ± 1	10 ± 7	4 ± 4	5 ± 2	6 ± 2	3 ± 1	7 ± 4	7 ± 2
PHOSPHATIDYL-ETHANOLAMINE (PE)	%	17.8 ± 2.8	17.2 ± 2.3	26.2 ± 5.0	21.4 ± 1.6	26.2 ± 2.0*	24.5 ± 2.7	27.3 ± 2.8	30.1 ± 3.0
	nmol/mg protein	46 ± 6	51 ± 8	35 ± 4	50 ± 10	115 ± 17*	78 ± 7*	68 ± 10*	76 ± 6
PHOSPHATIDIC ACID	%	4.9 ± 1.9	3.4 ± 1.5	2.8 ± 0.9	7.0 ± 1.8	3.0 ± 1.1	3.7 ± 1.6	3.5 ± 1.3	7.2 ± 2.1
	nmol/mg protein	13 ± 5	10 ± 4	7 ± 4	11 ± 3	9 ± 3	17 ± 8	17 ± 8	14 ± 5
PHOSPHATIDYLINOSITOL	%	2.5 ± 1.8	1.9 ± 0.4	3.4 ± 2.2	3.2 ± 1.5	1.7 ± 0.8	2.0 ± 0.8	1.5 ± 0.6	1.7 ± 0.6
	nmol/mg protein	9 ± 7	5 ± 1	12 ± 11	5 ± 2	7 ± 3	11 ± 6	5 ± 2	4 ± 1
PC/PE		2.4 ± 0.4	2.4 ± 0.4	1.1 ± 0.1	1.7 ± 0.3	1.3 ± 0.1*	1.6 ± 0.2	1.3 ± 0.2	1.4 ± 0.2

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high versus low cholesterol
ND, not detected

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

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CHAPTER 8

DIETARY OMEGA-3 FATTY ACIDS AND CHOLESTEROL MODIFY ENTEROCYTE MICROSOMAL MEMBRANE PHOSPHOLIPID AND CHOLESTEROL CONTENT AND PHOSPHOLIPID ENZYME ACTIVITIES IN DIABETIC RATS[7]

8.1 Introduction

The absorption of nutrients is increased in streptozotocin diabetes in the rat (Thomson, 1980, 1981, 1983, 1988), and this altered function is associated with changes in the phospholipid composition of the intestinal brush border membrane (BBM) (Keelan et al., 1985, 1987). The presence of cholesterol or the type of fatty acid in the triglycerides in the diet (saturated beef tallow or polyunsaturated omega-3 fatty acids in fish oil) influences the intestinal uptake of D-glucose and lipids (Brasitus et al., 1989; Thomson et al., 1987a), and also modifies the BBM content of phospholipids and cholesterol (Brasitus et al., 1989; Keelan et al., 1991, see Chapter 7). The ratio of BBM phosphatidylcholine:phosphatidylethanolamine (PC:PE) may be influenced by the BBM activity of phosphatidylethanolamine methyltransferase (PEMT), but is also likely related to the activity of PEMT or of cholinephosphotransferase (CPT) in the enterocyte microsomal membrane (EMM). We hypothesize that 1) changes in dietary omega-3 fatty acids or cholesterol modify the content of cholesterol and the amount and type of phospholipids in EMM of control and diabetic rats; and 2) these alterations are the result of variations in the EMM activity of CPT and of PEMT.

8.2 Methods and Materials

8.2.a Animals and Diets

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Female Wistar rats weighing 200-250 g were divided into two groups. The first group was rendered hyperglycemic by the intravenous injection of the pancreas β-cell cytotoxic agent streptozotocin (65 mg/kg body weight). The second group was injected with saline and served as non-diabetic controls. Both groups were initially fed a standard rat chow (Purina®) diet for two weeks to allow

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stabilization of the diabetes. Then the control and diabetic groups were each subdivided further into four groups of 20 rats each, and were fed one of four semi-synthetic diets for two weeks: beef tallow with low cholesterol (BT), beef tallow with high cholesterol (BTC), fish oil with low cholesterol (FO) or fish oil with high cholesterol (FOC). These semisynthetic diets were isocaloric and nutritionally adequate, providing for all known essential nutrient requirements. The composition of the diets is given in Appendix I.

Food intake was monitored every two to three days. Animals were weighed weekly. Blood glucose concentrations were determined one week post-injection and at the time of sacrifice. Animals were allowed *ad libitum* access to food and water until the time of their sacrifice. Food intake, weight gain and blood glucose levels have been reported (Keelan et al., 1991; See Chapter 7, Table 7.1).

8.2.b Membrane Preparation and Analysis

Animals were sacrificed by intraperitoneal injection of Euthanyl® (sodium pentobarbial, 450 mg/kg). Two 40 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with ice cold saline. The intestine was then opened along its mesenteric border and the mucosal surface carefully washed with cold saline to remove mucus and debris. The mucosal surface was blotted with lint-free tissue to remove excess moisture. The mucosal surface was removed by gently scraping with a microscope slide and then snap frozen in liquid nitrogen and stored at -80°C for later EMM preparation.

Enterocyte microsomal membranes (EMM) were prepared by a modification of the methods described by Lindeskog et al. (1986). Intestinal mucosal scrapings were homogenized in Buffer A (0.25 M sucrose - 0.1 M KH_2PO_4 - 1 mM EDTA - 1 mM dithiothreitol - 66.7 mM NaF - 0.2% trypsin inhibitor, pH 7.2) and were centrifuged for 10 min at 800 x g to remove cellular debris. The supernatant was centrifuged for 30 min at 15,000 x g to remove mitochondrial membranes. The resultant supernatant was then centrifuged 30 min at 48,000 x g to remove brush border and basolateral membranes. The final supernatant was then centrifuged for 60 min at 105,000 x g to pellet the microsomal membranes, which were then resuspended in Buffer B (0.25 M sucrose - 0.15 M KCl - 0.04 M KH_2PO_4, pH 7.4).

Glucose-6-phosphatase activity was determined to assess the purification of the microsomal preparation, which was enriched 10 fold over homogenates (Freedland and Harper, 1957). Lipids were extracted and analyzed for free and esterified cholesterol content, phospholipid content and composition as described in the methods section of Chapter 7.

8.2.c Cholinephosphotransferase Activity

Cholinephosphotransferase (CPT) activity was determined according to the method of Pelech and Vance (1984). Samples containing 200-400 µg protein are added to the assay mixture (50 mM Tris-HCl, pH 7.6 - 6 mM cysteine-HCl - 25 mM $MgCl_2$ - 1 mM EDTA) and

preincubated for 5 min at 37°C. The reaction was initiated with the addition of 300 μM [Me-C]-CDP-choline probe solution and incubated for 10 min at 37°C. The addition of $CHCl_3$: MeOH : 2 N HCl (2:1:0.1) terminates the reaction. The lipids are then extracted into the chloroform layer with the addition of MeOH : 0.1 M KCl (1:1). The choroform layer is dried down into liquid scintillation vials, Scintiverse II scintillant added, and counted on a Beckman 5801 scintillation counter.

8.2.d. Phosphatidylethanolamine Methyltransferase Activity

Phosphatidylethanolamine methyltransferase (PEMT) activity was assayed according to the method of Dudeja et al. (1986) and of Fonlupt et al. (1981). Samples containing 200 μg protein are added to the assay mixture (50 mM Tris-HCl, pH 9.5 - 10 mM $MgCl_2$) and preincubated for 5 min at 37°C. The reaction was initiated with the addition of 200 μM [methyl-^{3}H]-SAM probe solution and incubated for 30 min at 37°C. The addition of $CHCl_3$: MeOH : 2 N HCl (6:3:1) terminates the reaction. The lipids are then extracted into the chloroform layer with the addition of MeOH : 0.1 M KCl (1:1), separated by thin-layer chromatography in chloroform: propionic acid : n-propanol : water (2:2:3:1). The spots are then scraped into liquid scintillation vials, Scintiverse II scintillant added, and counted on a Beckman 5801 scintillation counter.

8.2.c Data Analysis

All data are expressed as mean ± standard error of the mean. Each of the eight groups contained 20 rats, and each sample represented material pooled from two animals, such that each group contained 10 samples. The results were tested statistically by analysis of variance and by the Student Neuman-Keul multiple range test.

8.3 Results

8.3.a EMM Lipid Composition

In the jejunal EMM of control rats, feeding fish oil (FO or FOC) was associated with a lower total phospholipid content, no change in the cholesterol content and a resultant decline in the ratio of phospholipid/cholesterol than in animals fed beef tallow (BT or BTC) (Table 8.1). In diabetic rats, jejunal EMM total phospholipid content was reduced and cholesterol content was unchanged, therefore the phospholipid/cholesterol ratio was also diminished when animals were fed FOC as compared with those fed BTC. Cholesterol supplementation did not significantly alter the jejunal EMM phospholipid and cholesterol content of control animals fed beef tallow or fish oil, although small changes resulted in a decline in the phospholipid/cholesterol ratio in animals fed beef tallow (BTC vs BT). In contrast, in the jejunal EMM of diabetic animals, cholesterol supplementation decreased the phospholipid content in animals fed fish oil, and increased the cholesterol content in animals fed either beef tallow or fish oil, which resulted in a reduced

phospholipid/cholesterol ratio. Diabetes was associated with an increase in jejunal EMM total phospholipid content in animals fed BTC, FO and FOC, and an increase in total cholesterol content in animals fed BTC and FOC as compared with controls. The ratio of phospholipid/cholesterol was significantly increased only in diabetic compared with control animals fed FO.

Ileal BMM phospholipid and cholesterol content was unaffected by fish oil feeding in both control and diabetic animals (Table 8.2). Although ileal EMM phospholipid content and phospholipid/cholesterol were unchanged, the cholesterol content was significantly increased when control animals fed beef tallow were supplemented with cholesterol. In diabetic animals fed fish oil, cholesterol supplementation did not significantly affect phospholipid content, but increased ileal EMM cholesterol content and reduced the resultant phospholipid/cholesterol ratio. Cholesterol supplementation did not alter the ileal EMM phospholipid and cholesterol content of control animals fed fish oil or diabetic animals fed beef tallow. Although diabetes was associated with an increase in ileal EMM cholesterol content in animals fed FOC, the total phospholipid and phospholipid/cholesterol ratio remained unchanged as compared with controls fed FOC. Diabetes did not significantly alter the ileal EMM phospholipid and cholesterol content of animals fed BT, BTC or FO as compared with controls.

8.3.b EMM Phospholipid Composition

Feeding FO was associated with a decline in sphingomyelin (SM), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in jejunal EMM of control animals as compared with those fed BT, while feeding FOC was associated with a reduction in PE and phosphatidylinositol (PI) as compared with feeding BTC (Table 8.3). In the jejunal EMM of diabetic animals, feeding FO was also associated with a decrease in SM when compared with feeding BT, while feeding FOC was associated with a decline in SM and PE when compared with feeding BTC. Cholesterol supplementation did not alter the phospholipid composition of jejunal or ileal EMM in control animals fed either beef tallow or fish oil. In the jejunal EMM of diabetic animals, cholesterol supplementation was associated with an increase in SM and PE in animals fed beef tallow, while in diabetic animals fed fish oil, cholesterol supplementation was associated with a reduction in PC and PE. Diabetes was associated with an increase in jejunal EMM sphingomyelin and phosphatidylethanolamine in animals fed beef tallow (BT or BTC). In animals fed FO, diabetes was associated with an increase in jejunal EMM phosphatidylcholine and phosphatidylethanolamine with a resultant reduction in sphingomyelin/phosphatidylcholine, while in animals fed FOC only phosphatidylethanolamine was increased.

In the ileal EMM of control animals, feeding FOC did not alter phospholipid composition as compared with feeding BTC, while feeding FO was associated with a reduction in PI as compared with feeding BT (Table 8.4). In diabetic rats, feeding FO did not alter the ileal EMM

phospholipid composition as compared with those fed BT, while feeding FOC was associated with a decline in SM and in the ratio of PC/PE as compared with feeding BTC. Ileal EMM phospholipid composition was unaffected by cholesterol supplementation in diabetic animals fed either beef tallow or fish oil. Diabetes did not alter the ileal EMM phospholipid composition of animals fed either beef tallow or fish oil.

8.3.c Phospholipid Enzyme Activity

Feeding fish oil (FO or FOC), as compared with feeding beef tallow (BT or BTC) was associated with a marked decline in jejunal but not in ileal EMM cholinephosphotransferase (CPT) of control and diabetic rats (Table 8.5). CPT was not significantly affected by adding cholesterol to the diet, or by diabetes. The EMM activity of phosphatidylethanolamine methyltransferase (PEMT) in the jejunum and ileum was not significantly affected by diabetes, dietary fatty acids or by cholesterol supplementation.

8.4 Discussion

Diabetes was associated with an increase in jejunal enterocyte microsomal membrane (EMM) total phospholipid content in rats fed BTC, FOC and FO, and this increase was primarily due to an enhancement in PE content in all these groups, although SM was also increased in animals fed BTC (Tables 8.1-8.4). The mechanism of this change is unclear, since there was no diabetes-associated reduction in the activity of phosphatidylethanolamine methyltransferase (PEMT), the enzyme which converts PE to phosphatidylcholine (PC), (Table 8.5). Future studies should examine the enzymes involved in the *de novo* synthesis of PE as a possible mechanism by which an increase in PE could be mediated in the EMM. The total cholesterol content of jejunal EMM was also higher in diabetic than in control rats, but only when they were fed a high cholesterol diet (BTC or FOC). Ileal EMM phospholipid and cholesterol content, and phospholipid composition were not influenced by diabetes (except for an increase in cholesterol when animals were fed FOC). Thus, diabetes was associated with alterations in jejunal EMM content of cholesterol, total phospholipids, SM and PE, but these changes in phospholipids could not be explained by differences in PEMT activities in the enterocyte microsomes.

The mechanism by which diabetes increases, while fish oil feeding reduces brush border membrane phospholipid content (Keelan et al., 1991, See Chapter 7) is not clear. Feeding fish oil also reduced the total phospholipid content of control jejunal EMM (Table 8.1), which resulted in a decrease in the ratio of phospholipid to cholesterol, with changes in individual phospholipids including SM, PC and PI (Table 8.3). Fish oil feeding does not appear to alter membrane cholesterol content. In diabetic animals, feeding fish oil reduced the total phospholipid content of jejunal EMM of animals fed the high cholesterol diet (FOC), but not those fed the low cholesterol

diet (FO). This decline in total phospholipids was a consequence of the reduction in SM and PE (Table 8.3).

The decrease in EMM PC observed with fish oil feeding (Table 8.3) led to a hypothesis which would examine enterocyte microsomal PC synthesis via the CDP-choline pathway and the phosphatidylethanolamine methyltransferase (PEMT) pathway. The decrease in PC observed in jejunal EMM of control rats fed FO was associated with a marked reduction in the activity of cholinephosphotransferase (CPT) (Table 8.5), the enzyme involved in the last step of PC synthesis via the CDP-choline pathway. The decline in PE in control rats fed FO or FOC, and in DM fed FOC (Table 8.3), could not be explained by differences in microsomal phosphatidylethanolamine methyltransferase (PEMT) activity (Table 8.5). Thus, supplementing the diet with omega-3 fatty acids from fish oil, rather than with saturated fats from beef tallow, had no effect on EMM content of cholesterol, but did reduce the total and individual phospholipids by a process that must involve a step other than by way of the marked reduction in CPT activity. The increase in EMM SM with diabetes in animals fed beef tallow and the marked reduction in EMM SM content with fish oil feeding suggests that future studies also consider examining the activity of sphingomyelinase. Insulin increases sphingomyelinase activity (Petkova et al., 1990), and therefore diabetes may be associated with a decline in the activity of this enzyme and lead to reduced EMM SM.

Diabetes is associated with an increase in cholesterol synthesis throughout the small intestine shortly after onset, and persists for at least 5 weeks (Feingold 1989). Intestinal *de novo* synthesis of cholesterol is increased 2-3 fold in streptozotocin-diabetic rats (Feingold et al., 1982), due to an increase in HMG-CoA reductase activity (Goodman et al., 1983; Nakayama and Nakagawa, 1977; Young et al., 1982). Other organs, including liver, do not exhibit any increase in cholesterol synthesis with diabetes. A greater increase in cholesterol synthesis occurs in the distal as compared with proximal small intestine (Feingold and Moser, 1984). In addition a gradient in cholesterol synthesis occurs along the crypt-villus axis: the greatest increase in synthesis in distal intestine is at the villus tip, while for proximal intestine it is at the crypt (Feingold and Moser, 1987). Cholesterol feeding decreases cholesterol synthesis in the small intestine (Feingold et al., 1983). Diabetes is associated with an increase in cholesterol absorption (Thomson and Rajotte, 1983). Since diabetes and cholesterol feeding did not alter BBM cholesterol content (Keelan et al., 1991, See Chapter 7), it was predicted that cholesterol supplementation of the diet would not increase the amount of cholesterol in EMM. Cholesterol feeding did not change in the jejunal EMM cholesterol of control animals (Table 8.1).

In a study by Chauton et al., (1989), rats were raised for 8 weeks on a 17% fat diet composed of 10% salmon oil or corn oil with or without 1% cholesterol supplementation. ACAT activity was increased with feeding salmon oil but not corn oil. Cholesterol supplementation

increased ACAT activity in animals fed corn oil, but could not increase activity any further in animals fed salmon oil. Field et al., (1987) fed 10% menhaden oil or cocoa butter with and without 1% cholesterol supplementation to rabbits for a period of 14 days. In addition to ACAT activity, HMGR levels were also examined in proximal, middle and distal small intestine. ACAT activity was increased in animals fed menhaden oil as compared with those fed cocoa butter, but could not be increased any further with cholesterol supplementation. HMGR activity was not influenced by dietary fatty acids, but was similarly reduced with cholesterol supplementation when animals were fed either cocoa butter or menhaden oil. Omega 3 fatty acids have been demonstrated to influence ACAT expression (Drevon et al., 1979). The microsomal phospholipid and cholesterol content in Chautan's study were not affected by dietary fatty acids or cholesterol and agree with the control jejunal microsome lipid composition in our study (Table 8.1).

The activity of cholesterol-metabolizing enzymes was not assessed in our present study, but it would appear that a process was in place in control animals to maintain a constant EMM cholesterol level, even in the face of a high dietary content of cholesterol. However, this homeostatic process of maintaining a constant EMM cholesterol level breaks down in diabetes, with an approximately 45% increase in jejunal EMM total cholesterol in diabetic animals fed BTC or FOC, as compared with BT or FO (Table 8.1). Feeding diabetic animals a high cholesterol diet had no effect on total phospholipids when the cholesterol was fed with beef tallow (BTC versus BT), but was associated with a decline in total phospholipids when fed with fish oil (FOC versus FO). Cholesterol supplementation does not alter membrane cholesterol in control animals, but significantly increases membrane cholesterol in diabetic animals. Dietary cholesterol does not reduce cholesterol synthesis in diabetes, possibly due to altered regulation of HMGR. Diabetes itself does not increase membrane cholesterol, but if cholesterol is added to the diet of a diabetic rat, membrane cholesterol is significantly increased. Cholesterol synthesis is enhanced in diabetes and is not reduced by increasing dietary sources of cholesterol, therefore abundant amounts of cholesterol are available in diabetes and likely result in an increase in membrane cholesterol. Thus, the presence of cholesterol in the diet does not influence the amount of cholesterol or phospholipids in the jejunal EMM in normal animals, but the as yet unknown mechanism by which this homeostasis is maintained becomes abnormal in diabetic animals.

Depending upon the type of dietary fatty acid or dietary content of cholesterol, diabetes was associated with increased EMM cholesterol, total phospholipids (Table 8.1), and individual phospholipids including SM, PC and PE (Table 8.3), without diabetes-specific changes in CPT or PEMT (Table 8.5). While the activity of some enzymatic processes is altered by the amount of cholesterol and/or phospholipids in the membrane (Keelan et al., 1985, 1987; Dudeja et al., 1986), this was not the case for CPT or PEMT, with no significant change in the activity of EMM PEMT despite variations in the EMM content of cholesterol and phospholipids. Feeding fish oil, however,

was associated with a marked (<95%) reduction in the activity of CPT, but there was no interactive or additive effect of diabetes or the cholesterol content of the diet. Thus, it remains to be determined the mechanism by which diabetes leads to a disturbance in the control of membrane cholesterol, or the control of the type of phospholipids inserted into the EMM. Future studies could examine the activity of enzymes involved in the synthesis of SM, PE and cholesterol.

Whereas the jejunal EMM was responsive to the effects of dietary fatty acids and cholesterol, and to diabetes, the ileal EMM showed relatively few changes: a decline in SM and an increase in PC/PE with fish oil feeding in diabetes (FOC vs BTC), an increased cholesterol content and reduced phospholipid/cholesterol ratio with cholesterol feeding in diabetes (FOC versus FO) (Table 8.4). Except for an increase in cholesterol content with diabetes in animals fed FOC, diabetes was not associated with any change in phospholipid, cholesterol and phospholipid composition. The activities of CPT and PEMT in ileal EMM did not respond to fish oil feeding (Table 5). It is possible that the EMM are inherently different for the ileum than for the jejunum, as is the case for microsomal desaturase activities (Garg et al., 1988). Alternatively, the dietary signal to adaptation is likely much stronger in the jejunum than in the ileum, and when ileal EMM composition is modified, the change is qualitatively similar to the jejunum.

Feeding a polyunsaturated fatty acid diet may reduce the enhanced uptake of glucose and lipids in diabetic rats (Thomson et al., 1987b, c). Alteration in the lipid composition and properties of the BBM have been postulated to explain this decline in nutrient absorption (Keelan et al., 1990, 1991; See Chapter 7). Diabetes is associated with an increase in total phospholipids due to an increase in PC and PE (Keelan et al., 1985). This diabetic-associated increase in PC and PE, and fish oil-associated decline in PC and PE led to the hypothesis that the synthesis of phospholipids in the enterocyte was influenced by diet and diabetes. In this study of EMM, fish oil feeding clearly reduced PC and CPT activity, while diabetes increased PC but only slightly (not significantly) increased CPT activity in animals fed FO. This diabetes-associated increase in PC in animals fed FO was not observed when the diet was supplemented with cholesterol (FOC). In both control and diabetic animals, the unsaturation of dietary fatty acids as well as the cholesterol content of the diet likely influence the synthesis of phospholipids, either by *de novo* synthesis or phospholipid interconversion. In diabetes, feeding a saturated fatty acid diet (BT) increases phospholipid content and composition, while feeding a polyunsaturated fatty acid diet (FO) reduces phospholipid content and composition. Cholesterol supplementation increases SM and PE in diabetic animals fed BT and decreases PC and PE in diabetic animals fed FO. This suggests that saturated fatty acid increases phospholipid synthesis while polyunsaturated fatty acids decrease phospholipid synthesis, but only in diabetic animals does this occur to a significant extent.

Until the actual signal for EMM adaptation in response to dietary manipulation is identified, it will be unknown what the mechanism is for membrane adaptation in response to changes in dietary lipids or to diabetes, and whether the variable responses between jejunum and ileum are on the basis of qualitative or quantitative differences.

Table 8.1 Effect of dietary ω3 fatty acids and cholesterol on jejunal enterocyte microsomal membrane lipid composition in control and diabetic rats

LIPID	CONTROL				DIABETIC			
(nmol/mg protein)	BT	BTC	FO	FOC	BT	BTC	FO	FOC
TOTAL PHOSPHOLIPIDS	278 ± 19	274 ± 17	160 ± 16+	137 ± 11+	324 ± 18	361 ± 15*	285 ± 19*	206 ± 18+#*
CHOLESTEROL								
TOTAL	91 ± 6	111 ± 7	95 ± 4	111 ± 6	101 ± 6	146 ± 7#*	92 ± 5	135 ± 8#*
FREE	88 ± 6	101 ± 3	85 ± 5	98 ± 5	92 ± 6	125 ± 6#	83 ± 6	112 ± 8
ESTERS	4 ± 1	15 ± 4	10 ± 4	13 ± 5	9 ± 2	21 ± 3	10 ± 3	23 ± 2
PHOSPHOLIPID/ CHOLESTEROL	31 ± 0.2	2.5 ± 0.2#	1.7 ± 0.1+	1.3 ± 0.1+	3.3 ± 0.2	2.3 ± 0.2#	3.3 ± 0.3*	1.6 ± 0.1+#

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, cholesterol supplementation
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.
The composition of the diets is given in Appendix I.

Table 8.2 Effect of dietary ω3 fatty acids and cholesterol on ileal enterocyte microsomal membrane lipid composition in control and diabetic rats

LIPID	CONTROL				DIABETIC			
(nmol/mg protein)	BT	BTC	FO	FOC	BT	BTC	FO	FOC
TOTAL PHOSPHOLIPIDS	203 ± 21	198 ± 15	170 ± 10	152 ± 11+	226 ± 16	228 ± 13	210 ± 9	165 ± 17
CHOLESTEROL								
TOTAL	98 ± 7	133 ± 12#	104 ± 5	123 ± 7	119 ± 9	136 ± 8	110 ± 6	156 ± 7#*
FREE	94 ± 7	109 ± 8	97 ± 6	109 ± 5	104 ± 5	110 ± 6	99 ± 5	123 ± 6
ESTERS	6 ± 2	24 ± 10	9 ± 3	15 ± 3	15 ± 5	26 ± 4	11 ± 3	33 ± 4#*
PHOSPHOLIPID/ CHOLESTEROL	2.1 ± 0.2	1.6 ± 0.1	1.7 ± 0.2	1.2 ± 0.1	1.9 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.1 ± 0.1#

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, cholesterol supplementation
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol. The composition of the diets is given in Appendix I.

Table 8.3 Effect of dietary ω3 fatty acids and cholesterol on jejunal enterocyte microsomal membrane phospholipid composition in control and diabetic rats

PHOSPHOLIPID	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
SPHINGOMYELIN								
%	15.96 ± 2.07	17.52 ± 1.73	19.44 ±2.21	21.52 ± 2.22	19.19 ± 2.08	20.71 ± 1.53	9.12 ± 1.07+*	16.02 ± 1.32#
nmoles/mg protein	46.20 ± 6.32	47.68 ± 4.97	28.79 ± 2.00+	28.47 ± 3.05	59.19 ± 4.95*	73.67 ± 6.05#*	25.16 ± 1.99+	32.10 ± 1.97+
PHOSPHATIDYLCHOLINE								
%	24.87 ± 2.87	19.24 ± 1.84	20.90 ± 1.70	21.68 ± 2.41	24.10 ± 2.10	17.17 ± 1.83	30.72 ± 2.13*	24.67 ± 2.08
nmoles/mg protein	76.10 ± 12.90	52.34 ± 5.49	34.78 ± 5.29+	30.34 ± 4.17	76.81 ± 8.02	62.13 ± 8.03	92.98 ± 11.18*	54.24 ± 8.20#
PHOSPHATIDYLSERINE								
%	2.29 ± 0.60	2.82 ± 1.12	0.70 ± 0.30	1.18 ± 0.42	1.89 ± 0.46	2.18 ± 0.49	1.44 ± 0.33	1.26 ± 0.70
nmoles/mg protein	7.18 ± 3.31	6.96 ± 2.36	1.04 ± 0.43	1.54 ± 0.56	6.11 ± 1.47	7.75 ± 1.77	3.72 ± 0.62	2.62 ± 1.27
PHOSPHATIDYL-ETHANOLAMINE								
%	46.00 ± 2.53	50.73 ± 1.61	48.45 ± 1.52	47.11 ± 2.14	45.49 ± 2.07	52.73 ± 1.84	48.40 ± 1.84	47.93 ± 1.73
nmoles/mg protein	119.8 ± 15.28	139.9 ± 11.27	77.48 ± 7.76+	65.09 ± 7.09+	146.6 ± 14.74	187.0 ± 10.18#*	140.6 ± 8.51*	100.5 ± 9.63+#*
PHOSPHATIDIC ACID								
%	0.51 ± 0.25	0.82 ± 0.40	1.04 ± 0.56	1.43 ± 0.59	0.26 ± 0.13	0.11 ± 0.08	0.70 ± 0.27	0.89 ± 0.62
nmoles/mg protein	1.62 ± 0.97	2.14 ± 1.02	1.17 ± 0.57	1.52 ± 0.54	0.77 ± 0.36	0.33 ± 0.22	1.67 ± 0.66	1.36 ± 0.99
PHOSPHATIDYLINOSITOL								
%	10.28 ± 0.95	8.52 ± 0.72	9.33 ± 2.23	6.60 ± 0.74	7.53 ± 0.59	6.72 ± 0.47	9.24 ± 0.72	7.55 ± 0.72
nmoles/mg protein	23.63 ± 4.73	23.64 ± 2.80	16.60 ± 4.32	8.78 ± 1.01+	23.70 ± 2.08	24.23 ± 2.39	28.08 ± 3.04	16.41 ± 2.89
CHOLINE/AMINE	0.89 ± 0.11	0.69 ± 0.04	0.83 ± 0.05	0.93 ± 0.08	0.98 ± 0.08	0.71 ± 0.06	0.83 ± 0.08	0.88 ± 0.07
PC/PE	0.58 ± 0.10	0.39 ± 0.05	0.43 ± 0.04	0.48 ± 0.06	0.55 ± 0.06	0.33 ± 0.05	0.66 ± 0.07	0.53 ± 0.06
SM/PC	0.73 ± 0.13	1.06 ± 0.19	1.06 ± 0.21	1.21 ± 0.24	0.92 ± 0.18	1.31 ± 0.17	0.34 ± 0.07*	0.72 ± 0.10

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, cholesterol supplementation
* $p < 0.05$, diabetic versus control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.
The composition of the diets is given in Appendix I.

Table 8.4 Effect of dietary ω3 fatty acids and cholesterol on ileal enterocyte microsomal membrane phospholipid composition in control and diabetic rats

PHOSPHOLIPID	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
SPHINGOMYELIN								
%	24.90 ± 1.55	27.55 ± 2.34	25.72 ± 2.99	27.21 ± 2.16	26.75 ± 2.57	29.72 ± 1.95	18.65 ± 3.21	25.34 ± 2.01
nmoles/mg protein	50.60 ± 5.28	53.19 ± 4.66	42.94 ± 6.11	40.26 ± 4.04	55.63 ± 3.63	69.26 ± 5.35	39.19 ± 6.26	45.54 ± 2.84+
PHOSPHATIDYLCHOLINE								
%	22.43 ± 3.70	15.13 ± 1.51	18.11 ± 2.01	17.90 ± 2.27	15.91 ± 1.73	15.41 ± 1.55	23.16 ± 1.54	24.40 ± 3.37
nmoles/mg protein	50.13 ± 10.63	31.19 ± 5.22	30.70 ± 4.41	26.87 ± 3.72	33.89 ± 3.78	36.00 ± 4.01	50.05 ± 3.79	46.60 ± 8.31
PHOSPHATIDYLSERINE								
%	2.42 ± 0.78	1.30 ± 0.43	1.70 ± 0.50	2.23 ± 0.73	3.35 ± 0.50	2.56 ± 0.48	2.78 ± 0.38	2.6 ± 0.58
nmoles/mg protein	4.67 ± 1.50	2.67 ± 0.88	2.94 ± 0.89	3.10 ± 1.02	7.46 ± 1.43	5.98 ± 1.01	6.18 ± 0.89	4.88 ± 1.09
PHOSPHATIDYL-ETHANOLAMINE								
%	37.01 ± 2.70	44.22 ± 1.58	46.60 ± 3.01	44.16 ± 2.58	46.22 ± 2.88	43.82 ± 1.96	46.29 ± 2.76	37.59 ± 2.42
nmoles/mg protein	78.47 ± 9.64	86.63 ± 5.93	76.34 ± 5.35	66.63 ± 8.13	102.3 ± 12.07	103.5 ± 9.39	100.9 ± 7.57	68.27 ± 4.44
PHOSPHATIDIC ACID								
%	1.71 ± 0.69	1.83 ± 0.64	1.90 ± 0.71	1.34 ± 1.04	0.59 ± 0.29	0.74 ± 0.27	1.28 ± 0.64	1.69 ± 0.52
nmoles/mg protein	3.45 ± 1.72	4.26 ± 1.92	3.15 ± 1.22	1.58 ± 1.17	1.04 ± 0.47	1.58 _ 0.52	2.50 ± 1.15	2.64 ± 0.97
PHOSPHATIDYLINOSITOL								
%	10.20 ± 2.00	7.65 ± 1.19	5.78 ± 1.50	5.37 ± 1.18	6.69 ± 0.57	6.49 ± 0.86	7.40 ± 1.14	8.17 ± 0.71
nmoles/mg protein	19.89 ± 3.64	14.49 ± 2.08	9.15 ± 2.36+	9.83 ± 2.17	14.02 ± 1.02	15.00 ± 1.94	15.10 ± 2.99	15.26 ± 1.90
CHOLINE/AMINE	1.26 ± 0.15	0.96 ± 0.08	0.97 ± 0.13	1.00 ± 0.09	0.93 ± 0.12	1.02 ± 0.12	0.97 ± 0.17	1.33 ± 0.16
PC/PE	0.64 ± 0.12	0.35 ± 0.04	0.41 ± 0.06	0.42 ± 0.06	0.36 ± 0.05	0.37 ± 0.06	0.54 ± 0.07	0.71 ± 0.13+
SM/PC	1.44 ± 0.28	2.11 ± 0.36	1.55 ± 0.23	2.03 ± 0.55	1.90 ± 0.28	2.05 ± 0.21	0.90 ± 0.22	1.28 ± 0.24

\+ $p < 0.05$, fish oil versus beef

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.
The composition of the diets is given in Appendix I.

Table 8.5 Effect of ω3 fatty acids and cholesterol on enterocyte phospholipid enzyme activity in control and diabetic rats

ENZYME	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
CHOLINEPHOSPHOTRANSFERASE (CPT), nmol/mg protein/min								
JEJUNUM	0.145 ± 0.027	0.104 ± 0.018	0.008 ± 0.005+	0.005 ± 0.002+	0.155 ± 0.019	0.137 ± 0.024	0.041±0.011+	0.024 ± 0.009+
ILEUM	0.052 ± 0.013	0.040 ± 0.009	0.019 ± 0.005	0.012 ± 0.003	0.059 ± 0.009	0.051 ± .016	0.021 ± 0.005	0.012 ± 0.005
PHOSPHATIDYLETHANOLAMINE METHYLTRANSFERASE (PEMT), pmol/mg protein/min								
JEJUNUM	0.042 ± 0.016	0.013 ± 0.007	0.044 ± 0.027	0.035 ± 0.021	0.014 ± 0.010	0.005 ± 0.003	0.011 ± 0.004	0.028 ± 0.017
ILEUM	0.076 ± 0.017	0.046 ± 0.018	0.060 ± 0.025	0.037 ± 0.014	0.030 ± 0.012	0.021 ± 0.006	0.034 ± 0.008	0.038 ± 0.021

\+ $p < 0.05$, fish oil versus beef

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol. The composition of the diets is given in Appendix I.

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CHAPTER 9

DIETARY OMEGA-3 FATTY ACIDS AND CHOLESTEROL MODIFY DESATURASE ACTIVITIES AND FATTY ACYL CONSTITUENTS OF RAT INTESTINAL BRUSH BORDER AND MICROSOMAL MEMBRANES OF DIABETIC RATS[8]

9.1 Introduction

Dietary omega-3 fatty acids and cholesterol modify the enterocyte microsomal membrane (EMM) phospholipid and cholesterol content and phospholipid-metabolizing enzyme activities in diabetic rats (Keelan et al., 1993, See Chapter 8). The intestinal brush border membrane (BBM) transport and digestive functions are enhanced in streptozotocin-diabetes (Thomson, 1980, 1981, 1983, 1988; Thomson and Rajotte, 1983a,b), and this is associated with alterations in BBM content of phospholipids and their fatty acyl constituents (Keelan et al., 1987, 1989, 1991). The enterocyte microsomes have Δ^5, Δ^6 and Δ^9-desaturase activities which are responsive to dietary alterations (Garg et al., 1988a,b, 1992). These studies were undertaken to test the hypothesis that the diet- and diabetes-associated alterations in enterocyte microsomal activities of desaturases are responsible for the changes in the fatty acyl constituents in the BBM phospholipids of rat intestine. The data demonstrate that although BBM lipids and enterocyte microsomal activities of desaturases are responsive to modifications in dietary fatty acids, cholesterol or diabetes, the alterations are not necessarily causally related.

9.2 Methods and Materials

9.2.a Animals and Diets

The guiding principles in the care and use of laboratory animals, approved by the Canadian Federation of Biological Societies, were observed in the conduct of this study. Female Wistar rats weighing 200-250 g were divided into two groups. The first group was rendered hyperglycemic by the intravenous injection of pancreas β-cell cytotoxic agent streptozotocin (65 mg/kg body weight). The second group was injected with saline and served as non-diabetic controls. Both groups were initially fed a standard rat chow (Purina®) diet for two weeks to allow stabilization of the diabetes. Then the control and diabetic animals were each subdivided further

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into four groups of 20 rats each, and were fed one of four semi-synthetic diets for two weeks: beef tallow with low cholesterol (BT), beef tallow with high cholesterol (BTC), fish oil with low cholesterol (FO) or fish oil with high cholesterol (FOC). These semisynthetic diets were isocaloric and nutritionally adequate, providing for all known essential nutrient requirements. The complete composition of the diets is given in Appendix I.

Food intake was monitored every 2 to 3 days. Animals were weighed weekly. Blood glucose concentrations were determined one week post-injection of streptozotocin, and at the time of sacrifice. Animals were allowed *ad libitum* access to food and water until the time of their sacrifice. Food intake, weight gain and blood glucose levels have been reported (Keelan et al., 1991, See Chapter 7, Table 7.1).

9.2.b Brush Border Membrane Preparation

Animals were sacrificed by intraperitoneal injection of Euthanyl® (sodium pentobarbial, 450 mg/kg). Two 40 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with ice cold saline. The intestine was then opened along its mesenteric border and the mucosal surface carefully washed with cold saline to remove mucus and debris. The mucosal surface was blotted with lint-free tissue to remove excess moisture. The mucosal surface was removed by gently scraping with a microscope slide and then snap frozen in liquid nitrogen and stored at -80°C for later membrane preparation. Intestinal brush border membranes (BBM) were prepared from mucosal scrapings by homogenization, $CaCl_2$ precipitation, differential centrifugation and density gradient centrifugation as described in Chapter 7.

9.2.c Enterocyte Microsomal Membrane Preparation

Enterocyte microsomal membranes (EMM) were prepared from mucosal scrapings by homogenization and differential centrifugation as described in Chapter 8.

9.2.d Desaturase Assay

Δ^9-, Δ^6-, and Δ^5-desaturase activity was determined in jejunal and ileal microsomes using [1-^{14}C]-16:0, [1-^{14}C]-18:2ω6, and [1-^{14}C]-20:3ω6 respectively as substrates (Garg et al., 1986). The assay mixture contained in a final volume of 1.0 ml: 5μmol ATP, 0.1 μmol CoASH, 0.5 μmol niacinamide, 1.25 μmol NADH, 2.25 μmol glutathione, 5 μmol $MgCl_2$, 62.5 μmol NaF, 62.5 μmol KH_2PO_4 (pH 7.4) and 200 nmol [1-^{14}C] substrate (0.1 μCi). The reaction was started with the addition of microsomal membrane suspension containing 2-5 mg protein in 200 μl, and incubated for 15 min at 37°C in a shaking water bath. The reaction was stopped by adding 1 ml of 20% (w/v) KOH in methanol. Lipids were saponified by heating at 85°C for 2 hr and acidified with 1 ml 8 N HCl, and fatty acids were extracted with hexane. Fatty acids were converted into methyl esters by heating for 1 h at 100°C with 14% (w/w) BF_3-methanol reagent. Fatty acid methyl esters were separated based on the degree of unsaturation using thin layer chromatography (TLC) plates impregnated with 10% (w/w) $AgNO_3$ in silica gel G. Carrier methyl esters of 16:0 and 16:1,

18:2ω6 and 18:3ω6, or 20:3ω6 and 20:4ω6 were spotted on the plate along with labelled acids. Plates were developed in toluene:acetone 95:5 v/v for separation of monoenes, dienes and trienes. The fatty acid spots were made visible under ultraviolet light after spraying with 8-anilino-1-naphthalene sulfonic acid, then were scraped off the TLC plate directly into scintillation vials. These were counted with 5 ml of scintillation fluor (Aquasol®, NEN) in a liquid scintillation counter (Beckman, Model LS 5801) with a counting efficiency of more than 90%. Raw data was corrected for recovery and the enzyme activity was expressed as pmol of desaturated product formed per min per mg of microsomal membrane protein.

9.2.e Phospholipid Fatty Acid Analysis

Jejunal and ileal BBM and EMM lipids were extracted with chloroform:methanol (2:1, v/v) containing 0.005% (w/v) butylated hydroxytoluene, according to a modification of the method of Bowyer and King (1977) and of Folch et al. (1957). Individual phospholipids were separated on thin-layer chromatography plates (silical gel H, 20 x20 cm, 250 μ thickness, Analtech) using a solvent system comprising chloroform:methanol:2-propanol:triethylamine:0.25% (w/v) KCl (15:4.5:12.5:9:3) (Touchstone et al., 1979; Vitiello and Zanetta, 1978). Phosphatidylcholine and phosphatidylethanolamine spots were scraped off the plates for transesterification of their fatty acid components using 14% (w/w) BF_3-methanol reagent:hexane (1.5:2) for 60 min at 100°C (Metcalfe and Schmitz, 1961). Fatty acid methyl esters were extracted with hexane and analyzed on the Varian model 6000 gas chromatograph interfaced with the Varian Vista 402 data system (Varian Instruments, Georgetown, Ontario) equipped with a 20 m fused silica capillary column (BP-20, 25 m x 0.25 mm i.d., Varian, Georgetown, Ontario) and flame ionization detectors (Innis and Clandinin, 1981). The column was an open tubular glass capillary column (18 m x 0.25 mm i.d. coated with SP1000/grade AA). Helium was used as a carrier gas at a flow rate of 1.8 ml/min and inlet pressure of 80 kPa. The inlet splitter was set at 4 to 1. Samples were injected at 175°C and after 3 min in the oven, temperature was increased at a rate of 2°C/min for 20 min. Chromatography was complete after 50 min. Fatty acid methyl ester peaks were identified by injecting authentic standard mixtures of fatty acid methyl esters using the method of equivalent chain length (Miwa et al., 1960).

9.2.f Data Analysis

All data are expressed as mean ± standard error of the mean. Each of the eight groups contained 20 rats and each sample represented material pooled from two animals, so that each group contained 10 samples. The results were statistically tested by analysis of variance and by the Student Neuman Keul multiple range test.

9.3 Results

9.3.a *EMM Desaturase Activity*

Feeding fish oil did not alter jejunal enterocyte microsomal membrane (EMM) Δ^9-desaturase activity in either non-diabetic control or in diabetic animals (Table 9.1). Cholesterol supplementation increased ($p<0.05$) EMM Δ^9-desaturase activity in control rats fed fish oil, but did not change in diabetic animals. Diabetes did not affect Δ^9-desaturase activity in animals fed BTC or FOC. Diabetes, fish oil feeding and cholesterol supplementation did not alter ileal Δ^9-desaturase activity (Table 9.2).

No significant changes in Δ^6-desaturase activity were observed in jejunal EMM due to diabetes, fish oil feeding or cholesterol supplementation (Table 9.1). In ileal EMM, fish oil feeding increased ($p<0.05$) Δ^6-desaturase of control animals fed the high cholesterol diet (Table 9.2). Cholesterol supplementation increased ($p<0.05$) Δ^6-desaturase activity of control animals fed fish oil. Only in animals fed FOC did diabetes produce a significant decline ($p<0.05$) in Δ^6-desaturase activity.

Feeding FOC increased ($p<0.05$) jejunal EMM Δ^5-desaturase activity in control and diabetic animals, as compared with those fed BTC. Cholesterol supplementation increased Δ^5-desaturase activity in both control and diabetic animals fed fish oil (FOC versus FO), but not in animals fed beef tallow (BTC vs BT). Diabetes did not alter jejunal EMM Δ^5-desaturase activity. As in the jejunum, so also in the ileum feeding fish oil increased ($p<0.05$) EMM Δ^5-desaturase activity in control animals fed a high cholesterol diet, and also increased ($p<0.05$) with cholesterol supplementation when control animals were fed fish oil. These changes were not observed in the ileum of diabetic animals. However, diabetes was associated with a reduction ($p<0.05$) in Δ^5-desaturase activity in animals fed FOC.

9.3.b *Fatty Acyl Constituents of BBM and EMM Phospholipids*

Phosphatidylcholine

In the jejunum and ileum of control animals, feeding fish oil reduced the ω6 fatty acids (18:2ω6, 20:4ω6) in BBM 50-60% and EMM 40-45%, but increased the content of ω3 fatty acids (20:5ω3, 22:5ω3, 22:6ω3) in BBM and EMM 6-10-fold (Tables 9.3-9.10). In the jejunum and ileum of diabetic animals, feeding fish oil increased the ω3 fatty acids 11-15-fold without a significant decline in 20:4ω6, although a 50% decrease in 18:2ω6 was observed. The resulting ω6/ω3 ratio was reduced 86-96% in BBM and EMM with fish oil feeding in both control and diabetic animals. The content of 18:1ω9 was not altered by feeding fish oil in the jejunal BBM and EMM of control animals, but was reduced in control ileal as well as diabetic jejunal and ileal BBM and EMM, thereby resulting in an increase in the ratio of 18:0/18:1ω9.

Cholesterol supplementation did not alter the PC fatty acid composition of jejunal and ileal BBM and EMM of control animals fed either beef tallow or fish oil, except for a 25% decline in ω3 fatty acids (22:6ω3) in the ileal BBM of control animals fed fish oil. In diabetic animals fed beef tallow, cholesterol supplementation increased 18:1ω9, the ratio of M/S and 18:2ω6/20:4ω6 in jejunal BBM and EMM, but only increased 18:1ω9 and reduced 20:4ω6 in ileal BBM. Cholesterol supplementation did not alter the PC in jejunal and ileal EMM and jejunal BBM of diabetic animals fed fish oil, while in ileal BBM 20:5ω3 and 22:6ω3 were increased 50% when the fish oil diet was supplemented with cholesterol.

Diabetes was not associated with any changes in jejunal and ileal BBM PC of animals fed BT, but diabetes did increase 18:1ω9 22-32% in jejunal and ileal BBM of those fed BTC. Diabetes did not alter jejunal BBM PC in animals fed FO or FOC, but reduced ω3 fatty acids 51% in ileal BBM PC of animals fed FO, and reduced 18:1ω9 33% and increased 22:6ω3 45% in ileal BBM of animals fed FOC. In jejunal EMM PC, diabetes decreased 20:5ω3 55-65% when animals were fed BT or BTC, while no effect was observed with diabetes when animals were fed FO or FOC. In the ileum of animals fed BT or BTC, diabetes did not alter the fatty acid composition of EMM PC. Diabetes reduced 18:1ω9 30% and increased 20:4ω6 35% and 22:6ω3 25-45% in ileal EMM of animals fed FO or FOC which resulted in a decline in the ratios of M/S, 18:2ω6/20:4ω6 and 20:5ω3/20:4ωw6.

Phosphatidylethanolamine

Feeding fish oil reduced BBM PE ω6 fatty acids 35% in jejunum and 45% in ileum, but increased ω3 fatty acids 10-fold in jejunum and only 2.5-3.5-fold in ileum of control animals. In diabetic animals, feeding fish oil reduced BBM PE 60-64% in jejunum and 50-60% in ileum, but increased ω3 fatty acids 6.5-8-fold in jejunum and only 3.5-4.5-fold in ileum. Fish oil feeding reduced 18:1ω9 50-65%, increased 18:0/18:1ω9, reduced ω6 fatty acids 60-75% and increased ω3 fatty acids 4-7-fold in jejunal and ileal EMM PE of both control and diabetic animals. The ratio of ω6/ω3 fatty acids was reduced 97% in jejunum and more than 80% in ileum of control and diabetic BBM and EMM PE.

Cholesterol supplementation does not significantly alter the jejunal BBM or jejunal and ileal EMM PE of control and diabetic animals fed either beef tallow or fish oil. In ileal BBM PE, cholesterol supplementation increased 20:5ω3 33% in control animals fed fish oil, increased 18:1ω9 35% and decreased 20:4ω6 45% in diabetic animals fed beef tallow, and reduced 22:6ω3 50% in diabetic animals fed fish oil.

Diabetes did not alter jejunal BBM PE of animals fed BT, BTC, FO or FOC, nor ileal BBM PE of animals fed BT. A 25% increase in 18:1ω9, 50% increase in 18:2ω6, and a 42% decrease in 20:4ω6 was observed with diabetes in ileal BBM PE of animals fed BTC. Diabetes increased ω3 fatty acids (22:5ω3, 22:6ω3) 2-fold in ileal BBM PE of animals fed FO, but reduced 22:5ω3 75% in

those fed FOC. In jejunal and ileal EMM PE fed BT or BTC, diabetes increased 18:2ω6 33-40%, while in those fed FO or FOC, diabetes decreased 18:1ω9 60-65% and 20:5ω3 15-30%.

9.4 Discussion

The enterocyte microsomal membrane (EMM) activity of the jejunal and ileal Δ^5, Δ^6 and Δ^9-desaturases was influenced by the type of the dietary fatty acids, by dietary cholesterol, and by the presence of diabetes: cholesterol feeding increased desaturase activities, fish oil feeding increased desaturase activities in control animals when control and diabetic animals were fed a high cholesterol diet, and diabetes reduced desaturase activity in ileum but not jejunum (Tables 9.1 and 9.2). These dietary modifications were also associated with alterations in the jejunal and ileal BBM and EMM content of fatty acyl constituents in phosphatidylcholine (PC) and in phosphatidylethanolamine (PE) (Tables 9.3-9.10). However, it is important to ask whether the alterations that were actually seen in the fatty acyl constituents of the phospholipids in the BBM and EMM were causally interrelated on the basis of altered activities of EMM Δ^9, Δ^6 and Δ^5-desaturases. The desaturases in the EMM are thought to be responsible for the fatty acids incorporated into PC and PE, which then traffic from the EMM to BBM.

Fish oil feeding did not alter the Δ^9-desaturase activity of jejunal and ileal EMM of control and diabetic animals, yet the content of 18:1ω9 was reduced by feeding fish oil in jejunal and ileal EMM PC and PE. A previous study on diet and intestinal microsomes did not find any changes in Δ^6-desaturase activity when comparing animals fed fish oil versus those fed a saturated fatty acid diet (Garg et al., 1992). Δ^6 and Δ^5-desaturase activities were increased with fish oil feeding in ileal EMM of control animals, and could help explain the decline in 18:2ω6 observed in ileal BBM and EMM PC and PE, yet the concomitant decline in jejunal BBM and EMM 18:2ω6 cannot be attributed to any change in jejunal EMM desaturase activity. This is directly opposite to the decline in rat liver microsomal desaturase activity observed with fish oil feeding (Dang et al., 1989; Garg et al., 1988c, d). Feeding fish oil increased Δ^5 and Δ^6-desaturase activities in the jejunum and ileum of control and diabetic animals fed a high cholesterol diet (FOC versus BTC). On this basis it was predicted that 18:2ω6 would be reduced while 20:4ω6 would be increased. The ω3 family of fatty acids would not be expected to be influenced by alterations in Δ^5 and Δ^6-desaturase activities, since 20:5ω3 and 22:6ω3 were directly available from the diet and were not synthesized from 18:3ω3. As expected, jejunal and ileal BBM and EMM PC and PE of both control and diabetic animals contained less 18:2ω6 when feeding FO to animals on a high cholesterol diet (FOC versus BTC), while none of the BBM phospholipids had an increase in 20:4ω6. This was possibly due to the greater affinity of acyltransferase enzymes for ω3 fatty acids present in the diet

rather than due to changes in desaturase activity alone (Dudeja and Brasitus, 1987; Garg et al., 1990).

In rat liver microsomes, cholesterol feeding increases Δ^9-desaturase activity and reduces Δ^6 and Δ^5-desaturase activites (Garg et al., 1986). Cholesterol feeding increased jejunal EMM Δ^9-desaturase activity when control animals were fed FO, and increased jejunal EMM Δ^5-desaturase activity when control and diabetic animals were fed FO. On the basis of these changes in desaturase activities, it was predicted that 18:1ω9 would be increased in control animals fed fish oil due to an increase in Δ^9-desaturase activity. In the ileum, cholesterol supplementation increased the activity of Δ^6 and Δ^5-desaturase of control animals fed fish oil. It was surprising that neither BBM or EMM 18:1ω9 or 20:4ω6 content was altered with cholesterol supplementation to fish oil diets. On the contrary, 18:1ω9 was increased only in the jejunal and ileal BBM PC and PE of diabetic animals fed beef tallow supplemented with cholesterol, while 20:4ω6 was reduced only in the ileal BBM of diabetic animals fed beef tallow supplemented with cholesterol.

Diabetes is associated with a decline in desaturase activity in rat liver, heart, kidney and aorta (Dang et al., 1989; Holman et al., 1983). Diabetes did not alter jejunal EMM desaturase activity in animals fed BT, BTC, FO or FOC. Diabetes was associated with a decline in ileal EMM Δ^6 and Δ^5-desaturase activities when animals were fed FOC, suggesting that a corresponding increase in 18:2ω6 and a decline in 20:4ω6 would be an expected result. This was not observed in either jejunal or ileal BBM or EMM PC or PE when animals were fed FOC.

Since all the diets contain the same amount of 18:2ω6, it is reasonable that BBM and EMM PC and PE should have comparable levels of 18:2ω6 and 20:4ω6. This holds true for jejunal BBM and jejunal and ileal EMM PC and PE. However, in ileal BBM PC and PE, diabetes was associated with an increase in 18:2ω6 and a decline in 20:4ω6 when animals were fed BTC, yet was not correlated with any significant change in desaturase activity.

The results of this study raise several questions that merit further investigation. What steps control the incorporation of fatty acids into phospholipids designated for EMM and BBM? A significant porrtion of the enterocyte pool of polyunsaturated fatty acids is derived from *de novo* synthesis from precursors such as 16:0, 18:2ω6, 18:3ω3 at the microsomal membranes. Since desaturase activity has not been detected in BBM, phospholipid polyunsaturated fatty acids must be derived from diet or microsomal membranes for plasma membrane synthesis. Dietary 18:2ω6 is predominantly used for phospholipid and triacylglycerol synthesis in the jejunum, while in the ileum, dietary 18:2ω6 is used for diacylglycerol synthesis and conversion to phospholipid (Garg et al., 1988b). All of the diets (BT, BTC, FO FOC) contained essential levels of linoleic acid which would be available for desaturation and elongation. The presence of dietary ω3 fatty acids (20:5ω3 and 22:6ω3) possibly inhibit the utilization of 18:2ω6 for arachidonic acid synthesis by feedback inhibition to the Δ^5 and Δ^6-desaturase enzymes, since both ω3 and ω6 fatty acids

compete for the same desaturases. Secondly, it is possible that ω3 polyunsaturated fatty acids preferentially bind to acyltransferases required for the synthesis of membrane phospholipids. This would explain the decline in 20:4ω6 content observed in both jejunal and ileal BBM and EMM PC and PE of control and diabetic animals. How is the 18:1ω9 content of phospholipids regulated? Changes in 18:1ω9 content are clearly not due solely to changes in Δ^9-desaturase activity. How does fish oil reduce 18:1ω9 incorporation? The omega-3 family of fatty acids may have a preferential ability to bind acyltransferases used in phospholipid synthesis. How does the addition of cholesterol to the diet increase 18:1ω9 when animals are fed BT? Although it is known that cholesterol may influence desaturase activity, it is also possible that cholesterol may influence various aspects of phospholipid syntheses. How does diabetes increase 18:1ω9 in ileal BBM of animals fed beef tallow plus cholesterol and decrease 18:1ω9 in jejunal and ileal EMM of animals fed fish oil with or without cholesterol? The differences in the fatty acid profile of BBM and EMM may be related to possible reacylation/deacylation enzymes that may be present in the BBM that allow fine tuning of the BBM composition for optimal transport function.

Thus, alterations in EMM desaturase activities do not explain all of the observed changes in BBM phospholipid fatty acid composition. This data argues therefore that while the activities of the desaturases are influenced by dietary fatty acids, dietary cholesterol, and by diabetes, and while the fatty acyl constituents of the BBM and EMM change with these treatments, the alterations are not necessarily causally related. In other words, while changes in the EMM desaturases may alter the fatty acyl constituents of the EMM themselves, some other step must be involved in the diet- and diabetes-associated changes in the fatty acyl constituents of the phospholipids in the BBM.

Table 9.1 Effect of dietary ω3 fatty acids and cholesterol on jejunal enterocyte microsomal membrane desaturase activity in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
delta-9 DESATURASE								
pmol/mg protein*min	9.2 ± 34	197 ± 41	55 ± 16	207 ± 49#	109 ± 25	48 ± 9	109 ± 33	103 ± 34
% conversion	0.7 ± 0.3	1.1 ± 0.2	0.3 ± 0.1	1.0 ± 0.2	0.09 ± 0.2	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
delta-6 DESATURASE								
pmol/mg protein*min	86 ± 35	120 ± 43	119 ± 16	276 ± 83	104 ± 21	120 ± 33	261 ± 212	155 ± 56
% conversion	0.4 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	1.1 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1.3 ± 0.5	0.8 ± 0.2
delta-5 DESATURASE								
pmol/mg protein*min	159 ± 54	154 ± 28	77 ± 24	422 ± 169+#	217 ± 48	126 ± 28	139 ± 38	352 ± 81+#
% conversion	1.0 ± 0.3	1.0 ± 0.2	0.8 ± 0.3	1.4 ± 0.4	1.8 ± 0.5	0.9 ± 0.2	0.8 ± 0.1	1.6 ± 0.2

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high cholesterol vs low cholesterol

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.2 Effect of dietary ω3 fatty acids and cholesterol on ileal enterocyte microsomal membrane desaturase activity in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
delta-9 DESATURASE								
pmol/mg protein*min	296 ± 88	147 ± 41	194 ± 92	251 ± 54	153 ± 42	108 ± 28	99 ± 21	92.4 ± 25.2
% conversion	1.1 ± 0.4	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 02
delta-6 DESATURASE								
pmol/mg protein*min	185 ± 56	366 ± 105	320 ± 96	878 ± 185+#	236 ± 84	198 ± 46	277 ± 100	102 ± 34*
% conversion	0.6 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	2.3 ± 0.4+#	1.0 ± 0.3	0.7 ± 0.2	0.8 ± 0.2	0.4 ± 0.1*
delta-5 DESATURASE								
pmol/mg protein*min	228 ± 83	410 ± 80	398 ± 113	939 ± 198+#	440 ± 122	373 ± 64	202 ± 46	441 ± 71*
% conversion	0.8 ± 0.2	1.4 ± 0.2	1.5 ± 0.4	2.3 ± 0.3	1.7 ± 0.2	2.3 ± 0.5	1.1 ± 0.2	1.9 ± 0.4

\+ $p < 0.05$, fish oil versus beef
\# $p < 0.05$, high cholesterol vs low cholesterol
* $p < 0.05$, diabetic vs control

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.3 Effect of dietary ω3 fatty acids and cholesterol on jejunal brush border membrane phosphatidylcholine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	14.7 ± 2.21	13.9 ± 1.89	19.1 ± 1.76	21.1 ± 1.42	17.4 ± 1.54	16.3 ± 1.72	15.9 ± 2.22	15.0 ± 1.85
18:0	31.7 ± 1.05	33.6 ± 1.60	35.2 ± 1.04	32.4 ± 1.07	30.8 ± 1.32	28.5 ± 1.47	30.3 ± 2.74	35.2 ± 1.58
18:1ω9	4.8 ± 0.26 ab	6.8 ± 0.52 b	3.3 ± 0.35 a	4.5 ± 0.33 ab	5.5 ± 0.67 ab	10.0 ± 1.22 c	2.6 ± 0.29 a	3.7 ± 0.23 a
18:2ω6	31.7 ± 1.52 a	30.8 ± 0.52 a	14.4 ± 0.27 b	15.0 ± 0.63 b	30.6 ± 1.65a	31.6 ± 1.08 a	15.2 ± 2.66 b	16.4 ± 0.42 b
20:4ω6	8.9 ± 0.95 a	7.4 ± 0.80 ab	4.1 ± 0.09 b	3.8 ± 0.35 b	10.2 ± 1.02 a	6.6 ± 0.73 ab	6.7 ± 0.52 ab	5.7 ± 0.63 ab
20:5ω3	0.2 ± 0.21 a	0.1 ± 0.07 a	13.5 ± 0.52 b	13.1 ± 0.98 b	0.1 ± 0.07 a	0.2 ± 0.07 a	10.9 ± 1.33 b	14.5 ± 0.89 b
22:5ω3	0.2 ± 0.11	0.2 ± 0.16	0.1 ± 0.07	0.2 ± 0.10	ND	ND	0.6 ± 0.21	0.2 ± 0.10
22:6ω3	1.2 ± 0.15 a	1.1 ± 0.17 a	3.0 ± 0.18 ab	2.7 ± 0.26 ab	1.0 ± 0.12 a	0.9 ± 0.08 a	3.0 ± 0.58 ab	4.1 ± 0.47 b
TOTAL SFA	47.3 ± 1.78	48.3 ± 0.52	55.7 ± 1.39	54.8 ± 1.09	49.2 ± 0.58	45.5 ± 0.65	52.8 ± 2.40	50.9 ± 1.94
TOTAL MUFA	8.1 ± 0.66 ab	9.7 ± 0.44 ac	7.0 ± 1.16 ab	8.2 ± 0.85 ab	6.9 ± 0.96 ab	12.9 ± 1.10 c	5.1 ± 0.58 b	6.4 ± 0.34 ab
TOTAL PUFA	44.5 ± 1.77	42.0 ± 0.82	37.3 ± 0.46	37.0 ± 1.21	44.0 ± 1.02	41.6 ± 1.25	42.1 ± 1.93	42.7 ± 2.00
TOTAL ω9	6.4 ± 0.59 a	7.3 ± 0.53 a	4.0 ± 0.67 a	5.2 ± 0.66 a	5.9 ± 0.70 a	11.3 ± 1.06 b	3.9 ± 0.60 a	4.3 ± 0.22 a
TOTAL ω6	42.9 ± 1.71 a	40.6 ± 0.76 a	20.8 ± 0.59 bc	21.0 ± 0.54 b	43.0 ± 1.02 a	40.4 ± 1.28 a	27.2 ± 1.77 c	23.6 ± 0.58 bc
TOTAL ω3	1.6 ± 0.34 a	1.5 ± 0.26 a	16.5 ± 0.61 b	16.0 ± 1.20 b	1.0 ± 0.19 a	1.2 ± 0.18 a	14.9 ± 1.57 b	19.1 ± 1.48 b
M/S	0.2 ± 0.02 a	0.2 ± 0.01 ab	0.1 ± 0.03 a	0.2 ± 0.02 a	0.1 ± 0.02 a	0.3 ± 0.02 b	0.1 ± 0.02 a	0.1 ± 0.01 a
P/S	1.0 ± 0.07	0.9 ± 0.03	0.7 ± 0.02	0.7 ± 0.03	0.9 ± 0.03	0.9 ± 0.04	0.8 ± 0.08	0.9 ± 0.07
ω6/ω3	31.6 ± 3.86 a	33.4 ± 4.82 a	1.3 ± 0.08 b	1.4 ± 0.11 b	47.2 ± 6.35 a	37.0 ± 4.14 a	2.0 ± 0.32 b	1.3 ± 0.08 b
18:0/18:1ω9	6.8 ± 0.54 a	5.2 ± 0.61 a	11.2 ± 1.39 b	7.6 ± 0.73 ab	6.1 ± 0.81 a	3.4 ± 0.61 a	12.0 ± 1.24 b	9.7 ± 0.74 b
18:2ω6/20:4ω6	3.8 ± 0.42 abc	4.4 ± 0.35 ac	3.5 ± 0.15 abc	4.2 ± 0.27 abc	3.2 ± 0.45 ab	5.2 ± 0.54 c	2.5 ± 0.47 b	3.1 ± 0.30 ab
20:5ω3/20:4ω6	ND	ND	3.3 ± 0.19	3.6 ± 0.30	ND	ND	1.8 ± 0.29	2.6 ± 0.17
UNSATURATION INDEX	123 ± 5 ab	115 ± 3 a	143 ± 3 ab	141 ± 6 ab	121 ± 3 a	115 ± 3 a	155 ± 8 b	165 ± 10 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.4 Effect of dietary ω3 fatty acids and cholesterol on ileal brush border membrane phosphatidylcholine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	7.9 ± 0.57	10.4 ± 2.96	8.0 ± 1.40	10.0 ± 1.14	10.6 ± 1.69	8.4 ± 4.37	13.9 ± 6.62	8.8 ± 0.40
18:0	35.4 ± 164	33.6 ± 2.13	38.8 ± 1.36	38.3 ± 2.39	32.7 ± 0.72	32.1 ± 2.28	30.1 ± 0.99	34.9 ± 1.26
18:1ω9	11.5 ± 0.50 a	11.8 ± 0.49 a	7.3 ± 1.02 bd	9.2 ± 0.40 ab	10.9 ± 0.48 a	15.2 ± 0.47 c	7.3 ± 1.08 bd	6.1 ± 0.79 d
18:2ω6	16.3 ± 0.73 ade	17.9 ± 1.85 ad	5.7 ± 0.88 b	9.6 ± 1.07 ab	18.8 ± 1.27 cd	24.7 ± 3.46 c	8.8 ± 1.77 be	10.3 ± 0.69 ab
20:4ω6	13.5 ± 0.91 ac	12.7 ± 1.37 ac	5.9 ± 1.09 b	6.1 ± 0.50 b	14.3 ± 1.34 c	10.2 ± 0.94 ab	5.7 ± 0.66 b	10.0 ± 0.79 ab
20:5ω3	0.4 ± 0.43 a	0.1 ± 0.10 a	13.3 ± 3.85 b	10.8 ± 1.20 b	0.1 ± 0.07 a	0.1 ± 0.10 a	4.8 ± 0.51 c	10.2 ± 0.80 b
22:5ω3	ND a	0.2 ± 0.13 a	1.9 ± 0.46 c	0.9 ± 0.30 b	0.1 ± 0.08 a	0.2 ± 0.03 a	1.4 ± 0.33 b	1.2 ± 0.31 b
22:6ω3	2.6 ± 0.14 a	2.7 ± 0.52 a	8.5 ± 2.52 b	5.9 ± 1.30 c	2.8 ± 0.30 a	1.8 ± 0.38 a	5.2 ± 1.25 c	10.4 ± 0.23 b
TOTAL SFA	47.5 ± 0.88 ab	47.6 ± 1.53 ab	53.0 ± 6.00 ab	53.1 ± 1.68 ab	46.1 ± 1.24 ab	42.5 ± 2.75 b	56.2 ± 7.45 a	46.2 ± 0.86 b
TOTAL MUFA	15.2 ± 0.55 ad	15.2 ± 1.20 ad	9.1 ± 1.58 b	11.3 ± 1.04 bc	13.3 ± 0.45 cd	17.2 ± 0.53 a	11.2 ± 2.18 bc	7.7 ± 1.08 b
TOTAL PUFA	37.0 ± 1.01 ab	35.5 ± 0.76 ab	37.2 ± 6.65 ab	34.5 ± 1.98 ab	37.6 ± 1.29 ab	37.8 ± 2.32 ab	30.5 ± 3.97 b	45.5 ± 0.93 a
TOTAL ω9	12.8 ± 0.42 ac	14.3 ± 1.10 a	7.3 ± 1.02 b	9.2 ± 0.40 be	12.9 ± 0.36 ac	18.0 ± 0.53 d	10.8 ± 3.02 ce	6.6 ± 0.74 b
TOTAL ω6	33.9 ± 0.91 a	32.5 ± 0.94 a	13.5 ± 0.28 b	16.9 ± 1.02 bc	34.6 ± 1.10 a	35.7 ± 2.46 a	19.1 ± 2.86 bc	23.8 ± 1.09 c
TOTAL ω3	3.0 ± 0.35 a	3.0 ± 0.53 a	23.7 ± 6.73 b	17.6 ± 2.15 c	3.0 ± 0.36 a	2.1 ± 0.36 a	11.5 ± 1.12 d	21.8 ± 0.95 bc
M/S	0.3 ± 0.02 ae	0.3 ± 0.04 a	0.2 ± 0.04 b	0.2 ± 0.02 bd	0.3 ± 0.01 ade	0.4 ± 0.04 c	0.2 ± 0.08 bde	0.2 ± 0.03b
P/S	0.8 ± 0.03	0.8 ± 0.03	0.7 ± 0.19	0.7 ± 0.05	0.8 ± 0.05	0.9 ± 0.12	0.6 ± 0.17	1.0 ± 0.03
ω6/ω3	11.6 ± 1.14 a	14.2 ± 4.64 a	0.7 ± 0.29 b	1.0 ± 0.16 b	12.4 ± 1.56 a	17.9 ± 3.19 a	1.7 ± 0.09 b	1.1 ± 0.08 b
18:0/18:1ω9	3.1 ± 0.29	2.9 ± 0.19	5.6 ± 0.99	4.2 ± 0.39	3.0 ± 0.19	2.1 ± 0.19	4.3 ± 0.63	6.3 ± 0.97
18:2ω6/20:4ω6	1.2 ± 0.07	1.6 ± 0.32	1.0 ± 0.13	1.6 ± 0.08	1.4 ± 0.20	2.5 ± 0.57	1.6 ± 0.32	1.1 ± 0.10
20:5ω3/20:4ω6	ND	ND	2.2 ± 0.44	1.8 ± 0.16	ND	ND	0.9 ± 0.09	1.1 ± 0.13
UNSATURATION INDEX	133 ± 2 a	129 ± 8 a	177 ± 36 bc	152 ± 11 abc	136 ± 6 ab	128 ± 4 a	135 ± 21 ab	198 ± 3 c

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.5 Effect of dietary ω3 fatty acids and cholesterol on jejunal brush border membrane phosphatidylethanolamine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	4.6 ± 1.28	5.1 ± 0.72	3.2 ± 0.60	4.4 ± 0.93	4.4 ± 0.87	3.2 ± 0.60	3.4 ± 0.88	4.8 ± 1.08
18:0	40.5 ± 2.21	39.8 ± 1.81	45.1 ± 2.33	35.2 ± 2.17	39.4 ± 0.65	38.1 ± 0.94	39.8 ± 2.16	39.3 ± 1.42
18:1ω9	6.3 ± 0.72 ab	8.2 ± 0.44 b	3.0 ± 0.45 a	4.4 ± 0.64 ab	5.6 ± 0.35 ab	7.9 ± 0.89 b	2.8 ± 0.44 a	3.6 ± 0.31 a
18:2ω6	17.2 ± 1.44 a	19.4 ± 0.67 a	5.9 ± 0.62 b	5.2 ± 0.54 b	16.9 ± 1.47 a	17.5 ± 1.08 a	5.9 ± 0.39 b	5.9 ± 0.56 b
20:4ω6	16.5 ± 2.47 a	19.0 ± 1.82 a	6.9 ± 0.48 b	6.1 ± 0.47 b	23.8 ± 1.84 a	20.4 ± 1.95 a	8.4 ± 0.47 b	8.0 ± 0.69 b
20:5ω3	0.2 ± 0.09 a	0.6 ± 0.23 a	13.5 ± 0.80 b	16.4 ± 1.00 b	0.7 ± 0.65 a	0.9 ± 0.52 a	13.8 ± 0.48 b	15.1 ± 1.16 b
22:5ω3	0.9 ± 0.42	0.3 ± 0.10	1.9 ± 0.25	2.6 ± 0.20	0.2 ± 0.13	0.4 ± 0.14	2.5 ± 0.23	2.1 ± 0.26
22:6ω3	1.6 ± 0.36 a	2.5 ± 0.29 a	18.0 ± 1.64 b	18.4 ± 0.87 b	3.2 ± 0.51 a	3.4 ± 0.41 a	18.1 ± 0.67 b	16.2 ± 1.62 b
TOTAL SFA	47.6 ± 1.57	45.4 ± 1.80	48.7 ± 2.69	40.5 ± 1.51	44.4 ± 1.18	42.3 ± 1.05	43.8 ± 1.70	44.6 ± 1.84
TOTAL MUFA	11.0 ± 1.85 a	10.1 ± 0.79 a	3.4 ± 0.66 b	7.8 ± 1.20 ab	6.7 ± 0.63 ab	11.0 ± 1.13 a	5.5 ± 0.80 ab	5.6 ± 0.90 ab
TOTAL PUFA	41.2 ± 2.53	44.5 ± 1.96	47.9 ± 2.63	51.7 ± 1.31	48.9 ± 1.53	46.6 ± 0.97	50.7 ± 1.50	49.8 ± 2.11
TOTAL ω9	7.2 ± 0.84 ab	9.1 ± 0.59 b	3.0 ± 0.45 a	5.6 ± 0.62 ab	6.0 ± 0.34 ab	9.8 ± 1.05 b	4.2 ± 0.26 a	4.3 ± 0.37 a
TOTAL ω6	37.8 ± 2.42 a	40.7 ± 1.71 a	14.5 ± 0.50 b	13.5 ± 0.76 b	44.6 ± 2.03 a	41.4 ± 1.31 a	16.1 ± 1.10 b	16.0 ± 1.32 b
TOTAL ω3	3.4 ± 0.45 a	3.6 ± 0.32 a	33.4 ± 2.55 b	38.2 ± 1.40 b	4.3 ± 1.37 a	5.2 ± 1.04 a	34.6 ± 0.77 b	33.9 ± 2.73 b
M/S	0.2 ± 0.04	0.2 ± 0.02	0.1 ± 0.07	0.2 ± 0.03	0.2 ± 0.01	0.3 ± 0.03	0.1 ± 0.02	0.1 ± 0.02
P/S	0.9 ± 0.08	1.0 ± 0.08	1.0 ± 1.01	1.3 ± 0.07	1.1 ± 0.06	1.1 ± 0.04	1.2 ± 0.08	1.1 ± 0.08
ω6/ω3	12.5 ± 1.77 a	12.0 ± 1.00 a	0.4 ± 0.03 b	0.4 ± 0.03 b	14.5 ± 2.93 a	10.8 ± 2.76 a	0.5 ± 0.03 b	0.5 ± 0.11 b
18:0/18:1ω9	6.8 ± 0.54 ab	5.0 ± 0.43 a	17.1 ± 3.10 c	9.2 ± 1.38 ab	7.2 ± 0.51 ab	5.3 ± 0.87 a	15.9 ± 2.87 c	11.5 ± 1.04 b
18:2ω6/20:4ω6	1.2 ± 0.27	1.1 ± 0.18	0.9 ± 0.14	0.9 ± 0.08	0.7 ± 0.10	0.9 ± 0.15	0.7 ± 0.05	0.8 ± 0.11
20:5ω3/20:4ω6	ND a	ND a	2.0 ± 0.04 b	2.9 ± 0.32 c	ND a	0.1 ± 0.04 a	1.7 ± 0.09 b	1.9 ± 0.15 b
UNSATURATION INDEX	143 ± 9 a	152 ± 8 a	232 ± 16 bc	256 ± 8 b	171 ± 8 ac	166 ± 6 ac	246 ± 6 b	240 ± 14 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.6 Effect of dietary ω3 fatty acids and cholesterol on ileal brush border membrane phosphatidylethanolamine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	4.9 ± 1.04 a	2.6 ± 1.30 a	8.3 ± 2.13 ab	5.5 ± 2.37 a	3.2 ± 1.10 a	8.1 ± 2.02 ab	4.6 ± 2.05 a	10.8 ± 3.04 b
18:0	31.8 ± 4.15	37.2 ± 1.87	35.5 ± 1.07	37.3 ± 2.72	38.8 ± 1.23	33.6 ± 0.70	39.6 ± 1.90	41.7 ± 2.27
18:1ω9	9.0 ± 1.49 ab	10.1 ± 1.31 b	7.7 ± 1.51 ab	8.1 ± 1.26 ab	8.9 ± 0.54 ab	13.6 ± 1.29 c	5.2 ± 0.83 a	5.9 ± 0.18 a
18:2ω6	7.9 ± 0.78 ac	9.7 ± 0.75 ac	6.6 ± 3.73 a	7.5 ± 1.21 ac	11.9 ± 0.87 bc	14.8 ± 1.59 b	4.5 ± 1.35 a	5.7 ± 0.54 a
20:4ω6	22.4 ± 3.20 a	24.8 ± 1.94 a	8.0 ± 3.04 b	9.9 ± 1.69 b	26.2 ± 2.60 a	14.4 ± 2.58 b	9.4 ± 0.98 b	7.5 ± 1.39 b
20:5ω3	1.7 ± 1.33 a	1.2 ± 0.70 a	6.3 ± 1.37 b	10.0 ± 1.58 c	0.1 ± 0.09 a	1.3 ± 0.50 a	7.8 ± 0.85 bc	8.0 ± 2.18 bc
22:5ω3	0.6 ± 0.38 a	0.8 ± 0.34 a	1.9 ± 1.03 a	6.2 ± 1.68 b	1.3 ± 0.22 a	0.6 ± 0.13 a	6.9 ± 1.73 b	1.7 ± 1.18 a
22:6ω3	4.3 ± 1.02 a	5.9 ± 1.05 a	8.4 ± 2.74 ab	11.3 ± 2.97 b	5.6 ± 1.01 a	2.8 ± 0.50 a	16.4 ± 4.01 c	8.0 ± 2.56 ab
TOTAL SFA	40.9 ± 4.33 a	40.8 ± 0.52 a	49.7 ± 4.49 ab	44.7 ± 4.89 a	42.2 ± 2.33 a	45.0 ± 2.42 a	46.5 ± 4.34 a	58.5 ± 4.24 b
TOTAL MUFA	13.1 ± 1.83 abc	12.0 ± 1.90 abc	16.1 ± 2.09 bc	10.1 ± 2.08 ab	10.3 ± 1.08 ab	16.7 ± 1.31 c	7.2 ± 1.37 a	7.9 ± 0.86 a
TOTAL PUFA	46.2 ± 5.71	45.6 ± 1.61	33.7 ± 4.97	45.0 ± 6.86	46.3 ± 3.19	36.5 ± 3.37	46.1 ± 5.86	33.3 ± 4.75
TOTAL ω9	12.4 ± 1.96 a	13.2 ± 1.48 ab	15.9 ± 2.01 bc	9.6 ± 1.67 ad	11.1 ± 0.76 ad	17.0 ± 1.36 c	6.8 ± 1.00 d	6.9 ± 0.37 d
TOTAL ω6	39.5 ± 4.77 a	37.7 ± 1.42 a	16.4 ± 6.52 b	17.4 ± 0.96 b	39.4 ± 2.33 a	30.9 ± 3.00 a	14.7 ± 0.62 b	14.5 ± 0.32 b
TOTAL ω3	6.8 ± 1.16 a	7.9 ± 0.49 a	17.3 ± 4.15 ab	27.6 ± 6.06 bc	6.9 ± 1.15 a	5.7 ± 1.06 a	31.4 ±6.28 c	18.8 ± 4.45 b
M/S	0.3 ± 0.06 ab	0.3 ± 0.05 ab	0.3 ± 0.04 ab	0.2 ± 0.03 ab	0.2 ± 0.02 ab	0.4 ± 0.02 b	0.2 ± 0.02 a	0.1 ± 0.02a
P/S	1.2 ± 0.21 a	1.1 ± 0.04 ab	0.7 ± 0.16 ab	1.1 ± 0.23 ab	1.1 ± 0.12 ab	0.8 ± 0.13 ab	1.0 ± 0.19 ab	0.6 ± 0.12 b
ω6/ω3	6.1 ± 0.53	4.8 ± 0.31	1.4 ± 0.82	0.8 ± 0.22	6.3 ± 0.89	6.4 ± 1.77	0.1 ± 0.21	0.9 ± 0.25
18:0/18:1ω9	3.8 ± 0.50	4.0 ± 0.57	5.1 ± 0.79	5.0 ± 0.76	4.4 ± 0.14	2.5 ± 0.22	8.0 ± 0.74	7.1 ± 0.18
18:2ω6/20:4ω6	0.4 ± 0.10	0.4 ± 0.06	0.8 ± 0.29	0.9 ± 0.27	0.5 ± 0.09	1.1 ± 0.22	0.6 ± 0.24	0.9 ± 0.27
20:5ω3/20:4ω6	0.1 ± 0.06 a	0.1 ± 0.04 a	1.2 ± 0.33 b	1.0 ± 0.08 b	ND a	0.1 ± 0.05 a	0.8 ± 0.01 b	1.0 ± 0.11 b
UNSATURATION INDEX	198 ± 26 ab	194 ± 8 ab	164 ± 19 ab	215 ± 36 ab	187 ± 15 ab	144 ± 13 a	230 ± 35 b	154 ± 27 a

Values with different letters are significantly different, $p<0.05$

ND, not detected

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.7 Effect of dietary ω3 fatty acids and cholesterol on jejunal enterocyte microsomal membrane phosphatidycholine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	21.6 ± 0.40	21.0 ± 0.79	22.3 ± 1.41	21.7 ± 1.30	22.0 ± 0.23	21.2 ± 0.25	20.7 ± 0.63	20.7 ± 0.80
18:0	26.4 ± 0.64	26.0 ± 1.09	31.6 ± 0.56	30.2 ± 0.89	24.9 ± 0.50	23.5 ± 0.36	30.7 ± 0.66	31.4 ± 1.75
18:1ω9	7.5 ± 0.43 ac	8.6 ± 1.02 a	3.3 ± 0.23 b	4.1 ± 0.17 c	7.2 ± 0.35 a	10.2 ± 0.60 d	2.6 ± 0.09 e	4.7 ± 1.15 be
18:2ω6	26.2 ± 0.99 a	26.1 ± 0.79 a	12.4 ± 0.35 b	13.7 ± 0.32 b	29.1 ± 0.71 a	30.0 ± 0.94 a	15.2 ± 0.54 b	15.0 ± 1.32 b
20:4ω6	8.8 ± 0.41 a	8.4 ± 0.43 ac	5.2 ± 0.27 b	4.9 ± 0.20 b	9.7 ± 0.37 d	7.2 ± 0.62 e	8.0 ± 0.49 c	6.1 ± 0.64 be
20:5ω3	0.3 ± 0.13	0.5 ± 0.16 a	13.3 ± 0.61 b	14.1 ± 0.41 b	0.1 ± 0.02 a	0.2 ± 0.03 a	12.9 ±1.40 b	11.5 ± 0.99 b
22:5ω3	0.1 ± 0.01	0.1 ± 0.02	0.5 ± 0.07	0.7 ± 0.08	0.1 ± 0.01	0.1 ± 0.01	0.4 ± 0.02	0.4 ± 0.04
22:6ω3	1.2 ± 0.06 a	1.3 ± 0.06 a	5.0 ± 0.27 b	4.7 ± 0.39 b	1.0 ± 0.05 a	1.1 ± 0.06 c	4.2 ± 0.23 b	4.8 ± 0.57 b
TOTAL SFA	50.8 ± 0.52	50.0 ± 0.81	56.1 ± 1.24	53.9 ± 0.70	49.4 ± 0.43	47.1 ± 0.39	53.4 ± 1.17	54.1 ± 2.30
TOTAL MUFA	10.1 ± 0.58 a	11.7 ± 1.24 a	6.7 ± 0.48 b	7.4 ± 0.14 b	8.8 ± 0.41	12.2 ± 0.78	5.0 ± 0.15	7.2 ± 1.00
TOTAL PUFA	38.1 ± 0.75	38.3 ± 1.05	37.2 ± 1.01	38.7 ± 0.65	41.8 ± 0.75	40.7 ± 0.92	41.6 ± 1.25	38.7 ± 3.26
TOTAL ω9	8.9 ± 0.88 ac	9.2 ± 1.04 ac	3.9 ± 0.20 b	4.5 ± 0.17 b	7.6 ± 0.38 a	10.7 ± 0.61 c	3.0 ± 0.10 b	5.0 ± 1.13 b
TOTAL ω6	36.4 ± 0.77 a	36.2 ± 1.07 a	18.1 ± 0. 61 b	19.0 ± 0.50 b	40.5 ± 0.78 a	39.2 ± 0.09 a	23.9 ± 0.94	21.8 ± 1.93 b
TOTAL ω3	1.8 ± 0.14 a	2.1 ± 0.21 a	19.1 ± 0.56 b	19.6 ± 0.68 b	1.4 ± 0.08 a	1.5 ± 0.09 a	17.7 ± 1.33 b	16.9 ± 1.44 b
M/S	0.20 ± 0.01 a	0.24 ± 0.03 a	0.12 ± 0.01 b	0.14 ± 0.01 b	0.18 ± 0.01 a	0.26 ± 0.02	0.09 ± 0.001	0.13 ± 0.01
P/S	0.8 ± 0.02	0.8 ± 0.03	0.7 ± 0.03	0.7 ± 0.02	0.9 ± 0.02	0.9 ± 0.02	0.8 ± 0.04	0.7 ± 0.07
ω8/ω3	21.7 ± 1.69 a	18.6 ± 1.80 a	1.0 ± 0.03 h	1.0 ± 0.05 b	31.4 ± 2.50 a	27.5 ± 1.57 a	1.6 ± 0.33 b	1.3 ± 0.05 b
18:0/18:1ω9	3.6 ± 0.24 a	5.4 ± 2.67 ac	10.2 ± 1.17 b	7.6 ± 0.40 b	3.6 ± 0.18 a	2.4 ± 0.15 a	11.7 ± 0.36 b	8.1 ± 0.65 c
18:2ω6/20:4ω6	3.1 ± 0.27 ab	3.2 ± 0.19 a	2.4 ± 0.09 b	2.8 ± 0.09 ab	3.1 ± 0.13 a	4.5 ± 0.41 c	2.0 ± 0.09 b	2.5 ± 0.13 b
20:5ω3/20:4ω6	0.04 ± 0.02 a	0.07 ± 0.03 a	2.6 ± 0.15 b	2.9 ± 0.18 b	0.02 ± 0.001 a	0.03 ± 0.001 a	1.8 ± 0.21 b	2.0 ± 0.10 b
UNSATURATION INDEX	115 ± 2 a	114 ± 2 a	154 ± 4 b	158 ± 3 b	119 ± 2 a	115 ± 2 a	162 ± 6 b	153 ± 11 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.8 Effect of dietary ω3 fatty acids and cholesterol on ileal enterocyte microsomal membrane phosphatidycholine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	30.2 ± 0.73	29.1 ± 0.76	27.6 ± 1.83	29.3 ± 0.41	26.3 ± 0.66	26.8 ± 1.01	27.9 ± 0.73	27.4 ± 0.82
18:0	23.0 ± 0.78	21.6 ± 0.26	27.4 ± 1.12	25.0 ± 0.49	22.7 ± 0.37	22.9 ± 0.56	27.2 ± 0.44	28.3 ± 0.71
18:1ω9	11.3 ± 0.28 a	12.5 ± 0.37 a	6.9 ± 0.51 bc	7.5 ± 0.29 b	10.8 ± 0.39 a	11.6 ± 0.84 a	5.0 ± 0.25 c	5.2 ± 0.29 c
18:2ω6	14.1 ± 0.44 a	15.8 ± 0.81 ac	9.9 ± 0.67 b	10.4 ± 0.64 b	19.8 ± 1.03 c	20.2 ± 1.19 c	10.8 ± 0.32 b	10.2 ± 0.42 b
20:4ω6	11.4 ± 0.36 a	10.3 ± 0.32 a	5.2 ± 0.30 b	5.5 ± 0.25 b	11.2 ± 0.52 a	9.1 ± 0.91 a	7.7 ± 0.29 c	8.6 ± 0.64 c
20:5ω3	0.2 ± 0.06 a	0.3 ± 0.07 a	10.7 ± 0.82 b	8.6 ± 0.28 bc	0.2 ± 0.07 a	0.2 ± 0.07 a	8.0 ± 0.86 bc	7.2 ± 6.00 c
22:5ω3	0.2 ± 0.06 a	0.2 ± 0.03 a	1.2 ± 0.12 b	0.9 ± 0.15 b	0.2 ± 0.02 a	0.2 ± 0.11 a	1.2 ± 0.08 b	0.9 ± 0.04 b
22:6ω3	1.7 ± 0.09 a	1.6 ± 0.07 a	4.7 ± 0.14 b	3.9 ± 0.19 b	1.8 ± 0.09 a	1.8 ± 0.13 a	6.4 ± 0.34 c	6.8 ± 0.47 c
TOTAL SFA	54.7 ± 0.47 a	52.1 ± 0.61 ab	56.7 ± 1.26 a	57.6 ± 1.13 a	50.3 ± 0.77 b	51.1 ± 1.48 b	56.5 ± 0.88 a	57.3 ± 0.91 a
TOTAL MUFA	15.3 ± 0.42 a	17.1 ± 0.61 a	10.5 ± 0.93 b	11.4 ± 0.46 b	13.8 ± 0.48 b	14.4 ± 0.86 a	8.5 ± 0.29 b	8.1 ± 0.58 b
TOTAL PUFA	29.9 ± 0.03 a	30.9 ± 0.66 a	32.8 ± 1.91 ab	30.9 ± 0.97 ab	35.9 ± 0.79 b	34.5 ± 1.17 b	35.0 ± 0.85 b	34.6 ± 0.87 b
TOTAL ω9	11.7 ± 0.22 a	12.8 ± 0.38 a	7.4 ± 0.57 b	7.9 ± 0.36 b	11.0 ± 0.39 a	11.8 ± 0.83 a	5.7 ± 0.27 c	5.7 ± 0.36 c
TOTAL ω6	27.5 ± 0.28	28.6 ± 0.69	15.6 ± 0.88	16.7 ± 0.86	33.7 ± 0.76	32.2 ± 1.06	19.2 ± 0.49	19.5 ± 0.74
TOTAL ω3	2.3 ± 0.12 a	2.3 ± 0.16 a	17.2 ± 1.19 b	14.3 ± 0.62 b	2.3 ± 0.10 a	2.3 ± 0.19 a	15.8 ± 0.74 b	151 ± 0.56 b
M/S	0.28 ± 0.01 a	0.33 ± 0.01 a	0.18 ± 0.02 bc	0.20 ± 0.01 b	0.28 ± 0.01 a	0.29 ± 0.02 a	0.15 ± 0.01 c	0.14 ± 0.01 c
P/S	0.6 ± 0.01 a	0.6 ± 0.02 ab	0.6 ± 0.05 a	0.5 ± 0.02 a	0.7 ± 0.03 c	0.7 ± 0.04 bc	0.6 ± 0.02 ab	0.6 ± 0.02 ab
ω6/ω3	12.0 ± 0.58 a	13.1 ± 1.12 a	0.9 ± 0.04 b	1.2 ± 0.10 b	15.0 ± 0.68 a	14.6 ± 1.01 a	1.3 ± 0.08 b	1.3 ± 0.07 b
18:0/18:1ω9	2.1 ± 0.11 a	1.7 ± 0.05 a	4.3 ± 0.64 b	3.4 ± 0.20 b	2.1 ± 0.11 a	2.1 ± 0.24 a	5.6 ± 0.30 b	5.7 ± 0.44 b
18:2ω6/20:4ω6	1.3 ± 0.08 a	1.6 ± 0.12 ab	1.9 ± 0.13 b	1.9 ± 0.07 b	1.8 ± 0.18 b	2.5 ± 0.34 c	1.4 ± 0.07 b	1.3 ± 0.12 ab
20:5ω3/20:4ω6	0.02 ± 0.01 a	0.03 ± 0.01 a	2.1 ± 0.08 b	1.6 ± 0.06 c	0.02 ± 0.01 a	0.02 ± 0.01 a	1.1 ± 0.11 d	0.9 ± 0.12 d
UNSATURATION INDEX	109 ± 1 a	110 ± 1 a	142 ± 7 b	130 ± 4 b	119 ± 2 a	113 ± 4 a	148 ± 4 b	147 ± 4 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.9 Effect of dietary ω3 fatty acids and cholesterol on jejunal enterocyte microsomal membrane phosphatidylethanolamine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	5.6 ± 0.26	5.6 ± 0.27	6.1 ± 0.41	5.2 ± 0.15	5.3 ± 0.14	5.5 ± 0.15	5.1 ± 0.26	5.2 ± 0.23
18:0	35.3 ± 0.88	34.1 ± 0.45	36.4 ± 0.78	35.1 ± 0.64	35.6 ± 0.44	32.4 ± 0.73	40.2 ± 1.15	37.6 ± 0.64
18:1ω9	8.5 ± 0.80 a	10.3 ± 0.48 a	3.6 ± 0.16 b	3.7 ± 1.14 b	7.5 ± 0.36 a	10.3 ± 0.66	2.7 ± 0.08	3.1 ± 0.11
18:2ω6	14.3 ± 0.97 a	14.7 ± 0.42 a	5.7 ± 0.14 b	5.6 ± 0.23 b	16.6 ± 0.51 ac	18.6 ± 0.79 c	5.8 ± 0.10 b	6.1 ± 0.14 b
20:4ω6	23.6 ± 0.51 a	22.3 ± 0.48 a	6.4 ± 0.20 b	6.1 ± 0.40 b	23.9 ± 0.69 a	20.8 ± 0.64 a	8.3 ± 0.43 c	6.5 ± 0.36 bc
20:5ω3	0.5 ± 0.13 a	0.4 ± 0.07 a	12.6 ± 0.56 b	12.4 ± 0.31 b	0.3 ± 0.05 a	0.4 ± 0.03 a	12.8 ± 0.53 b	12.6 ± 0.50 b
22:5ω3	0.6 ± 0.04 a	0.6 ± 0.04 a	3.7 ± 0.18 b	3.8 ± 0.21 b	0.6 ± 0.06 a	0.7 ± 0.04 a	2.4 ± 0.16 b	2.9 ± 0.13 b
22:6ω3	4.3 ± 0.17 a	4.1 ± 0.16 a	20.2 ± 1.41 b	22.2 ± 0.66 b	4.0 ± 0.29 a	4.3 ± 0.23 a	18.1 ± 0.90 b	21.5 ± 1.22 b
TOTAL SFA	42.3 ± 0.75	41.3 ± 0.36	43.7 ± 0.87	41.8 ± 0.65	42.3 ± 0.58	39.4 ± 0.67	46.4 ± 1.37	44.1 ± 0.73
TOTAL MUFA	10.3 ± 1.06 a	12.2 ± 0.57 a	6.3 ± 0.42 b	6.0 ± 0.18 b	8.8 ± 0.35 a	11.8 ± 0.79 a	4.9 ± 0.21 c	5.0 ± 0.17 c
TOTAL PUFA	47.2 ± 0.65	46.3 ± 0.56	50.0 ± 1.26	52.2 ± 0.68	48.9 ± 0.77	48.8 ± 0.69	48.7 ± 1.47	50.9 ± 0.78
TOTAL ω9	9.1 ± 0.89 a	10.9 ± 0.52 a	4.5 ± 0.25 b	4.7 ± 0.20 b	8.0 ± 0.36 a	10.8 ± 0.70 a	3.4 ± 0.11 c	3.8 ± 0.13 bc
TOTAL ω6	41.5 ± 0.70 a	40.8 ± 0.49 a	13.3 ± 0.17 b	13.1 ± 0.67 b	43.8 ± 0.78 a	43.0 ± 0.68 a	15.0 ± 0.37 b	13.7 ± 0.35 b
TOTAL ω3	5.7 ± 0.36 a	5.4 ± 0.26 a	36.7 ± 1.30 b	39.1 ± 0.86 b	5.1 ± 0.39 a	5.8 ± 0.30 a	33.7 ± 1.25 b	37.2 ± 0.91 b
M/S	0.25 ± 0.03 ac	0.30 ± 0.01 a	0.14 ± 0.01 b	0.14 ± 0.001 b	0.21 ± 0.01 c	0.30 ± 0.02 a	0.11 ± 0.001 b	0.11 ± 0.001 b
P/S	1.1 ± 0.02	1.1 ± 0.02	1.2 ± 0.05	1.3 ± 0.03	1.2 ± 0.03	1.2 ± 0.03	1.1 ± 0.06	1.2 ± 0.03
ω6/ω3	7.6 ± 0.60 a	7.6 ± 0.37 a	0.4 ± 0.02 b	0.3 ± 0.02 b	9.0 ± 0.70 a	7.6 ± 0.43 a	0.5 ± 0.02 b	0.4 ± 0.02 b
18:0/18:1ω9	4.4 ± 0.40 a	3.4 ± 0.22 a	10.1 ± 0.51 b	9.7 ± 0.44 b	4.8 ± 0.24 a	3.3 ± 0.26 c	14.9 ± 0.37 e	12.4. ± 0.57 d
18:2ω6/20:4ω6	0.6 ± 0.05 a	0.7 ± 0.03 a	0.9 ± 0.04 b	0.9 ± 0.04 b	0.7 ± 0.06 a	0.9 ± 0.06 b	0.7 ± 0.04 a	1.0 ± 0.07 b
20:5ω3/20:4ω6	0.02 ± 0.01 a	0.02 ± 0.001 a	2.0 ± 0.11 b	2.1 ± 0.15 b	0.01 ± 0.001 a	0.02 ± 0.001 a	1.6 ± 0.07 b	2.0 ± 0.06 b
UNSATURATION INDEX	179 ± 3 a	175 ± 2 a	251 ± 7 b	263 ± 4 b	178 ± 2 a	177 ± 2 a	239 ± 8 b	254 ± 5 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

Table 9.10 Effect of dietary ω3 fatty acids and cholesterol on ileal enterocyte microsomal membrane phosphatidylethanolamine fatty acid composition in control and diabetic rats

	CONTROL				DIABETIC			
	BT	BTC	FO	FOC	BT	BTC	FO	FOC
16:0	6.9 ± 0.33	6.5 ± 0.41	5.9 ± 0.43	6.3 ± 0.42	5.8 ± 0.22	5.7 ± 0.16	5.9 ± 0.32	5.9 ± 0.31
18:0	31.3 ± 0.65	30.2 ± 0.70	33.1 ± 1.66	31.5 ± 0.84	30.9 ± 0.48	30.2 ± 0.45	32.6 ± 0.68	31.9 ± 0.54
18:1ω9	11.3 ± 0.33 a	11.8 ± 0.45 a	5.3 ± 0.31 b	6.1 ± 0.20 b	9.3 ± 0.43 c	10.9 ± 0.54 a	3.7 ± 0.28 d	3.8 ± 0.18 d
18:2ω6	8.1 ± 0.24 a	8.5 ± 0.26 a	4.4 ± 0.27 b	5.4 ± 0.27 b	11.2 ± 0.58 c	14.0 ± 0.61 d	5.1 ± 0.19 b	5.4 ± 0.17 b
20:4ω6	24.2 ± 0.62	23.3 ± 0.41	7.9 ± 0.27	8.7 ± 0.35	24.9 ± 0.78	22.3 ± 0.62	8.9 ± 0.32	9.3 ± 0.51
20:5ω3	0.5 ± 0.14 a	0.6 ± 0.18 a	11.7 ± 0.32 b	11.2 ± 0.43 b	0.5 ± 0.13 a	0.3 ± 0.04 a	9.9 ± 0.38 c	8.8 ± 0.58 c
22:5ω3	1.0 ± 0.08 a	1.0 ± 0.09 a	8.0 ± 0.85 b	8.0 ± 0.60 b	1.1 ± 0.41 a	1.0 ± 0.07 a	7.9 ± 0.39 b	7.4 ± 0.35 b
22:6ω3	5.6 ± 0.25 a	5.7 ± 0.36 a	17.3 ± 0.78 b	14.5 ± 0.67 b	6.1 ± 0.42 a	6.0 ± 0.40 a	19.4 ± 0.78 bc	21.4 ± 1.28 c
TOTAL SFA	40.0 ± 0.63	38.1 ± 0.67	40.2 ± 1.91	40.0 ± 1.82	47.9 ± 0.63	36.8 ± 0.48	39.8 ± 1.01	38.9 ± 0.67
TOTAL MUFA	13.6 ± 0.52 ac	14.8 ± 0.48 a	7.8 ± 0.36 bd	8.8 ± 0.51 b	11.2 ± 0.58 c	13.0 ± 0.61 ac	6.3 ± 0.48 bd	6.0 ± 0.33 d
TOTAL PUFA	46.2 ± 0.63	46.9 ± 0.80	52.0 ± 1.74	51.2 ± 1.65	50.9 ± 0.89	50.1 ± 0.55	53.9 ± 1.41	55.2 ± 0.79
TOTAL ω9	12.1 ± 0.40 a	12.5 ± 0.47 a	6.4 ± 0.36 be	7.5 ± 0.39 e	9.9 ± 0.48 c	11.6 ± 0.61 ac	5.1 ± 0.44 bd	4.9 ± 0.21 d
TOTAL ω6	38.3 ± 0.66 a	38.7 ± 0.60 a	14.3 ± 0.42 b	16.2 ± 0.72 b	42.3 ± 1.13 a	42.0 ± 0.32 a	15.4 ± 0.33 b	16.5 ± 0.50 b
TOTAL ω3	8.0 ± 0.29 a	8.2 ± 0.62 a	37.7 ± 1.70 b	35.0 ± 1.56 b	8.6 ± 0.67 a	8.1 ± 0.49 a	38.4 ± 1.39 b	38.7 ± 0.92 b
M/S	0.34 ± 0.02 a	0.39 ± 0.01 a	0.20 ± 0.01 b	0.23 ± 0.02 b	0.30 ± 0.02 a	0.35 ± 0.02 a	0.16 ± 0.01 bc	0.15 ± 0.01 c
P/S	1.2 ± 0.03	1.2 ± 0.04	1.3 ± 0.10	1.3 ± 0.09	1.4 ± 0.04	1.4 ± 0.03	1.4 ± 0.07	1.4 ± 0.04
ω6/ω3	4.9 ± 0.21 a	5.0 ± 0.43 a	0.4 ± 0.02 b	0.5 ± 0.03 b	5.2 ± 0.39 a	5.4 ± 0.36 a	0.4 ± 0.02 b	0.4 ± 0.02 b
18:0/18:1ω9	2.8 ± 0.12 a	2.6 ± 0.14 a	6.5 ± 0.56 bd	5.2 ± 0.23 d	3.4 ± 0.19 a	2.8 ± 0.16 a	9.2 ± 0.55 c	8.6 ± 0.49 bc
18:2ω6/20:4ω6	0.3 ± 0.02 a	0.4 ± 0.01 a	0.6 ± 0.05 bc	0.6 ± 0.03 be	0.5 ± 0.02 c	0.6 ± 0.05 d	0.6± 0.03 d	0.6 ± 0.05 cd
20:5ω3/20:4ω6	0.02 ± 0.01 a	0.03 ± 0.01 a	1.5 ± 0.05 b	1.3 ± 0.06 b	0.02 ± 0.01 a	0.02 ± 0.00 a	1.1 ± 0.04 b	1.0 ± 0.05 b
UNSATURATION INDEX	193 ± 3 a	196 ± 3 a	260 ± 9 b	248 ± 8 b	203 ± 3 a	195 ± 3 a	267 ± 8 b	273 ± 5 b

Values with different letters are significantly different, $p<0.05$
ND, not detected
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/S, monounsaturates/saturates; P/S, polyunsaturates/saturates

The diets were: BT, beef tallow low cholesterol; BTC, beef tallow high cholesterol; FO, fish oil low cholesterol; FOC, fish oil high cholesterol.

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CHAPTER 10

GENERAL DISCUSSION

The first membrane that nutrients must cross is the BBM of the enterocytes that line the villi of the small intestine. Modifications in the lipid composition of the BBM may be associated with changes in intestinal nutrient transport. The passive movement of lipids across the BBM, as well as the function of BBM transport proteins (SGLT1, GLUT5) and binding proteins (NHE_3/$FABP_{pm}$, cholesterol transfer protein) may be influenced by their lipid environment. Lipid composition characterizes the fluidity of a membrane, a property which describes the relative motional freedom of molecules within the membrane. Alterations in membrane lipid composition -- cholesterol or phospholipid content, phospholipid composition or phospholipid fatty acid composition -- may alter the conformation of binding sites on proteins, as well as permeation of molecules through the membrane. A model is proposed to describe the diet- and diabetes-mediated changes in the enterocyte (Figure 10.1). This model is based on the studies presented in this thesis.

10.1 Mechanisms of Nutrient Absorption

In order to understand the mechanisms by which diet and diabetes modify the uptake of lipids and sugars, it was first necessary to examine the mechanisms by which the uptake of these nutrients occurs. Dietary lipids are solubilized in bile salt micelles, and then diffuse across the intestinal unstirred water layer. The role of bile salt micelles in the delivery of lipids to the BBM may be different for fatty acids versus cholesterol. Using BBMV, it appears that cholesterol partitions directly from the micelle into the BBM (collision model), whereas fatty acid uptake occurs both by direct partitioning as well as partitioning first into an aqueous phase and uptake then from this aqueous environment into the BBM (aqueous/dissociation model) (Proulx et al., 1984c; Westergaard and Dietschy, 1976; Chapter 3). An equilibrium exists between the micellar and monomeric concentration of fatty acid in the unstirred water layer in the intestinal lumen. The BBM Na^+/H^+ exchanger, NHE_3, maintains an acid microclimate in the unstirred water layer immediately adjacent to the BBM; this acid microclimate promotes protonation and permeation of fatty acid monomers into the BBM (Shiau, 1990). As the monomeric form of the lipid permeates into the membrane, there is further partitioning of fatty acid from the micelle. At the BBM, fatty acid may cross the lipophilic membrane bilayer by passive diffusion, or with the aid of the $FABP_{pm}$ to the cytoplasm of the cell (Stremmel, 1988).

A small portion of the fatty acid taken up into the BBM may remain in a free fatty acid pool present in the membrane. The membrane free fatty acid pool comprises up to 15% of the membrane lipid (Keelan et al., 1985a-d, 1986a; Pind and Kuksis, 1987; Dudeja et al., 1989), and may also include end products formed from deacylation reactions resulting from the activity of phospholipases, such as the Ca^{2+}-independent phospholipase A_2 in the BBM. The physiological role of the Ca^{2+}-independent phospholipase A_2 is not clear. This phospholipase has a high specificity for PE, and may provide a constant source of long chain polyunsaturated fatty acids to the free fatty acid pool. Although the size of the free fatty acid pool is significant, it is not clear what physiological role that it plays. The free fatty acid pool could be very important for cell signal transduction, as well as for the fine-tuning of the lipid composition of the BBM. The changes in BBM FFA reflect the alterations in dietary lipids, although to a lesser degree than triglycerides and phospholipids in the enterocyte (unpublished observations, 1996).

Most of the free fatty acid taken up by the BBM passes through the membrane bilayer. Once across the bilayer, FFA exits the cytosolic leaflet of the BBM by binding to the I- or L-$FABP_c$ for transport to the EMM and other organelles within the enterocyte, and may influence nuclear as well as microsomal events.

10.2 Changing Dietary Fatty Acids Plus Diabetes Alters Nutrient Absorption and BBM Lipid Composition

Dietary fatty acids are modulators of BBM phospholipid fatty acid composition and intestinal function (Keelan et al., 1990a, Thomson et al., 1986,1987c). Generally, saturated dietary lipids (palmitic and stearic acid) were associated with increases in intestinal nutrient transport and increases in the saturation of BBM phospholipid fatty acids, while ω6 polyunsaturated dietary lipids were associated with a decline in intestinal nutrient transport and an increase in the unsaturation of BBM phospholipid fatty acids. The degree by which intestinal nutrient transport may be altered is influenced by the number of double bonds present in the dietary fatty acids. Feeding a diet enriched with 18:1ω9 (OA) decreased glucose uptake, as compared with feeding a saturated fatty acid diet enriched with 16:0 and 18:0 (SFA) (Thomson et al., 1987c). Feeding a diet enriched with 18:3ω3 (LNA) reduced glucose uptake with respect to feeding SFA or OA, but not to the same degree as feeding 18:2ω6 (LA) which reduced glucose uptake to the greatest extent (Thomson et al., 1987c). Feeding a diet enriched with ω3 fatty acids derived from fish oil (20:5ω3 and 22:6ω3; FO) reduced glucose uptake even further than observed with feeding LA (Thomson et al., 1988b).

BBM phospholipid composition was not altered significantly in animals fed diets of different fatty acid unsaturation (Keelan et al., 1990a; Thomson et al., 1986, 1987d). Changes in the fatty acid composition of BBM PC and PE were observed with feeding diets differing in fatty acid unsaturation, although these changes were not as dramatic as those reported for the membrane phospholipids of other tissues (Foot et al., 1982; Innis and Clandinin., 1981; Croft et al., 1984: Clandinin et al., 1985). In the intestine, BBM phospholipid composition changed in a number of models of intestinal adaptation such as aging, streptozotocin-induced diabetes, chronic ethanol feeding, intestinal resection, and following external abdominal irradiation (Keelan et al, 1985a,b,c,d,1986a). Thus, modification of BBM phospholipids explains intestinal adaptation in only some models of adaptation, such as diabetes mellitus, but not with changes in dietary triglycerides.

Streptozotocin-induced diabetes was associated with changes in intestinal nutrient transport, as well as BBM phospholipid composition and BBM phospholipid fatty acid composition (Dudeja et al., 1990; Debnam et al., 1990; Fedorak et al., 1987, 1989, 1990). *In vitro* glucose and lipid uptake into the intestine were increased with diabetes, and were mediated in part by a decline in the effective resistance of the UWL and increases in BBM passive permeability (Thomson, 1980, 1983). Diabetes was associated with an increase in BBM phospholipid content due to an increase in PC and PE (Keelan et al., 1985a), and as well there were alterations in the fatty acid composition of PC and PE (Keelan et al, 1990b). Membrane lipid composition changes alter the lipophilic properties of the BBM, which may result in variations in the permeability properites of the BBM. It is possible, however, that the changes in lipid uptake were only partially explained by alterations in the lipophilic properties of the BBM. There may have been additional contributions to changes in the protein-mediated components of lipid uptake, although there is no direct information as to whether diet or diabetes modifies the activity of NHE_3 or $FABP_{pm}$.

10.3 Changing Dietary Fatty Acids Plus Diabetes Alters *in vitro* and and *in vivo* Nutrient Uptake

Feeding animals a diet enriched in ω6 polyunsaturated fatty acids (linoleic acid [LA]) reduced the enhanced uptake of glucose associated with diabetes (Thomson et al., 1987a,b). In non-diabetic control rats, the largest reduction in D-glucose uptake was observed with animals fed a diet enriched with FO, and may be even more successful than LA in decreasing the enhanced nutrient uptake associated with diabetes (Thomson et al., 1988b). This was confirmed with the in vitro studies in BBMV (Chapter 4) and intestinal sheets (Chapter 5), as well as with *in vivo* perfusion (Chapter 6). The V_{max} for D-glucose uptake into jejunal BBMV of diabetic rats was 9-

fold greater than in non-diabetic rats fed BT, and 6-fold greater than in non-diabetic rats fed FO (Chapter 4). A 50% decline in glucose uptake was observed in jejunal BBMV isolated from diabetic animals fed FO as compared with those fed BT. Diabetes and diet did not influence the K_m of D-glucose uptake into jejunal BBMV. *In vitro* intestinal sheet studies confirmed that jejunal and ileal D-glucose uptake is increased in diabetic as compared with non-diabetic animals fed either BT or FO, and may be significantly reduced in diabetic animals fed FO as compared to those fed BT (Chapter 5). Unlike the *in vitro* BBMV studies, diabetes was associated with an increase in jejunal V_{max} with *in vivo* perfusion studies in animals fed FO, and a decline in V_{max} in animals fed BT (Chapter 6). Ileal D-glucose uptake was increased with diabetes in the *in vivo* perfusion studies in animals fed either BT or FO. Feeding FO to diabetic animals, however, significantly reduced jejunal and ileal D-glucose uptake following *in vivo* perfusion, as compared with diabetic animals fed BT. The intestinal uptake of D-glucose was generally reduced in diabetic animals fed FO as compared with those fed BT (Chapters 4,5,6), and was associated with a decline in fasting plasma glucose levels observed with long-term feeding (Lakey et al., 1993). Thus, these dietary changes in transport occur both *in vitro* and *in vivo* and have important metabolic effects in the diabetic rats.

Intestinal lipid uptake was also increased with streptozotocin induced diabetes (Thomson et al., 1988a). Feeding diabetic animals a diet enriched in ω3 fatty acids derived from fish oil for 10 weeks reduced the HgA_1C concentration and prevented the enhanced uptake of stearic acid, linoleic acid and cholesterol which was observed in diabetic animals fed a diet enriched in saturated fatty acids (Lakey et al., 1993). The decline in lipid uptake was associated with a fall in fasting plasma cholesterol, but not fasting plasma triacylglycerol concentrations. These observations suggest that feeding FO may be beneficial for the treatment of hyperlipidemia observed with diabetes.

Data from *in vivo* studies do not always correlate with the *in vitro* results of nutrient uptake, because of the additional variables involved with *in vivo* studies which can be controlled or eliminated when uptake studies are performed *in vitro*. *In vivo* intestinal perfusion studies of nutrient uptake include the effect of the unstirred water layer and the acidic microclimate, permeability through the BBM, tight junctions and Peyer's patches, as well as metabolism and exit from the enterocyte into the lymph and portal blood. *In vitro* intestinal sheet studies of nutrient uptake exclude permeability through Peyer's patches (these are avoided when cutting intestinal sheets), minimize the effect of the unstirred water layer (by stirring the fluid exposed to the luminal surface) and exit from the enterocyte (since the lymph and portal circulation are no longer intact), but do include the effects of the acidic microclimate, permeability through the BBM and tight junctions, as well as metabolism within the enterocyte. *In vitro* BBMV studies assess only the nutrient transport across the BBM, since all other variables are eliminated. Because the effective

resistance of the UWL is minimized with *in vitro* intestinal sheet studies, the correlation of these studies with *in vitro* BBMV studies is likely due to the fact that alterations in uptake are primarily occurring due to changes in the permeability properties of the BBM. Differences in UWL resistance observed between diabetic and control animals with in vivo studies may obscure the changes in V_{max}, K_m and P_d that have been observed with *in vitro* studies (Hotke et al., 1985).

In vivo intestinal perfusion studies include the effect of the resistance of the UWL which is influenced by diabetes and by diet. Previous *in vivo* studies suggest that the resistance of the UWL is increased with diabetes (Hotke et al., 1985). In the present studies, diabetes was associated with a similar UWL resistance as compared with non-diabetic animals fed BT, but a decrease in UWL resistance as compared with non-diabetic animals fed FO. Feeding FO was associated with an increase in jejunal UWL resistance in non-diabetic animals as compared with those fed BT. In diabetic animals, feeding FO was associated with a decrease in ileal UWL resistance as compared with those fed BT. The effective resistance of the UWL was not assessed in the present *in vitro* intestinal sheet studies, although previous experiments have demonstrated that the effective resistance of the UWL is decreased with diabetes (Thomson, 1980,1981,1983).

The passive permeability coefficient (P_d) was also influenced by diabetes and by diet. Previous studies report a decline in the value of the apparent P_d for galactose with diabetes (Hotke et al., 1985). When the apparent P_d was corrected for the effects of the UWL in the present *in vivo* perfusion studies, the jejunal and ileal P_d for L-glucose was greater in diabetic than in control animals fed BT, but unchanged in those fed FO. Jejunal and ileal P_d was increased in non-diabetic animals fed FO as compared with those fed BT. In diabetic animals, feeding FO reduced jejunal P_d as compared with feeding BT, while the ileal P_d was unchanged with diet. The present *in vitro* studies did not correct for the effects of the UWL, and suggest that the apparent jejunal P_d is decreased with diabetes in rats fed BT, and is unchanged in animals fed FO. Ileal P_d is reduced with diabetes in animals fed FO, and unchanged in those fed BT. Previous in vitro studies have reported an increase in P_d with diabetes when corrected for the effects of the UWL (Thomson, 1980,1983). Thus, diabetes alters both the active and the passive components of nutrient uptake.

Feeding ω3 fatty acids derived from fish oil was associated with an increase in the animal's body weight gain, despite a decline in food intake as compared with animals fed a saturated fatty acid diet. A hypertrophic effect on the intestine due to FO feeding has been reported previously (Thomson et al., 1988b), and was confirmed in the present feeding studies (Chapter 6). The reason for this hypertrophic effect is not clear. Variations in food intake, weight gain and mucosal mass, however, did not explain the observed differences in nutrient uptake.

10.4 Dietary Fatty Acids Influence Enterocyte Microsomal Lipid Synthesis

At the EMM, absorbed FFA are activated by fatty acyl CoA synthetase to form acyl-CoAs; these acyl-CoAs are subsequently utilized for re-esterification of absorbed cholesterol and lysophosphatidylcholine (Brindley and Hubscher, 1966; Mansbach, 1977). Fatty acids are also transferred from acyl-CoAs to diacylglycerols, which may be used for the synthesis of triacylglycerols and phospholipids. In addition, fatty acyl-CoA acylates *sn*-glycerol-3-phosphate to form phosphatidic acid, the precursor for the *de novo* synthesis of PC and PE. Choline- and ethanolamine phosphotransferases (CPT and EPT) have higher affinities for diacylglycerol than diacylglycerol acyltransferase, which acylates phosphatidate-derived diacylglycerol to triacylglycerol. This ensures that at low fatty acid availability and low rates of diacylglycerol formation, diacylglycerol is directed to phospholipid synthesis to maintain membrane turnover.

The specificity of the fatty acyl Co-A synthetases may contribute to a distinctive mixture of fatty acids for metabolism in the enterocyte. Although not yet described in the enterocyte, broad and narrow specificities have been described for fatty acyl Co-A synthetases in T-lymphocytes (Taylor et al., 1985). The rate at which fatty acid synthetase converts fatty acids to acyl CoA thioesters is dependent upon the availability of free fatty acids and of CoASH, as well as feedback inhibition by the amount of acyl-CoA.

Lipid synthesis may be influenced by the type of dietary lipid presented to the enterocyte. Long chain fatty acids (18:0, 18:2) may be desaturated and elongated to form long-chain polyunsaturated fatty acids. Desaturases use fatty acyl-CoA as a substrate, although Δ^5-desaturase can act directly on the phospholipid substrate eicosatrienoyl phosphatidylcholine to form arachidonyl phosphatidylcholine. The ω3 fatty acids present in FO compete with ω6 fatty acids for the *sn*-2 position on phospholipids, and result in the enrichment of ω3 fatty acids in phospholipids of animals fed a FO diet. The decrease in 18:2ω6 in ileal and jejunal EMM observed when feeding ω3 fatty acids, despite equal 18:2ω6 concentrations in each diet, may be due to a greater affinity of acyltransferase enzymes for ω3 fatty acids than for 18:2ω6 (Garg et al., 1992).

Diet-associated variations in membrane phospholipid fatty acid composition may be mediated by alterations in EMM desaturase activity. Feeding fish oil enriched diets did not alter jejunal or ileal Δ^9-desaturase activity, despite the fact that an increase in the ratio of 18:0/18:1ω9 was observed for BBM and EMM PC and PE of control and diabetic animals (Chapter 9). Jejunal and ileal Δ^6- and Δ^5-desaturase activities were unaffected by feeding FO, except in the presence of cholesterol in the diet (FOC vs BTC): ileal Δ^6-desaturase activity was increased in control

animals, while jejunal and ileal Δ^5-desaturase activity was increased in control and diabetic animals; this did not correlate with the observed reduction in 20:4ω6 in the jejunal and ileal BBM and EMM PC and PE of control animals, and EMM PE of diabetic animals. Since 18:2ω6 content was maintained at approximately 10% in the semisynthetic diets, far above the minimum requirement of 1-2%, 20:4ω6 content should not have been affected. The presence of 20:5ω3 and 22:6ω3 may inhibit Δ^6- and Δ^5-desaturase activities by end-product inhibition and thereby substitute for 20:4ω6 in membrane phospholipids. However, it is not clear why Δ^6- and Δ^5-desaturase activities would increase with FO feeding. It is possible that the membrane boundary lipids surrounding the desaturase proteins may be altered with diet, and may thereby change the conformation of these proteins and their resultant activities; or, perhaps ω3 fatty acids affect the gene expression of the desaturase enzymes.

Changes in desaturase activities associated with dietary cholesterol supplementation were not consistent with membrane phospholipid fatty acid composition. No significant differences in 18:0/18:1ω9 were observed with cholesterol supplementation in jejunal or ileal BBM or EMM PC or PE of either control or diabetic animals; however, Δ^9-desaturase activity was increased in the small intestine of control animals fed FO (Chapter 9). 18:1ω9 content was increased in jejunal and ileal BBM and EMM PC and PE of diabetic animals fed BT supplemented with cholesterol, although no difference in Δ^9-desaturase activity was observed. Cholesterol supplementation increased intestinal Δ^6-desaturase activity only in control animals fed FO, and increased Δ^5-desaturase activity in control and diabetic animals fed FO. This was not associated with any alterations in 20:4ω6 content and the ratio of 18:2ω6/20:4ω6 in control animals, but was associated with a decline in 20:4ω6 content yet no change in 18:2ω6/20:4ω6 in diabetic animals. In contrast, cholesterol supplementation stimulates Δ^9-desaturase activity, but reduces Δ^6- and Δ^5-desaturase activties in rat liver microsomes (Garg et al., 1986., Muriana et al., 1992). Alterations in microsomal fatty acid composition were not consistent with desaturase activites in the liver of diabetic BB rats (Mimouni and Poisson, 1992). This was particularly true for Δ^9-desaturase and suggests there is no direct cause-effect relationship.

Diabetes was not associated with any changes in Δ^9-desaturase activity, although a decline in Δ^6- and Δ^5-desaturase activity was observed in the small intestine of animals fed FOC (Chapter 9). This differs from the effect of diabetes on desaturase activities for rat liver. In diabetic BB rats, Δ^9-, Δ^6- and Δ^5 -desaturases are depressed, with Δ^9-desaturase more markedly depressed than Δ^6 and Δ^5 (Mimouni and Poisson, 1992). This suggests that intestinal desaturases may be subject to different regulatory mechanisms than liver desaturases.

Diabetes-associated changes in membrane phospholipid composition may be mediated by alterations in phospholipid metabolism, specifically in PC synthesis. Accordingly, these studies were designed to examine the synthesis of PC by two pathways: *de novo* synthesis by the CDP-

choline pathway, and by the PEMT pathway which converts PE to PC. The CDP-choline pathway was assessed by the activity of cholinephosphotransferase (CPT), while the synthesis of PC from PE was assessed by the activity of phosphatidylethanolamine methyltransferase (PEMT). Feeding FO was associated with a decline in jejunal EMM CPT activity of 95% in control animals, and of up to 82% in diabetic animals, without any alterations in PEMT activity (Chapter 8). This data supports the findings of Houweling and coworkers (1995) in mutant Chinese hamster ovary cells which state that methylation of PE to PC cannot substitute for an impaired CDP-choline pathway. This suggests that PE and PC composition does not require the methylation of PE. PEMT activity is reduced in liver microsomes of alloxan diabetic rats (Hoffman et al., 1981). No changes in intestinal CPT or PEMT activity were observed with diabetes or with dietary cholesterol supplementation, suggesting that intestinal and hepatic enzymes of phospholipid synthesis may be regulated differently or in a manner that may be tissue specific.

At the nucleus, lipids may indirectly, via second messengers, or directly influence gene expression of mRNA, which could thereby modify expression of EMM lipid metabolizing enzymes. Preliminary data suggests that dietary fatty acids modify transporter gene expression in enterocytes (Wild et al., unpublished observations). There is a literature indicating that dietary fatty acids as well as carbohydrates, vitamins and minerals do alter gene expression in other tissues (Clarke and Abraham, 1992; Jump et al., 1994, 1995; McGrane and Hansen, 1992). If cytosolic fatty acids influence gene expression with an increase in the mRNA for phospholipid and fatty acid metabolizing enzymes, then this altered abundance in response to diet and diabetes would lead to changes in the abundance and therefore the activity of EMM enzymes involved in lipid synthesis. The influence of dietary lipids on the molecular control of the activity of lipid metabolic enzymes needs to be established.

10.5 Dietary Lipids and Diabetes Alter Enterocyte Membrane Lipid Composition

Previous studies have demonstrated changes in BBM PC and PE content with diabetes (Keelan et al., 1985), and very little changes in BBM phospholipid composition with changes in dietary fat unsaturation (Thomson et al, 1986). Since BBM lipids are synthesized in the EMM, the question arose as to whether alterations in BBM phospholipid composition are mirrored in the phospholipid composition of the EMM.

Diabetes was associated with an increase in the total phospholipid content in both BBM and EMM, and was reflected by an increase in PE for both membranes; in EMM, SM content was also increased (Chapters 7,8). Enzymes in the *de novo* pathway for PE synthesis,

(ethanolaminephosphotransferase, cytidylyltransferase) may be influenced by diabetes. Sphinomyelinase is increased with insulin (Petkova et al., 1990). In the diabetic animal which may have depressed insulin levels, SM synthesis might be increased. Feeding FO reduced the total phospholipid content in both BBM and EMM, but produced different changes in phospholipid composition of these two membranes. SM content was reduced in the BBM, while PC, PE, and PI were reduced in the EMM. Dietary cholesterol supplementation did not alter the cholesterol content or phospholipid composition of either BBM or EMM in control animals fed either BT or FO. In diabetic animals, cholesterol supplementation did not alter BBM phospholipid composition, but was associated with changes in EMM phospholipid composition. EMM cholesterol content was increased in animals fed either BT or FO. In addition, in the diabetic animals fed FO, cholesterol supplementation reduced EMM total phospholipid content due to a decline in SM, PC, and PE. Thus, dietary lipids do result in alterations in EMM lipids without necessarily being associated with changes in BBM lipids. The mechanism of the fish oil and cholesterol associated decline in phospholipid content is unknown, but may involve down-regulation of the enzymes involved in phospholipid synthesis.

The ω3 fatty acids present in FO compete with ω6 fatty acids for the *sn*-2 position of phospholipids, and result in the enrichment of ω3 fatty acids in phospholipids of animals fed a FO diet. This was observed in the present studies, although the fatty acid composition of PC was influenced by diet to a much greater degree than PE. In addition, the jejunal and ileal PC responded similarily to differences in dietary fat unsaturation, while PE did not. The ratio of ω6/ω3 fatty acids was reduced by an average of 90% with fish oil feeding in both BBM and EMM PC in the jejunum and ileum of control and diabetic animals (Chapter 9). Ileal PE ω6/ω3 was reduced by only 80%, as compared to the 97% decline observed in the jejunum of control and diabetic animals. PC and PE may have different diacylglycerol acyl chain specificity during *de novo* synthesis. Diacylglycerol acyl chain specificity for cholinephosphotransferase and ethanolaminephosphotransferase enzymes has been described in Saccharomyces cerevisieae (Hjelmstad et al., 1994). PC remodelling at the sn-1 and sn-2 positions might also explain some of the differences between PC and PE fatty acid composition. This type of remodelling has been described for rat hepatocytes (Tijburg et al., 1991).

The diet-associated alterations in phospholipid fatty acid composition were also different in control and diabetic animals. In the jejunum and ileum of control animals, feeding FO was associated with an approximate 50% decline in 18:2ω6 and 20:4ω6 in BBM and EMM PC, while ω3 fatty acids were increased 6- to10-fold . Feeding FO to control animals reduced ω6 fatty acids by 35% in jejunal and 45% in ileal BBM PE, and increased ω3 fatty acids 10-fold in the jejunum but an average of only 3-fold in the ileum. In the jejunum and ileum of FO fed diabetic animals, only 18:2ω6 was decreased 50%, while 20:4ω6 was not altered, and ω3 fatty acids were

increased 11-15-fold in BBM and EMM PC. In contrast, EMM PE 18:1ω9 was reduced by up to 65%, ω6 fatty acids were decreased up to 75%, and ω3 fatty acids were increased 4-7 fold in the jejunum and ileum of control animals and diabetic animals with FO feeding. BBM and EMM PC 18:1ω9 was reduced in control ileum, and diabetic jejunum and ileum of animals fed FO. The data suggests that it is likely a multitude of factors that contribute to the final composition of the BBM and EMM, including acyl exchange, B-oxidation and prostaglandin synthesis (Mimouni and Poisson, 1991.)

Cholesterol supplementation did not alter jejunal BBM and EMM or ileal EMM PC and PE of control animals (Chapters 7 and 8). Control ileal BBM PC contained less 22:6ω3, while control ileal BBM PE contained less 20:5ω3 with cholesterol supplementation of FO diets (FOC vs FO). Cholesterol supplementation to BT fed diabetic animals increased 18:1ω9 and decreased 20:4ω6 in jejunal and ileal BBM and EMM PC. In diabetic ileal BBM PE, cholesterol supplementation increased 18:1ω9 and decreased 20:4ω6 when animals were fed BT, and reduced 22:6ω3 when animals were fed FO. In liver, Δ^9-desaturase activity is increased, while Δ^6 and Δ^5 are decreased with cholesterol feeding. This was not observed in the intestine, and suggests that hepatic desaturases are regulated differently than those from the intestine.

Diabetes was associated with alterations in BBM and EMM phospholipid fatty acid composition. Ileal BBM PC and PE ω3 fatty acid content declined when animals were fed FO, but reduced jejunal and ileal BBM and EMM PC 18:1ω9 and increased ileal 22:6ω3 when animals were fed FOC. EMM PE 18:2ω6 was increased with diabetes in animals fed BT or BTC, and decreased 18:1ω9 when animals were fed FO or FOC. These changes in fatty acid composition do not reflect the expected changes in desaturase activity observed in the intestine with diabetes, possibly due to other factors that control membrane lipid composition.

10.7 Post-microsomal Modification of Membrane Lipids

The polarized sorting and traffic of membrane constituents in epithelial cells has been reviewed (Simons and Wandinger-Ness, 1990; Rothman and Orci, 1992). We do not have specific information on the mechanisms and avenues of the traffic of lipids from EMM to BBM and BLM. During this process of sorting, post-microsomal modification of the EMM lipids may occur in the trans-Golgi network (TGN) when lipids move directly to the BBM or BLM, or may occur in the TGN and/or BLM and/or in a transcytosis vesicle when lipids move indirectly from the TGN to the BLM and then to the BBM. Post-microsomal modification may also occur at the destination membrane. Evidence for post-microsomal modification of enterocyte membrane lipid composition is supported by the differences in BBM, BLM and EMM phospholipid and fatty acid composition (Garg et al, 1990; Chapters 7,8,9). EMM contain much less cholesterol than BBM, as indicated by

phospholipid:cholesterol ratios of 2:1 and 1:1, respectively. Jejunal EMM is similar to BBM and BLM except for an increase in 18:0 and a decrease in 18:2ω6 in BBM and BLM as compared with EMM. BBM have less 16:0 and more 20:4ω6 than BLM or EMM. Intestinal plasma membranes (BBM, BLM) appear to rely on diet or EMM for membrane PUFA, since desaturase activity was not detected in the plasma membranes (Garg et al., 1990). Do changes in EMM desaturase activity occur prior to changes in membrane lipid composition? Garg et al. (1990) suggests that this is the case, since the fasting-associated increase in Δ^6-desaturase activity did not coincide with any changes in ileal EMM fatty acid composition, which remained essentially unaltered. Enterocyte desaturases are localized to the EMM, and respond to physiological changes such as fasting, alterations in dietary fat composition, and diabetes. However, intestinal desaturases respond differently to these physiological changes than do the liver desaturases (Garg et al., 1990; Chapter 9), and therefore are likely regulated by different factors.

No clear relationship has been found between the lipid composition of EMM and BBM. If the EMM lipids were directly incorporated into the BBM lipids, then one would expect that diet and diabetes would have a similar effect on EMM and BBM lipids. This did not occur. In addition to the possibility that post-microsomal metabolism of these lipids by way of BBM acyltransferases/PEMT, BBM phospholipases may have removed some fatty acids from the phospholipids and contributed to the BBM free fatty acid pool. It is unknown whether the BBM free fatty acid could exit from the membrane into the UWL, although the magnitude of such an exit process is likely to be small, because of the unfavourable concentration gradients, unfavourable H^+ gradients, and the presence of at least two membrane proteins (NHE_3 and $FABP_{pm}$) which would tend to rapidly return fatty acids into the BBM. It has been suggested that every type of membrane may possess its own complement of enzymes in order to tailor the fatty acyl molecular species of membrane phospholipids, as well as the type of phospholipids in the membrane to the functions of the membrane (Lynch and Thompson, 1984). A gradual metabolic repositioning of important fatty acids does occur in murine myeloid cells (Kannagi et al., 1982). The repositioning could facilitate the placement of fatty acids (ie. 20:4ω6 or 20:5ω3) into phospholipids (ie. phosphatidylinositiol) that may be hydrolyzed in response to an intracellular stimulus.

Changes in membrane composition could be due to alterations in the activity of acyltransferases or phospholipid transferase enzymes present at each membrane. These findings support the concept that membrane phospholipids are first synthesized at the microsomes and are then used for membrane biogenesis where minor adjustments are made on location at the site of the membrane by deacylation/reacylation enzymes. The BBM does have deacylation enzymes such as phospholipase, and likely does have reacylation enzymes, although acyltransferase activity has not yet been specifically demonstrated for the BBM. In addition,

adjustments in membrane lipid composition may also be achieved through the activity of enzymes within the BBM, such as PEMT.

10.8 Summary

The model described in this thesis proposes a mechanism by which BBM nutrient uptake and lipid composition may be altered with diabetes and diet. Diabetes and diet alter the activity of EMM enzymes involved in lipid synthesis, which subsequently changes the lipid composition of the EMM and newly synthesized lipids, such as phospholipids, that traffic from the EMM to the BBM. Alterations in the composition of bulk and boundary lipids result in the respective modifications in BBM passive and protein-mediated nutrient transport, as well as enzyme activity (i.e. alkaline phosphatase). The lack of changes in BBM cholesterol in response to diet or diabetes suggests that the BBM cholesterol level is tightly controlled. In contrast, the EMM cholesterol level is influenced by diet and endogenous synthesis, since the EMM acts as a distribution control center for the incorporation of cholesterol into lipoproteins and other organelle membranes. At the BBM, local tailoring of membrane phospholipid composition may take place through the activity of acyltransferases and PEMT.

Numerous pieces of evidence from this thesis support the proposed model:

1) Feeding control animals a fish oil (FO) diet as compared with feeding a beef tallow diet (BT) was associated with a decline in jejunal EMM CPT activity, EMM PC content, and the EMM ratio of phospholipid/cholesterol (Chapter 8). No alteration in the PC content of BBM was observed. Some additional fine tuning may have taken place locally at the BBM, possibly through PEMT activity which can convert PE to PC.

2) Feeding control and diabetic rats a FO diet as compared with feeding a BT diet was associated with an increased content of ω3 fatty acids (EPA and DHA) in jejunal and ileal EMM and BBM (Chapter 9), with a corresponding decline in ω6 fatty acids (LA and AA). This change in BBM fatty acids was associated with reduced hexose and lipid uptake (Chapters 4-6). I propose that the dietary lipids modify the gene expression of BBM and EMM proteins and thereby alter mRNA and/or protein abundance, which changes sugar transporter activity and lipid metabolizing enzyme activity. This results in changes in sugar transport and in membrane lipid composition, respectively. The altered membrane lipid composition results in changes in membrane fluidity and the lipophilic properties of the membrane, which may alter BBM lipid permeability and which additionally may modify membrane protein activity (i.e. BBM sugar transporter, BBM lipid transporter).

3) Cholesterol supplementation in control animals fed a FO diet was associated with an increase in jejunal EMM Δ^9-desaturase activity and 18:1ω9 content of EMM PC, but not BBM PC

(Chapter 9). I suggest that local deacylation/reacylation reactions at the BBM modified the fatty acid composition of BBM PC.

4) Cholesterol supplementation of either BT or FO diets increased EMM cholesterol content of diabetic EMM (Chapter 8) but not BBM (Chapter 7). I suggest that the additional cholesterol was retained at the EMM and did not traffic to the BBM, or that a local mechanism removed cholesterol from the BBM in order to maintain the cholesterol content of the BBM at a constant level.

5) Diabetes increased jejunal EMM and BBM sphingomyelin content of animals fed BT (Chapters 7 and 8). I suggest that increased EMM sphingomyelin synthase activity explains the observed increases in EMM and BBM sphingomyelin, and that this may serve as a second messenger in regulating signal transduction.

6) Diabetes increased jejunal EMM and BBM PE in animals fed either a BT or FO diet supplemented with cholesterol (Chapters 7 and 8). The increased phospholipid content of diabetic BBM was associated with a dramatic increase in lipid and hexose uptake. I suggest that elevated EMM and BBM PE arises from an increase in the *de novo* PE synthesis (CTP:phosphoethanolamine cytidylyltransferase, ethanolamine phosphotransferase).

The signals for these diabetes- and diet-associated changes are unknown. I propose that diabetes and diet produce the changes in EMM lipids, BBM lipids and BBM uptake as a result of the unknown signals being primarily effective on the activities of EMM lipid enzymes or on the gene expression of EMM lipid enzymes and BBM transport proteins. Changes in EMM lipid enzymes activities may be mediated by dietary lipids acting to modify signal transduction by second messengers (cAMP, Ca^{2+}, diacylglycerol, PI-derived AA) which may alter mRNA expression, or may play a role in the local fine-tuning of membrane lipid composition.

These studies also suggest that there are post-microsomal steps associated with the diabetes- and diet-associated changes in BBM lipid composition. The presence of deacylation/reacylation enzymes at the BBM which are able to utilize the resources of the large free fatty acid pool present in the BBM, may play an important role in fine-tuning BBM phospholipid fatty acid composition. I speculate that this is the explanation for the reflection of similar EMM phospholipid changes in BBM phospholipids, but dissimilar changes being observed in phospholipid fatty acid composition.

While diabetes and diet are associated with alterations in the BBM lipids, the changes in nutrient uptake are likely due both to changes in membrane lipids as well as to pre- or post-translational modification of BBM proteins (SGLT1, GLUT5, NHE_3/$FABP_{pm}$, cholesterol transfer protein, PEMT and acyltransferases). Our preliminary results demonstrate that the diabetes-associated changes in nutrient carrier protein is not explained simply by alterations in mRNA abundance (unpublished observations, 1996).

An expected change in EMM desaturase was not always seen in conjunction with an observed alteration in EMM lipids. For example, in diabetic jejunum, there was no change in Δ^6- or Δ^5-desaturase, yet there were changes in EMM PC 20:4ω6. This may represent selective enrichment of EMM but not BBM with 20:4ω6, with the 20:4ω6-containing PC directed to lipoproteins or other organelle membranes. With the fish oil-associated increase in Δ^5-desaturase, an increase in EMM PC 20:4ω6 to at least control levels was expected, but not observed. It is possible that the increase in Δ^5-desaturase activity was insufficient and unable to overcome the depressing effect of the high levels of 20:5ω3 and 22:6ω3 on both Δ^6- or Δ^5-desaturase activities. Changes in EMM PC 18:1ω9 without alterations in EMM Δ^9-desaturase activity may have resulted from selective enrichment of the EMM PC with dietary 18:1ω9.

Modifications in dietary lipids produce changes in EMM lipids and BBM lipids, but the changes did not simply reflect the alterations in dietary lipids. Thus, the lipid composition of these membranes is controlled. I did not always find that changes in EMM phospholipid and fatty acid metabolizing enzymes were reflected in all of the anticipated changes in EMM lipids, nor did we always find that changes in EMM lipids were reflected by alterations in BBM lipids. As discussed above, this may have been due to post-microsomal processing of lipids. In addition, however, it is only recently been recognized that a greater proportion of dietary lipid is taken up from BBM from enterocytes from the upper as compared with the lower portion of the villus (Fingerote et al, 1994). Similar findings have been observed for sugars and amino acids (Haglund et al., 1973; Smith, 1981; Thomson, 1995). This raises the concept of the heterogeneity of the villus, so that it is possible that since I used homogenates of enterocytes from along the CVA, part of the dissociation between dietary lipid and diabetes associated changes in EMM and BBM lipid have been the result of heterogeneous distribution of EMM CPT, PEMT, and desaturases. A recent study from our laboratory would lend support to this suggestion: Δ^5-desaturase activity is greatest in the upper portion of the villus (unpublished observations, 1996). Given this new information, a better correlation between lipid composition and enzyme activities might be obtained in future studies using enterocytes collected from along the length of the villus.

While the saturation index of lipid varies between the villus tip and crypt cells (Dudeja et al, 1990; Meddings et al, 1990), there is as yet no published data on the likely gradient along the villus axis in phospholipid fatty acid composition. The diet- and diabetes-associated changes in BBM lipid composition produce alterations in the lipophilic properties of the BBM which are partially responsible for the increased uptake of lipid in diabetes, and in animals fed the saturated as compared with the polyunsaturated fish oil diet. However, changes in the activity of NHE_3 and $FABP_{pm}$ may also contribute to changes in lipid uptake. Given that there may be a heterogeneity in expression of EMM enzymes and lipids, there is likely also a heterogeneity in the expression of BBM PEMT, BBM lipid, NHE_3 and $FABP_{pm}$. If a heterogeneity of lipid composition occurs, then

one would anticipate differences in lipid uptake between the upper as compared with the lower portion of the villus. At least in rabbit studies, this has been observed, but there is no data linking this gradient of lipid uptake with a gradient in BBM lipid composition or in the abundance and/or activity of NHE_3 or $FABP_{pm}$.

I have presented abundant experimental data which supports the proposed model for intestinal adaptation in response to dietary lipids and diabetes. There is much work to be done in the future to further investigate the role of dietary lipids and second messengers on translational and post-translational events within the enterocyte. Some of these studies are proposed in Chapter 11.

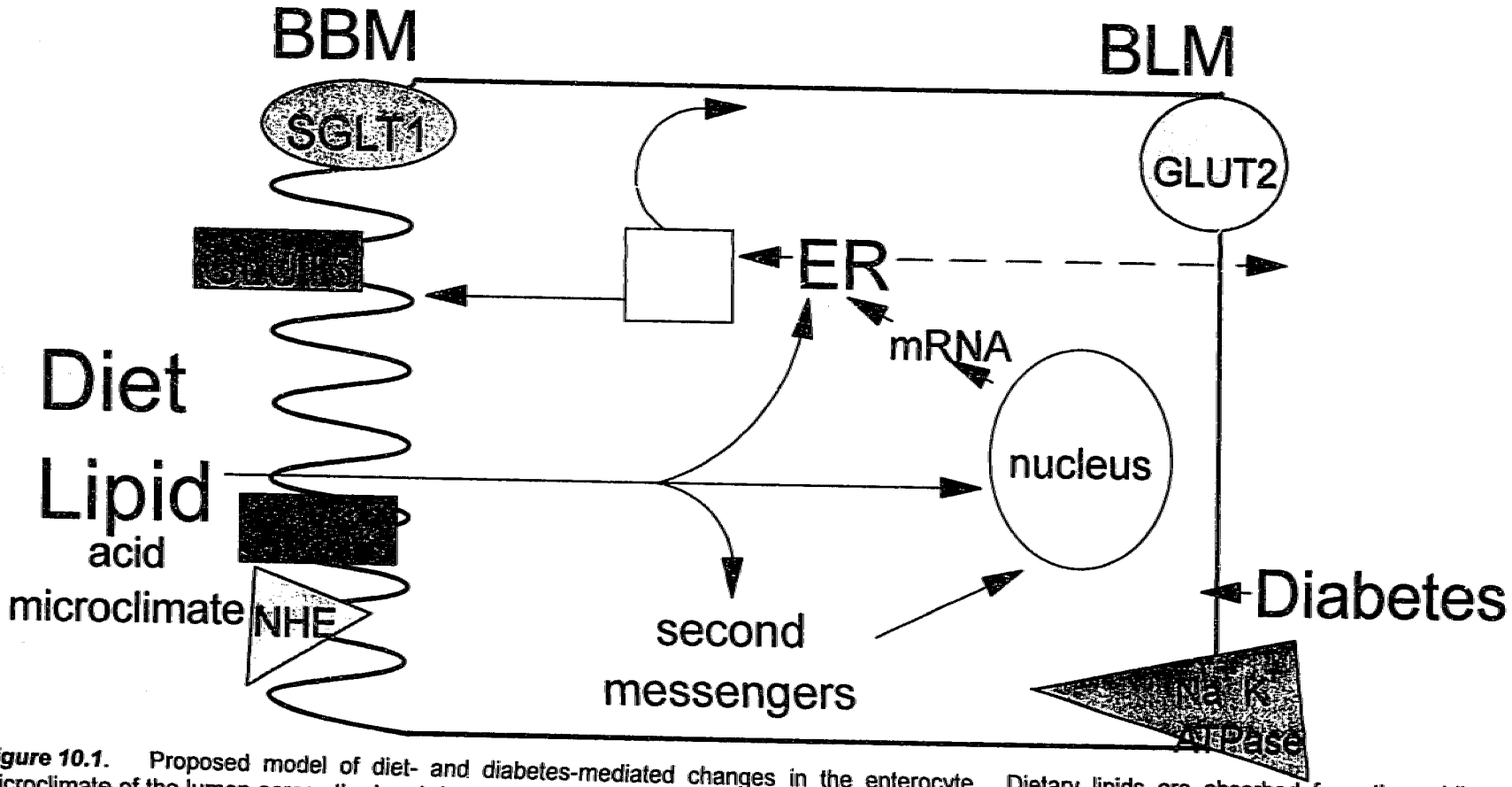

Figure 10.1. Proposed model of diet- and diabetes-mediated changes in the enterocyte. Dietary lipids are absorbed from the acidic microclimate of the lumen across the brush border membrane (BBM) into the enterocyte where they may act directly on the nucleus or indirectly on the nucleus via second messengers to alter the gene expression of nutrient transporters or lipid enzymes. Dietary lipids may be able to act directly on the endoplasmic reticulum (ER) to alter lipid synthesis or processing of transporters. Alterations in lipid synthesis at the ER would produce changes in BBM and basolateral membrane (BLM) lipid composition, which may in turn alter the properties of the membranes and result in changes in transporter function and membrane permeability. Diabetes produces a systemic influence from across the BLM to mediate changes in enterocyte membrane lipid composition and function.

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CHAPTER 11

FUTURE STUDIES

Future studies must examine the effect of diet and diabetes on the relationship between protein activity, protein abundance and gene expression (mRNA abundance). It is only by examining the different enterocyte populations that we will be able to discover the true relationship between protein function and gene expression.

The following questions will direct future studies:

1. Does diet and/or diabetes modify the activity of the proteins involved in fatty acid transport, NHE_3 or $FABP_{pm}$, along the villus? Are the changes pre- or post-translational? Can changes in activity be explained by alterations in mRNA and/or protein abundance?
2. Does diet and/or diabetes modify the activity of the BBM sugar transport proteins, SGLT1 and GLUT5, along the crypt-villus axis? Are the changes pre- or post-translational? Can changes in activity be explained by alterations in mRNA and/or protein abundance?
3. Does diet and/or diabetes modify the activity of the BLM sugar transport protein, GLUT2, and the activity of Na^+K^+-ATPase? Are the changes pre- or post-translational? Can changes in activity be explained by alterations in mRNA and/or protein abundance?
4. Does diet and/or diabetes modify the activity of enzymes involved in phospholipid synthesis along the crypt-villus axis; enzymes such as CPT, phosphocholine cytidylyltransferase, EPT, ethanolamine cytidylyltransferase, PEMT, or sphinogmyelin synthase?
5. Does diet and/or diabetes modify the activity of enzymes involved in long chain polyunsaturated fatty acid synthesis: Δ^9-, Δ^6-, Δ^5-desaturases along the crypt-villus axis?
6. Does diet and/or diabetes modify the activity of enzymes involved in long chain polyunsaturated fatty acid incorporation into phospholipids?
7. Is BBM composition further refined locally by tailoring enzymes? Do deacylation/reacylation enzymes exist at the BBM? How are they distributed along the crypt-villus axis?
8. How is BBM cholesterol content so tightly controlled? What is the distribution of HMGR and ACAT along the crypt-villus axis: HMGR?
9. Does diet and/or diabetes alter the gene expression of lipid metabolizing enzymes and transport proteins directly, or indirectly through signal transduction by second messengers such as calmodulin, Ca^{2+} concentration, protein kinase C (activated by Ca^{2+} and diacylglycerol generated by hydrolysis of PIP_2), inositol triphosphate, adenylate cyclase

(increases cAMP to activate protein kinase A), phospholipase C (catalyzed the hydrolysis of PI to diacylglycerol), PI-derived arachidonic acid, G-proteins (cGMP), nuclear transcription factors (c-myc, erg, jun, fos)?

A greater understanding of the mechanisms by which the intestine may adapt to diet and diabetes will provide the direction for future studies to examine how dietary manipulations could be used therapeutically to modulate diseases, such as diabetes, by preventing unwanted and enhancing desirable intestinal adaptation.

APPENDIX I

COMPOSITION OF SEMI-SYNTHETIC DIETS

Macronutrient Composition of the Semisynthetic Diets

Ingredient	Concentration g/kg diet
Fat + +	200
Casein	270.5
Cornstarch	378
Non-nutritive fiber	80
Vitamin mix*	10
Mineral mix#	50
L-methionine	2.5
Choline	2.75
Inositol	6.25

* A.O.A.C. vitamin mix (Teklad Test Diets, Madison, WI) provided the following per kilogram of complete diet: 20,000 IU of vitamin A; 2,000 IU of vitamin D; 100 mg of vitamin E; 5 mg of menadione; 5 mg of thiamine-HCl; 8 mg of riboflavin; 40 mg of pyridoxine-HCl; 40 mg of niacin; 40 mg of pantothenic acid; 2,000 mg of choline; 100 mg of myoinositol; 100 mg of p-aminobenzoic acid; 0.4 mg of biotin; 2 mg of folic acid; and, 30 mg of vitamin B12.

Bernhart Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH) was modified to provide 77.5 mg of Mn and 0.06 mg Se per kilogram of complete diet.

+ + All diets were nutritionally adequate (National Research Council: Subcommittee on Laboratory Animal Nutrition (1978) Nutrient Requirements of Laboratory Animals #10. Natl. Acad. Sci., Washington, D.C.).

Preparation of Dietary Fat

Ingredient g/kg total diet	BT	BTC	FO	FOC
Beef Tallow	180	180	80	80
Safflower Oil	20	20	20	20
Fish Oil	-	-	100	100
Cholesterol	0.6	20.6	- *	20

* Fish oil contains cholesterol (0.6%, w/w), and therefore no additional cholesterol is added to the fish oil low cholesterol diet. Thus, the final cholesterol content of low and high cholesterol diets was 0.06% (w/w) and 2.06% (w/w), respectively.

BT, beef tallow low cholesterol diet
BTC, beef tallow high cholesterol diet
FO, fish oil low cholesterol diet
FOC, fish oil high cholesterol diet

Fatty Acid Composition of the Diets

Fatty Acid % of total	BT	BTC	FO	FOC
14:0	3.8	3.8	5.6	5.5
15:0	0.6	0.6	0.4	0.4
16:0	27.5	28.9	17.9	19.1
16:1ω7	0.6	0.6	6.3	5.8
17:0	2.1	2.1	0.9	1.0
18:0	52.3	51.9	25.2	27.5
18:1ω9	5.3	5.0	7.1	7.1
18:1ω7	0.1	0.1	1.3	1.2
18:2ω6	6.9	6.8	7.6	8.1
18:3ω3	-	-	3.2	2.8
20:5ω3	-	-	15.1	14.0
22:5ω3	-	-	1.3	1.2
22:6ω3	-	-	5.6	5.1