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Aging and Intestinal Adaptation

by

Trudy Dawn Woudstra 

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

Department of Medicine

Edmonton, Alberta

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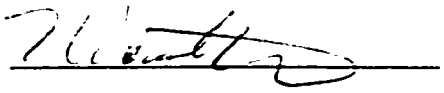

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University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Aging and Intestinal Adaptation submitted by Trudy Dawn Woudstra in partial fulfillment of the requirements for the degree of Master of Science.

Dr. A.B.R. Thomson

Dr. C.I. Cheeseman

Dr. M.T. Clandinin

Dr. A. Sclater

Dr. B.R. Yacyshyn

January 9, 2002

Abstract

The intestine undergoes modification of its morphology and nutrient absorption in a variety of conditions such as diabetes mellitus, following abdominal radiation, following resection of the small intestine and with aging. Aging is associated with a decline in the absorptive capacity of the small intestine for several nutrients, including carbohydrates and amino acids. We tested the hypothesis that aging was associated with a decline in lipid absorption in the F344 rat, and that this decline could be corrected by manipulating the fat composition of the diet by feeding a diet of higher saturated fatty acid (SFA) content. In addition, we hypothesized that decreased lipid absorption would be due to a decline in the abundance and/or expression of selected fatty acid binding proteins and their mRNAs in the intestine.

Aging is associated with changes in lipid uptake in the jejunum and ileum. However the direction and magnitude of these alterations is dependent on the method used to express the rate of lipid uptake. For example, when the rate of uptake of fatty acid 16:0 in the ileum is expressed on the basis of weight of the intestine there is a decrease in uptake, but when expressed on the basis of surface area there is no change in the rate of uptake. Regardless how rate of lipid uptake was expressed the changes were not explained by alterations in the abundance of the proteins of I-FABP or ILBP; or by the mRNA expression of L-FABP of ILBP.

While the ileum does not compensate for loss of function of the jejunum in aging, the ileum of 24-month old rats demonstrates a higher degree of adaptive response to alterations in dietary fat than does the jejunum. Old animals adapt differently to

alterations in dietary fat than do mature animals, with a polyunsaturated fatty acid (PUFA) diet resulting in increased absorptive function on the basis of surface area in old animals and a SFA diet increasing absorption in 9-month old animals. SFA results in increased intestinal surface area in mature animals, while PUFA increased the surface area of the old intestine to a similar magnitude. This suggests an altered response in aging rather than a reduced response to dietary changes. This finding underscores the importance of research being conducted in aging animals, because treatment effects cannot necessarily be predicted by findings observed in young or mature animals.

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List of Abbreviations

AP-1	activator protein-1
BBM	brush border membrane
BLM	basolateral membrane
CCK	cholesystokinin
cDNA	complementary deoxyribonucleic acid
CR	caloric restriction
EGF	epidermal growth factor
ER	endoplasmic reticulum
F344	Fischer 344
FABP	fatty acid binding protein
FAT	fatty acid translocase
FATP	fatty acid transport protein
GIP	gastric-inhibitory peptide
GLUT2	sodium-independent glucose and fructose transporter in BLM
GLUT5	sodium-independent fructose transporter in BBM
GRP	gastrin –releasing peptide
IDDM	insulin dependent diabetes mellitus
I-FABP	intestinal-fatty acid binding protein
ILBP	ileal lipid binding protein
IR	immunoreactive
Km	Michaelis-Menten affinity constant
L-FABP	liver-fatty acid binding protein

MDR	multidrug resistance protein
mRNA	messenger ribonucleic acid
mtDNA	mitochondrial deoxyribonucleic acid
MTP	microsomal triglyceride transport protein
NBB	neutral brush border
NF-KB	nuclear factor-KB
NIDDM	non-insulin dependent diabetes mellitus
ODC	ornithine decarboxylase
PKA	protein kinase A
PKC	protein kinase C
PPAR	peroxisome-proliferator activator receptor
PPRE	peroxisome-proliferator response elements
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SGLT1	sodium-dependent transporter in BBM
SR-B1	Scavenger receptor of class B type 1
TG	triglyceride
TGF	transforming growth factor
TJ	tight junctions
TTBS	tween tris buffered saline
UWL	unstirred water layer
Vmax	maximal transport rate

CHAPTER ONE

Literature Review

1.1 Introduction

Developed nations are faced with the demographics of an aging population. In Canada, over 12 % of the population is older than 64 years, and this percentage is expected to increase by 2 % within 11 years (Statistics Canada, 1999). Aging is associated with an increased prevalence of many medical conditions such as diabetes, heart disease and malnutrition (Halter, 1999; Morley, 1998). Some of these age-associated conditions may benefit from dietary modification. However, little is known about the effect of age on the intestinal absorption of nutrients, or the effect of dietary modification on nutrient absorption.

The intestine undergoes modification of its morphology and nutrient absorption in a variety of conditions such as diabetes mellitus, following abdominal radiation, following resection of the small intestine, and with aging. Aging is associated with a decline in the absorptive capacity of the small intestine for carbohydrates (Ferraris *et al.*, 1993), amino acids (Chen *et al.*, 1990), calcium (Morris *et al.*, 1985) and lipids (Thomson, 1980, Peachey *et al.*, 1999). Alterations in the dietary content of carbohydrates (Ferraris and Diamond, 1989) and lipids (Thomson *et al.*, 1986) influence the function of the intestine. For example, young animals fed diets rich in saturated fatty acid (SFA) have increased intestinal uptake of sugars and lipids when compared to animals fed a polyunsaturated fatty acid (PUFA) rich diet (Thomson *et al.*, 1986). The mechanisms of these changes are not known, and it is unknown if the same influences of diet are seen in older animals.

The complexity of lipid absorption may make it highly susceptible to the effects of aging (Keelan and Thomson, 2000). Factors such as the pH of the microclimate adjacent to the BBM (Shiau, 1985), membrane fluidity (Wahnon *et al.*, 1989), the effective resistance of the unstirred water layer (UWL), and the contribution of fatty acid binding proteins may be subject to age-associated alterations.

Cytosolic lipid binding proteins found in the intestinal epithelium include the liver-fatty acid binding protein (L-FABP), the intestinal-lipid binding protein (I-FABP) and the ileal lipid binding protein (ILBP). L-FABP is a 14.1 kDa protein located in the duodenum and jejunum, with maximal expression in the proximal jejunum (Table 1.1). I-FABP is a 15.1 kDa protein that is expressed throughout the small intestine, with maximal expression in the distal jejunum (Halden and Aponte, 1997). It is likely that L-FABP and I-FABP play different roles in the absorption of lipids. For example, the fatty acid transfer from I-FABP occurs by direct collisional interaction with the phospholipid bilayer, and it has been speculated that I-FABP may play a role in the uptake or subcellular targeting of fatty acids (Hsu and Storch, 1996). In contrast, L-FABP may transfer fatty acids in an aqueous diffusion-mediated process, and may act as a cytosolic buffer for fatty acids (Hsu and Storch, 1996). Both I-FABP and L-FABP bind long chain fatty acid with high affinity. Rats treated with clofibrate (a hypolipidemic drug) have increased expression of L-FABP protein and mRNA, with no change in the expression of the I-FABP counterparts (Bass *et al.*, 1985). The mRNA expression of I-FABP and L-FABP is increased in rats fed a diet rich in polyunsaturated fats (Poirier *et al.*, 1996). The ileal lipid binding protein (ILBP) is a 14 kDa cytoplasmic protein that binds bile acids (Lin *et al.*, 1991). ILBP is structurally related to the FABP family, and is located

predominantly in the distal ileum (Kramer *et al.*, 1998). It is unknown if ILBP changes with alterations in the diet or with aging.

Several membrane-associated proteins may also contribute to lipid absorption in the intestine, such as the fatty acid binding protein-plasma membrane (FABPpm), fatty acid translocase (FAT) (Abumrad *et al.*, 1984), and the fatty acid transport protein-4 (FATP4) (Stahl *et al.*, 1999).

This study was undertaken to test the hypotheses that 1) aging is associated with a decrease in the absorption of lipid in Fischer 344 (F344) rats; 2) the decreased absorption of lipid is due to a fall in the passive permeability properties of the brush border membrane as well as reduced contribution of lipid transporters; 3) the decreased uptake of lipids is due in part to the reduced abundance of fatty acid binding proteins; 4) the decreased abundance of transporters is the result of a decline in the mRNA for lipid transporters and binding proteins; and 5) the decreased gene expression, protein abundance and absorption can be reversed by feeding a saturated rather than a polyunsaturated enriched diet.

1.2 Aging

1.2.1 Definitions

Life span is defined as the time spent living by any organism, and maximum life span is based on the life span of organisms living under favorable conditions determined by the longest-lived member or of the top percentile (Smith, 1993). Life expectancy is a summary statistic that estimates the average time yet to be lived by an individual at a given age (Carnes *et al.*, 1999). Life expectancy is often expressed from birth, and the terms life span and life expectancy are often used interchangeably.

The maximum life span of the human population is about 120 years, but significant changes in life expectancy have occurred through the ages (Hazzard, 1999). Up until the time of Christ, life expectancy did not usually exceed 22 years. During the following centuries this number doubled to 45 years. The past century has witnessed the greatest increase in life expectancy, with an increase from 45 years to the current 75 years (Smith, 1993). However, men and women do not have the same life expectancies. At birth, in Canada, life expectancy of males is 75.8 years and for females it is 81.4 years (Statistics Canada, 1999). The gap between life expectancies of the two genders has been narrowing over the past two decades, from a peak of 7.5 years in 1978 to its current difference of 5.6 years in 1997. Life expectancy in undeveloped countries is much lower, but is the same for men and women. In the past century, women have benefited dramatically from improved prenatal and obstetrical care. Women in developed nations have also had enhanced access to food and education. As a result of these improvements for women, the gender longevity difference has grown in the United States. It is not clearly understood why women outlive men, but estrogen has been implicated in this phenomena (Hazzard, 1999).

Death from heart diseases (including myocardial infarction) is almost twice as prevalent in men, and is the leading cause of death in Canada. Cardiovascular diseases account for the second largest number of deaths in Canada, with a 3:2 ratio of deaths among men as compared to women. Cancers, particularly lung cancer, are the third leading cause of death in Canada, with lung cancer resulting in death in men at about twice the rate of women (Statistics Canada, 1999).

When is a person "old"? Who are the "elderly"? While population statistics report occurrences of deaths and diseases by chronological age, it is far more difficult to delineate young and old by chronological age. Anecdotally, we are probably all able to think of someone who is "young" for their age; their health and lifestyle are major factors in their apparent youthfulness. Perhaps in considering the differences between these two arbitrary extremes {young versus old}, it is more useful to consider "biological-age" and the "processes" of aging and senescence.

The term "old" is used to describe virtually all time-dependant changes from molecules to ecosystems (Medawar, 1952). Frolkis suggests that aging is a biological process, which limits the adaptive possibilities of an organism, and thereby reduces life span. For example, the adaptive response of the intestine to dietary changes is altered in aging (Holt and Kotler, 1987; Chambon-Savonovitch *et al.*, 1999). Masoro proposed that aging refers to deteriorative changes that occur over time, and reduce the ability of the organism to survive. However, Arking and Dudas suggest that when we have reached a more sophisticated understanding of aging, we will be able to eliminate the word "time" from our definition, and instead use the physiological processes (Arking *et al.*, 1989). Finch (1990) stresses the importance of avoiding the implications associated with the word "time". Use of the word aging is therefore limited and instead changes observed over a life span are referred to as "age-related" changes.

The term senescence is used interchangeably with aging by some authors, while others prefer to reserve its usage to describe changes that occur during the functional decline of an organism's life (Finch, 1990). It is beyond the scope of this paper to consider the implications associated with word usage. I will use the terms aging, age-

related and senescence interchangeably, and will use these terms to describe physiological changes that occur over the lifespan of the Fischer 344 rat.

1.2.2 Mechanisms of Aging

Nature or nurture, which one makes the greatest contribution to longevity? Twin studies have suggested that 25% of the variation in lifespan can be attributed to nature (Herskind *et al.*, 1996). Studies of human progerias and the characterization of age genes in *Caenorhabditis elegans* suggest a possible larger contribution of genes. Several processes which are thought to contribute to the aging process include genetic programs, oxidative damage, genome instability and cell death.

1.2.2.1 Genetic Programs

The classic evolutionary theory of aging proposes that organisms age because of the weakening of the force of natural selection. This means that natural selection favorably selects organisms with the capacity to survive infancy through to the reproductive years. For a species to survive, reproduction is required, and so there is less selective pressure progressively through a lifetime (Medawar, 1952). Carnes and coworkers further elaborate on this theory by considering the selective pressures for a given gene during the three partitions of the individual's lifespan. Selection is highly effective during the first partition, the pre-reproductive period, and follows an age-gradient decline in effectiveness during the second partition, the reproductive phase. During the final partition, the post-reproductive phase, there is no selective pressure for alleles whose effects, either harmful or beneficial, are limited to the post-reproductive phase (Carnes *et al.*, 1999; Smith, 1993). This theory implies that alleles with post-reproductive deleterious effects would accumulate in the genome if they also confer

reproductive advantage early in life (Williams and Birnbaum, 1988). Supporting this link with reproduction is the finding that *Drosophila* that reproduce at a lower rate and have a later onset of the reproductive period have increased life spans (Lints and Hoste 1974; Luckinbill and Clare,1985).

A mutation of the age-1{hx546} gene found in *C. elegans*, a nematode, increased the life span of the mutants by 2- to 4-fold (Friedman and Johnson, 1988). Subsequent findings have suggested that several genes may effect the life span of *C. elegans*, such as 3 *daf* genes which control early development (reviewed by Johnson *et al.*, 1999). The daf genes encode proteins similar to an insulin receptor and are involved in dauer formation in C. elegans. Together these findings suggest that while genes that confer longevity *per se* are not naturally selected, genes that confer advantages in the prereproductive and reproductive phases of life are naturally selected, and these genes may also have an effect on longevity. Therefore, a genetic contribution to longevity is in keeping with the laws of natural selection.

1.2.2.2 Oxidative stress

The oxidative stress theory suggests that while oxygen is required by aerobic organisms, oxygen is easily converted to highly reactive forms (primarily O_2^- and also H_2O_2 and •OH). These reactive species can cause oxidative damage to polyunsaturated fatty acid chains, nucleic acids and proteins {reviewed by Sohal and Weindruch, 1996}. *C. elegans age-1* mutants are less susceptible to oxidative damage, as are the *Drosophila* mutants *methusela* (Lin *et al.*, 1998). The mitochondria are particularly sensitive to oxidative damage. It is hypothesized that there is a "vicious cycle" of accumulated damage. The vicious cycle consists of three steps: (1) the oxidants cause damage to

mitochondrial DNA {mtDNA}, resulting in defective proteins; (2) the defective proteins cause defective electron transport and the generation of mutagenic oxidants; and (3) the mutagenic oxidants cause greater damage to mtDNA (Beckman and Ames, 1999; Wong and Cortopassi, 1997). In keeping with this hypothesis, it has been shown that oxidative damage increases exponentially with age. The exponential increase in oxidative damage may be further enhanced by reduced repair mechanisms within the senescent cell, such as a reduction in cytochrome C oxidase activity. This would tend to increase the oxidizability of up-stream respiratory components, and therefore increase the oxidative mutagens (Sohal, 1992). In *C. elegans* age-1 mutants mtDNA damage accumulates more slowly (Melov *et al.*, 1995), possibly due to phosphatidyl-inositol 3 kinase (Morris *et al.*, 1996).

1.2.2.3 Genome instability

1.2.2.3.1 ***Telomere shortening***

The theory of "telomere shortening" suggests that telomeres may play a central role in the senescence of proliferating cells (reviewed by Von Zglinicki, 1998). Because of the semiconservative nature of DNA replication, the distal 3' end of the lagging strand of linear DNA cannot be replicated (Olovnikov, 1973). Immortal cell lines, germline cells and unicellular organisms are able to extend the 3' lagging end of the telomere by way of an activated telomerase (Greider and Blackburn, 1985; Von Zglinicki, 1998). Minimal telomerase activity has been found in some human stem cells, but not in somatic cells (Kim, 1997). It has been demonstrated that the rate of telomeric shortening of fibroblasts *in vitro* could be increased by oxidative stress (Von Zglinicki *et al.*, 1995). This may be the result of single-stranded breaks in the telomeres, which are not repaired

as is the rest of the chromosome. It is suggested that the telomeres may trigger cell cycle arrest, possibly through the activation of a tumor suppressor gene p53 (Brown *et al.*, 1997). As with the theory of oxidative damage, telomere shortening may contribute to the aging process in an environment-dependant manner. Due to this dependency, it is possible to modify the effects of nature (genetic limitations) by manipulating the environment. This concept has been clearly established in calorie-restricted animals that have a lower rate of incidence of age-associated diseases, as well as an increased lifespan (see section 1.2.5 this review).

1.2.2.3.2 ***Mitotic Misregulation***

Fibroblasts from normal old donors as well as from patients with the rare pediatric progeria, Hutchinson–Gilford syndrome, demonstrate a dramatic decrease in the expression of genes involved in cell cycle progression, and an up-regulation of genes involved in the maintenance and remodeling of the extracellular matrix (Ly *et al.*, 2000). These changes may result in an increase in the rate of somatic mutations that result in the aging phenotype. Werner syndrome, an adult progeroid disease, is due to a mutation to the WRN gene, which encodes a DNA helicase (Yu *et al.*, 1996). Werner syndrome results in a greater frequency of chromosomal deletions and rearrangements (reviewed by Ellis, 1997).

1.2.2.4 Cell Death

Cell death may play two distinct roles in mitotic versus postmitotic cells. In postmitotic cells, such as cardiac myocytes and cortical neurons, there is an age-associated decline in the number of functioning cells. However, in mitotic cells the inability to undergo apoptosis has been linked to the increased rate of cancer seen in

senescence. The mitochondria control apoptosis through production of radical oxygen species, disruption of the electron transport chain, and by the release of caspase activators such as cytochrome C. The mitochondria may contribute to altered aging via the same mechanisms (Sohal, 1993). For instance, a reduction in cytochrome C seen in aging would decrease apoptosis and thereby increase the risk of developing cancer with advancing age.

1.2.3 Successful Aging

In 1987 Rowe and Kahn suggested that the effects of aging had been exaggerated and that the modifying effects of diet, exercise, personal habits and psychosocial factors were underestimated. For instance, Zavaroni and coworkers (1986) evaluated the correlation between age and glucose intolerance, as well as extrinsic factors such as obesity, physical activity and family history. They found that the contribution of age to glucose intolerance was relatively modest, as compared to other age-related environmental factors. Rowe and Kahn (1987) observed that gerontological researchers had primarily distinguished between pathological and non-pathological states in the aged. They suggested that researchers should also focus on the "normal" or non-pathological aged, and focus on the differences between "usual" and "successful" aging. This distinction emphasizes the link between intrinsic and extrinsic factors, including the relationship between the physiological and the psychosocial aspects of the individual (Rowe and Kahn, 1987). Because extrinsic factors play a major role in "successful aging", nutrition is an important modifiable factor in enhancing the health of the aged.

1.2.4 Nutritional Needs of the Elderly

The nutritional needs of humans change throughout their life span, and several age-related changes influence the nutritional needs and intakes of the elderly. First, there is a decrease in energy requirements. This is due partially to a decline in physical activity, and is also due to a reduction in muscle mass. The loss of muscle mass, known as sarcopenia, is caused in part by reduced activity, but also by biological changes, such as the loss of motor neurons and decreased hormonal influences associated with aging, as well as pathological states causing catabolic stress such as congestive heart failure (Fiatarone and Rosenberg, 1999). There is a life-long, age-related reduction in muscle mass and strength, with a more pronounced reduction in males than in females. The primary reduction in muscle mass is in the type II or "fast twitch" fibers. The mass of type II fibers is selectively reduced by disuse, and their mass can be increased by strength building exercise, specifically resistance training (Frontera *et al.*, 1991). The fall in muscle mass reduces both the metabolic rate and the thermogenic effect, and reduces energy requirements by about 100 kilocalories per decade (Fiatarone and Rosenberg, 1999). Sarcopenia may also reduce insulin sensitivity. Decreased muscle mass is often associated with a decrease in energy input, and with this there is often a reduction in micronutrient intake. With inadequate micronutrient intake, there may be an increased risk of infection and immune dysfunction (Fiatarone and Rosenberg, 1999).

The second factor influencing nutritional needs in the elderly is an increased protein requirement. The equilibrium between protein synthesis and degradation may be disrupted in aging (reviewed by Gariballa and Sinclair, 1998). Protein-calorie malnutrition is estimated to affect 11% to 22% of community dwelling elderly outpatients

(Miller *et al.*, 1989). The recommended daily allowance of protein is 0.8 g/kg of body weight (National Academy of Science, 1989). Based on a nitrogen balance study, Campbell and colleagues recommend that protein intake should be increased to 0.91+/- 0.043 g/kg/day in healthy older men and women (Campbell *et al.*, 1994). A study by Meridith and coworkers found that an increase in muscle was significantly enhanced in those elderly persons who were supplemented with protein during strength training, as compared with their non-supplemented counterparts (Meridith *et al.*,1992). However, protein supplementation without exercise has been shown to have little effect on improving muscle mass (Blumberg, 1997).

A third change to nutritional needs with aging is an increase in micronutrient requirements. Some physiological factors influencing the increased need for micronutrients include a reduction in the intestinal absorption of calcium, vitamin B12, iron and folic acid; decreased metabolic utilization of vitamin B6; diminished synthesis of vitamin D; and a decline in immune function which may respond to vitamin E and to other antioxidants (Blumberg, 1997; Fiatarone and Rosenberg, 1999). In contrast to the increased need for micronutrients, there is a greater risk of micronutrient toxicity. This may be due to decreased metabolization of the micronutrients, particularly vitamin A, and to increases in adipose deposits, which store fat-soluble vitamins (Fiatarone and Rosenberg, 1999).

Finally, as suggested earlier, there is an overall decrease in nutrient intake by the elderly. Several factors may contribute to this reduction, such as decreased taste and olfactory perception, difficulty eating, anorexia, as well as difficulty in obtaining and preparing food. Anorexia presenting *de novo* in the older adult, called *anorexia tardive,*

may be due to social factors, alcoholism, Alzheimer's' disease and drugs (Gazewood and Mehr, 1998). Davis and coworkers (1990), in a study of 4964 persons aged 55 years and older, found that those who lived alone consumed fewer calories than those living with others. Medications may cause the sensation of nausea, or they may reduce a person's ability to taste foods. Several medical illnesses are associated with weight loss and anorexia. Interleukins and tumor necrosis factor contribute to the anorexia experienced in some cancer patients, and some common gastrointestinal disorders such as peptic ulcer disease and gastroesophogeal reflux disease may also contribute to anorexia (Gazewood and Mehr, 1998).

Difficulty in eating, despite a good appetite, may be caused by oral problems, functional impairments, or swallowing disorders. In the frail elderly, dental problems may be the best predictor of weight loss (Gazewood and Mehr, 1998; Sullivan *et al.*, 1993). Presbyesophagus is a common cause of dysphagia and decreased food intake in the elderly. Dysphagia may result from neurological disorders such as stroke and Parkinson's disease. It may also represent structural lesions or central nervous system (CNS) disorders such as hypo/hyperthyroidism (Castell, 1994).

1.2.5 Obesity and Weight Change

Obesity has clearly been shown to negatively influence health by increasing the risk of disease and by reducing life span. In a 32-year study of 1741 university alumni, smoking, higher body-mass index and poor exercise patterns were associated with poorer health, as well as with decreased survival (Vita *et al.*, 1998). In keeping with these findings, caloric restriction (CR) is the most effective method to extend life span and to reduce the deleterious effects of aging. In laboratory rodents as well as other mammalian

and non-mammalian species, CR has repeatedly been shown to extend the life span by as much as 40% (Roth *et al.*,1999; Weindruch and Walford, 1998). The mechanism for this extension is a reduction in metabolic rate in the CR animal, and hence a reduction in oxidative stress (Sohal and Weindruch, 1996). Oxidative stress is the damage of DNA and proteins caused by highly reactive forms of oxygen. The reactive oxygen species are the consequence of aerobic metabolism (Beckman and Ames, 1999; Sohal and Weindruch, 1996). In early reports of CR primates, there appeared to be a reduction in Type II diabetes and in cardiovascular disease, two diseases that are typically considered to be age-related (Roth *et al.*, 1999). In humans, caloric restriction may also contribute to life span extension. On the Japanese island of Okinawa, inhabitants consume 40% fewer calories than those on the mainland. This may contribute to the greater proportion of centenarians living on the island versus the mainland (Kagawa, 1978).

As well, there are benefits to increased weight in the elderly. Increased weight is associated with higher bone mineral density and a lower fracture rate (Reynolds *et al.*, 2000). Increased weight is also associated with higher lean muscle mass, with greater isometric strength and enhanced mobility. As well, excess weight may supply a reserve during periods of catabolic stress, such as illness. Some findings suggest that in the elderly, a more accurate predictor of mortality is "weight change" rather than obesity. In a study of community-dwelling women, those with a change in weight status (either increase, decrease or fluctuations) were at greater mortality risk than those women who maintained a steady weight (Reynolds *et al.*, 2000).

1.2.6 Diabetes

The Canada Study of Health and Aging, reported by Rockwood and colleagues (1998), found that there was a rate of overall prevalence of diabetes mellitus of 12.4% among the nations' elderly, aged 65-100 years. In this population, the prevalence of diabetes mellitus is higher among the institutionalized-elderly than among the community-dwelling elderly population (17.5% and 12%, respectively). In an American report, the prevalence of diabetes, elevated fasting glucose and glucose tolerance were reported in about 20% of those in the over 60 year old group (Halter, 1999). The presence of diabetes is associated with an increased risk of several other age-related disease processes. For instance, the risk of stroke, cardiovascular disease and renal disease is double in the diabetic, as compared with the non-diabetic population (reviewed by Halter, 1999). The risk of blindness is 25-fold higher for the diabetic patient due to the complication of diabetic retinopathy (Fam, 1999). Diabetes accelerates the aging process, increasing the biological age of the diabetic by 10 years over the chronological age (Morley, 1999). Aging, *per se*, only accounts for 20% of the hyperglycemia in the aged. However, other changes often associated with aging also contribute to hyperglycemia, such as an increased body mass, increased adiposity, and decreased physical activity (Halter, 1999).

1.2.6.1 Type 1 Diabetes Mellitus

Type 1 or insulin-dependent diabetes mellitus (IDDM) is an autoimmune disorder that may first present at any age, including the elderly patient. It is associated with a destruction of the insulin-producing beta cells of the endocrine pancreas. The disease is associated with markers of immune destruction of pancreatic beta cells, with resulting

islet cell antibodies or insulin antibodies (Halter, 1999). Human leukocyte antibodies are also associated with type 1 diabetes. In cases of severe insulin deficiency, there is increased mobilization of fatty acids resulting in an enhanced production of ketoacids. This can lead to the life-threatening condition of diabetic ketoacidosis (DKA). DKA is the classic presentation of type 1 diabetes in young persons, yet not in the elderly who commonly present with a more indolent course of type 1 diabetes.

1.2.6.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), is not associated with the markers of autoimmunity which are seen in type 1 diabetes. Type 2 diabetes accounts for 85% of the diabetes worldwide (Weir and Leahy, 1997). NIDDM is common in older patients, and is associated with increased hyperglycemia and not with the development of DKA. There is evidence suggesting a genetic component for the development of NIDDM. A British study of 44 non-diabetic twin subjects followed for a 15 year period found the rate of concordance for the development of type 2 diabetes or abnormal glucose metabolism to be 76% and 96%, respectively (Medici *et al.*, 1999). In a recent review of type 2 diabetes, Vaag (1999) suggests that some of the environmental factors contributing to the disease include low birth weight, low physical activity, high fat diet, the development of obesity, as well as elevated tissue and plasma free fatty acid levels.

1.2.6.3 Diabetes and the Gastrointestinal Tract

Altered gastric motility is a frequent complication of diabetes mellitus, and is present in 30% to 50% of randomly selected diabetics (reviewed by Kong and Horowitz, 1999). The most common finding is delayed gastric emptying. This can lead to

gastrointestinal symptoms, poor glycemic control, changes in oral drug absorption, and postprandial hypotension (Kong and Horowitz, 1999). The rate of gastric emptying is slower during periods of hyperglycemia than during hypoglycemia. The mechanism of this altered metabolism may be via the central nervous system. Hyperglycemia suppresses vagal cholinergic activity, and affects the secretion of a number of gastrointestinal hormones including motilin, pancreatic polypeptide, somatostatin, glucagon, and gastric inhibitory polypeptide (Barnett and Owyang, 1988; Hilstead, 1982; Schavarez *et al.*, 1997). Postprandial hypotension in diabetes may be due to impaired regulation of splanchnic blood flow, and to the release of gastrointestinal hormones (Jones *et al.*, 1998).

1.3 Digestion and Absorption

1.3.1 Small Intestine Morphology

The main function of the small intestine is nutrient absorption. In humans, the small intestine is approximately 6 meters in length in humans (Thomson *et al.*, 1997), and 1.1 meters in mature Fischer 344 rats (Holt *et al.*, 1984). The most proximal region is the duodenum, followed by the jejunum, and most distally the ileum. The interior of the small intestine is the lumen, surrounded by the intestinal mucosa. Numerous mucosal villi increase the absorptive surface area by 7- to 14- fold (Madara and Trier, 1994). The height of the villi in humans ranges from 0.5 mm to 0.8 mm (Madara and Trier, 1994), and ranges from approximately 0.3 mm to 0.5 mm in F344 rats (Holt *et al.*, 1984). The mucosa may be divided into three layers, (1) the muscularis mucosa, which is a thin layer of smooth muscle cells and separates the mucosa from the submucosa; (2) the lamina propria, which is a continuous sheet of connective tissue abundant in many

immunologically important cells such as plasma cells, lymphocytes and macrophages, and as well contains blood and lymph vessels; and (3) the epithelial layer, which can be further subdivided into two regions, the crypt and villous epithelium. The crypts of Lieberkühn are located at the base of the villi, and contain a number of cells such as mucus secreting cells, endocrine cells, as well as immature and undifferentiated cells (Madara and Trier, 1994). The immature cells leave the crypt and migrate to the villous tip over a period of approximately 2-3 days in rats and 4-5 days in humans. During this time they differentiate into fully functional cells. The villous epithelium is a single cell layer, which contains a large number of absorptive cells known as the enterocytes. The enterocytes are highly polarized cells that facilitated vectorial transport of nutrients. The apical membrane is characterized by microvilli, and is known as the brush border membrane (BBM). It is estimated that the BBM increases the absorptive surface area of the enterocyte by 14- to 40- fold (Madara and Trier, 1994).

Several hormones also regulate the development, maintenance and adaptation of the gastrointestinal tract, as well as aid in nutrient digestion and absorption (Walsh, 1994). Age-related changes in the activity and abundance of several of these hormones are briefly reviewed below.

1.3.2 Digestive hormones

1.3.2.1 Gastrin

Gastrin is a hormone produced primarily in the gastric antrum as well as in the human duodenum. Gastrin regulates gastric acid secretion and gastric mucosal cell proliferation. The hormone is produced and secreted by "open"-type endocrine cells, the principal cell being the G-cell located in the glands of the antral mucosa. The cells are

located in such a manner that their surface is in contact with the intestinal luminal contents.

Nutrients such as peptides, amino acids and calcium cause the release of gastrin from G-cells. The most effective amino acids are tryptophan and phenylalanine. Glucose and fats, when administered without amino acids, cause no measurable increase in serum gastrin concentrations. Another stimulant of gastrin release is bombesin, or the mammalian equivalent gastrin-releasing peptide (GRP). GRP is a neural regulator of gastrin release, and involves the lateral hypothalamus (Miller *et al.*, 1989). Gastrin release is inhibited by the acidification of the luminal contents below a pH of 3, and by the action of the gut hormone somatostatin.

The effects of aging on gastrin levels appear to differ between species. In humans, there is a modest age-related increase on serum gastrin concentrations (Trudeau and McGuiggan, 1970). Sandstom and El-Salhy (1999) reported an age effect in the number of gastrin-immunoreactive (IR) cells. When compared with the 20-29 year old group, both the 1-2 and 60-69 year old groups demonstrated an increase in gastrin-IR cells. In contrast in rodents, aging is associated with decreased serum gastrin levels (Khalil *et al.*, 1988), gastrin protein levels and gene expression (Kogire *et al.*, 1993; Kyo *et al.*, 1996; Majumdar *et al.*, 1988; El Salhy and Sandstom, 1999). Short-term caloric restriction (CR) of young, mature and old rats results in a reduction of antral gastrin content and gene expression in all age groups. While CR results in a reduction in serum gastrin concentrations in young and mature rats, no further reduction in serum gastrin levels was found in the CR-old rats over their control counterparts (Kyo *et al.*, 1996).

1.3.2.2 Somatostatin

Somatostatin is a neuropeptide which is widely distributed in the autonomic and central nervous system (Walsh, 1994). Somatostatin in the intestine has been identified in enteroendocrine cells, in D-cells and in enteric neurons. In humans, circulating somatostatin is derived mainly from the small intestine (Ensinck *et al.*, 1990). First identified by its action to inhibit the release of growth hormone from the pituitary (Hall *et al.*, 1978), somatostatin has subsequently been shown to inhibit the secretion of many gut hormones, as well as to inhibit nutrient absorption (Walsh, 1994). Gastric acidification causes the release of somatostatin (Holst *et al.*, 1992). Dietary protein and fats also stimulate somatostatin release (Polansky *et al.*, 1983).

In mice, there is an age-related decline in tissue somatostatin in the antrum, duodenum and colon (El Salhy and Sandstom, 1999). There is also an increase in the number of somatostatin-IR cells in 40-49 year olds, as compared with 20-29 year olds (Sandstom and El Salhy, 1999).

1.3.2.3 Cholesystokinin

Cholesystokinin (CCK) is expressed in endocrine cells in the mammalian duodenal and jejunal mucosa (Rehfeld, 1978), as well as in the central and peripheral nervous systems (Walsh, 1994). CCK stimulates pancreatic enzyme secretion and gallbladder contraction. CCK slows gastric emptying, stimulates somatostatin release, and inhibits gastric acid secretion. CCK also increases satiety, possibly by the activation of CCK receptors in the peripheral nerves that transmit satiety signals to the brainstem (Walsh, 1994). Studies in humans have shown that by blocking the CCK receptor using a specific CCK-A antagonist, satiety was decreased (Wolkowitz *et al.*, 1990). There is an

increased plasma CCK concentration in the elderly, suggesting that this hormone may play a role in the decreased appetite of these persons (MacIntosh *et al.*, 1999). There is also an increase in CCK-IR cells in those 60-69 years old over younger counterparts (Sandstom and El Salhy, 1999)

1.3.2.4 Secretin

Secretin-producing cells are located primarily in the small intestine, with greatest concentrations in the duodenum (Miller *et al.*, 1978). Secretin stimulates pancreatic bicarbonate secretion, and also potentiates the action of CCK on pancreatic enzyme secretion. Secretin also stimulates the secretion of pepsin into the gastric juice of cats and dogs (Magee and Nakajima, 1968; Stening *et al.*, 1969). Secretin levels in aged mice are not significantly different from mature mice, but are higher than in young animals (El Salhy and Sandstrom, 1999). Other investigators have reported decreased pancreatic bicarbonate and enzyme secretion associated with aging in humans (Laugrer *et al.*, 1991).

1.3.2.5 Gastric-Inhibitory Peptide (GIP)

GIP is predominantly found in the duodenum and jejunum, with lower amounts in the ileum and gastric antrum (Polack, 1973). GIP inhibits gastric secretion and stimulates intestinal secretion. Glucose and galactose stimulate GIP release. GIP release is also stimulated by hydrolyzed triacylglycerol, specifically long-chain fatty acids (Ross and Shaffer, 1981). GIP release reduces water and sodium absorption in the jejunum (Helman and Barbezat, 1977). GIP enhances pancreatic insulin release in the presence of hyperglycemia (Elahi, 1979). Cheeseman and O'Neill (1998) report an increase in phloridzin-insensitive D-glucose uptake into enterocytes following vascular perfusion of

GIP. Levels of GIP release are reported to be higher in obese and in diabetic individuals (Ebert *et al.*, 1979). While no significant decrease in the levels of circulating GIP occur in the aged (Chung *et al.*, 1993), normal aging results in a decline in beta-cell sensitivity to GIP during modest hyperglycemia. This may contribute to the glucose intolerance associated with aging (Meneilly *et al.*, 1998).

1.3.2.6 Enteroglucagons

The enteroglucagons are produced from the same gene as glucagon, located on chromosome 2 in humans (Tricoli *et al.*, 1984). Post-translational processing of proglucagon in the small intestine results in two main glucagon-like peptides (GLP), GLP-1 and GLP-2 (Holst *et al.*, 1992). GLP-1 stimulates insulin release (Holst *et al.*, 1987) and the plasma concentration of GLP-1 in humans is not altered in aging (MacIntosh *et al.*, 1999). GLP-2 induces intracellular trafficking of SGLT-1 from an intracellular pool into the BBM of the enterocyte (Cheeseman, 1997). Vascular infusion of GLP-2 increases phloridzin-insensitive glucose transport (Cheeseman and O'Neill, 1998). However, this increased transport was significantly reduced by brefeldin A, which is an inhibitor of intracellular trafficking. These findings suggest that GLP-2 may also up-regulate GLUT2 activity by way of protein trafficking within the cell.

1.3.3 Intestinal nutrient absorption

Dietary nutrients or drugs are absorbed from the intestinal lumen by the transcellular and paracellular routes. Enterocytes are joined by tight junctions (TJ), which restrict the free movement of molecules from the intestinal lumen to the blood stream via the paracellular route. As well, the TJs maintain the polarity of the

enterocytes with an apical brush border membrane (BBM) and a basolateral membrane (BLM) (Fasano, 2000; Balda and Matter, 1998).

In hamsters the presence of D-glucose within the enterocyte causes a contraction of the cell and dilation of the TJ, as well as increased paracellular glucose transport (Madara and Pappenheimer, 1987). Studies with human jejunal tissue do not demonstrate increased TJ permeability induced by Na+ dependent glucose transport (Fine *et al.*, 1993). In dogs, the paracellular route of glucose absorption represents 4% - 7% of the total absorption of glucose at physiological concentrations (Lane *et al.*, 1999). Thus, the contribution of the paracellular route to the transport of glucose is likely to be small.

The transcellular route of absorption involves passage through the enterocyte BBM and BLM. Transmembrane proteins facilitate the movement of many molecules across the cell membrane. Absorption via a transport protein is a saturable process, and is dependent on the number of transporters available. Therefore, a change in the value of the maximal transport rate (Vmax) may be due to an alteration in the number of transporters, or their rate of turnover. A change in the value of the Michaelis-Menten affinity constant (Km) represents an alteration in the affinity of a transporter for its substrate (Thomson and Wild, 1997).

1.3.3.1 Carbohydrates

Dietary D-glucose is transported from the intestinal lumen by an active transport mechanism in the BBM, the sodium-glucose transporter, SGLT. Glucose transport across the BBM is driven by a Na+ gradient maintained by the Na+/K+ pump in the BLM. The SGLT1 transporter is an 84 kDa protein, and both D-glucose and D-galactose are substrates for SGLT1 (Dodson *et al.*, 1996). The stoichiometry for SGLT1 transport is 1

glucose molecule, 2 Na+ ions and 210 molecules of H_20 (Meinild *et al.*, 1998). While there are several possible phosphorylation sites located on the cytoplasmic side of SGLT1, there is no evidence that these sites are actually phosphorylated (Wright *et al.*, 1998). The functional SGLT1 cotransporter exists as a homotetramer (Stevens *et al.*, 1990). The density of SGLT1 is highest in the jejunum, intermediate in the ileum, and lowest in the duodenum (Kennelly *et al.*, 1991). The abundance of SGLT1 is either constant along the villus (Gould and Holman, 1993), or increases from the crypt-villus junction to the villous tip (Yoshida *et al.*, 1995). Immunohistochemical studies using a polyclonal antibody against SGLT1 demonstrate a uniform distribution of SGLT1 immunoreactivity in the BBM of adult rat jejunal enterocytes (Smith *et al.*, 1992). This is also true for SGLT1 mRNA levels (Smith *et al.*, 1992). However, the activity of the transporter increases toward the tip of the villus (Koepsell and Seibicke, 1990; Davidson *et al.*, 1992). It may be that SGLT1 becomes functionally activated only in the upper portion of the villus. This increased post-translational activity may be due to the formation of a homotetramer of SGLT1 proteins, the presence of regulatory and or catalytic subunits, or the action of protein kinases (Thomson and Wild, 1997). Protein kinase A (PKA) and protein kinase C (PKC) may regulate functional activity by protein trafficking between endosomal and plasma membranes (Hirsch *et al.*, 1996). PKA may also increase the stability and the expression of SGLT1 mRNA (Peng and Lever, 1995; Clancey and Lever, 2000). Human SGLT1 activity is increased by PKC, while rabbit and rat SGLT1 activity is decreased by PKC when expressed in *Xenopus laevis* oocytes (Hirsch *et al.*, 1996). In addition, PKC may also inhibit glucose transport by reducing the turnover rate of the transporter (Vayro and Silverman, 1999).

Transport of sugar by SGLT1 depends on the maintenance of the Na+ gradient by the Na+/K+ ATPase (Wright *et al.*, 1992). The Na+/K+ ATPase αl and β1subunits are located in the BLM of the enterocytes (Wild *et al.*, 1994). The activity of the Na+/K+ ATPase increases toward the tip of the villus, as does the mRNA expression of the αl and β1subunits (Wild and Murray, 1992). Phosphatidylinositol 3-kinase may play an important role in the activation of the Na+/K+ ATPase and the transport of D-glucose (Alexander and Carey, 2001).

GLUT2 and GLUT5 are members of a large family of facilitative hexose transporters. GLUT5, a 50 kDa protein, is responsible for D-fructose transport across the BBM of the enterocyte. The Km of GLUT5 for fructose is approximately 15 mM (Corpe *et al.*, 1999). GLUT2 transports D-fructose, D-galactose and D-glucose across the BLM (Wright *et al.*, 1998). GLUT2 may also be located in the BBM (Kellett and Heliwell, 2000; Kellet). Kellett (2001) proposes that glucose is initially transported by SGLT1, which activates PKC βII, which may then cause a rapid trafficking of GLUT2-containing vesicles to the BBM. GLUT2 has the highest Km of all the transporters for glucose, measured between 15 to 40 mM (Zeirler, 1999). This high Km value may reflect the role of GLUT2 in cells where intracellular glucose concentration exceeds that of the plasma concentration (Zeirler, 1999). GLUT5 and GLUT2 are expressed proximally to distally in the small intestine, with the highest level of expression being in the proximal small intestine. Along the crypt to villous axis, expression increases from the crypt-villous junction to the villous tip, but is absent in the crypts (Thomson and Wild, 1997).

1.3.3.2 Lipids

The majority of dietary fat is in the form of triacylglycerol (TG), which is hydrolyzed by lingual, gastric and pancreatic lipases to form monoacylglycerol and free fatty acids (reviewed by Phan and Tso, 2001). Digestion of phosphatidylcholine occurs in the small intestine by the action of pancreatic phospholipase A2. Dietary cholesterol esters are hydrolyzed by the action of cholesterol esterase in the intestinal lumen, and are absorbed in the small intestine as free cholesterol and fatty acids (reviewed by Dawson and Rudel, 1999). Cholesterol esters may also be subject to protein-mediated uptake (Compassi *et al.*, 1995). The absorption of fatty acids occurs primarily in the proximal intestine, and in the upper portion of the villi (Fingerote *et al.*, 1994). In this review, the absorption of lipids will be considered in four stages; (1) diffusion across the intestinal unstirred water layer (UWL); (2) transport across the BBM; (3) intracellular binding and trafficking; and (4) microsomal transport and lipoprotein formation.

Lipids diffuse across the intestinal UWL before contacting the BBM. The effect of the resistance of the UWL is to reduce the concentration of the lipid presented to the enterocyte. Therefore, it is important to correct for the effect of the UWL on absorption in order to assess the true permeability properties of the BBM (Proulx *et al.*, 1984, Westergaard and Dietschy, 1974). Failure to correct for the effective resistance of the UWL will result in underestimation of the true permeability properties of the BBM. The fatty acid partition coefficient increases with its chain length by a factor corresponding to a decrease in the incremental change in free energy moving from an aqueous to a lipid

phase (Thomson and Dietschy, 1981). Phospholipids are found in bile micelles together with cholesterol and bile salts. Bile acid micelles greatly enhance the uptake of fatty acids and cholesterol from the small intestine by helping to overcome the resistance of the UWL and by increasing monomer concentration at the aqueous-membrane interface (Westergaard and Dietschy, 1976).

Three models of passive lipid uptake have been proposed (Thomson and Dietschy, 1981): (1) the entire mixed micelle is absorbed by the BBM. However, no experimental evidence supports this model (Wilson and Dietschy, 1972); (2) the micelle collides with the BBM, allowing for the uptake of lipids to occur. Evidence for this model is suggested by the linear relationship between cholesterol uptake and bile acid concentration (Proulx *et al.*, 1984, Burdick *et al.*, 1994); (3) the lipids dissociate from the micelle into the aqueous compartment of the UWL before being taken-up by the BBM (Westergaard and Dietschy, 1976). Support for this latter model is suggested by the finding that fatty acid uptake decreases with an increase in the number of bile acid micelles, where fatty acid concentration is kept constant (Westergaard and Dietschy, 1976).

The dissociation of lipids from bile acid micelles is under the influence of the acidic microclimate adjacent to the BBM (Shiau, 1990). Under these acidic conditions the critical micellular concentration increases and fatty acids become protonated; protonation increases their rate of permeation across the BBM (Shiau, 1990). An increase in the fluidity of the BBM also increases the rate of permeation of lipids (Higgins, 1994). Other factors influencing the rate of lipid uptake may be the luminal lipid composition, fatty

acid binding proteins, and the membrane potential. For instance, polyunsaturated fatty acids (such as 18:1, 18:2, 18:3, 20:4) and phosphatidylcholine may inhibit cholesterol absorption, possibly by shifting the partition coefficient of cholesterol away from the cell membrane back to the micelle, thereby preventing the uptake of lipid (Hollander and Morgan, 1980).

Several proteins have been described in the BBM that may contribute to fatty acid and cholesterol transport (Table 1.1). A 43 kDa protein, known as the plasma membrane fatty acid binding protein ($FABP_{pm}$), was identified in the BBM and BLM of intestinal cells (Stremmel *et al.*, 1985). This protein binds long chain fatty acids (LCFA), monoglycerides and cholesterol. Incubation of rabbit jejunal BBM vesicles with anti-$FABP_{pm}$ antibody results in a reduction in oleic acid uptake (Schoeller *et al.*, 1995).

The scavenger receptor of class B type I (SR-BI) is a 57 kDa integral membrane protein and is located in the BBM (Schulthess *et al.*, 2000). High-density lipoproteins, the physiological ligand for this receptor in the liver, inhibit the uptake of free and esterfied cholesterol into the BBM. SR-BI may act as a docking receptor for donor particles, such as bile acid micelles, followed by the transfer of lipids to the BBM (Hauser *et al.*, 1998). SR-BI may also play a role in cholesterol absorption, as well as lipoprotein transport (Cai *et al.*, 2001). SR-BI is present on both the apical and basolateral surfaces of the jejunum villus, with little SR-BI being detectable on either apical or basolateral membranes in the ileum (Cai *et al.*, 2001).

Caveolin-1 is a 22 kDa integral membrane protein located in detergent-resistant microdomains of the BBM. With the influx of oleic acid or cholesterol, plasma

membrane cholesterol is transported to the endoplasmic reticulum, is esterified by acyl-CoA:cholesterol acyltransferase, and is part of the secreted lipoprotein particle. With the influx of micellar cholesterol, plasma membrane cholesterol moves to these microdomains and is transported to the ER (Field *et al.*, 1998). Newly synthesized cholesterol replenishes the plasma membrane cholesterol. The caveolins may act as a plasma membrane storage of cholesterol, and may play a role in the sterol-sensing component of the BBM (Field *et al.*, 1998). Caveolin-1 also exhibit a binding affinity for long chain fatty acids (Trigatti *et al.*, 1999). The role of caveolin-1 in the intestine has not been established, but may be involved in the intracellular targeting of lipids (Uittenbogaard and Smart, 2000).

Fatty acid translocase (FAT) is an 88 kDa transmembrane glycoprotein located in the BBM of enterocytes (Abumrad *et al.*, 1984). Rat FAT is 85 % homologous to the human scavenger receptor CD36, which is found in platelets, lactating mammary epithelium, monocytes and adipocytes. FAT null mice are viable, but have reduced uptake of triglycerides into adipocytes. FAT mRNA is expressed primarily in the BBM and in the upper two thirds of the intestinal villi. Dietary fat rich in polyunsaturated fatty acids up-regulates the expression of intestinal FAT mRNA (Poirier *et al.*, 1996).

The fatty acid transport protein (FATP) is a 63 kDa membrane protein which is expressed in adipose tissue, heart and skeletal muscle (Schaffer and Lodish, 1994). FATP increases the uptake of oleate in fibroblast cell lines. There is a large family of FATPs (Hirsch *et al.*, 1998), but only FATP4 is present in appreciable levels in the intestine. FATP4 mRNA is expressed in the enterocytes of the jejunum, ileum, and at lower levels in the duodenum. Expression of FATP4 mRNA is absent from both the crypts of the

small intestine and from the colon. Fatty acids containing 10-26 carbon atoms are thought to be substrates for FATP4 (Stahl *et al.*, 1999).

The passive absorption of sterols occurs as a result of collision between mixed bile salt micelles and the BBM, but BBM vesicle transport is reduced following membrane digestion with proteases, suggesting the existence of a transport protein (Thurnhofer and Hauser, 1990). This "cholesterol transport protein" is an integral membrane protein, with at least one hydrophobic domain (Boffelli *et al.*, 1997). Long chain triacylglycerols may be transported across the BBM by a protein, perhaps by the same protein as cholesterol. The multidrug resistance protein (MDR; MDR1 in humans), may be involved in the uptake of cholesterol into intestinal epithelial cells (Tessner and Stenson, 2000). A sterol glycoside derivative specifically binds to the BBM of enterocytes, and blocks the absorption of cholesterol (Hernandez *et al.*, 2000, Detmers *et al.*, 2000). A 145 kDa integral membrane protein in the BBM of rabbit enterocytes has also been identified, and may contribute to intestinal cholesterol absorption (Kramer *et al.*, 2000).

A family of cytosolic fatty acid binding proteins has been identified, including three located in the small intestine: the intestinal FABP (I-FABP), the liver-FABP (L-FABP) and the ileal lipid binding protein (ILBP). L-FABP is a 14.1 kDa protein located in the duodenum and jejunum, with maximal expression in the proximal jejunum. I-FABP is a 15.1 kDa protein that is expressed throughout the small intestine, with maximal expression in the distal jejunum. L-FABP and I-FABP proteins are present along the crypt-villous axis. However, L-FABP is absent in the villous tips (Halden and

Aponte, 1997). The mRNAs for I-FABP and L-FABP are expressed throughout the small intestine and along the length of the villi (Poirier *et al.*, 1996).

The tertiary structure of the FABP consists of 10 antiparallel β strands containing a ligand-binding cavity (Halden and Aponte, 1997). Both I-FABP and L-FABP bind long chain fatty acids with high affinity. Rats treated with clofibrate (a hypolipidemic drug) have increased expression of L-FABP protein and mRNA, with no change in the expression of the I-FABP counterparts (Bass *et al.*, 1985). The mRNA expression of I-FABP and L-FABP is increased in rats fed a diet rich in polyunsaturated fats from sunflower-oil (Poirier *et al.*, 1996). The transfer of fatty acid from I-FABP to membranes occurs by direct collisional interaction with the phospholipid bilayer, which suggests a possible role in the uptake or subcellular targeting of fatty acids (Hsu and Storch, 1996). In contrast, L-FABP may transfer fatty acids in an aqueous diffusion-mediated process, and may act as a cytosolic buffer for fatty acids (Hsu and Storch, 1996). However, in I-FABP knockout mice, fatty acid uptake is maintained, demonstrating that the I-FABP protein is not required for intestinal lipid uptake (Vassileva *et al.*, 2000). While it is still possible that I-FABP is involved in the uptake and sorting of lipids, it appears that this task may be carried out by other proteins as well.

Peroxisome-proliferator activator receptors (PPAR) may play an obligatory role in up-regulating the expression of L-FABP and I-FABP genes (Motojima, 2000). The livers of mice fed bezafibrate, a PPAR hypolipidemic drug, showed a four-fold increase in L-FABP protein and mRNA (Besnard *et al.*, 1993). In addition, bezafibrate increases the mRNA expression of FAT, suggesting a complementary role of the FAT and FABP proteins in lipid absorption (Boffelli *et al.*, 1997).

The ileal lipid binding protein (ILBP) is a 14 kDa cytoplasmic protein that binds bile acids (Lin *et al.*, 1991). Molecular cloning and expression of ILBP in Cos-7 cells demonstrates a saturable binding of photolabeled 7,7-azo[3H]taurocholate (Gong *et al.*, 1994). ILBP may be functionally active as a homotetramer in association with a homotetramer of a integral 93 kDa BBM protein. Together these two homotetramers comprise the ileal Na+/bile acid co-transporter. ILBP is structurally related to the FABP family, and is located predominantly in the distal ileum. The sterol ring of the bile acids bind deep within the binding cleft of the protein (Kramer *et al.*, 2001). Binding of bile acid to ILBP increases the affinity of binding additional bile acid, suggesting the possibility of a secondary binding site (Kramer *et al.*, 1998).

Mansbach and coworkers have suggested that the rate-limiting step in lipid absorption is the trafficking of triacylglycerol from the endoplasmic reticulum (ER) to the Golgi (Mansbach and Dowell, 2000). Specifically, the rate-limiting step may be the formation of a prechylomicron vesicle that transports the developing chylomicron from the ER to the Golgi.

The microsomal triglyceride transport protein (MTP) is an ER-localized cofactor required for the assembly of apolipoprotein B (apo B). MTP is a heterodimer consisting of a 58 kDa subunit of protein disulfide isomerase (a multifunctional ER protein), and a unique 97 kDa subunit (Gordon *et al.*, 1995). MTP is essential for the transfer of TG and cholesterol esters into the hydrophobic core of apo B (Leiper *et al.*, 1994). A defect in MTP in humans results in the disease abetalipoproteinemia. (Wetterau *et al.*, 1992). In human fetal jejunal and colonic tissue, the MTP protein is expressed along the crypt-villous axis by the 13^{th} gestational week (Levy *et al.*, 2001).

It is unclear what is the relative contribution of these lipid-binding proteins to the total lipid absorbed by the enterocyte, or whether changes in the abundance of these proteins plays a role in the adaptation of lipid uptake such as that which occurs with aging.

1.3.3.3 Amino Acids

The topic of amino acid transport has been reviewed (Palacin, 1998) and the intestinal amino acid transporters are summarized below. The NBB (neutral brush border) or B system is a Na+ dependent system of broad specificity responsible for the transport of zwitterionic amino acids and di- and tripeptides. IMINO is located in the BBM, and transports proline and *N*-methylated glycine (Na+ dependent). The BBM rBAT / bo+ system transports cationic amino acids and cystine (Na+ independent) as well as zwitterionic amino acids (Na+ dependent). A defect in this system is responsible for the condition of cytinuria. CAT-1 is a widely expressed cationic (Na+ independent) and zwitterionic (Na+ dependent) amino acid transporter. EAAT-3 is highly expressed in the small intestine, and transports anionic amino acids (Na+/K+ dependent). PepT-1 transports di- and tripeptides, and is driven by an inwardly directed H+ gradient (Pan *et al.*, 2001).

1.4 Intestinal Adaptation

Intestinal adaptation is defined as the ability of the intestine to change functionally and/or morphologically in response to alterations in environmental stimuli. Generally, the adaptive changes are beneficial, such as increased nutrient absorption following small intestinal resection, chronic alcohol ingestion, or sub-lethal abdominal radiation (Thomson and Wild, 1997). In contrast, the enhanced absorptive adaptation observed in diabetes contributes to the hyperglycemia, hyperlipidemia and to the obesity that is associated with this metabolic disorder.

Depending on the nutrients available, the intestine adapts to variations in dietary load and composition (Diamond, 1991). For example, increased dietary carbohydrates cause an increase in the Vmax for intestinal glucose uptake (Ferraris and Diamond, 1989). Nonessential and nontoxic amino acids are up-regulated on high protein diets, and this response may be impaired in aging (Ferraris and Vinnakota, 1993). Potentially toxic substances such as some essential amino acids, iron and calcium may be down-regulated by a dietary increase in these respective substrates (Diamond and Karasov, 1987; Ferraris and Vinnakota, 1993). Animals fed isocaloric diets enriched with saturated fatty acid (SFA) have greater glucose uptake, as compared to those animals fed polyunsaturated (PUFA) diets (Thomson *et al.*, 1986).

Morphological changes are often associated with intestinal adaptation. Following bowel resection, hyperplasia of the remaining gut is associated with increased nutrient, water and electrolyte absorption (Dowling and Booth, 1967). However, hyperplasia does not necessarily correlate with altered absorption (Thomson *et al.*, 1986). For example, in

the rat jejunum, the uptake of glucose is enhanced by dietary fats, but is not associated with increased mucosal surface area. Following small bowel resection, the mass of the remnant intestine was increased to 50-70% of its pre-resection level, but glucose uptake was restored to only 33% (O'Connor *et al.*, 1999). These findings suggest that morphological changes do not necessarily accompany alterations in absorption, and *vice versa*.

The dietary induction of intestinal BBM glucose transport takes place in the developing enterocytes in the intestinal crypts (Ferraris and Diamond, 1992); mice were switched from a high carbohydrate diet to a no carbohydrate diet, or *vice versa*. Glucose-protectable phlorizin binding was used as a measure of glucose transport site density. An increase or decrease of phlorizin site density first appeared in the crypts, and then over the course of three days extended to the villous tips (Ferraris and Diamond, 1992). This suggests that dietary alterations result in a reprogramming of the developing enterocytes in the crypts, and that changes in uptake are observed as these cells migrate toward the villous tips. Cell dynamics such as the cell turn-over rate, crypt cell production rates, and enterocyte migration rates may be key determinants in the process of intestinal adaptation. Aging results in a higher proliferative rate of the crypt cells, possibly reducing the number of mature transporting enterocytes, and thereby reducing nutrient absorption (Jenkins and Thompson, 1994).

Intestinal adaptation, which is associated with alterations in glucose uptake, is usually due to changes in the value of Vmax (Thomson and Wild, 1997; Diamond and Karasov, 1984; Ferraris and Diamond, 1989). This change in Vmax may result from: (1) an alteration in the absorptive surface area; (2) a change in the lipid membrane fluidity

that alters the exposure of substrate binding sites; (3) variation of transporter gene transcription and a parallel change in protein abundance; (4) post-translational events leading to a change in the number of functional transporters per enterocyte; (5) an alteration in the pattern of distribution of transporters along the villus, with more transporters near the villous tip but without necessarily an alteration in the total number of transporters; (6) insertion of pre-existing transport proteins from intracellular vesicles into the brush border membrane, or removal of the proteins by endocytic retrieval; and (7) covalent modification of the transporter by reversible phosphorylation (Thomson and Wild, 1997; Jenkins and Thompson, 1994). Intestinal adaptation in the chronic diabetic rat involves changes at the transcription level as well as a posttranscriptional event, leading to increased Na+ coupled sugar absorption (Wild *et al.*, 1999). After inducing acute hyperglycemia in rats, there is a rapid up-regulation of glucose transport across the BLM of the enterocyte (Cheeseman and Maenz, 1989). In rats, both vascular and luminal glucose infusion causes an increase in glucose transport capacity across the BLM (Tsang *et al.*, 1994). However, no significant increase in BLM cytochalasin B binding or in GLUT2 protein abundance was observed, suggesting that there may be a post-translational event that increases the number of GLUT2 proteins available for transport.

1.5 Brush Border Membrane (BBM)

The lipid composition of cell membranes alters the passive permeability properties and transporter activity across the membrane (Spector and Yorek, 1985). Enhanced fluidity of the BBM increases the transport of lipids and glucose, but decreases the activity of leucine aminopeptidase in the BBM (Brasitus *et al.*, 1985, Wahnon, 1989).

Low BBM fluidity reflects a low phospholipid-to-cholesterol ratio. Fluidity is greater in the proximal than in the distal small bowel, and decreases as enterocytes migrate from the crypt to the villus (Meddings and Thiessen, 1989).

In the BBM of starved rats, there is an increased ratio of phospholipids (*w/w*) and an increased double-bond index (Waheed *et al.*, 1998). Also, during starvation there is a decreased ratio of cholesterol/phospholipids, protein/lipid and free fatty acids (*w/w*). This suggests that membrane fluidity is increased during starvation, and may facilitate increased glucose transport across the BBM in spite of decreased levels of glucose transporter protein during the starved state. Diets enriched in saturated fatty acids increase the saturation of BBM phospholipids, and PUFA enriched diets increase the percent of unsaturated BBM phospholipids (Thomson *et al.*, 1986). However, alteration of dietary cholesterol intake does not change membrane cholesterol content, suggesting that the cholesterol content of the BBM is controlled.

1.6 Age-associated changes

Of concern, in Canada 59% of elderly patients admitted to a tertiary care facility were found to be malnourished or at high risk for malnourishment (Azad *et al.*, 1999). Many factors contribute to this high rate of malnourishment such as poor dentition, medication use, and psychosocial issues. Aging may also result in a decline in nutrient absorption, and this reduction also contributes to poor nutritional status.

1.6.1 Carbohydrates

An age-associated decline in D-glucose absorption has been found in mice (Ferraris and Ravi, 1993). D-xylose absorption assessed from the urinary excretion of this sugar after oral intake decreases in aging humans. However, D-xylose excretion is dependent on renal function, and when renal function is taken into consideration, there is only a modest reduction in xylose absorption associated with aging (Hosoda, 1992). When fed a meal containing 100 g carbohydrate, one-third of subjects over 65 years had excess breath hydrogen, suggesting malabsorption (Feibusch and Holt, 1982). However, this is a large amount of carbohydrate for one meal, and this reduced absorptive capacity may have minimal nutritional impact for persons consuming lesser amounts of carbohydrate. In addition, breath hydrogen tests can be falsely positive in the presence of bacterial overgrowth of the small intestine. It should also be noted that bacterial overgrowth may occur more frequently among the elderly, and anaerobic bacteria can produce proteases that interfere with disaccharidases in the BBM, thereby resulting in reduced carbohydrate absorption (Riepe *et al.*, 1980). Thus, aging may be associated with a fall in carbohydrate absorption, but the mechanism of this decline remains unknown.

1.6.2 Lipids

In aging there may be reduced gastric lipase and bile acid secretion, decreased lipid solubilization, and thus a decline in lipid absorption (Holt and Balint, 1993). In a study of dietary fat intake and fecal fat output, the digestibility of fatty acids was reduced in old as compared to younger cats (Peachy *et al.*, 1999). *In vivo* perfusion studies in rats showed an increase in fatty acid and cholesterol uptake associated with aging when uptake was expressed on the basis of the length of the intestine (Hollander and Dadufalza, 1983).

However, *in vitro* uptake studies done with rabbit jejunal discs demonstrated a decline in the uptake of fatty acids and cholesterol with age when uptake was expressed on the basis of the weight of the intestine (Thomson, 1980). Radiolabeled fat breath tests have confirmed that lipid absorption is reduced in mature as compared with suckling rats (Flores *et al.*, 1989).

A decrease in intestinal BBM phospholipid composition and membrane fluidity have been reported in aging rats, and this may contribute to the reduced lipid absorption in the aged (Wahon *et al.*, 1989). In contrast, aging is associated with a decrease in the thickness and resistance of the UWL (Thomson, 1980), which would tend to increase the net absorption of nutrients, possibly to partially counteract the decreased permeability of the BBM to lipids with aging.

Older studies suggested that the lower serum chylomicron levels following a fatty meal indicate a reduced absorptive capacity among the aged (Becker *et al.*, 1950). In a study of only the healthy aged, there was no correlation between age and 72 hour fecal fat excretion (Arora *et al.*, 1989). However, the absorption of fat may take longer in the elderly, and this method of measuring serum chylomicron levels may not reflect total uptake of lipids (Arora *et al.*, 1989). There is also a reduction in postprandial serum bile acids levels in elderly humans, suggesting that bile acids are absorbed less effectively (Salemans *et al.*, 1993).

In total, these data suggest that lipid absorption is lower in the older as compared with the younger individual; but the extent depends on the method used to express the data. The mechanisms of this impairment remain unknown.

1.6.3 Amino Acids, Vitamins and Minerals

The absorption of tyrosine, arginine and aspartic acid declines in senescent rodents (Chen and Hellstrom, 1990). In *in vivo* perfusion studies with rats, vitamin A absorption increases in a linear fashion with age (Hollander and Morgan, 1979). Reduced calcium absorption is reported in aging, and may result from attenuated vitamin D metabolites (Morris *et al.*, 1985). The pH microclimate of the rat jejunum is less acidic with aging, and this change may play a role in the reduced intestinal absorption of nutrients, particularly amino acids, lipids, and calcium (Ikuma *et al.*, 1996).

1.6.4 Morphology

Changes in the morphology of the small intestine may also contribute to age-associated alterations in nutrient absorption. In studies of F344 rats, there was no age-related change in the density of the villi in the small intestine, or in the size of the enterocytes (Holt *et al.*, 1984). However, with aging there is an increase in the width of the villi throughout the intestine, an increase in duodenal and jejunal crypt depth, as well as increased ileal villous height, ileal mass and ileal DNA content. The number of villi per unit area of intestine decreases in aged rats, suggesting that the overall surface area of the intestine may fall without an alteration in villous height or width (Clarke, 1972). The height, width, depth and number of villi are incorporated into a measurement of surface area, and it may not be appropriate to predict changes in mucosal surface area based only on alterations in the height of the villi (Ecknauer *et al.*, 1982). In fact, there would appear to be an age-related decline in mucosal surface area in rabbit jejunum (Keelan *et al.*, 1985). In the jejunum of human subjects, no significant difference between young

and old enterocyte height and jejunal surface area-to-volume ratio was found (Corazza *et al.*, 1986).

Morphological changes associated with aging fail to fully explain altered absorptive capacity in laboratory animals. For example, increased saturated fatty acid uptake was observed in the jejunum of mature as compared to young rabbits, and yet surface area decreased (Keelan *et al.*, 1985). These findings suggest that other factors may be responsible for the altered lipid uptake. For example, differentiation of the enterocytes as they migrate from crypt to villous tip is delayed in aging animals. There is a greater crypt cell proliferative rate, as well as a broadened zone of proliferation within the crypts of old rats (Holt *et al.*, 1998). The immunohistochemical expression of the proliferation cell nuclear antigen is markedly increased in the duodenal villi and crypts of humans over 65 years, as compared with younger persons (Corazza *et al.*, 1998). This suggests that the abnormal proliferation pattern may explain the coexistence of "normal morphology", and yet impaired absorptive function in the elderly (Corazza *et al.*, 1998). In aged rats refed a high protein diet following 48 hours of starvation, there is a 35% increase in villous height in the ileum as well as an increased ileal aminopeptidase activity (Raul *et al.*, 1988). This suggests that the ileum may compensate for any age-associated loss of surface area of the jejunum. It is speculated that the ileum of aged rats may be exposed to a greater luminal load due to decreased uptake in the proximal small intestine, thereby causing hyperplasia of the ileum. Supporting this suggestion, ileal-jejunal transposed rats showed an increase in the mucosal mass of the transposed ileum in young, mature and old animals (Tsuchiya *et al.*, 1995). Thus, there may be a modest decline in the surface area of the intestine in some species, but this by itself does not explain functional alterations.

1.6.5 Adaptive response

The adaptive response of the gut is impaired in aging. Therefore, in times of stress such as injury or illness, malnutrition may result more readily (Yoshinaga *et al.*, 1995; Hebuterne *et al.*, 1995). For example, dietary restriction initiated in aged rats resulted in a dramatic weight loss, with no weight stabilization after 12 weeks of reintroduction of a normal diet. In addition, the intestine of the animals was atrophied and ileal hydrolase activity was decreased (Chambon-Sanovitch *et al.*, 1999). Following a period of under–feeding, elderly men continued to under feed themselves for a period of 9-10 days, while their younger counterparts increased their energy intake (Roberts *et al.*, 1994). This suggests that following a period of illness, during which nutrient intake is reduced, the elderly may continue to underfeed themselves despite their recovery from the illness. Thus, the reduction in nutrient absorptive capacity of the small intestine that occurs with aging, plus the reduced adaptive response, will put the older patient at high risk for becoming malnourished.

1.6.6 Possible signals of adaptation:

It has previously been shown that there is a higher rate of enterocyte proliferation in the aging intestine. In gastric mucosa increased expression in ornithine decarboxylase (ODC), epidermal growth factor (EGF) receptor, transforming growth factor- α (TGF-α), activator-protein 1 (AP-1) (*c-fos/c-jun)* and nuclear factor- κB (NF- κB) may contribute to this hyperproliferation (Xiao and Majumdar, 2000). It has been suggested that NF-κB, AP-1 proteins (*c-fos* and *c-jun*) are activated in response to intrinsic factors such as oxidative stress and extrinsic factors such as dietary components (Papaconstantinou, 1994). In addition AP-1 proteins may play an important role in the development of the

intestine in the postnatal development of the intestine (Blais *et al.*, 1996), and in models of intestinal adaptation (Holt and Dubois, 1991; Tappenden,*et al.*, 1997). EGF has also been shown to increase glucose uptake and surface area of the small intestine following small bowel resection (Hardin *et al.*, 1999), possibly by activating the AP-1 proteins (Hodin *et al.*, 1995). ODC is the first enzyme of polyamine synthesis which is essential for nucleic acid and protein synthesis (Heby and Persson, 1990). Control over polyamine synthesis may be less rigidly controlled in aging and ODC activity may increase in aging rats (Yoshinaga *et al.*, 1993). The role of these proteins in intestinal adaptation associated with aging and dietary lipids has not been investigated. Additional proteins of cell cycle regulation such as cyclin dependent-kinase inhibitor p16ink4α and transcription factor p53 may also play a role in age-associated and diet-induced changes in the small intestine. This speculation has not been examined experimentally (Thullberg *et al.*, 2000; Shin *et al.*, 1999).

There are three PPARs (α,δ and γ) which are single polypeptide nuclear receptors, and these PPARs are activated by fatty acids (Jump and Clarke, 1999). PPARs bind to peroxisome proliferator response-elements (PPRE) located upstream from genes involved primarily in lipid metabolism (Poirier *et al.*, 2001) such as FAT and L-FABP expression. PPAR-δ contributes to dietary lipid induced adaptation of the small intestine (Poirier *et al.*, 2001). Aging is associated with an increased expression of PPAR in the liver of old rats as compared to young rats (Tollet-Egnell, 2001). The effect of age on intestinal PPAR expression has not been examined.

1.7 Summary

Malabsorption of carbohydrates, lipids, amino acids, minerals and vitamins has been described in the elderly. The ability of the intestine to adapt may be impaired in the elderly, and this may lead to further malnutrition. Dietary manipulation may prove to be useful to enhance the needed intestinal absorption with aging. There is an age-associated increase in the prevalence of dyslipidemia as well as diabetes. These conditions may benefit from nutritional intervention targeted at reducing the absorption of some nutrients. With the continued characterization of proteins involved in sterol and fatty acid absorption, therapeutic interventions to modify absorption may become available in the future.

CHAPTER TWO

Hypothesis

Hypothesis

1. Aging is associated with a decline in the absorption of lipids in Fischer 344 (F344) rats;
2. The reduced absorption of lipids is due to a fall in the passive permeability and a decreased contribution of lipid transporters, which is reflected by a reduced abundance of fatty acid binding proteins and their mRNAs;
3. The age-associated changes in lipid uptake can be reversed by feeding a saturated rather than a polyunsaturated enriched diet; and
4. The adaptive response to changes in dietary lipids is impaired in old animals when compared to young and mature animals.

CHAPTER THREE

Methods and Materials

3.1 Animals

The principles for the care and use of laboratory animals, approved by the Canadian Council on Animal Care and the Council of the American Physiological Society, were observed in the conduct of this study. Male Fischer 344 rats, aged 1, 9 and 24 months were obtained from the National Institute of Aging colony and Harlan Laboratories, Maryland, D.C.. Pairs of rats were housed at a temperature of 21°C, with 12 hours of light and 12 hours of darkness. Water and food were supplied *ad libitum*.

Animals were fed standard Purina® rat chow for one week, and then fed one of three diets for a further two weeks: standard Purina® rat chow, or a semi-purified diet containing 20% (w/w) fat and enriched with either saturated (SFA) or polyunsaturated (PUFA) fatty acids (Thomson *et al.*, 1986). The isocaloric semi-purified diets were nutritionally adequate, providing for all known essential nutrient requirements. Animal weights were recorded weekly during the study period.

3.2 Uptake Studies

3.2.1 Probe and marker compounds

The [^{14}C]-labelled probes included cholesterol (0.05 mM) and fatty acids lauric (12:0), palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) (0.1 mM). The labeled and unlabeled probes were supplied by New England Nuclear and Sigma Co. (St Louis, MO) respectively. The probes were prepared by solubilizing them in 10 mM taurodeoxycholic acid (Sigma Co., St Louis, MO) in Krebs-bicarbonate buffer, with the exception of 12:0, which was solubilized in Krebs-bicarbonate buffer only. [^{3}H]-inulin was used as a non-absorbable marker to correct for adherent mucosal fluid

volume. Probes were shown by the manufacturer to be more than 99% pure by high performance liquid chromatography.

3.2.2 Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl® (sodium pentobarbitol, 240 mg/100 g body weight). The whole length of the intestine was rapidly removed and rinsed with 150 ml cold saline. The proximal third beginning at the ligament of Treitz was termed the "jejunum", and the distal third was termed the "ileum"; the middle third of the small intestine was discarded. The intestine was opened along its mesenteric border, and pieces of the segment were cut and mounted as flat sheets in the transport chambers. A 5 cm piece of each segment of jejunum and ileum was gently scraped with a glass slide to determine the percentage of the intestinal wall comprised of mucosa. The chambers were placed in preincubation beakers containing oxygenated Krebs-bicarbonate buffer (pH 7.2) at 37 °C, and the tissue discs were preincubated for 15 minutes to allow the tissue to equilibrate at this temperature. The rate of uptake of lipids was determined from the timed transfer of the transport chambers to the incubation beakers containing [^{3}H]-inulin and ^{14}C-labelled probe molecules in oxygenated Krebs-bicarbonate (pH 7.2, 37 °C). Preincubation and incubation chambers were mixed with circular magnetic bars at identical stirring rates, which were precisely adjusted using a strobe light. A stirring rate of 600 revolutions per minute was selected to achieve low effective resistance of the intestinal unstirred water layer (Lukie *et al.*, 1974; Westergaard *et al.*, 1976).

3.2.3 Determination of uptake rates

After incubation of the intestinal discs in labelled solutions for 6 min, the experiment was terminated by removing the chamber and rinsing the tissue in cold saline for approximately 5 seconds. The exposed mucosal tissue was then cut out of the chamber with a circular steel punch, placed on a glass slide, and dried overnight in an oven at 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M 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 (Lukie *et al.*, 1974).

The rates of uptake were expressed as nmol 100 mg tissue $^{-1}$ min $^{-1}$, nmol 100 mg mucosal tissue $^{-1}$ min $^{-1}$, nmol cm $^{-2}$ mucosal surface area min $^{-1}$, and nmol cm $^{-2}$ serosal surface area min $^{-1}$ (Table 3.1).

3.3 Morphology, protein and messenger RNA analysis

3.3.1 Tissue preparation

For Western blotting, Northern blotting, morphological analysis and immunohistochemistry, animals were raised and sacrificed similarly as for the uptake studies. A 5 cm portion of proximal jejunum and distal ileum was quickly harvested following rinsing, was snap-frozen in liquid nitrogen, and stored at –80°C for later mRNA isolation. The remaining intestine was opened along the mesenteric border. A 20 cm segment of proximal jejunum and distal ileum was gently scraped with a microscope slide to remove the mucosal tissue, was snap-frozen in liquid nitrogen, and stored at –80°C for later isolation of cellular components. Two 1 cm pieces of each

section were mounted on a styrofoam block, and were preserved in 10 % formalin for later paraffin block mounting to be used in morphology and immunohistochemistry analysis.

3.3.2 Morphological analysis

In order to determine the surface area of the intestine based on its 3-dimensional architecture, a transverse and a vertical section were prepared for each site. Hematoxylin stained slides were prepared from paraffin blocks. Crypt depth and villous height, as well as villous width, depth and density were obtained using a projection microscope, and the projected images were calibrated and measured. The measurements of villous height, villous width at half height, villous width at base and crypt depth were obtained from vertical sections. The measurement of villous depth was obtained from transverse tissue sections. Group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of 4 animals in each group. Mean values and standard errors of the mean were used for statistical analyses.

3.3.3 Protein analysis

Brush border membranes (BBM), basolateral membranes (BLM) and cytosol were isolated from rat intestinal mucosal scrapings (minimum of 6 animals per group) using homogenization, differential centrifugation, and Ca^{2+} precipitation (Maenz and Cheeseman, 1986; Orsenigo *et al.*, 1987; Orsenigo *et al.*, 1985). Each mucosal scraping was homogenized (Polytron® at setting 8) two times for 30 seconds each, in Membrane Suspension Solution (MSS) buffer (sucrose, Tris-HCl, phenylmethyl-sulfonyl fluoride, and distilled H_2O at pH 7.4). The homogenates were centrifuged for 15 minutes at 2400 x

g. Supernatant was centrifuged for 20 minutes at 43 700 x g and 1 hour at 88 100 x g. Samples were stored at -80°C for Western immunoblotting.

The protein concentration of the samples was determined using the Bio-Rad Protein Assay (Life Science Group, Richmond, CA). Samples were incubated at room temperature for 10 minutes and absorbance was read at 600 nm on a SLT 340 ATTC photometer. Cytosol aliquots, containing 20 μg of protein, were solubilized in Sample Buffer Dye (0.125 M Tris-HCl, pH 6.8, 20 % glycerol, 4 % SDS, 10 % b-mercaptoethanol, 0.025% Bromophenol Blue) and boiled for 5 minutes. Aliquots were stored at -80°C until use.

Proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), using a modification of the method developed by Laemmli (1970). Gels were prepared in a multicaster chamber (Hoefer Scientific Instruments, San Francisco, California), and were stored at 4° C overnight.

Electrophoresis of samples and Kaleidoscope Prestained Standards (Bio-Rad laboratories, Hercules, Canada) was carried out in a Hoefer electrophoresis tank. Gels were oriented vertically, and were submerged in a tank containing electrophoresis buffer (0.025 M Tris, pH8.3, 0.192 M glycine, 0.1 % SDS). Electrophoresis was run at room temperature at a constant voltage of 100 Volts for 30 minutes through the staking gel (4% gel, 0.123 M Tris, pH 6.8), and 200 volts for 2.5 hours through the resolving gel (15%gel, 0.375 M Tris, pH 8.8).

After migration, proteins were immobilized on a solid support by electroblotting to a nitrocellulose membrane (Towbin *et al.*, 1979). Gel was put in contact with a nitrocellulose membrane, and placed in a Hoefer transfer cassette. Cassettes were placed

in a Hoefer transfer tank between two electrodes panels, and were totally submerged in freshly prepared Transfer Buffer (25mM Tris, 192 mM glycine, 20% methanol). Electrotransfer was carried out at 1 Ampere for 160 minutes.. Transfer efficiency was tested by Ponceau S (3- hydroxy- 4 - (2- sulfo- 4- [4- sulfophenylazo]- phenylazo) -2, 7- naphtalenediasulfonic acid) staining of membranes and Coomassie Blue staining of gels (Coomassie Blue R250, methanol, deionized water and glacial acetic acid). Membranes were destained with deionized water until no further trace of Ponceau S was visible. Membranes were blocked by incubation overnight in BLOTTO (Bovine Lacto Transfer Technique Optimizer) containing 5% w/v dry milk in Tween Tris Buffered Saline (TTBS) (0.5% Tween 20, 30 mM Tris, 150 mM NaCl).

3.3.3.1 Primary antibody incubation.

Membranes were washed in TTBS (3 x 10 minutes each) with constant agitation. Membranes were probed with specific rabbit anti-rat antibodies against I-FABP (Vassileva *et al.*, 2000) and ILBP (Labonte and Agellon, unpublished data, 2001), which detect cytosolic proteins of 14 kDa. Incubation was carried out at room temperature, for 2 hours with antibodies diluted in 2 % dry milk in TTBS at a dilution of 1:500. Following this primary incubation, membranes were washed with TTBS to remove the residual unbound primary antibody. Membranes were then incubated for one hour with goat anti-rabbit antibody (1:20000 in 2 % dry milk in TTBS) conjugated with horseradish peroxidase (Pierce, Rockfort, Illinois, USA). Membranes were washed again in TTBS to remove residual secondary antibody, and incubated for less than 5 minutes with Supersignal® Chemiluminescent-HRP Substrate (Pierce, Rockfort, Illinois, USA) composed of 50 % Stable Peroxide Solution and 50 % of Luminol/Enhancer Solution.

Membranes were exposed to X-OMAT AR films for various times. The relative band densities were determined by transmittance densitometry using Bio-Rad Imaging Densitometer (Model GS-670, Imaging densitometer, Biorad Laboratory, Mississauga, Ontario).

3.3.4 Messenger RNA expression

3.3.4.1 RNA isolation

The intestinal pieces (minimum of 6 animals per group) were homogenized in a denaturing solution containing guanidium thiocyanate, using a Fast Prep cell disruptor (Savant Instruments Inc., Holbrook, New York). Following addition of 2 M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase containing the RNA was collected. RNA was precipitated with isopropanol overnight at -80° C, with a final wash with 70% ethanol. The concentration and purity of RNA was determined by spectrophotometry at 260 and 280 nm. Samples were stored at -80° C until use for Northern blot or RT-PCR (reverse transcriptase- polymerase chain reaction).

3.3.4.2 Preparation of probes for Northern blotting

DH5A bacteria were transformed and plasmid isolation was carried out using a Boehringer Mannheim High Pure Plasmid Isolation Kit. To make cDNA probes, the DNA insert was cut by 2 specific restriction enzymes (Gibco BRL, Life Technologies, USA). A DIG labeled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the in vitro DNA synthesis using the Klenow fragment of a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labeled DNA (Roche Diagnostics, Quebec, CA).

Fifteen (15) μg of total RNA was loaded and electrophoresed for 5 hours at 100 volts (HLB12 Complete Horizontal Long Bed Gel System, Tyler, Edmonton, Alberta,) in a denaturing agarose gel (1% agarose, 0.66M formaldehyde gel). Ethidium bromide (10 mg/ml) was added so that the integrity of the RNA could be determined by visualizing the 28 S and 18 S ribosomal bands under UV light. Capillary diffusion was used to transfer the RNA to a nylon membrane (Roche Molecular Biochemicals, Manheim, Germany) and RNA was fixed to the membrane by baking at 80° C for 2 hours.

Membranes were pre-hybridized (30 minutes) with DIG Easy Hyb solution (Roche Diagnostics, Quebec, CA) in order to reduce non-specific binding. Probes were heat denatured for 10 minutes at 100° C, and were added to prewarmed DIG Easy Hyb. The membranes were incubated overnight in solution containing the labeled probe. After stringency washes in SSC/SDS solutions, membranes were blocked in 1 x blocking solution for 30 minutes in order to reduce non-specific binding. The membranes were then incubated for 30 minutes with an anti-digoxigenin-alkaline phosphatase conjugate antibody (Roche Diagnostics, Quebec, CA) and were washed twice for 15 minutes with a 1x washing buffer.

Following the pH equilibration with a detection buffer (0.1 M Tris HCl, 0.1 M NaCl) for 5 minutes, detection of the bound antibody was performed using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10 to 20 minutes.

The density of the mRNA bands was determined by transmittance densitometry (Model GS-670, Imaging densitometer, Biorad Laboratory, Mississauga, Ontario).

Quantification of the 28 S ribosomal units from the membranes was used to account for loading discrepancies.

3.3.5 Expression of results

The results were expressed as mean ± standard error of the mean, as determined using Lotus1-2-3. The statistical significance of the differences between the three age groups was determined by analysis of variance (ANOVA) ($p<0.05$). Individual differences between ages were determined using a Student-Neuman-Keuls multiple range test. The statistical significance for diet effect (SFA versus PUFA) was determined using Student's t-test ($p<0.05$). Statistical significance was accepted for values of $p \leq 0.05$.

CHAPTER FOUR

Results

4.1 Animal Characteristics

In animals fed chow, the rate of body weight change fell between 1 and 24 and between 9 and 24 months (Figure 4.1). The rate of body weight change also fell between 1 and 9, and between 1 and 24 months in rats fed SFA or PUFA. At each age, the body weight change was greater with SFA than with PUFA. Food intake was not influenced by the age of the rats, regardless of whether they were fed chow, SFA or PUFA (data not shown).

In the jejunum, neither age nor diet had an effect on the weight of the intestine, the weight of the intestinal mucosa, or on the percentage of the intestinal wall comprised of mucosa (Table 4.1). In contrast, in the ileum of rats fed chow, the weight of the intestine and the weight of the intestinal mucosa was approximately twice as high at 24 as compared with 1 month (Table 4.1). In rats fed SFA, the ileal weight was lower at 24 than 9 months, but not as compared with 1 month. In rats fed PUFA, the mean ileal weight was lower at 24 than at 1 month. Therefore, the weight of the mucosa had to be taken into account when expressing the rate of uptake of the lipids.

There were no differences in the heights of the villi of the jejunum or ileum of rats aged 1, 9 or 24 months (Figure 4.2). In animals fed chow, there were no significant differences in the jejunal or the ileal mucosal surface area at 1, 9 or 24 months (Figure 4.3). In contrast, in animals fed SFA, the jejunal and ileal mucosal surface areas were lower at 9 and 24 months as compared with 1 month (Figure 4.4). In those fed PUFA, both the jejunal and ileal mucosal surface area was lower at 24 as compared with 1 and 9 months. In the jejunum, diet had no effect on villous surface area at 1, 9 or 24 months. In the illeum of 9-month old animals the SFA diet decreased the mucosal surface area

when compared to 9-month animals fed PUFA. However, in 24-month SFA fed animals, Mucosal surface area was increased when compared to same age PUFA fed animals. The length of the intestine was greater at 9 and 24 months when compared to 1 month, and serosal surface area (length x width) was greatest at 9 months (Table 4.2).

4.2 Uptake of Lipids

In order to answer the question "is the absorption of lipids altered by age or by dietary lipids?," the jejunal and ileal uptake of fatty acids and cholesterol was first expressed on the basis of the weight of the entire wall of the intestine (nmol 100 mg $^{-1}$ min $^{-1}$) (Table 4.3).

In animals fed chow, the jejunal and ileal uptake of 18:0 fell between 1 and 9 months, the ileal uptake of 16:0 fell between 1 and 9, and 1 and 24 months.

In animals fed SFA, there was reduced jejunal uptake of 18:0, 18:1 and 18:2 between 1 and 9 months and in 18:0 between 1 and 24 months. In animals fed PUFA, there was reduced jejunal uptake of 18:0 between 1 and 24 months. In 9-month old animals fed a SFA diet there was increased ileal uptake of 18:2, and decreased uptake of 18:3 when compared to 9-month PUFA fed animals. At 24 months there was decreased ileal uptake of 18:0 in SFA fed animals when compared to PUFA fed animals.

The rate of uptake was also expressed on the basis of the weight of the mucosa (nmol 100 mg mucosal tissue $^{-1}$min $^{-1}$). In animals fed chow, there was reduced jejunal uptake of 18:0 between 1 and 9 months (Figure 4.5), reduced ileal uptake of 16:0 and 18:0 between 1 and 9 and between 1 and 24 months, and reduced ileal uptake of 18:2 between 1 and 24 months (Figure 4.6).

In animals fed SFA, there was reduced jejunal uptake of 18:0 between 1 and 9 and 1 and 24 months, as well as reduced jejunal uptake of 18:2 between 1 and 9 months. Feeding SFA was associated with reduced ileal uptake of 18:0 between 1 and 24 months. Feeding PUFA reduced jejunal uptake of 18:0 between 1 and 24 months and increased ileal uptake of 18:3 between 1 and 24 months. There was no effect of feeding a SFA diet versus a PUFA diet on lipid uptake within the 1 or 9 month animals. In the ileum of 24-month animals, a PUFA diet was associated an increase uptake of 18:3 when compared to SFA fed 24-month animals.

In animals fed chow the rate of jejunal uptake (nmol · 100 mg · mucosa^{-1} · min^{-1}) of 12:0 was higher at 24 than 9 months, whereas in the ileum the rate of uptake decreased between 1 and 9 months (Figure 4.7).

In rats fed SFA the ileal uptake of 12:0 was lower at 9 versus 1 or 24 months. In the jejunum and ileum of animals fed PUFA there was an increase in uptake of 12:0 between 1 and 24 months and 9 and 24 months. In the ileum of 1-month old animals a SFA diet increased 12:0 uptake when compared to PUFA fed animals. At 24 months the SFA diet decreased 12:0 uptake compared to the 24-month PUFA fed animals.

In animals fed chow, when expressed as nmol cm $^{-2}$ serosal area min $^{-1}$, there was higher jejunal uptake of 18:3 at 9 versus 1 month (Table 4.4). In rats fed SFA and PUFA, there was no age effect on the uptake of lipids. In the jejunum of SFA fed 24-month animals 18:1 uptake was increased when compared to animals fed PUFA.

Because the surface area of the intestine changed with age in animals fed SFA and PUFA (Figure 4.4), the rate of uptake was also expressed on the basis of the mucosal surface area (nmol cm^{-2} min $^{-1}$). In animals fed chow, the jejunal uptake of 18:2 increased

between 1 and 9 and 1 and 24 months 2 and the uptake of 18:3 increased between 1 and 9 months (Figure 4.8). The ileal uptake of cholesterol increased between 1 and 9 months (Figure 4.9).

In animals fed SFA, there was increased jejunal uptake of 18:1, 18:2 and 18:3 between 1 and 24 months, and increased uptake of cholesterol between 1 and 9 and 24 months. In the ileum there was increased uptake of 16:0, 18:0, 18:1, 18:2 and 18:3 and cholesterol between 1 and 9 months, and increased uptake of 18:1 and 18:3 between 1 and 24 months. In animals fed PUFA there was a decrease in jejunal uptake of 18:0 between 1- an 9-months, and increased uptake of 18:2 between 1- and 24-months. There was also increased ileal uptake of 16:0, 18:0, 18:2, 18:3 and cholesterol between 1- and 24-months.

In 9- and 24-month animals, feeding a diet high in SFA resulted in increased uptake of cholesterol when compared to the same age PUFA fed animals. In 9-month animals, fed SFA there was increased ileal uptake of 16:0, 18:0, 18:1, 18:2 and cholesterol when compared to PUFA fed animals. However, SFA fed 24-month old animals had reduced ileal uptake of 16:0, 18:0, 18:3 and cholesterol compared to their PUFA fed counterparts. Similarly, at 1-month, the uptake of 16:0 and 18:0 was lower in the SFA fed animals compared to the PUFA fed animals

4.3 Intestinal Lipid Binding Proteins

The expression of L-FABP mRNA in the jejunum and ileum was not affected by the age of the animals fed chow or PUFA (Figure 4.10). In those fed SFA, the expression of L-FABP mRNA in the jejunum was higher at 24 as compared with 1 or 9 months. ILBP expression in the ileum was unaffected by age in animals fed SFA or PUFA, but in those fed chow the mRNA expression of ILBP was higher at 24 months as compared with 9 months (Figure 4.11). In contrast, the abundance of ILBP protein was unaffected by age or by diet and the abundance of I-FABP protein was also unaffected by age or by diet (Figures 4.12 and 4.13).

CHAPTER FIVE

Discussion

Weight changes

In this study, the 24-month old animals fed chow lost weight during the observational period and this was not explained by reduced food intake. The F344 rat is expected to gain weight from birth to approximately 18 months, at which time the weight plateaus at about 450 g and then gradually declines (Masoro, 1980) (Figure 5.1). Both the animal weights and the weight changes of the chow-fed animals in this study were similar to these findings. However, by feeding a diet enriched in fatty acids, the weight change pattern was altered. The chow diet in this study contained 4.5 % weight from fat (12% total calories), and the SFA and PUFA diets were 20% weight from fat (40% total calories). In animals fed a diet high in SFA, 9- and 24-month old animals lost weight during this period. The reduced jejunal uptake of 18:0, based on mucosal weight, may have contributed to weight loss in these groups. However, the PUFA fed 24-month-old animals experienced weight gain, and yet there was a decrease in jejunal uptake of 18:0. This effect was shown previously: in adult female Wistar rats, a PUFA enriched diet resulted in greater weight gain than a chow or SFA enriched diet (Thomson *et al.*, 1986). The decline in body weight that typically begins at 18 months in the F344 rat may represent a period of functional decline for the animal, marked by the onset of pathologies such as leukemia or pituitary adenoma (Turturro *et al.*, 1999). The PUFA enriched diet may influence this period of decline by slowing or reversing the age-associated weight loss. In senescence-accelerated mice, a safflower enriched diet (low n-3/n-6 ratio) increased mean life span from 357 to 426 days over perrila oil (high n-3/n-6 ratio) fed animals. However, in the safflower fed group, there was a higher incidence of tumors as well as higher concentrations of serum total cholesterol, HDL cholesterol,

triacylglycerol and phospholipids levels (Umezawa *et al.*, 2000). It is not known what are the short- and long-term effects of feeding a PUFA enriched diet compared to a SFA enriched diet on life span in the F344 rat. However, the short-term feeding of a PUFA diet may provide a therapeutic option to mitigate the weight loss associated with aging. This possibility requires further investigation.

Methods of Expression of Uptake

The simplest way of expressing the rate of *in vitro* uptake of nutrients is on the basis of the weight of the full thickness of the intestine. However, if a treatment alters the weight of the intestine, then there may be variations in the rate of nutrient uptake which are understandable in the light of there simply being more mucosal tissue. For this reason, where there are treatment-associated variations in mucosal mass or in the surface area of the villous membrane, as was observed in this study (Figure 4.4), then it is more appropriate to express uptake on the basis of the mass of the transporting mucosal tissue or the villous surface area. It is still possible, of course, that nutrient uptake may alter without a change in mucosal mass or villous surface area, with uptake responding to a change in the distribution of transporters along the villus, or adapting to an alteration in the brush border membrane permeability. Thus, while aging is associated with a decline in the uptake of some lipids in animals fed chow, SFA or PUFA when expressed on the basis of intestinal or mucosal weight, when age-associated alterations in surface area are taken into account, there is no change in the uptake of these lipids.

Does aging alter lipid uptake?

Our findings show that there are changes in the rate of lipid uptake in the small intestine associated with aging. However, it depends on the method used to express the results as to whether there is an increase, decrease, or no alteration in the rate of uptake (Table 5.1). For example, when our results are expressed on the basis of serosal surface area, there is a decline in the jejunal rate of uptake of 18:3 between 9 and 24 months in chow fed animals. When using an *in vivo* perfusion technique and expressing lipid uptake on the basis of the length of the intestine, the uptake of cholesterol and fatty acid was shown to increase in aging rats (Hollander, 1979; Hollander, 1983). When our results are expressed on the basis of the weight of the entire wall of the intestine, we find an increase in the rate of jejunal uptake of 18:0 and 18:2 in the 24- as compared to 9-month old chow fed animals. In contrast, in the ileum there was decreased uptake of 16:0 at 9 and 24 months when compared to 1 month. In an *in vivo* perfusion study, Holt and Dominguez (1981) found a decrease in lipid uptake in 21- as compared to 4-month old rats when uptake was expressed on the basis of intestinal weight. Methodology differences and the use of *in vitro* versus *in vivo* techniques may explain these contrasting findings. The jejunal and ileal uptake of 18:0 is lower in the 9-month as compared to the 1-month animals fed chow when results are expressed on the basis of intestinal weight. Thomson (1981) also showed a decline in lipid uptake in the jejunum of 11- compared to 1-month old rabbits. It is difficult to establish the pattern of changes in lipid uptake observed throughout the lifetime of the animal based on current literature. Differences in methodologies, units of expression, and ages of comparison clearly play a critical role in the conclusion of any findings.

It has been suggested that the ileum compensates for the loss of function of the jejunum in aging (Raul *et al.*, 1988); for example, an increased villous height and aminopeptidase activity was found in the ileum of rats refed a high protein diet following a period of starvation (Raul *et al.*, 1988). In our study, chow fed animals had no age-associated change in ileal villous height or in villous surface area of the 24-month animals compared to the 9- or 1-month animals (Figures 4.2 and 4.3). There was also no increase in the ability of old animals to absorb fatty acid or cholesterol; in fact, there was a decrease in the rate of uptake of 16:0, 18:0 and 18:2 (nmol 100 mg mucosal tissue $^{-1}$ min $^{-1}$) in the ileum of the 24-month chow fed animals (Tables 5.1, Figure 4.6). These findings do not support the suggestion that the ileum compensates for loss of function of the jejunum associated with aging. However, the ileum of the old animals did respond to dietary lipid more dramatically than did the jejunum of the young or mature animals. In the PUFA fed 24-month old animals, there was a decrease in the surface area of the jejunum of about 50 % compared with the 1 or 9-month animals, and in the ileum this difference was more dramatic at about 75% (Figure 4.4). The rate of uptake is not reduced in the ileum when expressed on the basis of serosal surface area or on the weight of the intestine in PUFA fed animals. The reduction in the surface area of these old animals did not result in a concomitant reduction in absorptive capacity. Therefore, changes in morphology or surface area of the small intestine may not accurately predict the ability of the aged intestine to absorb nutrients. While it has been suggested that increased ileal height may result in increased functional capacity of the ileum in aging (Raul *et al.*, 1988), we show this may not be the case; i.e. increased intestinal surface area does not necessarily result in increased absorptive capacity.

Does aging impair the adaptive possibilities of the intestine?

Investigators have previously shown an altered adaptive response associated with aging. In old F344 rats that are refed, following a period of starvation, there is an exaggerated increase in enzyme activity when compared to same aged controls and young refed animals (Holt and Kolter, 1987). This exaggerated response may suggest impairment in protein degradation in the old animal. In addition, villus cell proliferation rate is not controlled as tightly in older rats as compared with younger rats in response to refeeding (Holt *et al.*, 1988). It has also been shown that aging reduces the adaptive response of the small intestine to changes in dietary carbohydrates and amino acids (Ferraris and Vinakota, 1993). By altering the type of dietary fat, we have shown that the old intestine is still capable of adapting to changes in dietary fat. However, this response is altered in the aging rats when compared to mature animals. Intestinal adaptation may be associated with morphological and/or functional change. In the current study, a change in the surface area of the ileum was observed in the mature and old animals fed SFA or PUFA. In the mature animals SFA reduced the surface area compared to the chow and PUFA fed animals. In the old animals, PUFA resulted in a reduction in the surface area. The magnitude of these changes was similar in the two age groups, suggesting an altered adaptive response in aging, and not a reduced adaptive response.

The effect of the Intestinal Unstirred Water Layer

The uptake of 12:0 is a reflection of the effective resistance of the intestinal unstirred water layer (UWL), with higher uptake reflecting lower resistance (Thomson, 1980). In chow fed animals, the jejunal uptake of 12:0 was increased between 9 and 24 months, suggesting lower effective resistance of the unstirred water layer in the 24-month rats. This lower resistance would help to increase uptake of diffusion-limited probes such as long chain fatty acids (Westergaard *et al.*, 1976). However, at 24 months the jejunal uptake of lipids was unchanged when expressed as nmol 100 mg mucosal tissue $^{-1}$ min $^{-1}$. Furthermore, in rats fed PUFA, the jejunal uptake of 12:0 increased between 1- and 24-months, reflecting lower unstirred layer resistance. The jejunal uptake of 18:0 fell between 1- and 24-months, indicating that the age-associated alterations in lipid uptake could not simply be explained by variations in the effective resistance of the intestinal UWL.

Passive lipid uptake

Passive uptake of lipids across the BBM may be affected by membrane fluidity and by the pH microclimate adjacent to the BBM (Shiau, 1990; Higgins, 1994). Previous work suggests that the lipid composition of the BBM may contribute to alterations in lipid uptake (Keelan *et al.*, 1996). In aging, there is an age-associated decrease in BBM fluidity (Wahnon *et al.*, 1989), which could contribute to the decreased lipid absorption with aging. The low pH of the microclimate adjacent to the BBM increases the bile acid critical-micellar concentration, resulting in a dissociation of lipids from bile acid micelles (Shiau, 1990). In aging there, is an increase in the pH of the microclimate, which would

contribute to reduced absorption in aging (Ikuma *et al.*, 1996). Measurements of the pH of the microclimate and the BBM composition were not analyzed in this study.

Protein-mediated lipid uptake

Reduced lipid absorption in aging could not be explained by alterations in the expression of the mRNA of the selected lipid binding proteins or in the abundance of these proteins. In I-FABP knockout mice, fatty acid uptake is maintained, demonstrating that the I-FABP protein is not required for intestinal lipid uptake (Vassileva *et al.*, 2000). In this study, in the ileum the rate of uptake of several fatty acids was reduced in the 24-month group as compared to the 1 month group (Tables 4.3 and 5.1, Figure 4.6), yet no difference was seen in the abundance of the binding proteins (ILBP,L-FABP and I-FABP) or expression of their mRNAs (Figures 4.10, 4.11, 4.12 and 4.13). These findings together with the observations in the knockout mice, suggest that lipid absorption is not dependent on the abundance of these lipid-binding proteins. It is possible that the distribution of transporters along the crypt-villous axis is altered in aging, without a change in the actual number of transporters. It may be that the increased rate of proliferation in the small intestine that is associated with aging results in fewer mature cells toward the villous tip (Jenkins and Thompson, 1994). It is also possible that the abundance of the lipid binding proteins is controlled post-transcriptionally or post-translationally. Finally, there are several other lipid binding proteins in the enterocyte, and these may play a role in lipid uptake. For example, the fatty acid transport protein (FATP4) (Stahl *et al.*, 1999), or the fatty acid translocase (FAT) in the BBM of enterocytes (Abumrad *et al.*, 1984). It has also been suggested that the rate-limiting step in lipid absorption is the formation of a prechylomicron transport vesicle in the

endoplasmic reticulum (Mansbach and Dowell, 2000). Thus, we cannot dismiss the possibility that some lipid-binding proteins not assessed in this study play some role in the control of lipid uptake, and may have contributed to the change in lipid uptake observed with aging.

Do alterations in dietary lipids change the rate of lipid uptake?

In mature animals SFA increased the rate of uptake of fatty acids and cholesterol in the ileum when expressed on the basis of mucosal surface area (Table 5.2). Previous work in mature rats has also shown an increase in the rate of uptake of lipids, cholesterol and carbohydrates in animals fed a SFA enriched diet (Thomson *et al.*, 1986; Thomson *et al.*, 1987). The jejunal rate of cholesterol uptake was also increased in the mature and old SFA fed animals. In contrast, in the ileum of young and old animals, SFA reduced the rate of uptake of some lipids when calculated on the basis of mucosal surface area. The higher effective resistance of the UWL in the ileum of the young animals fed SFA may have contributed to the reduced uptake of lipids in the 1-month group. However, in the ileum of old animals fed SFA, the effective resistance of the UWL is reduced. This would increase the concentration of lipid at the membrane aqueous interface and increase lipid uptake (Thomson and Dietschy, 1981). Thus, the adaptive response to the composition of dietary lipid is age-dependant. In the ileum of 9-month old animals, SFA increases the rate of lipid uptake, and yet in 24-month old animals SFA decreases the rate of lipid uptake. While we hypothesized that a diet enriched in SFA might help to correct the age–associated loss of function of the small intestine, the current study suggests that a PUFA enriched diet may be more effective at reversing this loss in old F344 rats. This unexpected finding underscores the necessity of performing research in aging animals,

because treatment effects found in young or mature animals may be altered by the aging process.

Possible signals of intestinal adaptation

It is possible that genes that signal the adaptive response to dietary lipids are also altered by the aging process. Some of the possible genes are (1) peroxisome proliferator activator receptors (PPARs), which may contribute to dietary lipid induced adaptation of the small intestine (Poirier *et al.*, 2001) and may increase in aging (Tollet-Egnell, 2001); (2) ornithine decarboxylase (ODC), which is required for protein and nucleic acid synthesis (Heby and Persson, 1990) may be less rigidly controlled in aging (Yoshinaga *et al.* 1993); (3) epidermal growth factor (EGF), as well as AP-1 proteins (c-jun/c-fos), which play a role in intestinal adaptation and development (Blais *et al.*, 1996; Hardin et al., 1999) and which may also be sensitive to oxidative stress such as that seen in aging (Papaconstantinou, 1994) and; (4) cell cycle regulators such as the p53 and p16ink4a which may also play a role in adaptation and may be altered in aging (Thullberg *et al.*, 2000; Shin *et al.*, 1999). Further studies are needed to determine the role of these proteins in models of intestinal adaptation and aging.

Summary

Aging is associated with changes in lipid uptake in the jejunum and ileum. However, the direction of these changes is dependent on the method used to calculate the uptake. While the ileum does not compensate for loss of function of the jejunum in aging the ileum of old rats does demonstrate a higher degree of adaptive response to alterations in dietary fat than does the jejunum. Old animals adapt differently to alterations in dietary fat than do mature animals, with a PUFA diet resulting in increased absorptive function in old animals and a SFA diet increasing absorption in mature animals. Changes in lipid uptake were not explained by the abundance of the proteins of I-FABP or ILBP; or the mRNA expression of L-FABP or ILBP.

Future Studies

1. The expression of other genes involved in lipid metabolism such as fatty acid translocase (FAT/CD36), PPAR and FATP4 need to be evaluated. This could be done on existing RNA samples using RT-PCR technology.
2. The levels of gene expression of proteins involved in cellular growth and differentiation should be examined (ODC, proglucagon, c-myc, c-fos, c-jun, p16ink4α and p53).
3. Using immunohistochemistry the distribution of transporters and binding proteins could be evaluated to determine if this distribution contributes to altered uptake. For example, where uptake is reduced there may be fewer transporters available in the upper third of the villi where transport takes place. Where antibodies are not available for the protein of interest it *in situ* hybridization for the corresponding mRNA could be utilized.
4. A time course study of animals that have been placed on one of the enriched diets for 1, 3, 5, 7, 14, or 28 days should be conducted in order to identify possible signals that initiate or sustain an adaptive response.
5. cDNA array assay should be utilized to identify other possible signals of intestinal adaptation.

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TABLES

Table 1.1 Intestinal lipid binding proteins

Protein	Molecular Weight (kDa)	Localization	Substrate
Caveolin-1	22	Small intestine	cholesterol and LCFA
SR-BI (scavenger receptor class B type I)	57	Liver, peripheral tissue	high-density lipoproteins, phospholipids, triacylglycerol, cholesterol and cholesterol esters
FABPpm(plasma membrane-fatty acid binding protein)	40	Adipose tissue, heart, liver, intestine	LCFA
FAT(fatty acid transporter)/ CD36	88	Adipose tissue, heart, skeletal muscle, spleen, intestine	LCFA, triglycerides
FATP4 (fatty acid transport protein-4)	63	Small intestine	LCFA (oleate)
cholesterol transport protein	145	Small intestine	Cholesterol
FABP (fatty acid binding protein)	14-15	Adipose tissue, muscle, heart, brain, kidney	LCFA
L-FABP (liver-fatty acid binding protein	14-15	Liver and small intestine	LCFA, heme, bile acids, acyl CoA
I- FABPc (intestinal-fatty acid binding protein)	14-15	Small intestine	LCFA
ILBP	14	Ileum (predominant in distal ileum)	Bile acids
PCTV (prechylomicron transport vesicle)		Small intestine	triacylglycerol

Table 1.2 Age-associated alterations in nutrient uptake

Nutrient	Experimental Method	Method of expressing uptake	Effect of increased age on uptake	Model	Reference
Triglyceride	*In vivo* perfusion	/unit wet intestinal weight	↓	S.D. Rat	Holt and Dominguez, 1981
Cholesterol	*In vivo* perfusion	/unit intestinal length	↑	S.D. Rat	Hollander and Dadufalza, 1979
Oleic acid	*In vivo* perfusion	/unit intestinal length	↑	S.D. Rat	Hollander and Dadufalza, 1983
Cholesterol	*In vitro* discs	/unit dry intestinal weight	↓	N.Z. Rabbit	Thomson, 1981
Fatty acids	*In vitro* discs	/unit dry intestinal weight	↓	N.Z. Rabbit	Thomson, 1981
Dietary fats	Fecal analysis	Fecal fat content	↓	Cat	Peachey *et al.*, 1999
Dietary fats	Fecal analysis	Fecal fat content	No change	Human	Arora *et al.*, 1989

S.D.= Sprague Dawley, N.Z.=New Zealand albino

Table 3.1. Formulas used for methods of expressing rate of uptake of fatty acids and cholesterol

Based on	Formula	Units
Weight of the wall of the intestine	(^{14}C dpm in tissue-((^{14}C dpm in 100 µl standard x ^{3}H dpm in tissue) ÷ (^{3}H dpm of 100 µl standard))) ÷((^{14}C dpm in 100 µl standard x time x dry tissue weight) ÷ (substrate concentration))	nmol 100 mg tissue $^{-1}$ min $^{-1}$
Weight of the intestinal wall comprised of mucosa	(^{14}C dpm in tissue-((^{14}C dpm in 100 µl standard x ^{3}H dpm in tissue) ÷ (^{3}H dpm of 100 µl standard))) ÷((^{14}C dpm in 100 µl standard x time x (dry tissue weight x % mucosa)) ÷ (substrate concentration))	nmol 100 mg mucosal tissue $^{-1}$ min $^{-1}$
Serosal surface area	(^{14}C dpm in tissue-((^{14}C dpm in 100 µl standard x ^{3}H dpm in tissue) ÷ (^{3}H dpm of 100 µl standard))) ÷((^{14}C dpm in 100 µl standard x time x 1 cm^{2} serosa) ÷ (substrate concentration))	nmol cm $^{-2}$ serosal surface area min $^{-1}$
Mucosal surface area	(^{14}C dpm in tissue-((^{14}C dpm in 100 µl standard x ^{3}H dpm in tissue) ÷ (^{3}H dpm of 100 µl standard))) ÷((^{14}C dpm in 100 µl standard x time x surface area) ÷ (substrate concentration))	nmol cm $^{-2}$ mucosal surface area min $^{-1}$

Surface area: number of villi/cm^{2} serosa x (2 x m x h) + (2m – a) x d + (2 x d ((a-m^{2}) + h^{2})$^{0.5}$ where h=villous height, m=villous width at half height, a= villous bottom width, d=villous thickness at half height.

Table 4.1. Effect of age and diet on intestinal weight

	1 Month			9 Months			24 Months		
Tissue weight (mg/cm)	**Chow**	**SFA**	**PUFA**	**Chow**	**SFA**	**PUFA**	**Chow**	**SFA**	**PUFA**
Jejunum	9.0 ±0.7	8.8 ±0.9	10.1 ±1.4	12.3 ±1.3	12.5±0.5	11.2 ±0.8	10.7 ±1.5	13.6 ±2.1	10.5 ±1.1
Ileum	6.4 ±0.7	6.8 ±0.6 #	9.4 ±0.8 *	7.8 ±0.8	10.4 ±1.9	7.6 ±1.1 a	11.1 ±1.7 a	8.5 ±0.9 b	5.4 ±1.1*a
Mucosal weight (mg/cm)									
Jejunum	4.0 ±0.5	4.6 ±0.7	5.5 ±0.9	6.3 ±1.2	6.3 ±0.6	5.9 ±1.0	5.6 ±1.1	7.2 ±1.5	5.6 ±0.8
Ileum	2.9 ±0.6	3.1 ±0.5	4.7 ±0.7	3.5 ±0.6	5.6 ±1.1	3.9 ±1.0	6.0±1.1ab	4.2 ±0.7	2.9 ±0.7
% of intestinal wall composed of mucosa									
Jejunum	44.5 ±2.9	51.7 ± 3.7	55.0 ±6.2	48.8 ±5.2	50.6 ±3.7	51.8 ±6.2	48.7 ±4.0	51.4 ±3.6	51.2 ±3.4
Ileum	38.3 ±4.7	45.6 ± 5.0	49.9 ±5.3	43.9 ±3.1	51.4 ±3.6	48.8 ±5.4	50.1 ±6.4	49.3 ±4.0	42.8 ±8.9

Values are Mean ± SEM

a significantly different from 1 month old rats $p \leq 0.05$

b significantly different from 9 month old rats $p \leq 0.05$

* significantly different than chow fed animals $p \leq 0.05$

\# significantly different from PUFA $p \leq 0.05$

Table 4.2. Effect of age on intestinal length and serosal surface area

	1 month	9 month	24 month
Total intestinal length (cm)	91.4 ± 2.1 a	104.0 ± 1.9 b	105.4 ± 1.7 b
Total serosal surface area (length x width) (cm^2)	90.2 ± 2.6 a	111.0 ± 8.0 b	103.8 ± 2.0 ab

Values are mean + SEM (n=6). Different letters denote a significant age effect ($p \leq 0.05$).

Table 4.3 Effect of age and diet on fatty acid uptake expressed as nmol 100 mg tissue^{-1} min^{-1}

	16:0 (0.1mM)	18:0 (0.1mM)	18:1 (0.1mM)	18:2 (0.1mM)	18:3 (0.1mM)	Cholesterol (0.05mM)
Jejunum						
1 month						
Chow	1.8±0.3	2.3±0.3 a	1.3±0.3	1.2±0.2 ab	1.3±0.2	1.9±0.8
SFA	1.7±0.4	1.9±0.3 a	1.4±0.3 a	1.4±0.2 a	1.5±0.4	1.5±0.2
PUFA	1.3±0.2	1.8±0.3 a	1.2±0.2	1.2±0.4	1.3±0.2	1.0±0.2
9 month						
Chow	1.1±0.1	1.0±0.2 b	1.3±0.3	0.8±0.1 a	2.1±0.5	1.1±0.1
SFA	1.3±0.5	0.7±0.1 b	0.6±0.1 b	0.7±0.1 b	1.7±0.5	1.9±0.5
PUFA	0.8±0.2	1.8±0.3 a	0.8±0.2	0.6±0.2	1.3±0.4	1.5±0.2
24 month						
Chow	1.2±0.3	1.7±0.3 a	0.8±0.3	1.8±0.3 b	1.3±0.2	1.3±0.2
SFA	0.9±0.2	0.9±0.1 b	0.9±0.1 ab	1.1±0.2 ab	1.5±0.3	1.4±0.2
PUFA	1.3±0.3	0.8±0.1 b	0.9±0.3	1.6±0.4	1.8±0.7	1.1±0.2
Ileum						
1 month						
Chow	2.1±0.4 a	2.1±0.3 a	1.8±0.5	1.6±0.2	1.7±0.3	1.0±0.2
SFA	1.8±0.4	1.4±0.3	1.0±0.2 ab	1.3±0.2	1.5±0.3	1.5±0.2
PUFA	2.1±0.3	1.6±0.3	0.9±0.1	1.1±0.2	1.1±0.2	1.6±0.4
9 month						
Chow	0.9±0.1 b	1.0±0.1 b	0.7±0.3	1.3±0.3	1.6±0.3	1.1±0.1
SFA	0.9±0.2	1.2±0.3	0.7±0.1 a	1.6±0.3 *	0.9±0.2*	1.9±0.3
PUFA	1.2±0.2	0.9±0.2	0.6±0.2	0.6±0.3	2.6±0.7	1.6±0.5
24 month						
Chow	1.2±0.3 b	1.3±0.4 ab	0.7±0.1	1.2±0.3	1.9±0.3	1.2±0.2
SFA	0.8±0.3	0.5±0.1 *	1.5±0.3 b	1.4±0.2	1.5±0.3	1.3±0.2
PUFA	1.3±0.3	1.2±0.2	1.2±0.4	1.4±0.3	2.6±0.5	1.5±0.2

Values are mean ± SEM. Values are Mean + SEM. Different letters denote a significant age effect;($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$)(n=8).

Table 4.4 Effect of age and diet on fatty acid uptake expressed as nmol cm^{-2} serosal surface area min^{-1}

	16:0 (0.1mM)	18:0 (0.1mM)	18:1 (0.1mM)	18:2 (0.1mM)	18:3 (0.1mM)	Cholesterol (0.05mM)
Jejunum						
1 month						
Chow	0.14±0.02	0.18±0.03	0.12±0.03	0.09±0.02	0.10±0.01 a	0.15±0.05
SFA	0.19±0.04	0.16±0.03	0.11±0.02	0.11±0.01	0.14±0.04	0.12±0.02
PUFA	0.15±0.02	0.16±0.02	0.14±0.03	0.11±0.03	0.15±0.03	0.12±0.03
9 month						
Chow	0.13±0.02	0.14±0.03	0.16±0.03	0.10±0.01	0.22±0.03 b	0.11±0.02
SFA	0.09±0.02	0.09±0.02	0.07±0.01	0.08±0.02	0.15±0.03	0.14±0.02
PUFA	0.08±0.04	0.08±0.02	0.14±0.03	0.07±0.01	0.12±0.03	0.11±0.01
24 month						
Chow	0.10±0.02	0.13±0.02	0.07±0.01	0.14±0.03	0.12±0.02 a	0.13±0.01
SFA	0.11±0.02	0.07±0.01	0.14±0.02*	0.12±0.02	0.15±0.02	0.14±0.01
PUFA	0.16±0.02	0.08±0.01	0.08±0.02	0.17±0.06	0.10±0.03	0.10±0.01
Ileum						
1 month						
Chow	0.14±0.04	0.15±0.02	0.13±0.04	0.12±0.02	0.13±0.02	0.08±0.02
SFA	0.12±0.04	0.10±0.02	0.07±0.02	0.10±0.01	0.10±0.02	0.12±0.02
PUFA	0.15±0.03	0.15±0.02	0.08±0.02	0.12±0.02	0.12±0.02	0.16±0.04
9 month						
Chow	0.08±0.02	0.09±0.03	0.08±0.02	0.10±0.02	0.12±0.02	0.14±0.02
SFA	0.11±0.02	0.10±0.02	0.07±0.01	0.19±0.04	0.09±0.02	0.16±0.04
PUFA	0.10±0.03	0.10±0.02	0.07±0.02	0.11±0.03	0.11±0.02	0.09±0.01
24 month						
Chow	0.12±0.02	0.12±0.02	0.07±0.01	0.12±0.02	0.17±0.03	0.13±0.01
SFA	0.08±0.02	0.07±0.01	0.11±0.02	0.11±0.03	0.18±0.04	0.13±0.02
PUFA	0.09±0.02	0.08±0.01	0.06±0.02	0.10±0.03	0.15±0.03	0.10±0.01

Values are mean ± SEM. Different letters denote a significant age effect ($p \leq 0.05$) (n=8). No significant diet effect (SFA v.s. PUFA) was found.

Table 5.1 Summary of the effect of aging on the rate of uptake of fatty acids and cholesterol in 9 and 24 month old chow fed F344 rats compared to 1-month old animals when uptake is calculated in different ways

	Jejunum								Ileum							
	Jd		Jm		JSA		JSAm		Jd		Jm		JSA		JSAm	
	9	24	9	24	9	24	9	24	9	24	9	24	9	24	9	24
16:0	-	-	-	-	-	-	-	-	↓	↓	↓	↓	-	-	-	-
18:0	↓	-	↓	-	-	-	-	-	↓	-	↓	↓	-	-	-	-
18:1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:2	-	-	-	-	-	-	↑	↑	-	-	-	↓	-	-	-	-
18:3	-	-	-	-	↑	-	↑	-	-	-	-	-	-	-	-	-
Choles-terol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-

↑ / ↓: increased / decreased compared to 1 month
9 / 24: 9- and 24- month old Fischer 344 rats
Jd: uptake based on weight of the wall of the intestine Jm: uptake based on weight of the wall of the intestine comprised of mucosa JSA: uptake based on serosal surface area JSAm: uptake based on the mucosal surface area

Table 5.2 Summary of the effect of diet on the rate of uptake of fatty acids and cholesterol in F344 rats when uptake is calculated in different ways

	Jejunum				Ileum			
	Jd	Jm	JSA	JSAm	Jd	Jm	JSA	JSAm
16:0	---	---	---	---	---	---	---	↓⇑▼
18:0	---	---	---	---	--▼	---	---	↓⇑▼
18:1	---	---	--▲	---	---	---	---	-⇑-
18:2	---	---	---	---	-⇑-	---	---	-⇑-
18:3	---	---	---	---	-⇓-	--▼	---	--▼
Choles-terol	---	---	---	-⇑▲	---	---	---	-⇑▼

Arrows indicate an increase or decrease in the rate of uptake in SFA fed animals compared to the same age PUFA fed animals.

↑ / ↓: increased / decreased in 1-month old animals

⇑ / ⇓: increased / decreased in 9-month old animals

▲ / ▼ : increased / decreased in 24-month old animals

Jd: uptake based on weight of the wall of the intestine Jm: uptake based on weight of the wall of the intestine comprised of mucosa JSA: uptake based on serosal surface area JSAm: uptake based on the mucosal surface area

FIGURES

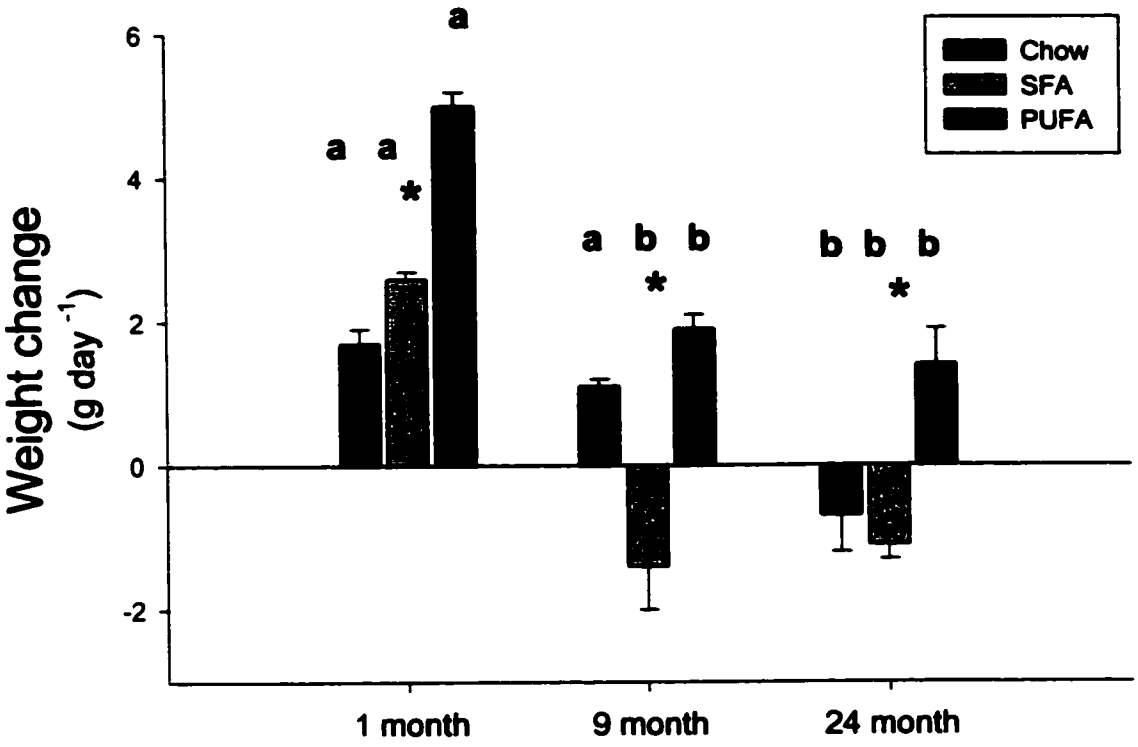

Figure 4.1. Effect of age and dietary lipids on body weight change of F344 rats.

Values are mean + SEM. Different letters denote a significant age effect ($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA) ($p \leq 0.001$)(n=14).

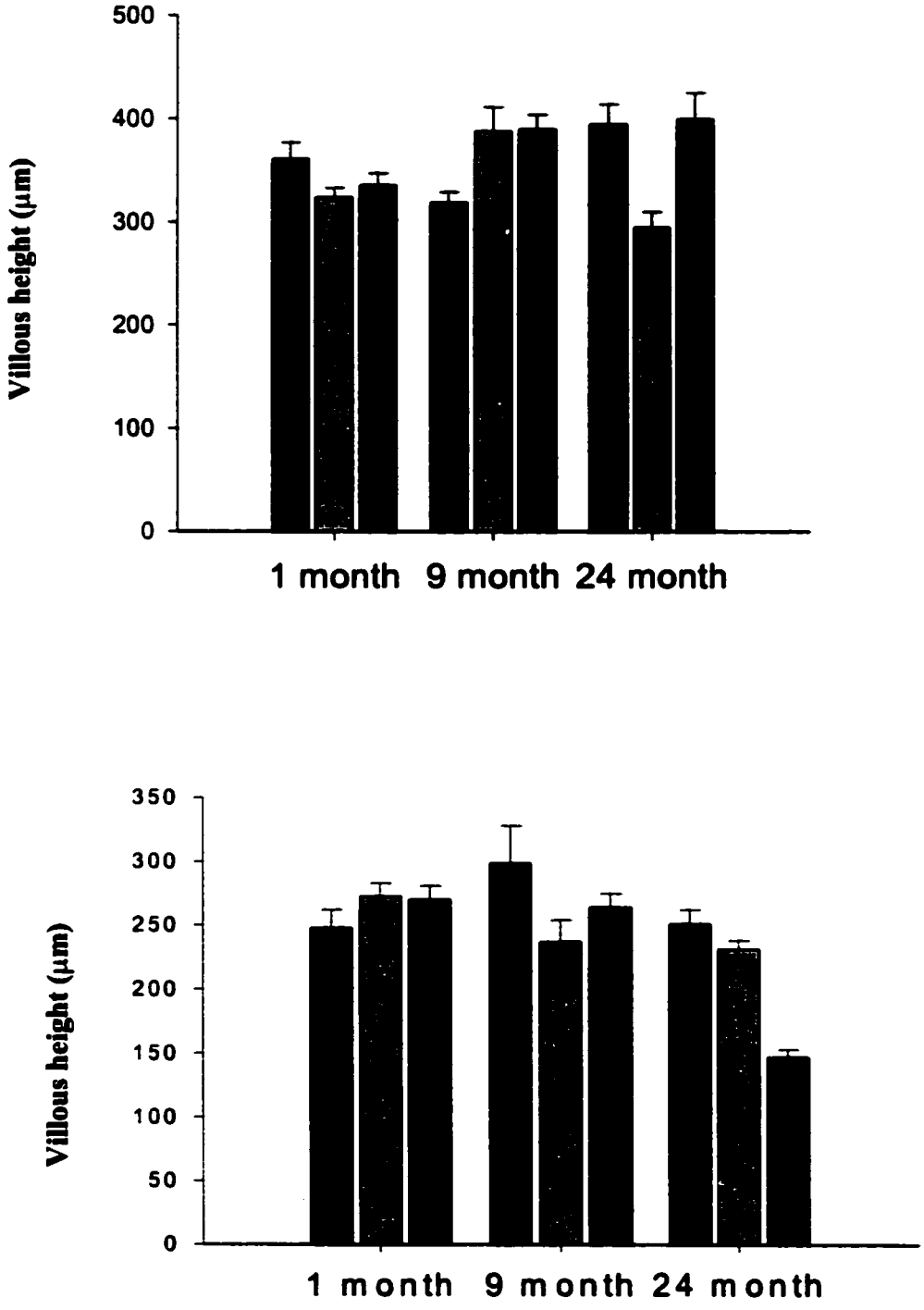

Figure 4.2. Effect of age and dietary lipids on villous height of F344 rats.

Values are mean + SEM. There are no significant differences between the groups ($p \leq 0.05$).

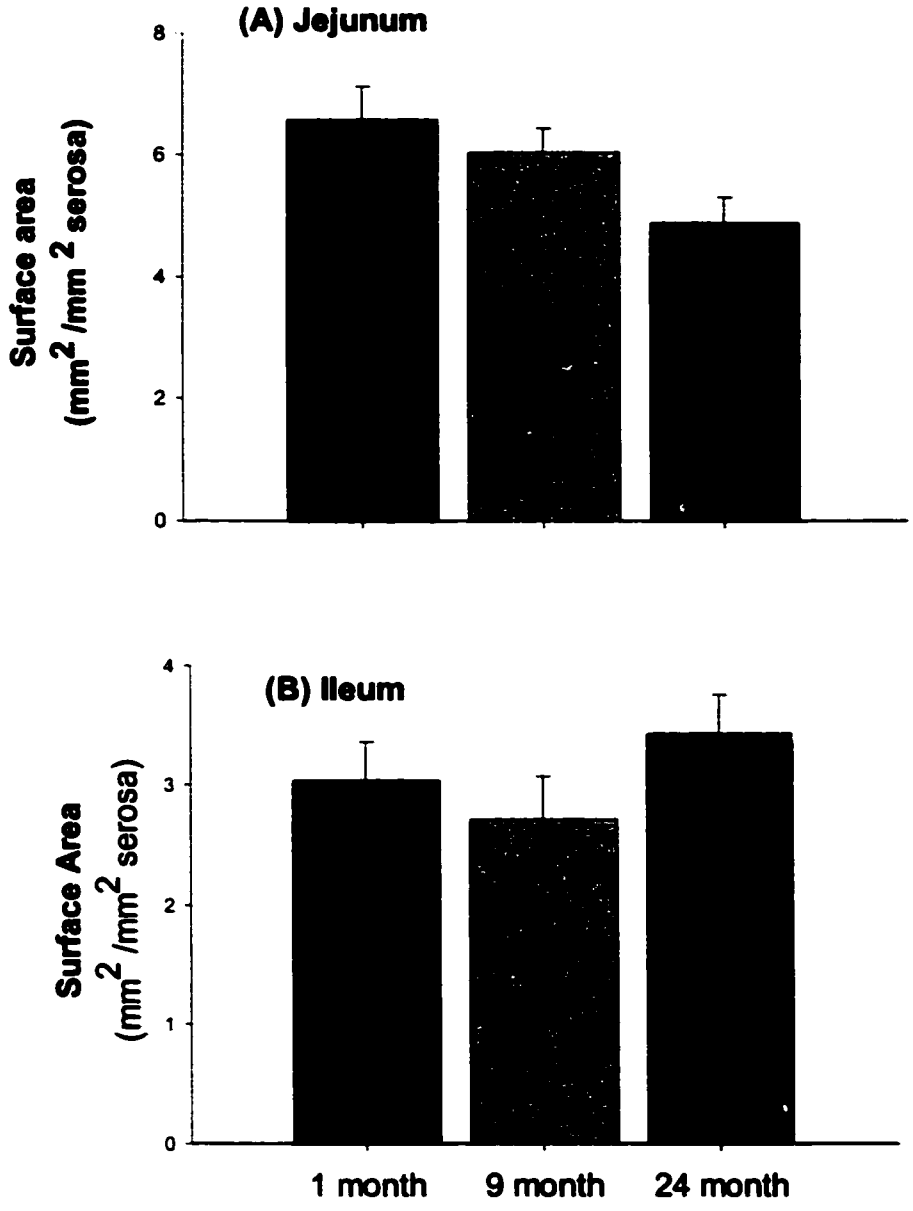

Figure 4.3. Mucosal surface area of the small intestine in chow fed F344 rats.

Values are mean +/- SEM. There are no significant differences between the groups ($p \leq 0.05$), and * indicates a significant diet effect ($p \leq 0.05$) (n=4).

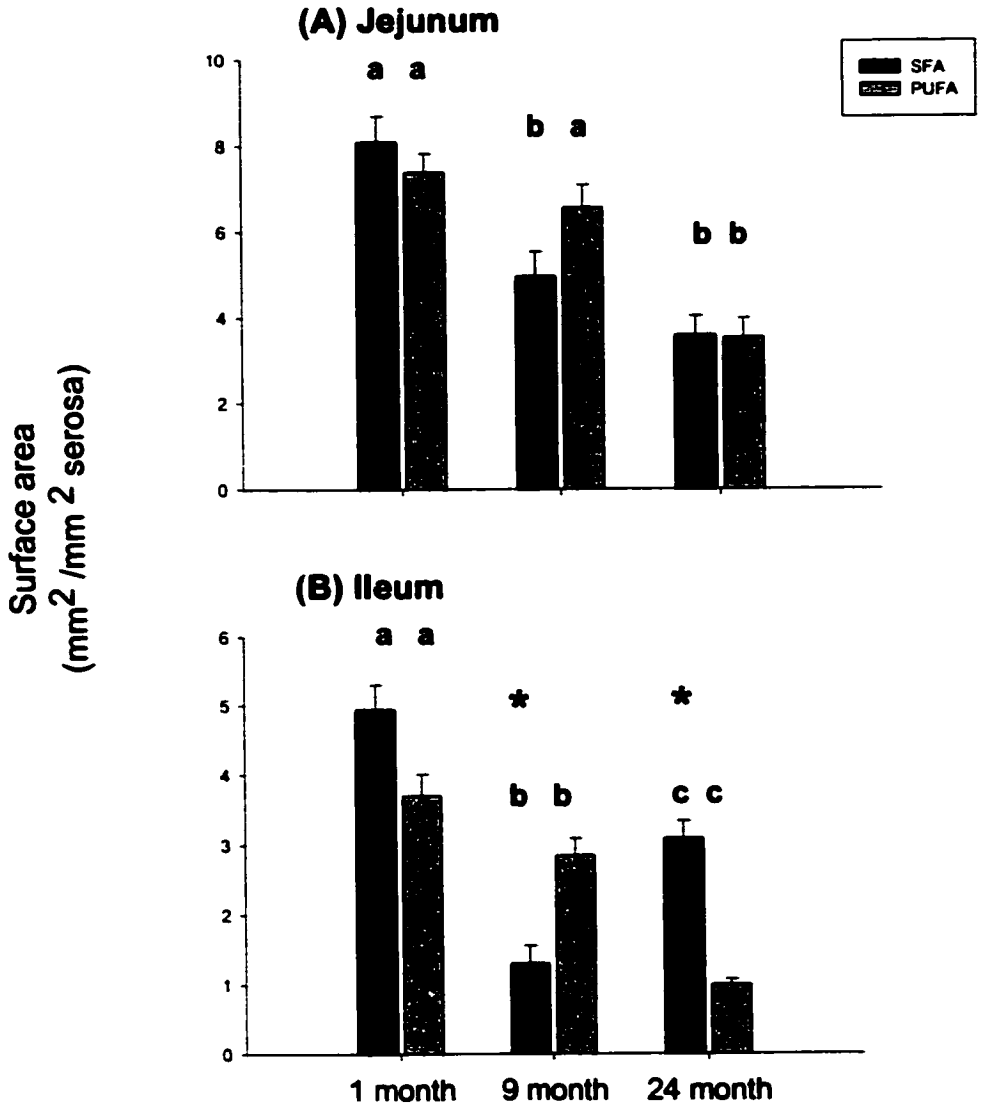

Figure 4.4. Mucosal surface area of the small intestine in SFA and PUFA fed F344 rats. Values are mean +/- SEM. Different letters indicate a significant difference between ages ($p \leq 0.05$), and * indicates a significant diet effect ($p \leq 0.05$) (n=4).

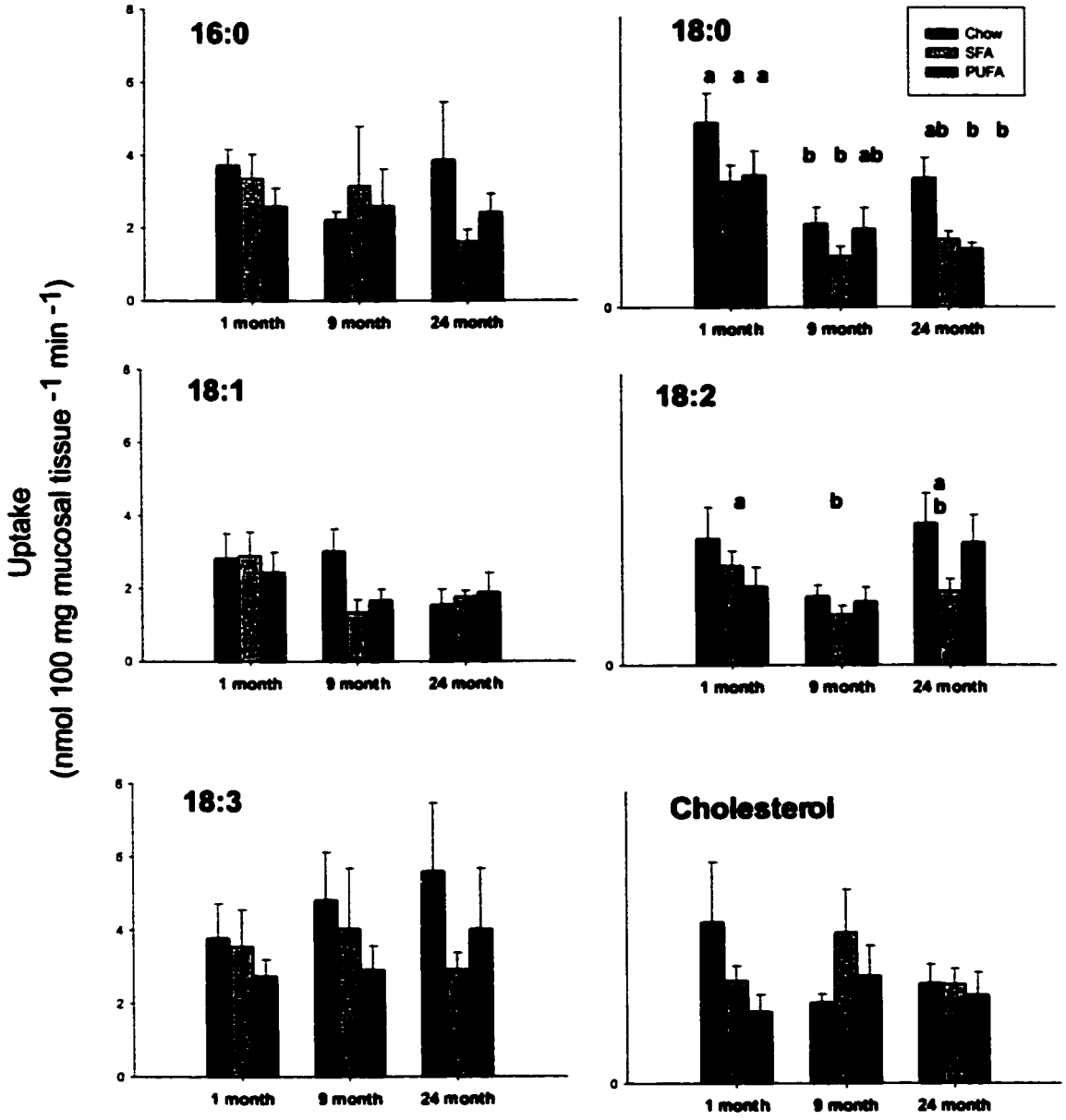

Figure 4.5. Jejunal uptake of fatty acids and cholesterol expressed as nmol 100 mg mucosal tissue -1min -1.

Values are mean +/- SEM. Different letters denote a significant age effect; ($p \leq 0.05$) significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$)(n=8).

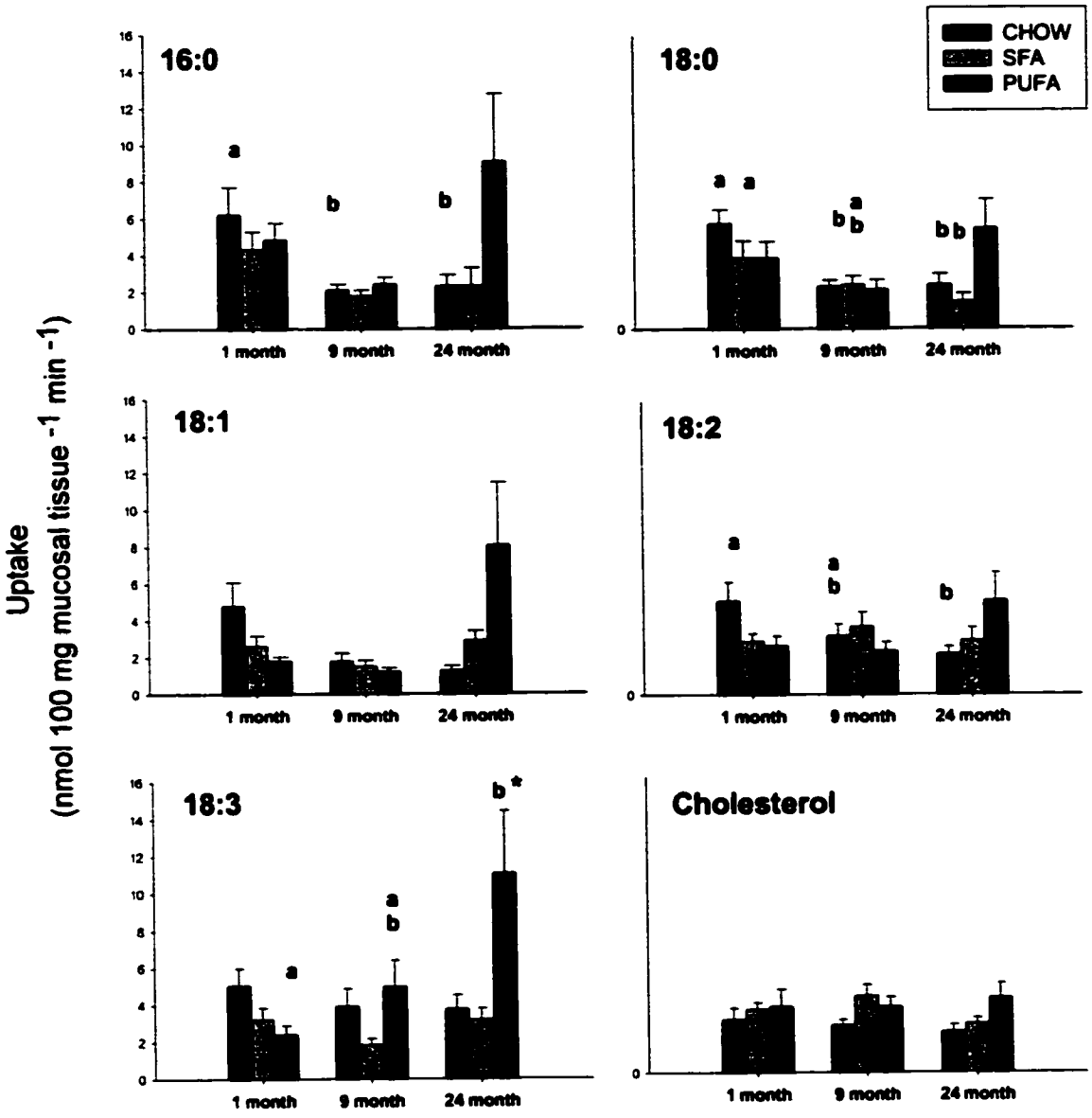

Figure 4.6. Ileal uptake of fatty acids and cholesterol expressed as nmol 100 mg mucosal tissue -1min -1.

Values are Mean + SEM. Different letters denote a significant age effect;($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$)(n=8).

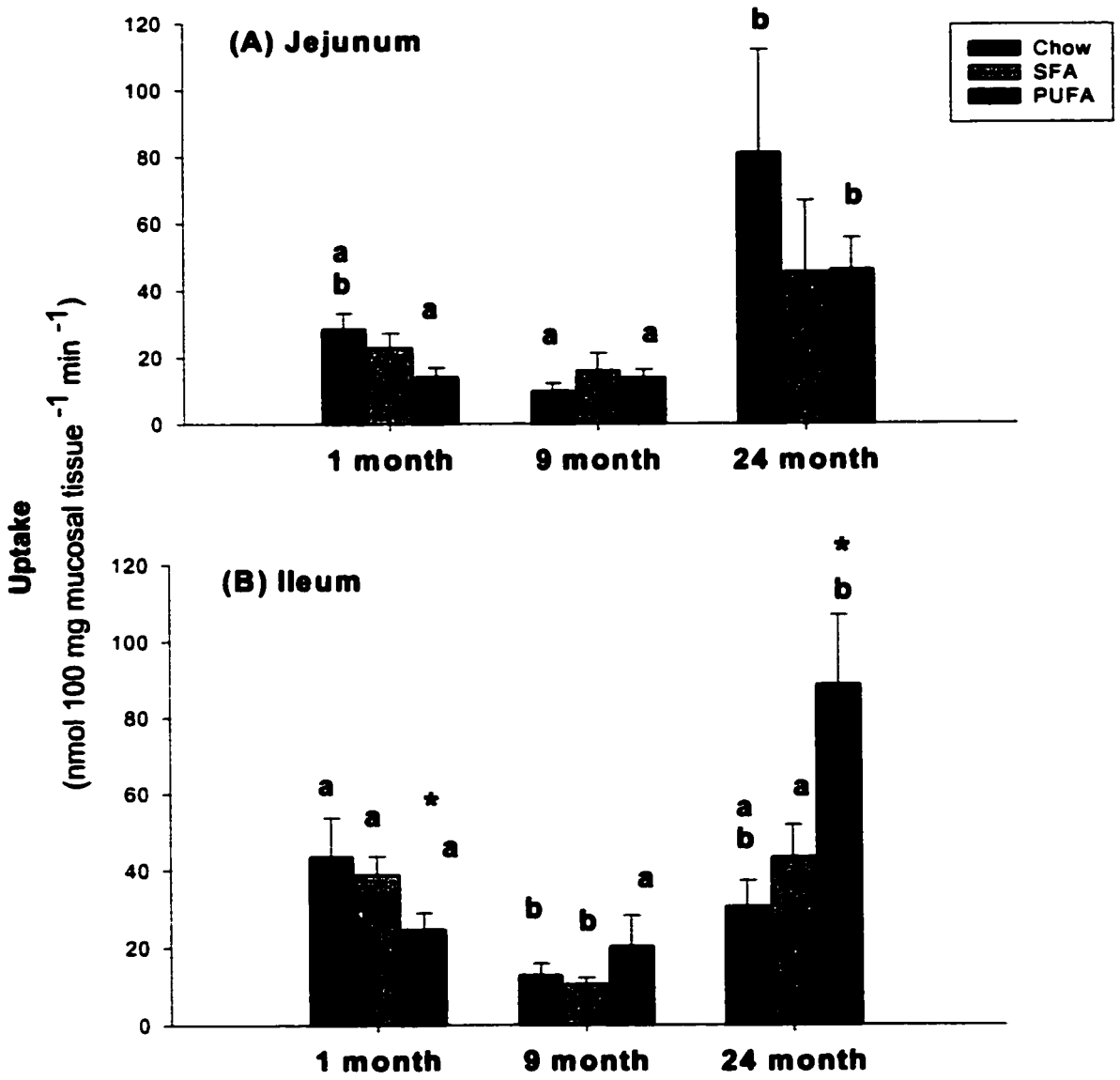

Figure 4.7. Uptake of fatty acid 12:0 expressed as nmol 100 mg mucosal tissue -1min -1. Values are Mean + SEM. Different letters denote a significant age effect;($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$)(n=8).

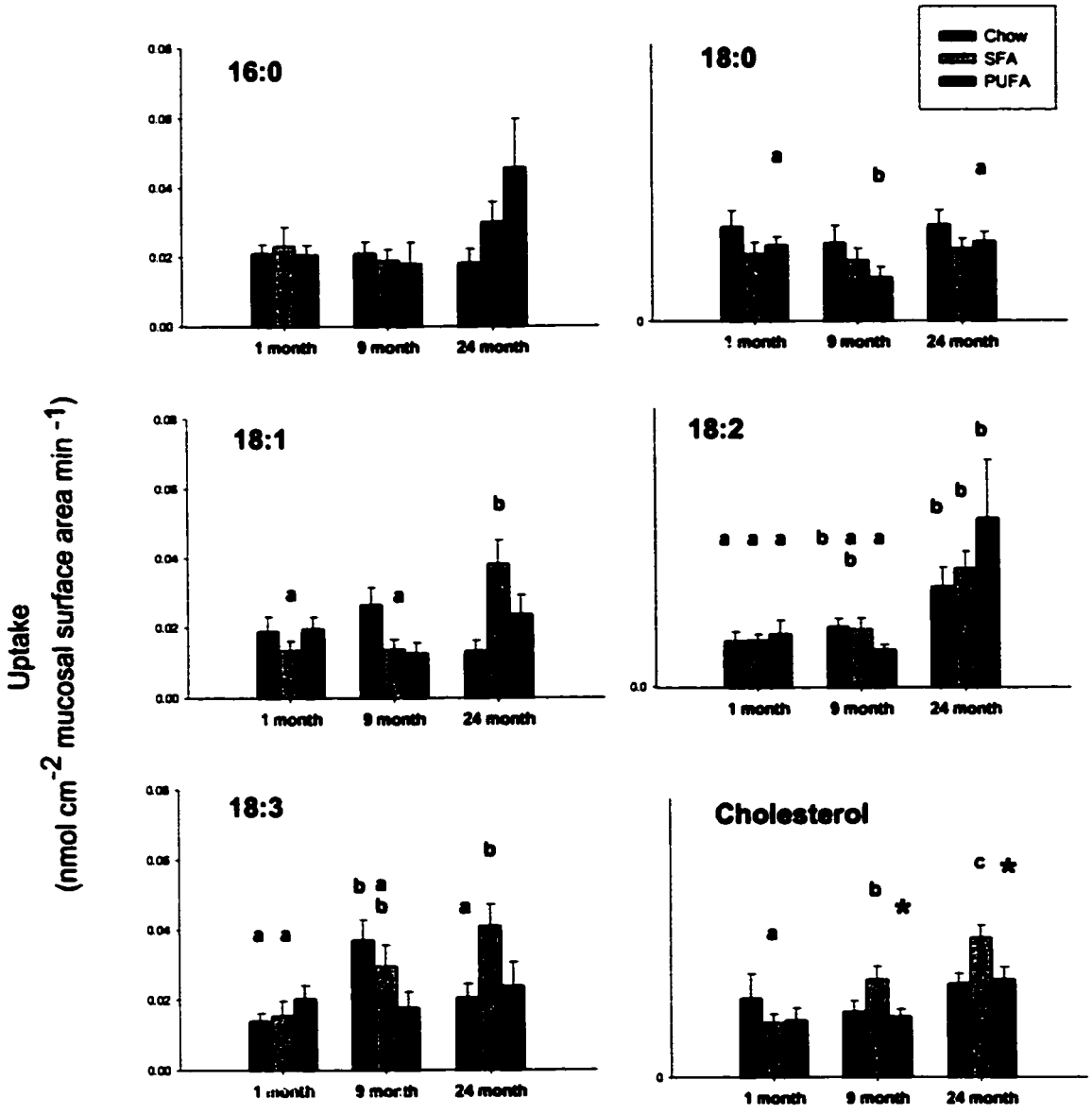

Figure 4.8. Jejunal uptake of fatty acids and cholesterol expressed on the basis of mucosal surface area.

Values are Mean + SEM. Different letters denote a significant age effect; ($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$) (n=8).

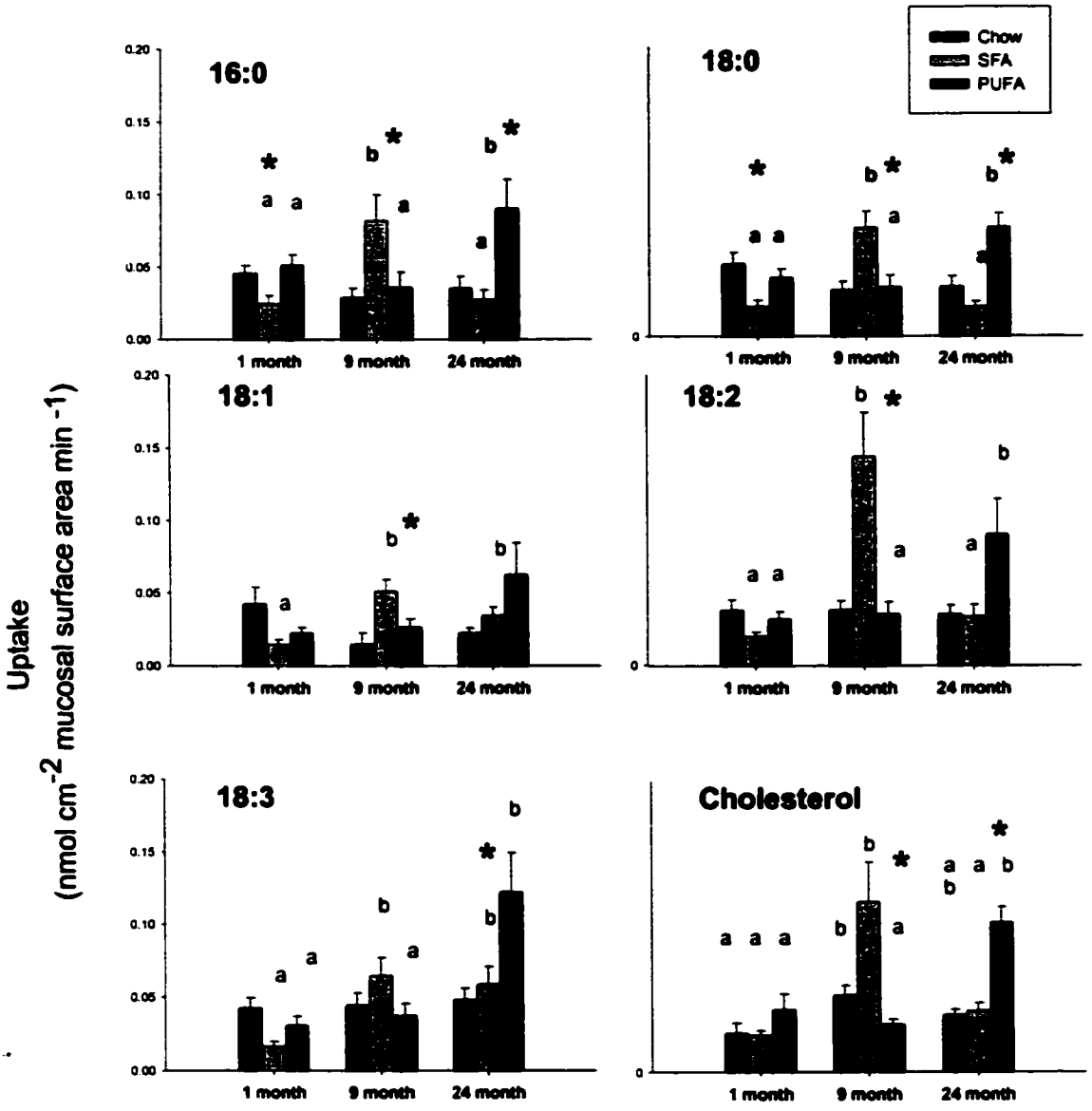

Figure 4.9. Ileal uptake of fatty acids and cholesterol expressed on the basis of mucosal surface area.

Values are Mean + SEM. Different letters denote a significant age effect; ($p \leq 0.05$), and * denotes a significant diet effect (SFA v.s. PUFA fed animals) ($p \leq 0.05$) (n=8).

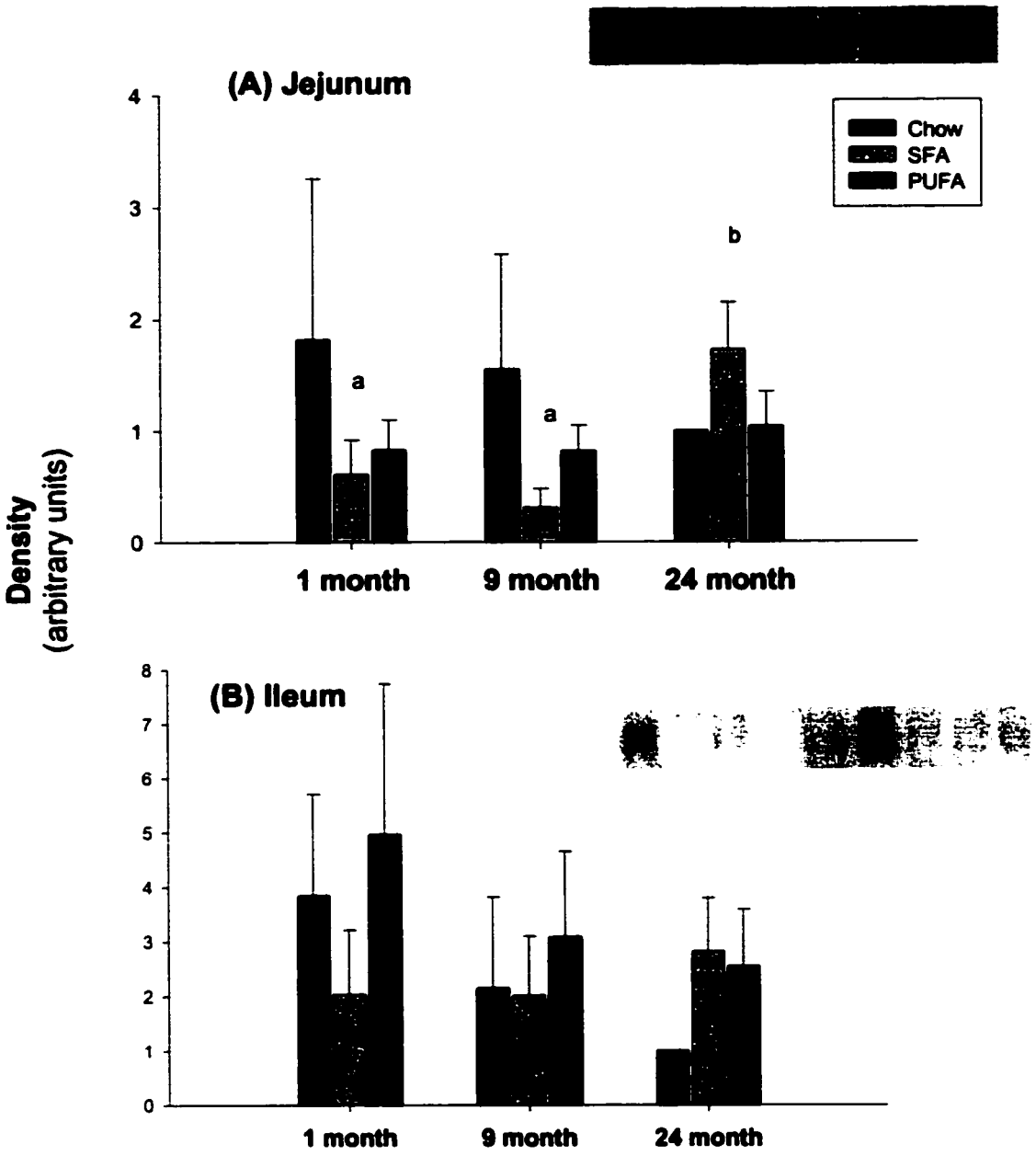

Figure 4.10. Expression of L-FABP mRNA as determned by Northern blot with values normalized to the 24 month chow fed group.

Values are mean + SEM. Different letters denote a significant age effect ($p \leq 0.05$). No significant diet effect was found (SFA v.s. PUFA fed animals) ($p \leq 0.05$) (n=4).

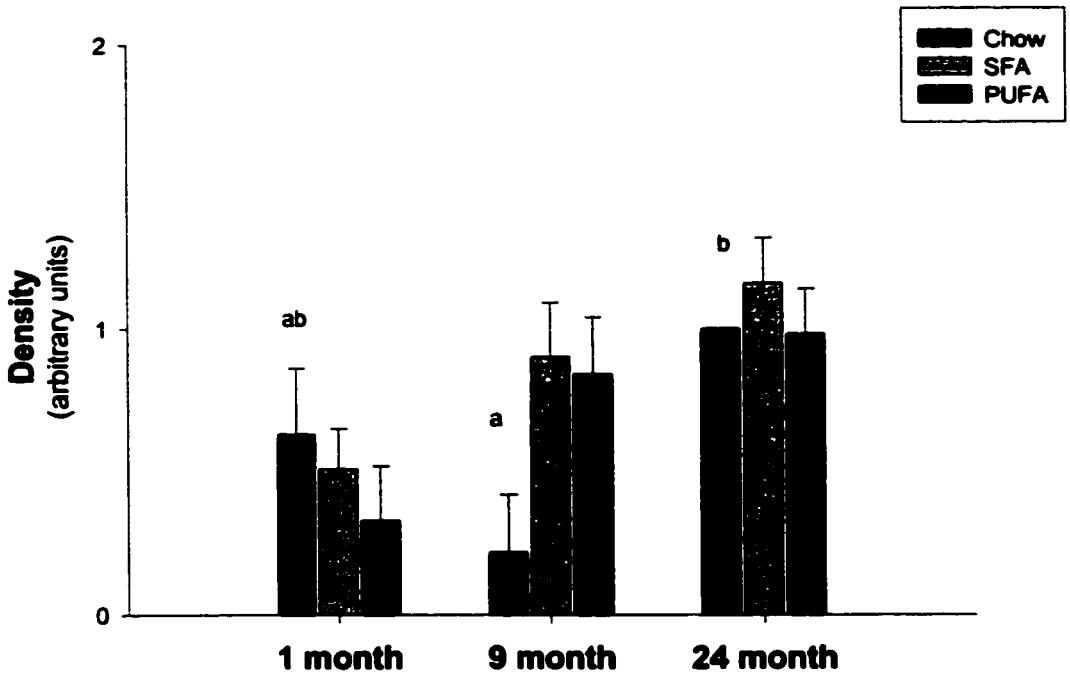

Figure 4.11. Expression of ILBP mRNA in the ileum as determined by Northern blot with values normalized to the 24 month chow fed group.

Values are mean + SEM. Different letters denote a significant age effect ($p \leq 0.05$). No significant diet effect was found (SFA v.s. PUFA fed animals) ($p \leq 0.05$) (n=4).

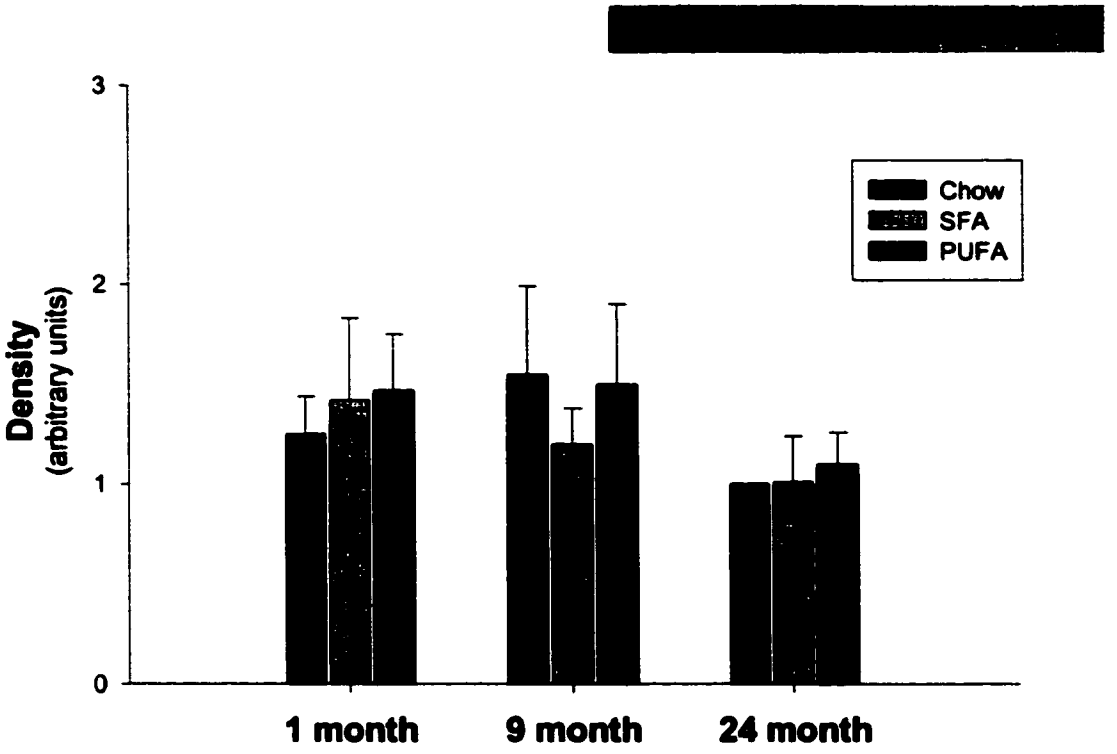

Figure 4.12. Abundance of ILBP in the ileum as determined by Western blot with values normalized to the 24 month chow fed group.

Values are mean + SEM. No significant age or diet effect ($p \leq 0.05$) (n=4).

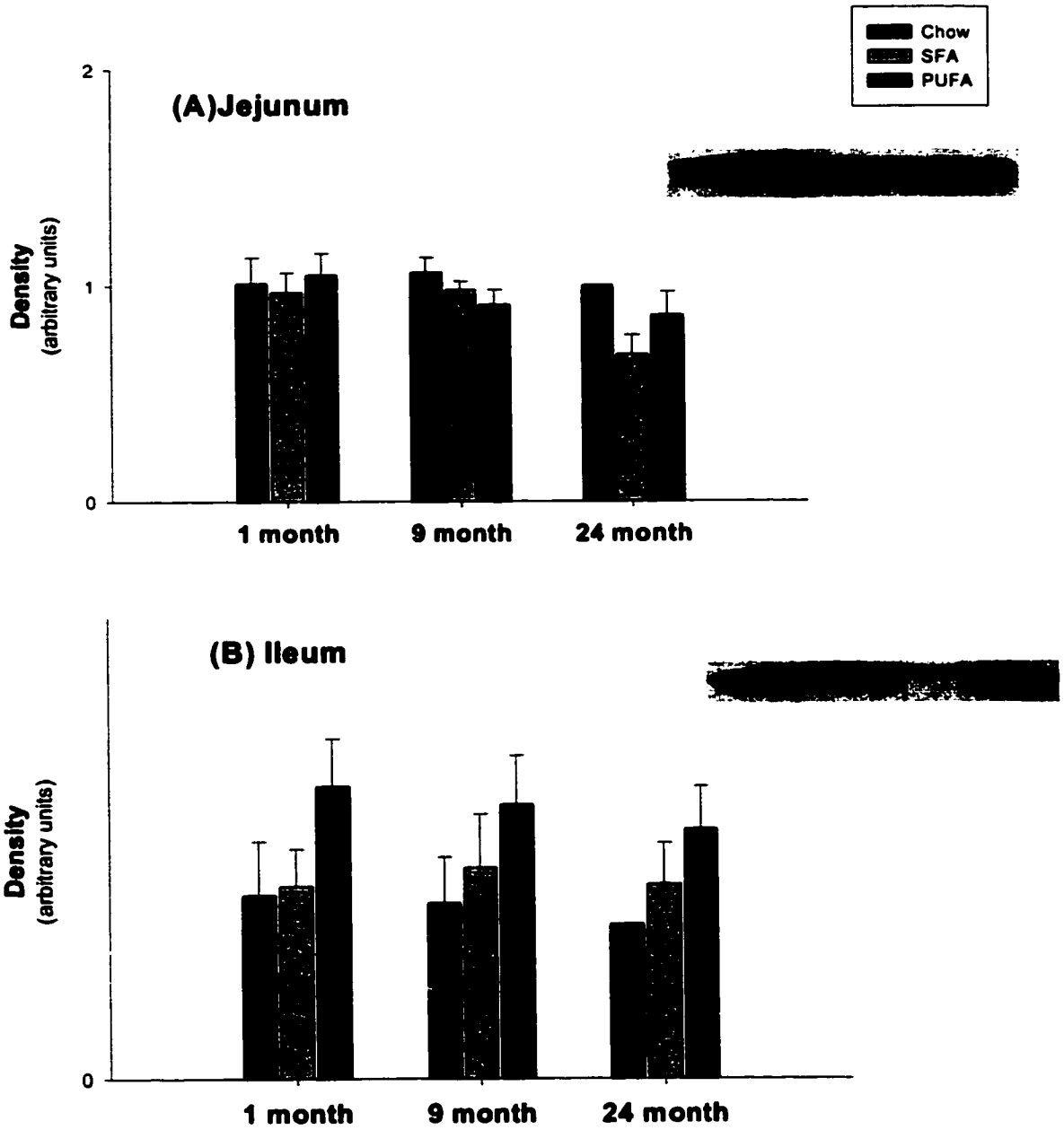

Figure 4.13. Abundance of I-FABP as determined by Western blot with values normalized to the 24 month chow fed group.

Values are mean + SEM. No significant age or diet effect ($p \leq 0.05$) (n=4).

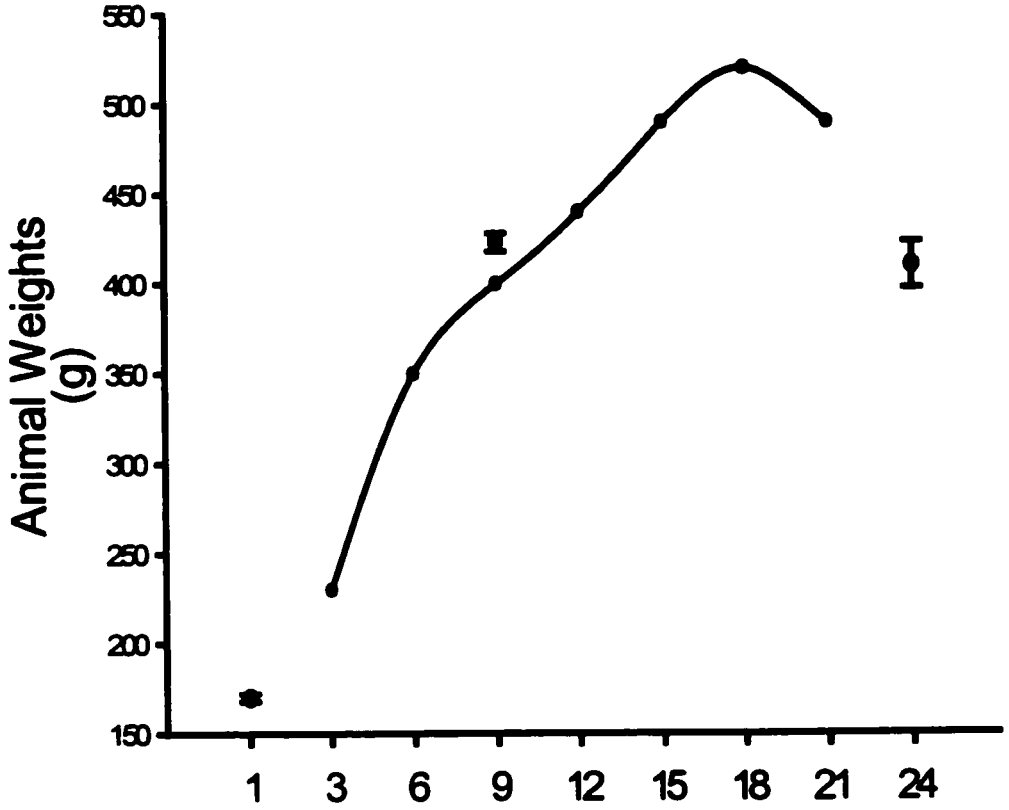

Figure 5.1. Weight change throughout the lifespan of the F344 rat

•—• represents previously reported values (Masaro, 1980).

represents mean ± SEM of current experimental data for chow fed animals.