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Use Of Deuterium Oxide To Measure Very Low Density Lipoprotein Triglyceride Synthesis In Normal Subjects Consuming Different Diet Fats

by

Stephanie Dawn Konrad

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

in

Nutrition and Metabolism 

Department of Agricultural, Food and Nutritional Science

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Date: March 20, 1997

ABSTRACT

The effect of dietary linoleic (C18:2) and palmitic acids (C16:0) on rate of hepatic *de novo* fatty acid synthesis was assessed in normal subjects. The diet was formulated to provide combinations of high and low levels of C18:2 and C16:0. After 21 days of diet treatment, plasma triglyceride level and incorporation of deuterium into the plasma very low density lipoprotein triglyceride (VLDL-TG) pool over 24 hours was measured. Plasma triglyceride levels were within the normal range. Increasing dietary intake of linoleic acid decreased plasma triglyceride level when subjects consumed a low level of dietary palmitic acid. The relative and net amount of *de novo* synthesized fatty acid in the plasma VLDL-TG pool was not influenced by the diet treatments. Two subjects experienced large changes in these parameters due to diet. A relationship between plasma triglyceride level and rate of hepatic *de novo* fatty acid synthesis was observed.

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LIST OF ABBREVIATIONS

ACC,	acetyl CoA carboxylase
apo,	apolipoprotein
CHD,	coronary heart disease
CHO,	carbohydrate
CM,	chylomicron
coA,	coenzyme A
DHA,	docosahexaenoic acid (C22:6 ω-3)
DNA,	deoxyribonucleic acid
$DNFA_n$,	net amount of *de novo* fatty acid in VLDL-TG
$DNFA_r$,	relative amount of *de novo* fatty acid in VLDL-TG
EDTA,	ethylene diamine tetraacetic acid
EPA,	eicosapentaenoic acid (C20:5 ω-3)
FA,	fatty acid
FAS,	fatty acid synthase
HDL,	high density lipoprotein
IDL,	intermediate density lipoprotein
kcal,	kilocalories
LDL,	low density lipoprotein
LPL,	lipoprotein lipase
MIDA,	mass isotopomer distribution analysis
mRNA,	messenger ribonucleic acid
MUFA,	monounsaturated fatty acid
NADPH,	reduced nicotinamide adenine dinucleotide phosphate
PUFA,	polyunsaturated fatty acid
RNA,	ribonucleic acid
S14,	Spot 14

SFA, saturated fatty acid

TG, triglyceride

VLDL, very low density lipoprotein

CHAPTER 1

INTRODUCTION

Lipids function as fuel sources, important components of biological membranes, fat-soluble vitamins, steroid and thyroid hormones, and various intracellular messengers. In contrast to these vital functions, lipid levels in plasma and levels stored in tissues cause concern when in excess. Since lipids are both essential and potentially detrimental, it is important to attain the proper balance between uptake of lipids and synthesis in the body. Understanding the mechanisms involved in metabolism and synthesis of lipids is central to prevention and treatment of disorders involving lipids.

Fatty acids present in the body have two sources, exogenous or dietary fatty acids and endogenous or *de novo* synthesized fatty acids. Modifying dietary fat intake or rate of endogenous fatty acid synthesis can alter plasma and tissue triglyceride levels, possibly leading to the imbalance of triglyceride present in disease states such as hypertriglyceridemia and obesity.

Altered rates of endogenous fatty acid synthesis can occur due to genetic, metabolic, hormonal and dietary factors. The effect of diet on endogenous fatty acid synthesis has been studied extensively in animals and *in vitro,* however, study of this pathway in humans has only recently been initiated due to the development of suitable methods.

1 LIPID METABOLISM

1.1 DIGESTION AND ABSORPTION

Dietary fats enter the digestive tract and are insoluble in this aqueous environment. Initially, large fat droplets form in the lumen of the small intestine. The mixing action of the intestine breaks these droplets up and the emulsifying action of bile salts prevents

smaller droplets from coalescing. Bile salts contain a lipid soluble portion, which adsorbs onto the surface of the droplet, and a water soluble portion which projects into the surrounding environment. This detergent action increases the surface area on which the digestive enzymes act. Although some digestion of lipids is initiated by lingual lipase and gastric lipase in the stomach (Hamosh, 1986), the majority of digestion is accomplished once the emulsion passes into the duodenum. Here, pancreatic lipase in conjunction with colipase, acts on TG to produce mainly 2-monoacylglycerols and free fatty acids (Borgstrom et al., 1957). Cholesterol esterase and phospholipase A_2 also act in the duodenum. The products of digestion combine with bile salts to form micelles, allowing transport through the aqueous chyme and access to the mucosal cells where the micellar contents are absorbed by passive diffusion. The bile salts are reabsorbed in the ileum and returned to the liver.

After absorption, these molecules are transported to the endoplasmic reticulum by a cytosolic fatty acid binding protein (Ockner & Manning, 1974). The rate limiting step in absorption of short and medium chain fatty acids occurs at the brush border membrane where a fatty acid binding protein may be involved in transport across this barrier (Clark & Armstrong, 1989). Unsaturated long chain fatty acids are absorbed more efficiently than saturated forms (Ockner et al., 1972).

After absorption is complete, medium chain fatty acids (6 to 10 carbons) are transported directly into the portal blood bound to albumin. Monoacylglycerol and some free glycerol are re-esterified with long chain fatty acids (12 to 18 carbons) to reform TG. This re-esterification occurs in the endoplasmic reticulum, either through action of the TG synthase complex or via the α-glycerophosphate pathway (Brindley, 1974). Cholesterol ester is also reformed, and assembled into lipoprotein transporters along with TG.

1.2 Lipoprotein Metabolism

Lipoproteins are spherical particles with hydrophobic cores composed of mainly TG and cholesterol ester, and a surface coat of phospholipid, cholesterol and apolipoproteins. Molecules on the lipoprotein surface are partially or completely hydrophilic, enabling the lipoproteins to be soluble in an aqueous environment. The known functions of the apolipoproteins are, to control lipoprotein synthesis, to act as cofactors to enzymes, and to function as ligands for lipoprotein receptors (Angelin & Rudling, 1992). Lipoproteins transport lipids in the blood and the processes involved in their metabolism can be divided into an exogenous and an endogenous cycle.

The exogenous cycle involves the transport of dietary lipids. After reassembly of lipids in the mucosal cell, a type of lipoprotein called a chylomicron is formed. The core of the CM is composed of mainly TG with some cholesterol ester, and the outer shell contains phospholipid, free cholesterol and apo A-I, A-IV and B-48 (Castelli, 1986; Gotto, 1990). Apo B-48 is a truncated form of hepatic apo B-100, formed by mRNA editing in the gut (Powell et al, 1987). The CM is secreted into the lymph and eventually reaches the plasma where it undergoes several transformations during circulation. It receives apo C-II and apo E from HDL (Castelli, 1986; Miller, 1990; Shepard & Krauss, 1991). The TG in the CM can be exchanged for cholesterol ester in HDL by the action of cholesteryl-ester transfer protein (Miller, 1990; Shepard & Krauss, 1991). Finally, the TG of the CM are hydrolyzed by the action of lipoprotein lipase. This enzyme exists at the capillary endothelial cell surface of various tissues, mostly in adipose and muscle tissue, and is activated by apo C-II on the CM surface. The hydrolysis products, monoacylglycerols and fatty acids, are released from the CM and are absorbed by the tissue cells. Upon entry into a cell, these products may be oxidized or re-esterified for storage (Breckenridge et al., 1978). In muscle cells, the fatty acids are primarily oxidized for energy, while in adipose tissue cells, the fatty acids are primarily used to synthesize

TG for storage of energy. As the CM loses TG, it releases free cholesterol, phospholipid and apo C molecules (Hamsten, 1988). HDL picks up these surface particles and the HDL serum concentration increases (Chung et al., 1989). After the lipolytic action of LPL on the CM, the remainder of the lipoprotein is called the chylomicron remnant. The removal of this remnant from circulation by the liver is dependent on the binding of apo E, on the lipoprotein surface, to binding sites on the hepatocyte surface (Barter, 1994).

Hepatic uptake of CM remnants initiates the endogenous cycle of the lipid transport system. The liver receives free fatty acids from the CM, from adipose tissue TG (Havel, 1961) and other free fatty acids in the plasma, and finally, from the *de novo* synthesis of fatty acids in the liver. From these fatty acids, along with monoacylglycerols and free glycerol from the CM, the liver reforms TG and packages it into VLDL. TG makes up the majority of the core of VLDL, although some cholesterol ester is also present. The surface of VLDL contains phospholipid and free cholesterol, along with apo B-100 and apoE (Castelli, 1986; Gotto, 1990). Once in circulation, VLDL particles undergo transformations similar to CM particles (Guerian et al., 1992). HDL donates apo C-II, TG is transferred from the VLDL to HDL in exchange for cholesterol ester via cholesteryl-ester transfer protein, and LPL hydrolyzes the core TG to release free fatty acids for uptake by cells. HDL again pick up released surface particles as the VLDL decreases in size and the VLDL remnant called IDL is formed. IDL is normally found at low concentrations in the plasma because it is either rapidly taken up by the liver, via LDL receptor binding to apo E on the surface of IDL, or it is converted to LDL by the action of hepatic lipase which further hydrolyses the TG in the IDL (Austin, 1989; Castelli, 1986; Shepard & Krauss, 1991). LDL is a cholesterol rich lipoprotein which is found in higher concentrations in the plasma because it is only slowly removed from the plasma by cell surface receptors called LDL or B/E receptors (Goldstein & Brown, 1977).

2 Triglyceride Levels

As part of the issue of lipid balance in the body, TG levels are becoming recognized as important contributors to disease processes. Due to the potential role of TG level as a risk factor in cardiovascular disease, investigating the causes of hypertriglyceridemia has become vital. Excessive storage of TG in adipose tissue leads to obesity, which is associated with many health risks. Research indicates that altered lipogenesis may be involved in the development of some cases of obesity.

2.1 Plasma Triglyceride Levels

While LDL cholesterol concentration is an established risk factor for atherosclerotic disease (NCEP, 1993) and HDL cholesterol concentration has been shown to be a negative risk factor (Tall, 1990), the relationship of TG rich lipoproteins, such as chylomicrons and VLDL, to coronary heart disease is not as well understood. Observational studies typically find elevated plasma TG in patients with CHD (Albrink & Man, 1959; Goldstein et al., 1973; Castelli et al., 1977; Carlson et al., 1979; Gotto et al., 1977; Cohn et al., 1976; Holmes et al., 1981). Epidemiological studies reveal a positive correlation between plasma TG levels and CHD risk (Gordon et al, 1977; Heyden et al., 1980; Bottiger & Carlson, 1980; Rhoads et al., 1976; Cambien et al., 1986; Brunner et al., 1977), however, the independence of this relationship is inconsistent when other lipid risk factors, especially HDL cholesterol levels, are taken into consideration (Austin, 1991). The close metabolic interrelationship of TG rich lipoproteins and HDL has been suggested as an explanation for why an independent association between TG levels and CHD is not seen (Austin, 1992).

There are two means by which plasma TG levels may be connected to CHD (Grundy & Vega, 1992). One mechanism may be atherogenicity of TG rich lipoproteins due to

altered size or lipid composition. The other possibility is that hypertriglyceridemia brings about metabolic changes, such as increased clearance of HDL and smaller, denser LDL, which increase CHD risk.

Hypertriglyceridemia is defined as fasting plasma TG concentration greater than 2.3 mmol/L in adults or 1.6 mmol/L in individuals under 20 years of age, and results from an accumulation of CM, VLDL or IDL in the circulation (Mancini et al., 1991). It is known that genetic abnormalities of lipoprotein metabolism can lead to hypertriglyceridemia, which can result in premature and severe atherosclerosis. Hypertriglyceridemia can also be acquired as a result of metabolic, hormonal or nutritional influences (Mancini et al., 1991). Non-insulin-dependent diabetes mellitus is the most common secondary cause of hypertriglyceridemia.

It has become widely accepted that diet plays a major role in the development of chronic disease, including CHD. This has prompted investigation into the physiological actions of dietary components. The influence of diet on plasma TG is receiving more attention since the recognition of possible links between TG level and CHD risk. The following discussion will outline the effects of specific classes of nutrients on plasma TG levels.

In general, dietary carbohydrate results in higher plasma TG concentrations than dietary fat (Knittle & Ahrens, 1964; Ahrens et al., 1961). A meta-analysis of 27 trials on dietary fatty acids and plasma lipoproteins done by Mensink and Katan (1992), revealed that substituting fat for carbohydrate lowered fasting plasma TG concentration. These investigators state that dietary saturates, monounsaturates, and ω-6 polyunsaturates all produce this decrease in plasma TG levels to about the same extent when replacing carbohydrates (Katan et al., 1994). The only class of dietary fat which produced a greater decrease in plasma TG levels was the ω-3 polyunsaturates. While these results suggest that the effect of most dietary fats is equal, other studies show that degree of unsaturation and location of double bonds can influence TG level response to dietary fat.

In comparison to MUFA, long-chain SFA do not raise plasma TG levels but medium-chain saturated fatty acids, such as caprylic, capric and lauric acid increase TG levels to about the same degree as carbohydrates (Hill et al., 1989; Hostmark et al., 1980; Van Heek & Zilversmit, 1988). When diets high in oleic acid were compared to SFA, the oleic acid diets did not increase plasma TG levels in normotriglyceridemic nor hypertriglyceridemic subjects (Mattson & Grundy, 1985; Bonanome & Grundy, 1988; Grundy, 1986). Diets high in oleic acid lowered plasma TG levels when replacing carbohydrate (Grundy, 1986). While ω-6 PUFA were found to decrease plasma TG levels when compared to SFA and carbohydrate in some studies involving hypertriglyceridemic subjects (Grundy, 1975; Chait et al., 1974), a consistent response has not been shown (Grundy & Denke, 1990). The ω-3 PUFA on the other hand, have been shown to greatly decrease plasma TG levels (Harris et al., 1983). Epidemiological studies revealed that populations consuming diets rich in fish had lower rates of cardiovascular disease (Bjerregaard & Dyerberg, 1988; Kromhout et al., 1985; Shekelle et al., 1985). This information led to a wide range of investigation into the effects of fatty fish, marine oils rich in ω-3 fatty acids, and ω-3 fatty acid supplements which has led to the conclusion that it is the ω-3 fatty acids in the fatty fish which are responsible for the plasma TG lowering effect (Herzberg, 1991). The TG lowering effect of ω-3 fatty acids is greater in hypertriglyceridemic patients, yet it does occur in normolipidemic subjects (Eritsland et al., 1995; Saynor & Gillott, 1992; Harris et al., 1984; Nestel et al., 1984; Sanders & Roshanai, 1983). Effective doses appear to be from 2-5 g ω-3 fatty acids per day (Harris,1989; Layne et al., 1996). A seven year open trial by Saynor & Gillott (1992) revealed that plasma TG levels were lowered in both normal and high baseline TG subjects and that this effect was sustained over 7 years on a dose of 1.8 g EPA and 1.2 g DHA per day. As habitual consumption of long chain ω-3 fatty acids reduces fasting plasma TG levels, it also reduces the magnitude of postprandial lipemia following ingestion of high fatty acid containing meals (Harris et al., 1988; Weintraub et al., 1988;

Brown & Roberts 1991). Of the different types of ω-3 PUFA, it appears that EPA (20:5ω-3) and DHA (22:6 ω-3) lower TG levels, whereas linolenic acid (18:3 ω-3) must be converted to 20:5 ω-3 before it has any TG lowering effect. Several studies have shown that flaxseed oil, a source of linolenic acid, does not possess hypotriacylglyceridemic effects (Sanders & Roshanai, 1983; Nettleton, 1991; Layne et al., 1996). Layne et al. (1996) suggest that either the flaxseed oil was not given in high enough doses to allow sufficient amounts of 18:3 ω-3 to be converted to 20:5 ω-3 or that this conversion was somehow limited. Diets high in linoleic acid (18:2 ω-6) could slow this conversion as this fatty acid competes with the 18:3 ω-3 for the same Δ-6 desaturase enzyme.

Research has revealed several mechanisms which are involved in the triglyceride lowering effect of long chain ω-3 fatty acids. Human studies have revealed that hepatic secretion of VLDL -TG is decreased by consumption of fish oils (Harris et al., 1984; Nestel et al., 1984; Sanders et al, 1985; Connor 1986). TG production in the liver appears to be reduced through inhibition of TG synthesizing enzymes (Rustan et al., 1988; Wong & Marsh, 1988), suppression of lipogenesis (Iritani et al, 1980; Yang & Williams, 1978), and increased oxidation of fatty acids (Nestel et al, 1984; Berge, 1993; Willumsen et al., 1993). Enhanced catabolism of VLDL in circulation may also contribute to the hypotriglyceridemic effect (Huff & Telford, 1989).

While dietary carbohydrate has been shown to raise plasma TG levels, in normotriglyceridemic individuals this effect is only mild, and may be only transitory (Antonis & Bersohn, 1961). It has also been suggested that a gradual transition to a low fat, high carbohydrate diet should not induce excessive plasma TG levels (Ullmann et al., 1991). With regards to specific carbohydrates, it has been shown that sucrose and fructose increase TG levels more than glucose when fed to various animals (MacDonald &

Roberts, 1967). Similar results were found in some human studies (MacDonald & Braithwaite, 1964; Kaufman & Kapitulnik, 1963; MacDonald, 1966).

While dietary fat lowers plasma TG levels when substituted for carbohydrate, high fat diets often lead to excess energy intake and weight gain. Such overnutrition is the most common cause of elevated TG levels, as TG synthesis in the liver and TG storage in adipose tissue are both increased in the overweight or obese state (Grundy & Denke, 1990).

The influence of various nutrients on plasma TG levels arises from effects on lipoprotein metabolism. There are several mechanisms by which the level of TG in circulation may be changed (Herzberg, 1991). The rate of entry of TG into the blood may be altered, either through changes in VLDL production by the liver or modified intestinal absorption of dietary lipid. Hepatic VLDL production in turn may be altered due to changes in amount of incoming dietary fat or changes in *de novo* TG synthesis rate. The other route by which circulating TG levels may be altered is through changes in the capacity for hydrolyzing the TG in the TG rich lipoproteins. Diet has the potential to affect all of these processes and thus alter TG levels (Grundy & Denke, 1990). Therefore, diet has the potential to affect the *de novo* synthesis of TG and alter plasma TG levels through this pathway.

2.2 Adipose Tissue Triglyceride Storage

Obesity is defined as the excessive accumulation of body fat. It can be classified by percentage overweight or body mass index; exceeding normal weight by at least 20% (Hunt & Groff, 1990) or having a body mass index greater than 27 kg/m^2 is considered obese (Food and Nutrition Board, Institute of Medicine, 1995). The accumulation of fat is in the form of TG stored in adipose tissue and there are many theories regarding the causes of this excess storage. It is widely accepted that both environmental and genetic factors interact to bring about the obese state. Along with investigating the effect of

environmental factors such as diet and lifestyle, it is important to understand the mechanisms by which genetic factors may cause increased fat storage. The *ob* gene and its protein product leptin are thought to play a role in the etiology of obesity through regulation of food intake (Rohner-Jeanrenaud & Jeanrenaud, 1996) and efficiency of energy utilization (Pelleymounter, 1996). Both genetic and environmental factors could increase rate of lipogenesis and thus increase accumulation of lipid.

The genetically obese mouse (*ob/ob*) (Clandinin et al., 1996) and Zucker fatty rat (*fa/fa*) (Zucker & Zucker, 1961; Cleary et al., 1980) develop obesity in a similar manner to humans. As models of human obesity, these animals give insight into the possible causes of this condition (Argiles, 1989). The enzymes involved in hepatic lipogenesis were shown to be elevated in *ob/ob* mice compared to lean mice (Clandinin et al., 1996). Researchers have found that *fa/fa* rats have increased activity of lipogenic enzymes in both liver and adipose tissue when allowed to eat ad libitum (Bray et al, 1970; Taketomi et al., 1975). Godbole and York (1978) suggest that the increased fatty acid synthesis results from the hyperphagia, hyperinsulinemia and increased mass of hepatic and adipose tissue seen in these rats. However, other research indicates that the *fa/fa* rat deposits more fat and becomes obese even when fed the same amount as lean control rats (Zucker, 1967; Bray et al., 1973; Cleary et al., 1980). Excess storage of TG in adipose tissue has been shown to develop in the first week of life, before hypertriglyceridemia and hyperphagia exist in these *fa/fa* rats (Boulange et al., 1979). These results suggest that nutrients are utilized in an abnormal way which channels them into lipid synthesis and storage (Bazin & Lavau, 1982). Research done by Bazin and Lavau (1982) suggests that adipose tissue may be the primary site of expression of the *fa/fa* genotype. There is an increased capacity for lipogenesis in the adipose tissue of these rats beginning during suckling, whereas there is no change in lipogenic enzymes in the liver at this point. The increased activity in adipose tissue would cause substrates such as glucose and amino acids to be directed towards lipogenesis in this tissue and therefore increase fat stores. These authors suggest that this channeling of nutrients could then induce hyperphagia as

well as hyperinsulinemia, which would then cause an increase in lipogenic activity in the liver. These ideas have led to research on the provision of lipogenic substrates. It has been shown that LPL, involved in regulating adipose tissue mass, and glyceraldehyde-3-phosphate dehydrogenase, involved in control of glycolysis, both have increased activity in the adipose tissue of *fa/fa* rats in the first week of life (Dugail et al., 1988a; Dugail et al., 1988b).

In addition to such genetic factors, rate of lipogenesis may be influenced by environmental factors. Research in artificially reared rat pups has shown that feeding a high carbohydrate formula during the suckling period results in increased hepatic lipogenic capacity compared to pups fed a high fat formula or suckling the dam's milk (Hiremagalur et al., 1992) This group of researchers has also shown that feeding a high fat formula providing only medium-chain TG results in the same increased hepatic lipogenic capacity as the high carbohydrate formula, while the inclusion of long-chain TG prevented this induction of lipogenic enzymes. Research done by Thekkumkara et al. (1990) suggests that feeding a high carbohydrate formula during suckling also has long term consequences such as increased lipogenic capacity in liver and adipose tissue, which may contribute to increased fat storage.

Therefore, altered rates of lipogenesis may be involved in the development of obesity in the rat. If these results have any application to humans, then researching the role of lipogenesis in the development of obesity in humans is important. Understanding the influence of diet on the regulation of lipogenesis may reveal a method of inhibiting lipogenic activity, providing a valuable preventative treatment.

3 LIPOGENESIS

De novo synthesis of long chain fatty acids from acetyl CoA is an essential metabolic pathway, enabling the body to store energy and provide structural components of cells

(Blennemann et al., 1992). The pathway is regulated by both dietary and hormonal factors, allowing varying amounts of fatty acids to be synthesized according to the body's needs or the necessity to store dietary energy in response to the amount and composition of dietary intake.

3.1 Pathway for Lipogenesis

The term lipogenesis refers to the *de novo* synthesis of fatty acids from acetyl CoA, and may also include the acylation of these fatty acids to a glycerol molecule to form TG (Hillgartner et al., 1995). Acetyl CoA is provided by carbohydrate via the glycolytic pathway, amino acids, or lactate (Hunt and Groff, 1990). Glucose is the primary substrate for fatty acid synthesis in most tissues. Therefore, the regulatory enzymes of glycolysis: glucokinase, 6-phosphofructo-1-kinase, and pyruvate kinase, are also potential sites for control of fatty acid synthesis (Hillgartner et al., 1995). After conversion of glucose to pyruvate, pyruvate dehydrogenase catalyzes its conversion to acetyl CoA in the mitochondria. Since fatty acids are synthesized in the cytosol and the mitochondria are impermeable to acetyl CoA, citrate is formed by condensing the acetyl CoA with oxaloacetate. Citrate is then transported to the cytosol and cleaved to produce acetyl CoA needed for fatty acid synthesis. The oxaloacetate reformed is returned to the mitochondria via reduction initially to malate and secondly to pyruvate. The conversion of malate to pyruvate is catalyzed by malic enzyme and the reaction produces NADPH. (Stryer, 1988) In addition to acetyl CoA, fatty acid synthesis requires NADPH. This substrate is provided by the malic enzyme reaction, as well as by the pentose phosphate pathway enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Hillgartner et al., 1995). Provided with acetyl CoA and NADPH, fatty acid synthesis is catalyzed by acetyl CoA carboxylase and fatty acid synthase (Stryer, 1988). These enzymes are located in the cytoplasm and catalyze a chain elongation reaction culminating in the formation of palmitate. Other enzyme systems then elongate or desaturate this major end product (Stryer, 1988). There is also mRNA encoding a

protein, designated the Spot 14 protein, which has been found in high concentrations in lipogenic tissues and which may be involved in regulation of lipogenesis (Jump et al., 1988). However, the function of Spot 14 protein has not been identified.

In summary, the key enzymes related to the *de novo* synthesis of fatty acids are considered to be glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, malic enzyme, acetyl CoA carboxylase, and fatty acid synthase (Noguchi et al., 1992).

3.2 Regulation of Lipogenesis

Flow through a metabolic pathway is controlled through short-term and long-term regulation (Hillgartner et al., 1995). Short-term regulation takes place through allosteric mechanisms or covalent modification of enzymes, such as phosphorylation-dephosphorylation. Long-term regulation involves a change in the capacity of the lipogenic pathway, usually by changing the concentration of the lipogenic enzymes. Changes in the amount and composition of macronutrients in the diet are communicated to the organs by humoral factors such as circulating fuels and hormones. These factors act as signals to alter the activity of regulatory enzymes, through a combination of short and long term mechanisms, in the appropriate cells (Hillgartner et al., 1995).

Most tissues synthesize fatty acids at low rates not associated with energy regulation, whereas liver and adipose tissue fatty acid synthesis rates are 10-1,000 times those in other tissues and are regulated by diet and hormones (Hillgartner et al., 1995). In adult humans, *de novo* synthesis of fatty acids takes place primarily in the liver (Bjorntorp and Sjostrom, 1978), while the primary site differs in other organisms (Hillgartner et al., 1995).

The effect of diet on rate of *de novo* lipogenesis has been studied extensively in animal models and *in vitro,* while research in *in vivo* humans has only recently been initiated due to the development of suitable methods.

3.2.1 ANIMAL MODELS AND IN VITRO RESEARCH

LIPIDS

Dietary fat decreases the rate of hepatic *de novo* fatty acid synthesis when compared to dietary carbohydrate (Herzberg & Janmohamed, 1980). Many investigators have shown that PUFA of the ω-3 and ω-6 families suppress lipogenesis in the liver, whereas SFA and MUFA do not (Allmann & Gibson, 1965; Flick et al., 1977; Jeffcoat & James, 1978; Toussant et al., 1981; Clarke et al., 1977; Shillabeer et al., 1990). The inhibitory effect of PUFA was not due to a decrease in carbohydrate content nor a reversal of essential fatty acid deficiency (Clarke et al., 1976). Further investigation has revealed that in order to have inhibitory abilities, fatty acids must be at least 18 carbons long and contain two double bonds at the 9 and 12 positions (Clarke et al., 1990b). It has also been found that fish oils are more potent inhibitors than vegetable oils (Clarke et al., 1988; Iritani et al., 1980) and that products of the Δ-6 desaturase are more potent inhibitors than the fatty acid precursors of this enzyme (Clarke & Jump, 1993b). Thus, fish oils rich in 20 and 22 carbon PUFA induce a greater response than the 18 carbon PUFA (Clarke & Jump, 1993a).

The ω-3 and ω-6 fatty acids suppress hepatic lipogenesis by decreasing the activities of the enzymes involved (Clarke & Abraham, 1992; Toussant et al., 1981). The lowered activity is due to decreased enzyme concentration resulting from inhibition of enzyme synthesis (Schwartz & Abraham, 1982). Further, this inhibition of enzyme synthesis results from a decrease in mRNA abundance in rodent liver, often due to inhibition of gene transcription (Clarke et al., 1990b; Jump et al., 1993). Jump et al. (1994) suggest that PUFA coordinately regulate the expression of several enzymes involved in lipogenesis.

In cultured hepatocytes it is apparent that these PUFA do not require peripheral metabolism to bring about the regulation of gene expression, but rather, act directly on the gene (Salati et al., 1988). Changes in the rate of gene transcription which result from

PUFA are too rapid to be explained by changes in hormone binding due to increased membrane fluidity (Clarke & Jump, 1993a). Clarke and Jump (1993a) propose the following model to explain the regulation of gene transcription by PUFA. Any 18 carbon PUFA must undergo desaturation by the Δ-6 desaturase before entering the nucleus along with the longer chain ω-3 and ω-6 PUFA. Once in the nucleus, these fatty acids may act as ligands or modifiers of a nuclear fatty acid receptor protein, PUFA-BP. This nuclear protein is considered a *trans* -acting regulatory protein, which means that it is a diffusible factor which can bind to a *cis* - acting nucleotide response element, a sequence within the same DNA molecule as the regulated gene (Goodridge, 1987). The *cis* - acting elements are usually upstream from the basal promoter elements and the start site for transcription, and allow for selective regulation by *trans* - acting protein factors (Hillgartner et al., 1995). In the model proposed by Clarke and Jump, the PUFA-BP will bind to a *cis* - acting response element, which governs gene transcription on the lipogenic enzyme gene, when the ω-3 or ω-6 fatty acid ligand is bound to it. Recent work done by Jump et al. (1993) has resulted in the localization of the PUFA-regulated *cis* -acting elements for the S14 gene, and current research is aimed at finding such elements in other lipogenic genes.

Other aspects of dietary lipids involved in the regulation of lipogenesis include the following. It was found that diets which are high in fat but deficient in linoleate, stimulated hepatic lipogenesis in rodents (Herzberg and Janmohamed, 1980; Herzberg, 1983). When rat hepatocytes were exposed to short chain fatty acids and medium chain fatty acids, acetyl-CoA carboxylase was activated (Geelen, 1994). Rats fed a diet rich in medium chain TG had increased activities of several lipogenic enzymes compared to rats fed a corn oil diet rich in ω-6 PUFA (Geelen et al., 1995). Dietary cholesterol has also been shown to increase the rate of fatty acid synthesis and increase the incorporation of these fatty acids into TG (Fungwe et al., 1994).

CARBOHYDRATES

While the lowest rate of lipogenesis occurs during long term starvation, the highest rate occurs by refeeding a high carbohydrate, low fat diet following starvation (Hillgartner et al., 1995). In rat hepatocytes, studies have shown that the activity of key enzymes of fatty acid synthesis is increased by the presence of glucose (Salati et al., 1988; Mariash and Oppenheimer, 1983; Katz and Ick, 1981; Decaux et al., 1989). Fructose was shown to cause higher rates of fatty acid synthesis and enzyme activity in rats than glucose (Volpe and Vagelos, 1974, Park et al., 1992). In diabetic rats, which would have decreased anabolic activity, feeding fructose restored fatty acid synthesis rates and enzyme activities to normal, while feeding glucose did not (Volpe and Vagelos, 1974; Fukuda et al., 1992a; Baker et al., 1952).

High fiber diets may also be beneficial in lowering elevated plasma TG levels (Anderson and Chen, 1979). A study by Mazur et al. (1992) suggests that fermentable fibers may decrease fatty acid synthesis, resulting in lower plasma TG concentrations.

As in the case of regulation by PUFA, carbohydrates can also act directly on gene expression. Actions of carbohydrates include direct control of gene transcription (Jump et al., 1990; Thompson & Towle, 1991; German et al., 1990; Rongnoparut et al., 1991), control of mRNA processing (Burmeister & Mariash, 1991), control of mRNA editing (Baum et al., 1990) and control of mRNA stability (Dozin et al., 1986). Different mechanisms of regulation are utilized for the various lipogenic genes (Clarke & Abraham, 1992). In contrast to regulation by PUFA, the action of carbohydrates on lipogenic enzymes is not limited to the liver (Coupé et al., 1990; Pape et al., 1988). While carbohydrates can act on gene expression independently of changes in hormone levels (Fukuda et al., 1992a), glucose requires insulin at least for its metabolism. Glucose and fructose appear to be metabolized before acting on gene transcription (Mariash, 1989). A carbohydrate response element has been found in the S14 gene (Jump et al., 1990), and

other genes have similar *cis* - acting elements (Thompson & Towle, 1991; German et al., 1990).

Meal Frequency

Other than macronutrient composition, another aspect of dietary intake which could influence lipogenesis is the frequency of consumption. Meal frequency has been suggested to influence body weight and composition of humans and animals (Fabry & Tepperman, 1970; Bray, 1972). However, other studies in humans (Wadhwa et al., 1973; Finkelstein & Fryer, 1971; Young et al, 1971), dogs (Romsos et al., 1978a) and pigs (Romsos et al., 1978b) suggest that meal frequency does not influence body weight nor body fat. A study in humans fed to energy balance in 2 versus 7 meals per day (Verboeket-van de Venne & Westerterp, 1991) found that meal frequency did not significantly affect total 24 hour energy expenditure or 24 hour nutrient utilization. However, feeding energy requirements in 2 meals per day resulted in a stronger diurnal periodicity of lipogenesis and lipolysis, compared to feeding over 7 meals per day.

Insulin

Insulin levels are increased in the fed state, and this hormone stimulates lipogenesis. It has been shown that an increase in circulating insulin levels corresponds to an increase in the rate of fatty acid synthesis and increased activities of lipogenic enzymes in rodent liver and adipose tissue (Owen et al., 1979; Szepesi et al., 1971). In diabetic animals, both lipogenesis and the activity of hepatic lipogenic enzymes are decreased, and treatment with insulin restores these functions to normal levels (Volpe and Vagelos, 1974; Nepokroeff et al., 1974) In isolated hepatocytes, insulin stimulates fatty acid synthesis (Goodridge, 1973; Ma et al., 1978; Topping and Mayes, 1982; Witters et al., 1979). Experiments performed with explants of rat tissues concluded that adipocytes and hepatocytes need both insulin and glucose to induce an accumulation of lipogenic enzyme mRNA (Foufelle et al., 1992b; Fukuda et al., 1992b).

GLUCAGON

Glucagon levels are increased in the fasting state, and this hormone inhibits lipogenesis. Treatment of normal rats with glucagon caused an inhibition of fatty acid synthesis and prevented the normal increase in fatty acid synthesis associated with refeeding starved rats (Klain, 1977; Lakshmanan et al.,1972). In isolated tissue preparations, glucagon was shown to inhibit fatty acid synthesis (Goodridge, 1973; Ma et al., 1978; Topping and Mayes, 1982; Witters et al., 1979).

THYROID HORMONE

Thyroid hormone levels are increased in the fed state and decreased in the fasting state in both rats and humans (Kaplan and Utiger, 1978; Merimee and Fineberg, 1976). Removing the thyroid from rats resulted in decreased activity of lipogenic enzymes, while replacement therapy restored normal activity levels (Tepperman and Tepperman, 1964; Mariash et al., 1980; Bottger et al., 1970). The effects of thyroid hormone have been found to be tissue specific. In the liver, thyroid hormone stimulates fatty acid synthesis (Freake et al. 1989; Roncari and Murthy, 1975). In white adipose tissue, the results are conflicting (Blennemann et al., 1992). In brown adipose tissue, fatty acid synthesis is stimulated in hypothyroid rats (Freake and Oppenheimer, 1987; Baht and Saggerson, 1988). For this stimulation to occur, it was found that an intact nerve supply was needed (Yeh and Freake, 1991). Therefore, it is thought that the stimulation of fatty acid synthesis in this situation is caused by sympathetic stimulation of brown adipose tissue in response to decreased metabolic rate in the hypothyroid state (Blennemann et al., 1992).

LACTATION

Another important aspect of hormonal regulation of lipogenesis is the redirection of fatty acid synthesis during lactation. Around the time of parturition, the rate of lipogenesis in

the mammary gland increases many fold (Vernon & Flint, 1983). Prolactin, together with insulin and cortisol, has been shown to increase the rate of lipogenesis in mammary explants of pregnant sheep (Barber et al., 1991), cows (Collier et al., 1977) and goats (Skarda et al., 1982), while decreasing the rate of lipogenesis in adipose tissue. Prolactin has been shown to increase mammary acetyl CoA carboxylase activity (McNeillie & Zammit, 1982) and decrease adipose tissue acetyl CoA carboxylase activity (Barber et al., 1992a). Growth hormone also plays an important role in maintaining lactation (Madon et al., 1986; Barber et al., 1992a; Flint et al., 1992). However, the mechanism by which it influences milk production is not clear, as growth hormone receptors have not been found on mammary epithelial cells (Barber et al., 1992b). While one theory suggests that growth hormone may act by stimulating the insulin-like growth factors (Prosser et al., 1990), Flint et al. (1992) have shown that neither insulin-like growth factor I nor insulin-like growth factor II could produce the effects of growth hormone on milk production in the rat.

Investigating the control of lipogenesis during lactation is important to the understanding of milk composition. Factors which influence the hormonal environment during lactation may alter the composition of the milk (Chappell & Clandinin, 1984).

Acetyl CoA Carboxylase

Changes in concentration of ACC during starvation and refeeding, as well as during weaning of rat pups, have been shown to be due to changes in the level of ACC mRNA brought about by regulation at a pretranslational level (Coupe et al., 1990; Foufelle et al., 1992a; Evans & Witters, 1988; Fischer & Goodridge, 1978; Pape et al., 1988). However, it has not yet been determined if this regulation is at gene transcription or another level of mRNA processing. Control of ACC synthesis by glucose and insulin is at a pretranslational level (Park & Kim, 1991), and some research indicates that insulin regulates transcription of the ACC gene (Pape & Kim, 1989). Although it is known that

PUFA act to decrease the level of ACC in the liver (Toussant et al., 1981), the precise level of regulation has not been shown.

Fatty Acid Synthase

Research indicates that the primary control of FAS concentration is through regulation of gene transcription. During starvation and refeeding, changes in FAS synthesis rate have been paralleled by changes in transcription rate of the hepatic FAS gene (Clarke et al., 1990a; Craig & Porter, 1973; Goodridge, 1986; Katsurada et al., 1990; Laux & Schweizer, 1990; Paulauskis & Sul, 1989). The suppression of hepatic FAS synthesis by PUFA has also been shown to occur at the level of gene transcription (Blake & Clarke, 1990; Clarke et al., 1990b; Shillabeer et al., 1990). The increase in FAS synthesis caused by insulin and the decrease resulting from glucagon are both associated with changes in the amount of FAS mRNA due to regulation of FAS gene transcription (Paulauskis & Sul, 1989). In culture, it has been suggested that the stimulation of FAS synthesis by thyroid hormone is also a result of increased gene transcription rates (Moustaid & Sul, 1991).

Spot 14 Protein

Data from several researchers has shown a strong correlation between spot 14 mRNA expression and lipid formation in the lipogenic tissues (Jump & Oppenheimer, 1985; Freake & Oppenheimer, 1987). Dietary carbohydrate and thyroid hormone have been shown to induce spot 14 mRNA expression, while PUFA, glucagon and catabolic states have been shown to inhibit expression (Jump et al., 1990; Kinlaw et al., 1988; Jump et al., 1993). Similar regulation of the spot 14 protein has also been shown (Kinlaw et al., 1989), and the protein has been shown to be located primarily in the nucleus (Kinlaw et al., 1992). Kinlaw et al. (1995) designed a study to test the hypothesis that spot 14 protein is involved in transduction of lipogenic signals. By transfecting an antisense RNA molecule to the spot 14 mRNA into cells expressing this gene, translation of the spot 14 mRNA is blocked. This procedure was used to assess the metabolic effects of inhibiting

the induction of spot 14 protein by thyroid hormone and glucose. Results showed that in antisense-transfected hepatocytes, glucose and thyroid hormone were not able to stimulate lipogenesis as usual (Kinlaw et al., 1995). Further, it was shown that the induction of several lipogenic enzymes was inhibited, and for malic enzyme in particular, decreased mRNA expression was shown (Kinlaw et al., 1995). These researchers hypothesize that blocking the translation of spot 14 mRNA blocks other pretranslational events, and suggest that spot 14 protein may be involved in integrating the regulation of lipogenesis by nutritional and hormonal factors.

3.2.2 Lipogenesis in Humans

Early studies of lipogenesis in humans utilized indirect calorimetry to measure net *de novo* synthesis of fatty acids. Acheson et al. (1984) studied net *de novo* lipogenesis in healthy young males after ingestion of a 500 g CHO load to meet 24 h energy requirements. Prior to the CHO load, body glycogen stores were modulated by dietary means. At 14 h after CHO loading, net lipogenesis amounted to 0.8 ± 0.5 g, 3.4 ± 0.6 g and 9 ± 1 g lipid (mean ± SEM) in subjects with depleted, maintained or increased glycogen stores respectively. In all subjects, however, there was no net gain of body fat from CHO at 24 h due to sufficient lipid oxidation. In a further study, Acheson et al. (1988) depleted glycogen stores in healthy young men and then provided excess calories through a diet composed of 86% kcal as CHO, 3% kcal as fat, 11% kcal as protein. After increasing glycogen stores by approximately 500 g, excess CHO was converted to fat. During the period of CHO overfeeding, approximately 150 g of lipid/day was synthesized and plasma TG levels increased from 1.3 ± 1.2 mmol/L to 8.6 ± 1.0 mmol/L (mean ± SD). Research by this group indicates that glycogen storage and high rates of glucose oxidation accomodate ingestion of large amounts of CHO. Net *de novo* lipogenesis can become a significant metabolic pathway, disposing of excess energy from massive CHO overfeeding once glycogen stores are full, and can contribute to increased plasma TG levels.

In order to understand the role of hepatic *de novo* lipogenesis more clearly, it is necessary to directly measure rate of synthesis rather than net synthesis. Stable isotopes are suitable tracers for direct measurement of biochemical processes in humans. A labelled precursor is introduced and the appearance of labeled end products is quantified (Zilversmit, 1943). In order to use these tracers to determine rate of hepatic *de novo* lipogenesis, the enrichment of the true intracellular precursor, cytosolic acetyl CoA, must be known in order to predict theoretical isotope content of the product fatty acid molecule. The fraction of newly synthesized fatty acid molecules in a mixture of plasma TG fatty acids can then be calculated after a discrete time interval. Input from many metabolic sources to the acetyl CoA pool can vary quantitatively, and cellular acetyl CoA is compartmentalized into pools used for different metabolic pathways (Hellerstein, 1996). Therefore, the specific acetyl CoA pool used for lipogenesis cannot be accessed to determine precursor enrichment. Two approaches have been developed to solve this problem and allow the direct measurement of hepatic *de novo* lipogenesis in humans.

Deuterium labelled water is not subject to compartmentalization but mixes freely across membranes. Extracellular fluids such as plasma can, therefore, be used to estimate intracellular deuterium enrichment. When *de novo* fatty acid sythesis occurs in the presence of labelled water, deuterium will enter the fatty acid during reduction reactions. Deuterium will only enter during synthesis or elongation of the fatty acid (Leitch & Jones, 1993). Deuterium will appear in the end product fatty acid in stable positions, attached directly to carbon atoms (Schoenheimer & Rittenberg, 1936). The theoretical isotope content of the end product can be predicted using a value for fraction of deuterium atoms incorporated into the fatty acid compared to hydrogen atoms. The enrichment of the end product can be measured by sampling plasma TG or more specifically VLDL-TG, as newly synthesized fatty acids are exported from the liver in VLDL-TG. The assumptions made in using this method are: that cell membranes are permeable to deuterium oxide, that a consistent fraction of deuterium atoms are

incorporated into fatty acids under various metabolic conditions, and that the VLDL-TG pool size remains constant over the time period studied (Leitch & Jones, 1991).

Leitch and Jones (1991) measured production rate of *de novo* TG-FA in healthy, free-living males by incorporation of deuterium from the plasma water pool. The contribution of *de novo* FA to total TG-FA pool was found to be 7.0% ± 0.5% (mean ± SEM) by a linear model or 7.3% ± 0.5% by an exponential model. In further research, values were found to be increased in subjects with apo E2-associated hyperlipidemia (14.3% ± 1.2%, mean ± SEM) compared to controls (8.2% ± 1.3%) (Jones et al., 1992). These researchers also studied lipogenic rate during feeding of 6 evenly spaced nutritionally complete liquid meals to healthy males (Leitch & Jones, 1993). Meals provided 32% kcal from fat, 53.3% kcal from CHO and 14.7% kcal from protein and met daily energy requirements. The contribution of *de novo* FA to total TG-FA pool was found to be 8.0 % ± 3.9 % (mean ± SD). In this study, a model for the calculation of net *de novo* TG-FA synthesis was developed; using a value of 300 mg/kg/day for VLDL-TG production rate, net TG-FA synthesis was estimated at 1.7 ± 0.8 g/day (mean ± SD). In 1995, Jones et al. studied the effect of meal frequency on lipogenic rate in healthy male subjects. A nutritionally complete liquid diet, with composition as given above, was fed as 3 or 6 meals per day to meet energy requirements. Contribution of *de novo* synthesis to total TG-FA production was not significantly different for subjects fed 3 meals (6.64% ± 2.08%, mean ± SEM) or 6 meals per day (7.88% ± 1.46%) for 3 days. Net TG-FA synthesis was estimated at 1.55 ± 0.53 g/day and 1.69 ± 0.30 g/day for subjects fed 3 and 6 meals per day respectively. In summary, research done using deuterium incorporation suggests that when healthy males consume diets containing 32-40% kcal from fat or self-selected diets, approximately 7-8% of TG-FA, or 2 g per day, is newly synthesized from CHO.

The other approach which has been developed to directly measure *de novo* lipogenesis in humans is mass isotopomer distribution analysis (MIDA), which applies probability analysis to mass isotopomer frequency distribution to calculate precursor enrichment.

Hellerstein et al. (1991) used MIDA to measure hepatic *de novo* lipogenesis in normal men. After an overnight fast, 0.91 ± 0.27% (mean ± SEM) of VLDL-palmitate and 0.37 ± 0.08% of VLDL-stearate were newly synthesized from CHO. Subjects were refed with either a constant infusion of glucose (7-10 mg/kg per min) for 8 h, hourly liquid mixed meal (Ensure, 53.7 % kcal as CHO, 31.5% kcal as fat, 14.8% kcal as protein) to deliver CHO at 7-10 mg/kg per min for 8 h, or a single high-CHO breakfast (3.5 g CHO/kg body weight). During CHO refeeding, contribution of *de novo* synthesized palmitate and stearate to VLDL -palmitate and VLDL-stearate was 1.64-1.97% (means) and 0.47-0.64% respectively. The authors conclude that most circulating fatty acids must be derived from reesterification of preformed free fatty acids and that lipogenesis is a quantitatively minor pathway under the conditions of the study.

Faix et al. (1993) fasted and refed a complete liquid diet (Ensure, 7mg CHO/kg per min for 7 hours) to a variety of subjects. Fraction of circulating VLDL-palmitate derived from endogenous synthesis was determined under fasting and fed conditions. Values in normal men ranged from 0.86 % ± 0.21% (mean ± SEM) in the fasted state to 4.94% ± 1.93% in the fed state. Values for normal women in luteal phase ranged from 1.5% ± 0.6% (fasted) to 3.5% ± 0.7% (fed), while values during follicular phase ranged from 4.2% ± 1.4% (fasted) to 9.7% ± 1.6% (fed). Obese men were found to have higher rates of synthesis with 6.9% ± 2.8% (fasted) to 16.5% ± 4.3% (fed) of VLDL-palmitate being derived from *de novo* synthesis.

A series of studies have been performed to investigate the effects of various dietary CHO manipulations on lipogenesis in humans (Hellerstein, 1996). Schwarz et al.(1993) found

that oral fructose (7 mg/kg per min for 6 h) increased fractional *de novo* lipogenesis from 2.8%± 0.8% (mean ± SEM) to 24.0% ± 1.7%.

Hudgins et al. (1996) studied the response of hepatic *de novo* lipogenesis to prolonged eucaloric low fat, high CHO diets. Normal subjects consumed either a low fat liquid formula (10% kcal as fat, 75% kcal as CHO) or a high fat liquid formula (40% kcal as fat, 45% kcal as CHO) for 25 d. Fraction of VLDL-palmitate derived from endogenous synthesis was determined by MIDA in the fasted and fed states. Values for subjects consuming the low fat diet ranged from 19.66% ± 5.37% (fasted) to 23.55% ± 5.62% (fed). For subjects consuming the high fat diet, values ranged from 3.63% ± 0.92% (fasted) to 7.89% ± 3.04% (fed). It was also found that VLDL-TG were enriched with palmitate and deficient in linoleate in subjects on the low fat diet. The authors suggest that the low fat diet stimulated *de novo* synthesis of palmitate and brought about palmitate enriched plasma TG. On the low fat diet, plasma TG levels peaked at 2.88 ± 1.30 mM (mean ± SD) compared to initial levels of 0.88 ± 0.27 mM. Plasma TG levels for subjects on the high fat diet peaked at 1.63 ± 0.63 mM.

In order to study the effect of overfeeding a low fat, high CHO diet, Schwarz et al. (1995) fed normal subjects a series of diets for 5 days each. In the fed state, the contribution of hepatic *de novo* lipogenesis to VLDL-palmitate was found to range from 3.3% ± 0.8% on a eucaloric diet, to 23.2% ± 3.3% on diets providing 25% excess energy as CHO, up to 30.4% ± 3.7% on diets providing 50% excess calories as CHO.

In summary of the research done using MIDA, in normal men and normal women in the luteal phase consuming Western diets, 1-8% of VLDL-palmitate is newly synthesized, representing < 1 g palmitate per day when net synthesis was estimated using published VLDL-TG production rates of 120 to 350 mg/kg/day. Stimulated synthesis was associated with obesity, women in the follicular phase, fructose intake, prolonged very

low-fat high-CHO diets, and CHO overfeeding. The authors estimate that even when *de novo* lipogenesis was the most highly stimulated, net production of lipids by this pathway was <10 g/day.

Although fatty acid synthesis generally occurs at low rates in humans consuming a mixed diet, increased rates due to physiological defects or specific dietary patterns could lead to increased plasma and adipose tissue levels of TG (Hillgartner et al., 1995).

4 Rationale and Hypotheses

Dietary influences on regulation of lipogenesis have potential to alter plasma and adipose tissue triglyceride levels. Research in animal models and in cells *in vitro* indicate that polyunsaturated fatty acids suppress hepatic lipogenesis while saturated and monounsaturated fatty acids do not. Examination of the influence of diet on lipogenesis *in vivo* in humans is now possible and important to the understanding of lipid metabolism and the etiology of several chronic disease states. The effects of different fatty acids in the diet on rate of hepatic *de novo* lipogenesis in humans is not known.

The objectives of this research are:

1) to investigate the effects of linoleic acid (C18:2) and palmitic acid (C16:0) in the diet on rates of hepatic *de novo* fatty acid synthesis in humans using deuterium incorporation;

2) to investigate the relationship between changes in rate of hepatic *de novo* fatty acid synthesis in humans and plasma TG level.

These objectives will be achieved by testing the following hypotheses:

1) A high dietary linoleic acid level suppresses lipogenic rate versus a low dietary linoleic acid level;

2) A high dietary palmitic acid level does not suppress lipogenic rate versus a low dietary palmitic acid level;

3) A high dietary linoleic acid level suppresses lipogenic rate at both high and low levels of dietary palmitic acid;

4) Changes in lipogenic rate are reflected in level of plasma TG.

An initial experiment will investigate the effects of a diet high in C16:0 and low in C18:2 versus a diet low in C16:0 and high in C18:2 on rate of hepatic *de novo* lipogenesis and plasma TG level. A second experiment will study the effects of changing between high and low levels of C18:2 at constant high or low levels of C16:0, on rate of hepatic *de novo* lipogenesis and plasma TG level.

CHAPTER 2

EXPERIMENTAL METHODS

1 EXPERIMENT 1

1.1 SUBJECTS AND DIETS

Subjects were recruited through advertisements at the University of Alberta. Six male and six female volunteers gave informed written consent to participate in this study after being screened for chronic disease, smoking, medication, supplements, and family history of diabetes or coronary artery disease. The study protocol was approved by the Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee, University of Alberta.

Subjects consumed three isoenergetic meals per day consisting of normal foods and designed to meet estimated energy requirements. Energy requirements for each subject were estimated using the Mayo Clinic Nomogram (Committee on Dietetics, Mayo Clinic 1954) to determine basal metabolic rate and multiplying this figure by activity coefficients between 1.7 and 2.0 (Table 1). Subjects were weighed daily to ensure stable body weight and adjustments in energy intake were made if sustained weight changes were observed.

The study consisted of two 21 day diet treatments, each consisting of a three day rotating menu. All meals were prepared in the Metabolic Research Kitchen and consumed at fixed times. Breakfast and lunch were consumed in a supervised dining room and supper was packaged to take out. Subjects were instructed not to consume any additional foods or supplements during the study period except energy and caffeine free beverages. For female subjects, diet treatments were coordinated with their menstrual cycles such that lipogenesis was measured during the luteal phase in order to be comparable to rates measured in male subjects (Faix et al., 1993).

Diets were formulated using published nutrient composition tables (US Department of Agriculture, Agriculture Handbooks 8-1-8-12, 1976) and canola oil data (Przybylake, 1994) to contain an average of 31 ± 2% of energy from fat, 55 ± 2% of energy from CHO, and 15 ± 1% of energy from protein. The diets were designed to provide low (2% kcal) or high (8% kcal) levels of palmitic acid (C16:0) and linoleic acid (C18:2) in the following combinations: low C16:0 and high C18:2, high C16:0 and low C18:2 (Table 2).

1.2 Experimental Design

Subjects were randomly placed on either the low C16:0/high C18:2 diet or the high C16:0/low C18:2 diet and fed for 21 days. After a washout period which varied from 0 days to 4 months, the second diet treatment was fed for 21 days. On day 21 of each treatment, a fasting blood sample (30 mL) was taken by venipuncture at 0730 h into vacutainer tubes containing EDTA. Subjects then consumed a priming dose of deuterium oxide (99.8 atom percent excess, ICN Biomedicals, Montreal, Canada) at 0.5g D_2O/kg estimated body water (60% of body weight). A maintenance dose of 1.0 g D_20/kg estimated body water was provided in a 2 L bottle of water to be consumed over the next 24 hours, in order to maintain plasma deuterium enrichment at plateau and compensate for unlabeled water obtained in the diet. Exactly 24 hours later (day 22), a second fasting blood sample (30 mL) was drawn into vacutainer tubes containing disodium EDTA (25 mL) or tubes containing no additive for the collection of serum (5 mL).

1.3 Analytical Methods

On day 22, serum was sent to the University of Alberta Hospitals for TG determination, which was performed enzymatically (Bucolo & David, 1973). The remaining blood from day 21 and 22 was centrifuged at 3000 rpm for 15 minutes at 4°C (Jouan refrigerated centrifuge, CR 4.11) to obtain plasma. Day 21 plasma was used to determine background deuterium enrichment of plasma water and VLDL-TG. Day 22 plasma was used to

measure the deuterium enrichment of plasma water and VLDL-TG 24 hours after deuterium oxide administration. Plasma water deuterium enrichment reaches a plateau by 12 hours and this enrichment is maintained over the 24 hour study period (Leitch & Jones, 1991). Half of approximately 10 mL of plasma obtained each day was used for this study, the other half was used for determination of deuterium incorporation into cholesterol.

LIPOPROTEIN SEPERATION

Two mL of plasma were used for lipoprotein separation, the remainder was stored in a -30°C freezer. Plasma VLDL fraction was isolated by sequential ultracentrifugation (Layne et al., 1996). The density gradient solutions were generated from a diluent containing: 0.372 g/L disodium EDTA, 0.132 g/L sodium azide, and 0.080 g/L thimerosal. Appropriate amounts of NaCl (or NaBr) and diluent were combined to prepare the density gradient solutions, which were brought to a pH of 7.0-7.5 using sodium hydrogen carbonate.

Two precalibrated, 1 mL thick walled ultracentrifuge tubes were each filled with 1 mL of plasma. Plasma samples were centrifuged in a Beckman TL-100 ultracentrifuge in a TLS-55 swing out rotor at 30,000 rpm for 15 minutes at 20°C. The chylomicron fraction seperated out as the upper opaque layer, visible by illumination with a white pen light. Since the blood sample was taken after subjects had fasted overnight, there was either no chylomicron fraction or very little. The bottom plasma layer was removed with a constricted pipet and transfered to another ultracentrifuge tube.

The volume of the removed plasma was made up to 1 mL with 0.196 molal NaCl, and the sample centrifuged in a Beckman TL-100 ultracentrifuge, TLA-100.2 angle head rotor at 100,000 rpm for 3 hours at 20°C. The upper opaque layer of VLDL was drawn off with a contricted pipet.

VLDL LIPID EXTRACTION

Lipids of the VLDL fraction were extracted by a modification of the method of Folch et al. (1957). Solvents were obtained from BDH Inc. and all were distilled. Duplicate samples of VLDL were dispensed into the same test tube containing 10 mL of chloroform:methanol 2:1 (v/v) and 0.05% (v/v) ethoxyquine as an antioxidant. Samples were vortexed and transfered to separatory funnels. Test tubes were rinsed with another 10 mL of chloroform:methanol 2:1 (v/v) and 0.05% (v/v) ethoxyquine, which was added to the separatory funnels, followed by 4.5 mL water. After shaking, the samples were left to separate into two phases. The lower phase containing the VLDL lipids from 2 mL of plasma in chloroform , was removed into screw cap test tubes and dried under nitrogen. The dried samples were then reconstituted with 1 mL chloroform and stored at -70°C.

THIN LAYER CHROMATOGRAPHY

The lipid classes of the VLDL fraction were separated by thin layer chromatography. Each sample of VLDL lipid was split into 2 replicates and spotted onto silica gel G plates (20x20 cm, FisherScientific) which had been washed with distilled hexane and heat activated for 1 hour at 110°C. A TG standard (Sigma) was also spotted on each G plate. Plates were developed in tanks lined with Whatman #1 filter paper and saturated with the solvent system containing petroleum ether:diethylether:glacial acetic acid 80:20:1 (v/v). Developed plates were air dried and the standard lane sprayed with 0.1% 8-anilino-1-naphthalene-sulfonic acid (ANSA), which binds with lipid classes to fluoresce under ultraviolet light. The triacylglycerol band was identified and scraped into screw cap test tubes. TG was extracted from the silica gel by washing 4 times with 5 mL hexane:diethyl ether 85:15 (v/v), followed by a final wash with 2mL methanol, 100 µL 6N HCl and 2 mL chloroform. Supernatant was pooled, dried under nitrogen and the dried TG sample

was transferred to 6 x 50 mm pyrex culture tube (Fisher brand) using 3 rinses of chloroform. Samples were dried under nitrogen, covered and stored at -30°C.

MEASUREMENT OF DEUTERIUM ENRICHMENT

VLDL-TG samples were prepared for measurement of deuterium enrichment. Cupric oxide wire (0.5g) and a 1x10 mm strip of silver foil were added to the pyrex tubes. The cupric oxide acts as the source of oxygen for combustion and the silver acts to remove any sulfur in the sample. The 1x10 mm pyrex tubes were placed into 15x10 mm pyrex tubes (Corning Glass Works, Corning, NY), which were evacuated to less than 50 mtorr before being sealed with a gas/oxygen flame. Samples of VLDL-TG were then combusted to H_2O (HDO) and CO_2 by placing the pyrex tubes in a furnace at 525°C for 4 hours and allowing to cool overnight. Pyrex tubes were attached to a vacuum manifold by means of flexible stainless steel tubing between Cajon fittings (DesMarais & Hayes, 1976), which allows the sealed tubes to be opened within a vacuum system. The exposed end of the pyrex tube was placed in liquid nitrogen (-196°C) to freeze the H_2O (HDO) and CO_2. The pyrex tube was then cracked by flexing the stainless steel tubing and air pumped out. The end of the pyrex tube was placed in a liquid nitrogen/acetone slush (-85°C) to keep the H_2O (HDO) frozen but allow the CO_2 to sublimate and be pumped out. The remaining water of combustion was vacuum-distilled into reaction tubes containing 60 mg zinc shot (University of Indiana, Bloomington).

Samples of plasma water were also prepared for measurement of deuterium enrichment. Plasma samples from Day 22 were diluted 20:1 with 5% bovine serum albumin in order to reduce the deuterium enrichment to within the analytical limits of the mass spectrometer; plasma samples from Day 21 were used directly. Plasma (7 µL) was pipeted into a test tube, attached to the vacuum manifold and frozen in liquid nitrogen. After pumping off

air, liquid nitrogen was removed and the plasma water was vacuum-distilled into reaction tubes containing 60 mg zinc.

Water from combusted VLDL-TG and plasma was reduced by zinc to hydrogen gas by placing the reaction tubes in a heating block at 470°C for 30 minutes (Coleman et al., 1982). Reaction tubes were attached directly to the mass spectrometer. Deuterium enrichment was measured by a Finnigan MAT 251 Isotope Ratio Mass Spectrometer (Bremen, Germany) against hydrogen prepared from a water standard (SMOW). The mass 3 abundance was corrected for H_3^+ contribution. Multiple analyses (n=13) of hydrogen produced from the reduction of a laboratory water standard demonstrated the analytical precision (coefficient of variation) of this instrument at 1%. All samples were analyzed in duplicate.

Fat Analysis of Diets

Diets were analyzed for total fat and fatty acid composition to compare with theoretical values. Duplicate meals for each day of the 3 day menu of each diet treatment were prepared for analysis. Meals were homogenized in a polytron (Brinkmann PT/10/35) with the addition of water to a smooth paste. After freezing at -30°C overnight, meals were freeze dried (Virtis 50-SRC, Gardiner, NY) at -60°C for approximately a week, until free of moisture. Weight of the freeze dried meal was recorded. A 2 g sample of each meal was weighed out in duplicate and transferred to a 150 mL beaker. One mL of tripentadecanoate (99%, Sigma) in chloroform (15.0 mg/mL) was added as an internal standard. Fat was extracted (Folch et al., 1957) by adding 40 mL chloroform:methanol 2:1 (v/v) and mixing with a magnetic stirrer for 5-10 minutes. Solvent was decanted into a separatory funnel through Whatman #1 filter paper and another 40 mL chloroform:methanol 2:1 (v/v) was added to repeat the extraction step. The beaker was rinsed with another 10 mL chloroform:methanol 2:1 (v/v) and all particles were transferred into the filter paper. After adding 20 mL water to the separatory funnel, the

contents were shaken and allowed to separate overnight in a cool room. The bottom layer was collected into a pre-weighed 50 mL culture tube. A 0.2 mL aliquot was taken in duplicate for fatty acid analysis and the remaining sample was dried under nitrogen until achieving a constant weight. This weight allowed for the calculation of total fat in the 2 g meal sample, which was then used to determine total fat per meal.

The 0.2 mL aliquots were dried under nitrogen prior to saponification (Bannon et al., 1982) and transesterification (Morrison & Smith, 1964). Saponification was carried out by adding 1.5 mL 0.5 N methanolic KOH and heating in a 110°C sandbath for 1 hour. After cooling, 1.5 mL boron trifluoride/methanol 14% (w/w) was added, followed by layering 2 mL hexane on top and heating in a 110°C sandbath for 1 hour. After cooling, 1 mL water was added, samples were vortexed and allowed to separate into two layers overnight. The top layer of hexane was pipeted into a microvial and dried under nitrogen. Fatty acid methyl esters were analyzed by an automated Gas-Liquid Chromatograph (GLC), Varian model 6000 equipped with a Vista 654 Data System and a Vista 8000 autosampler (Varian Instruments, Georgetown, Ontario). The system used a bond-phase fused silica capillary column, BP20: 25 mm x 0.25 OD SCG product. Helium was used as the carrier gas at a flow rate of 1.5 mL/minute, using a splitless injector. The GLC oven temperature was programmed for a two stage increase from an initial temperature of 90°C to 170°C at 20°C/minute and held for 15 minutes followed by a second stage temperature increase to 220°C at 2°C/minute for a total anaylsis time of 40 minutes. Fatty acid methyl esters were identified by comparison of retention data with that of authentic standards (Supelco Canada and Sigma Chemical Co.) and quantitated by peak area comparison with internal standards (C17:0, Sigma Chemical Co.).

After obtaining total fat and fatty acid composition data for each meal in duplicate, values for the 3 meals within one day were added together to obtain per day totals. Therefore,

analyzed values in Table 2 are means of values for each day of the 3 day menu cycle in duplicate (n=6).

2 EXPERIMENT 2

2.1 SUBJECTS AND DIETS

Subjects were recruited through advertisements at the University of Alberta. Eight male volunteers gave informed written consent to participate in this study after being screened for chronic disease, smoking, medication, supplements, and family history of diabetes or coronary artery disease. The study protocol was approved by the Faculty of Agriculture, Forestry and Home Economics Human Ethics Review Committee, University of Alberta.

Subjects consumed three isoenergetic meals per day consisting of normal foods and designed to meet estimated energy requirements. Energy requirements for each subject were estimated using the Harris-Benedict equation for basal metabolic rate (Harris & Benedict, 1919) multiplied by activity coefficients between 1.7 and 2.0 (Table 4). Subjects were weighed daily to ensure stable body weight and adjustments in energy intake were made if sustained weight changes were observed.

The study consisted of four 21 day diet treatments, each consisting of a three day rotating menu. All meals were prepared in the Metabolic Research Kitchen and consumed at fixed times. Breakfast and lunch were consumed in a supervised dining room and supper was packaged to take out. Subjects were instructed not to consume any additional foods or supplements during the study period except energy and caffeine free beverages.

Each of the four diet treatments were formulated using Food Processor II nutrient analysis computer software (Esha Research, Salem, Oregon) and fatty acid content from published nutrient composition tables (US Department of Agriculture, Agriculture Handbook No. 8-4, 1979) to contain an average of 29 ± 2 % energy as fat, 56 ± 2 %

energy as carbohydrate, and 16 ± 1% energy as protein (Table 5). The diets were also balanced for ω-3 fatty acids, cholesterol and fiber content. The four diets were formulated to provide combinations of high (10-12% kcal) and low (3% kcal) levels of palmitic acid (C16:0) and linoleic acid (C18:2) as follows: low C16:0 and low C18:2, low C16:0 and high C18:2, high C16:0 and low C18:2, high C16:0 and high C18:2 (Table 5).

2.2 Experimental Design

Each diet treatment was consumed for 21 days followed by a washout period of at least 21 days. Subject consumed the four diet treatments in the same order. On day 21 of each treatment, a fasting blood sample (30 mL) was taken by venipuncture between 0715 and 0830 h. Blood was drawn into vacutainer tubes containing disodium EDTA (25 mL) and Serum Separator Tubes (SST) (5mL) . Subjects then consumed a priming dose of deuterium oxide (99.8 atom percent excess, ICN Biomedicals, Montreal, Canada) at 0.5g D_2O/kg estimated body water (60% of body weight). A maintenance dose of 1.0 g D_20/kg estimated body water was provided in a 2 L bottle of water to be consumed over the next 24 hours, in order to maintain plasma deuterium enrichment at plateau and compensate for unlabeled water obtained in the diet. Exactly 24 hours later (day 22), a second fasting blood sample (30 mL) was drawn into vacutainer tubes containing disodium EDTA (25mL) and SST tubes (5mL).

2.3 Analytical Methods

On day 21, serum was sent to the University of Alberta Hospitals for TG determination and creatinine determination as an index of hydration status. Serum creatinine was again determined on day 22 samples. TG was determined enzymatically (Bucolo & David, 1973), and serum creatinine levels were determined by the kinetic Jaffe reaction (Narayanan & Appleton, 1980; Moss et al., 1975). The remaining blood from each day was centrifuged at 3000 rpm for 15 minutes at 4°C (Jouan refrigerated centrifuge, CR

4.11) to obtain plasma. Day 21 plasma was used to determine background deuterium enrichment of plasma water and VLDL-TG. Day 22 plasma was used to measure the deuterium enrichment of plasma water and VLDL-TG 24 hours after deuterium oxide administration. Half of approximately 10 mL of plasma obtained each day was used for this study, the other half was used for determination of deuterium incorporation into cholesterol.

The remaining analytical methods, including lipoprotein separation, VLDL lipid extraction, thin layer chromatography, measurement of deuterium enrichment and fat analysis of the diets, are identical to those in experiment 1. Results of the diet analyses for experiment 2 are found in Table 5.

3 Calculations and Statistical Analyses

3.1 Calculation of relative amount of de novo fatty acid in VLDL-TG ($DNFA_r$)

Sample enrichment was measured as the relative difference in deuterium abundance from standard mean ocean water (SMOW). This difference is termed delta (δ) and expressed in parts per thousand ($‰$). Delta is also defined in the following equation,

$$\textit{equation 1} \qquad \delta\,^{2}\mathrm{H}\,(‰) = \left(\frac{\mathrm{R}_{sample}}{\mathrm{R}_{std}} - 1\right) \times 1000$$

where R is the isotope ratio of deuterium to hydrogen and the standard is SMOW. The maximum possible enrichment of VLDL-TG on Day 22 would be equal to the enrichment of plasma water after deuterium oxide administration, corrected for the maximum number of deuterium atoms which could enter the newly synthesized fatty acids. Jungas (1968) found that 0.87 g-atoms ^{3}H per g-atom C was incorporated into adipose fatty acid during synthesis. In the VLDL fraction, an average TG molecule has three monounsaturated fatty acids with 17 carbon atoms (Layne et al, 1996). Using the ratio of carbon to hydrogen for an average TG molecule and correcting for the presence of glycerol carbon

atoms (Leitch & Jones, 1991) the appropriate correction factor for plasma enrichment can be found as follows:

equation 2

$$\frac{0.87D}{C_{fa}} \times \frac{51C_{fa}}{54C_{glycerol+fa}} \times \frac{54C_{glycerol+fa}}{93H_{fa}} = 0.48\frac{D}{H_{fa}}$$

The ratio between the actual enrichment of VLDL-TG over 24 hours and the maximum enrichment possible, represents the relative amount of the VLDL-TG pool which is made up of *de novo* synthesized fatty acids ($DNFA_r$). Expressed in terms of percent, this relationship is described in the following equation:

equation 3

$$DNFA_r\,(\%) = \frac{\delta \text{ VLDL TG}_{\textit{test day}} - \delta \text{ VLDL TG}_{\textit{background day}}}{(\delta \text{ plasma}_{\textit{test day}} - \delta \text{ plasma}_{\textit{background day}}) \times 0.48} \times 100$$

3.2 ESTIMATION OF VLDL-TG POOL SIZE

To estimate the net amount of newly synthesized fatty acids in the VLDL-TG pool, an estimate of this pool size is needed. Layne et al. (1996) conducted a study involving 26 subjects and four diet treatments at two different polyunsaturated to saturated fatty acid ratios. Parameters measured included total fatty acids in the VLDL-TG, LDL-TG and HDL-TG fractions, as well as plasma TG level. An estimate of VLDL-TG fatty acid was made by subtracting the HDL-TG and LDL-TG total fatty acid from plasma TG values. This estimate correlates very well with the actual measured VLDL-TG total fatty acid ($r = 0.7$, $p < 0.00001$). The equation for this relationship is:

equation 4

$$y = 0.29x + 36.2$$

where y is the measured VLDL-TG fatty acid (mg/L) and x is estimated VLDL-TG fatty acid (mg/L). It was also found that total plasma TG correlates with the sum of LDL-TG and HDL-TG fatty acids ($r = 0.4$, $p < 0.00001$). The equation of this regression line is:

equation 5 $y = 0.07x + 79.8$

where y is HDL-TG + LDL-TG fatty acid (mg/L) and x is plasma TG level (mg/L).

3.3 Estimation of net de novo fatty acid in VLDL-TG ($DNFA_n$)

Using these two regression equations, a reliable estimate of the VLDL-TG pool size for subjects in experiments 1 and 2 can be easily made. Plasma TG level was used to estimate HDL-TG + LDL-TG fatty acid using equation 5. An estimate of VLDL-TG fatty acid was made by subtracting HDL-TG + LDL-TG fatty acid from the plasma TG and using the difference as the x value in equation 4. An estimate of plasma volume was also made for each subject at 37.5 mL/kg for females and 45 mL/kg for males (Dagher et al., 1965). For each subject, an estimate was made of the net amount of fatty acid in the VLDL-TG pool that arises from *de novo* synthesis ($DNFA_n$). The equation for this estimate is as follows *(equation 6)*:

$DNFA_n$ (mg) = ($DNFA_r$ (%)/100) x VLDL-TG fatty acid (mg/L) x plasma volume (L)

3.4 Statistical Analyses

All regression analyses were performed using Microsoft Excel (Microsoft Corporation). In experiment 1, the effect of diet on plasma TG level, $DNFA_r$ and $DNFA_n$ was assessed using a blocked one-way analysis of variance (SAS software, SAS Institute, Inc, Cary, NC). The effect of palmitic and linoleic acids on these parameters in experiment 2 was assessed by a blocked two-way analysis of variance. Significant differences between interaction least-squares means were determined by t-tests. Values where given in the text for comparison represent the least-squares means ± SEM. Using proc univariate in SAS, boxplots of various data sets were formulated to reveal potential outliers. Data points further away than 3 interquartile ranges were considered outliers. One-way analysis of

variance was used to determine effect of day on creatinine levels and effect of diet on total fat and fatty acid content of diets.

CHAPTER 3

RESULTS

1 EXPERIMENT 1

Demographics for the twelve subjects who participated in the first experiment are shown (Table 1). Subjects were 24.7 ± 4.6 years of age (mean ± SD), 171.9 ± 11.5 cm in height, 69.3 ± 12.2 kg in weight and consumed 2750 ± 576 kcal/day. Compliance with diet treatments was high as determined by monitoring of meals consumed in the supervised dining room and all subjects completed both diet treatments. Changes in weight did not exceed 1.3% of body weight during a diet treatment.

The analyzed total fat and fatty acid composition of diet treatments are shown (Table 2). Total fat content was close to formulated values and did not significantly differ between diet treatments. Linoleic acid content was lower than formulated for the low 16:0/high 18:2 diet. The level of C18:3 ω-3 was higher in the low 16:0/high 18:2 diet than in the high 16:0/low 18:2 diet, while the intake of longer chain ω-3 fatty acids was low in both diets.

Normal plasma TG values for this age group are considered to be 0.60 - 2.30 mmol/L. All values measured during this study were within or below normal, ranging from 0.39 - 1.73 mmol/L (Figure 1). Plasma TG level for subject 12 on the high 16:0/low 18:2 diet is missing. Diet did not significantly affect plasma TG level, although there was a trend ($p < 0.09$) toward higher TG levels after consuming the high 16:0/low 18:2 diet (1.05 ± 0.06 mmol/L) than after consuming the low 16:0/high 18:2 diet (0.89 ± 0.06 mmol/L; Table 3).

The change in plasma deuterium enrichment from background to test day was not significantly different between the diet treatments. The mean difference between duplicate

TABLE 1 SUBJECT DEMOGRAPHICS EXPERIMENT 1

Subject	Gender	Age *years*	Height *cm*	Mean Weight *kg*	Energy Intake *kcal/day*
1	M	28	180.0	84.6 ± 0.8	3000
2	M	31	179.0	77.0 ± 0.2	2900
3	F	22	154.0	50.0 ± 0.5	2100
4	M	27	180.0	83.8 ± 0.5	3000
5	F	35	165.1	63.6 ± 0.3	2200
6	F	24	181.6	64.3 ± 0.5	2500
7	M	21	177.8	73.3 ± 0.8	3400
8	M	22	191.8	82.4 ± 0.5	3700
9	F	21	167.6	52.9 ± 0.6	2300
10	F	21	162.6	66.4 ± 1.8	2300
11	M	22	167.6	77.3 ± 1.5	3500
12	F	22	154.9	56.6 ± 0.7	2100
mean ± SD		25 ± 5	171.9 ± 11.5	69.3 ± 12.2	2750 ± 580

Weight was determined daily and mean weight represents the mean of all diet treatment periods. Values are means ± SD. Energy requirements were determined using Mayo Clinic Nomogram and activity coefficients.

TABLE 2 COMPOSITION OF DIETS FOR EXPERIMENT 1

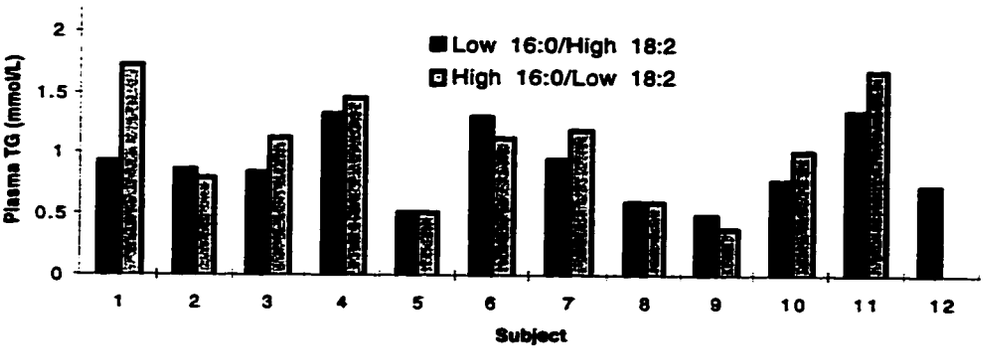
	Diet	
	Low 16:0/High 18:2	**High 16:0/Low 18:2**
Total Fat *(% kcal)*	32 ± 2	32 ± 2
C14:0 *(% kcal)*	0.2 ± 0.1	1.2 ± 0.1
C16:0 *(% kcal)*	2.1 ± 0.1	8.1 ± 1.0
C18:0 *(% kcal)*	1.1 ± 0.1	1.8 ± 0.2
C18:1 *(% kcal)*	14.4 ± 1.2	7.5 ± 1.7
C18:2 ω6 *(% kcal)*	6.0 ± 1.0	2.0 ± 0.5
C18:3 ω3 *(% kcal)*	1.7 ± 0.2	0.2 ± 0.1
C20:5 ω3 *(% kcal)*	0.03 ± 0.04	0.01 ± 0.01
C22:6 ω3 *(% kcal)*	0.04 ± 0.01	0.05 ± 0.05

Diets were formulated using nutrient composition tables (US Department of Agriculture) and canola oil data to contain to contain 31 ± 2% of energy from fat, 55 ± 2% of energy from CHO, and 15 ± 1% of energy from protein. The diets were designed to provide low (2% kcal) or high (8% kcal) levels of palmitic acid (C16:0) and linoleic acid (C18:2). Meals were analyzed in duplicate and values pooled to obtain per day values. Values are means ± SD of 3 day menu in duplicate, n = 6. Fatty acids were analyzed as methyl esters by gas liquid chromatography. Total fat was determined by the method of Folch et al., 1957.

analyses of deuterium enrichment for VLDL-TG on the test day was 19 ‰ ± 2 ‰ (mean ± SEM). $DNFA_r$ ranged from 0.2 - 6.6 % (Figure 2) and was not significantly different between the high 16:0/low 18:2 diet (2.2 % ± 0.4 %) and the low 16:0/high 18:2 diet (1.6 % ± 0.4 % ; Table 3). $DNFA_n$ ranged from 1.1 - 108.2 mg (Figure 3) and was not significantly different between the high 16:0/low 18:2 diet (21.5 ± 7.4 mg) and the low 16:0/high 18:2 diet (11.4 ± 6.8 mg; Table 3). A significant relationship was found between $DNFA_r$ and plasma TG level ($r = 0.5$, $p < 0.008$) as well as between $DNFA_n$ and plasma TG level ($r = 0.7$, $p < 0.0004$).

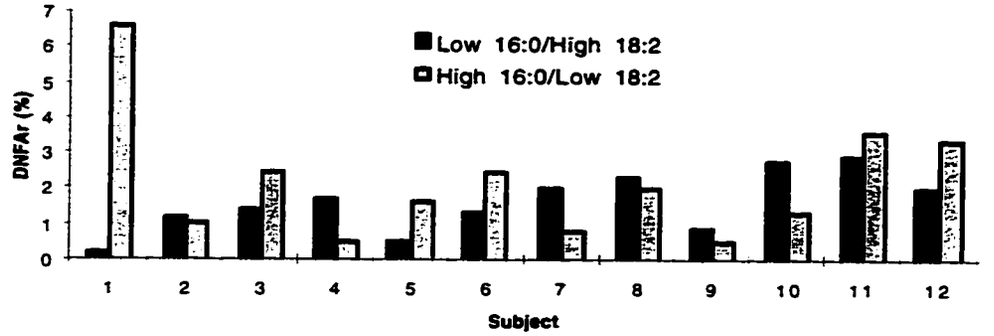
The data sets of all $DNFA_r$ and $DNFA_n$ values, and the changes in these parameters due to diet treatment were analyzed to reveal potential outliers. Subject 1 was an outlier in the data sets of change in $DNFA_r$, $DNFA_n$ after consuming the high 16:0/low 18:2 diet, and change in $DNFA_n$. Subject 11 was an outlier in the data set of $DNFA_n$ after consuming the high 16:0/low 18:2 diet. Removing the outliers did not alter the statistical effects of diet treatment on parameters given in Table 3. The relationship between $DNFA_r$ and plasma TG level became non-significant, however a significant relationship between $DNFA_n$ and plasma TG level ($r = 0.6$, $p < 0.009$) remained.

FIGURE 1 EFFECT OF DIET TREATMENT ON PLASMA TG LEVEL FOR EXPERIMENT 1



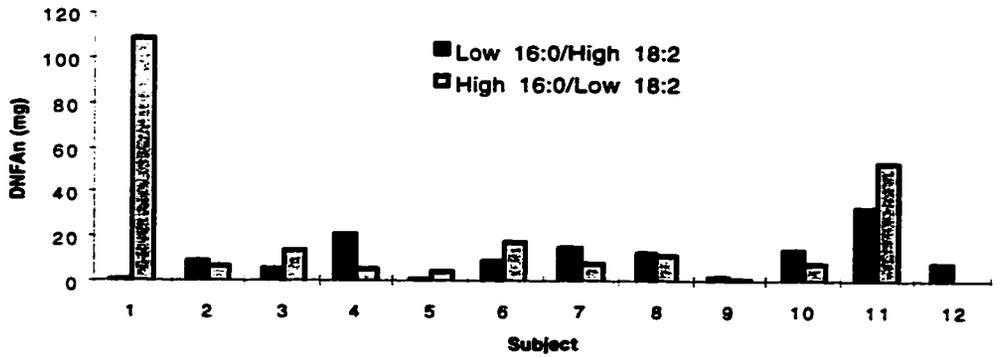
Blood was drawn on day 22 of each diet treatment and TG level was determined enzymatically.

FIGURE 2 EFFECT OF DIET TREATMENT ON $DNFA_r$ FOR EXPERIMENT 1



The ratio between the actual enrichment of VLDL-TG over 24 hours and the maximum enrichment possible, represents the relative amount of the VLDL-TG pool which is made up of *de novo* synthesized fatty acids ($DNFA_r$).

FIGURE 3 EFFECT OF DIET TREATMENT ON $DNFA_n$ FOR EXPERIMENT 1



An estimate was made of the net amount of fatty acid in the VLDL-TG pool that arises from *de novo* synthesis ($DNFA_n$) using the $DNFA_r$ value and an estimate of VLDL-TG pool size for each subject.

TABLE 3 EFFECT OF DIET TREATMENT ON PLASMA TG LEVELS, $DNFA_r$ AND $DNFA_n$ IN EXPERIMENT 1

Diet	Plasma TG (mmol/L)	$DNFA_r$ (%)	$DNFA_n$ (mg)
Low 16:0/High 18:2	0.89 ± 0.06	1.6 ± 0.4	11.4 ± 6.8
High 16:0/Low 18:2	1.05 ± 0.06	2.2 ± 0.4	21.5 ± 7.4

Values are lsmeans ± SEM (n= 12). Diet treatment effects were determined by blocked one-way analysis of variance. No significant difference was found between diet treatments for any parameters ($p>0.05$). Plasma TG level was determined enzymatically. The ratio between the actual enrichment of VLDL-TG over 24 hours and the maximum enrichment possible, represents the relative amount of the VLDL-TG pool which is made up of *de novo* synthesized fatty acids ($DNFA_r$). An estimate was made of the net amount of fatty acid in the VLDL-TG pool that arises from *de novo* synthesis ($DNFA_n$) using the $DNFA_r$ value and an estimate of VLDL-TG pool size for each subject.

2 *EXPERIMENT 2*

Demographics for the 8 subjects who participated in the second experiment are illustrated (Table 4). Subjects were 23.9 ± 4.0 years of age (mean ± SD), 182.1 ± 6.0 cm in height, 80.2 ± 16.8 kg in weight and consumed 3600 ± 302 kcal/day. Compliance with diet treatments was high as determined by monitoring of meals consumed in the supervised dining room. Four subjects completed all four diet treaments, 2 subjects completed 3 diet treatments and 2 subjects completed 2 diet treatments. Weight changes did not exceed 1% of body weight during a diet treamtent. Creatinine levels did not significantly change between background and test day.

The analyzed total fat and fatty acid composition of test diets are shown, along with formulated values (Table 5). Total fat content was close to formulated values and was not significantly different between diet treatments. Palmitic acid content was slightly lower than formulated in the high 16:0/low 18:2 diet. Linoleic acid content was slightly lower than formulated in the low 16:0/high 18:2 and high 16:0/high 18:2 diets. The level of 18:3 ω-3 did not differ across diet treatments and the intake of longer chain ω-3 fatty acids was low in all diet treatments.

Plasma TG levels were all within or below normal range, varying from 0.19 - 2.10 mmol/L (Figure 4). Palmitic acid had no effect on plasma TG level, while increasing linoleic acid decreased plasma TG level ($p < 0.05$). Performing t-tests on the interaction lsmeans revealed that the effect of linoleic acid occurs at low palmitic acid levels only (Table 6).

The change in plasma deuterium enrichment from background to test day was not significantly different between diets. The mean difference between duplicate analyses of deuterium enrichment for VLDL-TG on the test day was 14 ‰ ± 3 ‰ (mean ± SEM). $DNFA_r$ ranged from 0.5 - 13.7% (Figure 5) and was not significantly affected by palmitic

TABLE 4 SUBJECT DEMOGRAPHICS FOR EXPERIMENT 2

Subject	Gender	Age *years*	Height *cm*	Mean Weight *kg*	Energy Intake *kcal/day*
1	M	20	175.3	66.7 ± 1.4	3400
2	M	32	180.0	68.0 ± 1.4	3200
3	M	26	185.4	69.2 ± 0.8	3400
4	M	21	182.9	74.3 ± 0.5	3600
5	M	25	182.9	112.9 ± 1.3	3800
6	M	20	188.0	75.5 ± 0.5	3600
7	M	23	172.5	75.4 ± 2.1	3600
8	M	24	190.0	99.2 ± 1.3	4200
mean ± SD		24 ± 4	182.1 ± 6.0	80.2 ± 16.8	3600 ± 300

Weight was determined daily and mean weight represents the mean of all diet treatment periods. Values are means ± SD. Energy requirements were determined using the Harris-Benedict equation and activity coefficients.

TABLE 5 COMPOSITION OF DIETS FOR EXPERIMENT 2

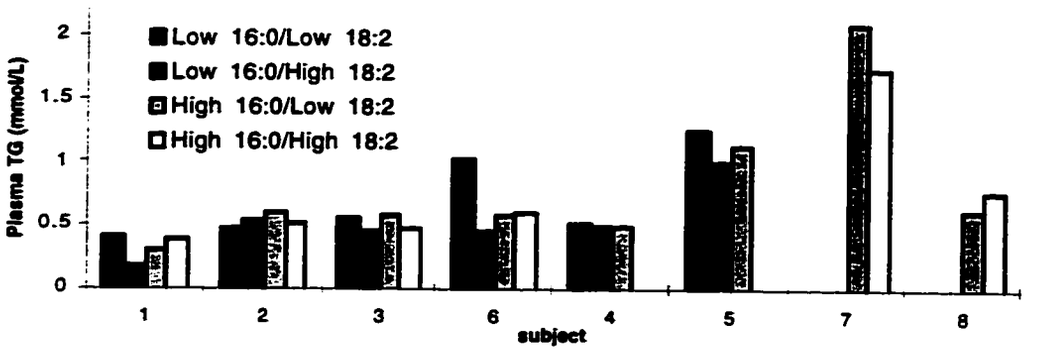
	Diet			
	Low 16:0 Low 18:2	Low 16:0 High 18:2	High 16:0 Low 18:2	High 16:0 High 18:2
Calories *(kcal)*	3070 ± 160	2990 ± 270	3030 ± 30	2990 ± 230
Protein *(% kcal)*	16 ± 3	17 ± 2	16 ± 1	16 ± 2
CHO *(% kcal)*	57 ± 5	57 ± 3	58 ± 1	54 ± 3
Total Fat *(% kcal)*	29 ± 2 (29.3 ± 2.1)	28 ± 1 (27.0 ± 1.8)	28 ± 1 (28.0 ± 3.4)	31 ± 1 (31.9 ± 2.1)
C14:0 *(% kcal)*	(0.07 ± 0.04)	(0.22 ± 0.2)	(0.82 ± 0.15)	(0.22 ± 0.01)
C16:0 *(% kcal)*	3.2 ± 0.2 (3.6 ± 0.2)	2.9 ± 0.2 (2.2 ± 0.2)	10.1 ± 0.4 (7.7 ± 0.6)	9.9 ± 0.2 (8.7 ± 0.4)
C18:0 *(% kcal)*	0.8 ± 0.1 (0.7 ± 0.2)	1.1 ± 0.1 (1.6 ± 0.1)	2.0 ± 0.1 (1.5 ± 0.1)	1.2 ± 0.1 (1.1 ± 0.1)
C18:1 *(% kcal)*	18.8 ± 0.9 (14.8 ± 1.4)	7.5 ± 0.1 (6.2 ± 1.1)	9.0 ± 0.2 (6.7 ± 0.8)	8.1 ± 0.3 (7.7 ± 0.7)
C18:2 ω6 *(% kcal)*	2.8 ± 0.6 (3.3 ± 0.2)	12.0 ± 1.4 (9.8 ± 0.9)	2.0 ± 0.2 (1.6 ± 0.2)	12.1 ± 0.6 (10.1 ± 0.5)
C18:3 ω3 *(% kcal)*	0.5 ± 0.1 (0.2 ± 0.0)	0.5 ± 0.1 (0.3 ± 0.1)	0.5 ± 0.1 (0.2 ± 0.1)	0.4 ± 0.0 (0.1 ± 0.0)
C20:5 ω3 *(% kcal)*	(0.05 ± 0.05)	(0.01 ± 0.01)	(0.01 ± 0.01)	(0.01 ± 0.01)
C22:6 ω3 *(% kcal)*	(0.06 ± 0.06)	(0.03 ± 0.02)	(0.01 ± 0.01)	(0.01 ± 0.02)

Diets were formulated using Food Processor II nutrient analysis software (Esha Research, Salem, Oregon). Values not contained in brackets are formulated values (means ± SD). Meals were analyzed in duplicate and values pooled to obtain per day values. Fatty acids were analyzed as methly esters by gas liquid chromatography. Total fat was determined by the method of Folch et al., 1957. Analyzed values in brackets are means ± SD of 3 day menu in duplicate, n = 6.

acid, linoleic acid or the interaction of palmitic and linoleic acids (Table 6). $DNFA_n$ ranged from 1.9 - 240.2 mg (Figure 6) and was not significantly affected by palmitic acid, linoleic acid or the interaction of palmitic and linoleic acids (Table 6). A significant relationship was found between $DNFA_r$ and plasma TG level ($r = 0.8$, $p < 0.00001$; Figure 7) as well as between $DNFA_n$ and plasma TG level ($r = 0.9$, $p < 0.00001$; Figure 8). When data from both experiments were combined, $DNFA_r$ ($r = 0.5$, $p < 0.0004$; Figure 9) and $DNFA_n$ values ($r = 0.7$, $p < 0.00001$; Figure 10) again correlated with plasma TG level.

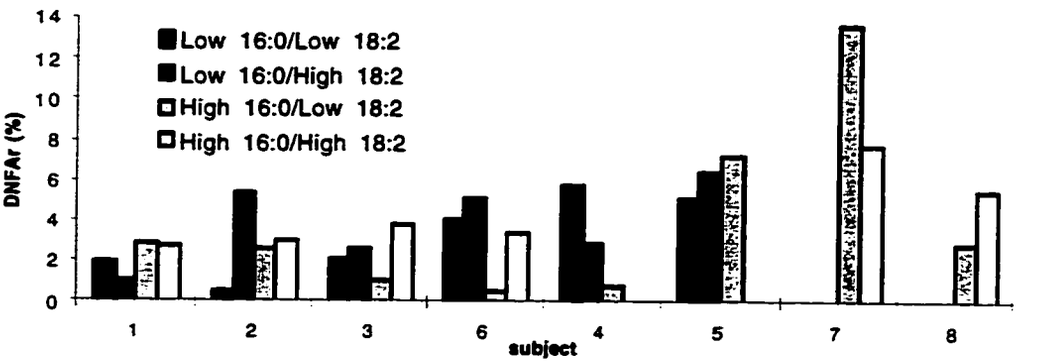
The data sets of all experiment 2 $DNFA_r$ and $DNFA_n$ values, and the changes in these parameters due to diet treatment were analyzed to reveal potential outliers. Subject 7 was an outlier in the data sets of $DNFA_n$, and change in $DNFA_n$. When this subject was removed from the statistical analyses of treatment effects, the effect of linoleic acid on plasma TG level became non-significant, while the interaction effect shown in Table 6 remained significant. A significant relationship remained between experiment 2 $DNFA_r$ and plasma TG level ($r = 0.7$, $p < 0.0002$; Figure 7) and between experiment 2 $DNFA_n$ and plasma TG level ($r = 0.9$, $p < 0.00001$; Figure 8). Using the data from both experiments with outliers removed, the relationship between $DNFA_r$ and plasma TG level became non-significant (Figure 9), while the relationship between $DNFA_n$ and plasma TG level remained significant ($r = 0.5$, $p < 0.0002$; Figure 10).

FIGURE 4 EFFECT OF DIET TREATMENT ON PLASMA TG LEVEL FOR EXPERIMENT 2



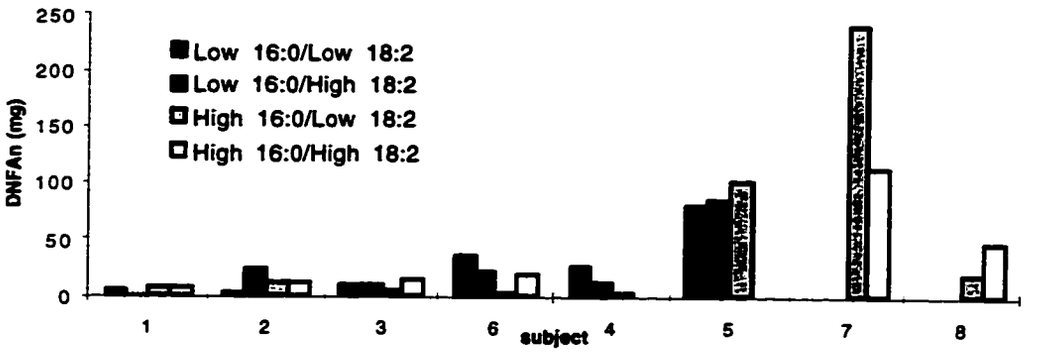
Blood was drawn on day 21 of each diet treatment and TG level was determined enzymatically.

FIGURE 5 EFFECT OF DIET TREATMENT ON $DNFA_r$ FOR EXPERIMENT 2



The ratio between the actual enrichment of VLDL-TG over 24 hours and the maximum enrichment possible, represents the relative amount of the VLDL-TG pool which is made up of *de novo* synthesized fatty acids ($DNFA_r$).

FIGURE 6 EFFECT OF DIET TREATMENT ON $DNFA_n$ FOR EXPERIMENT 2



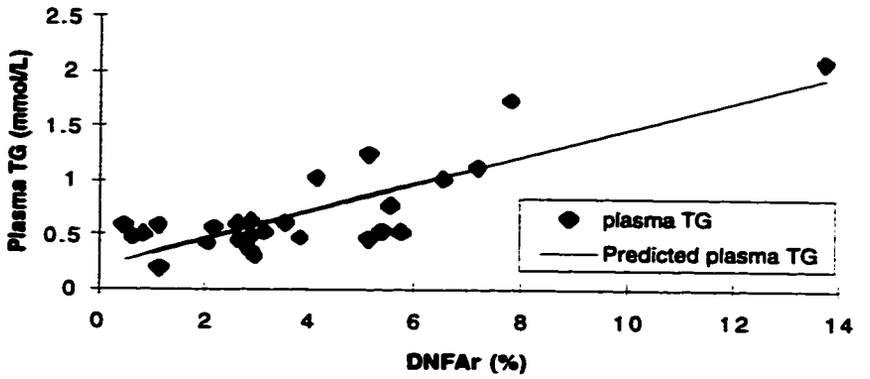
An estimate was made of the net amount of fatty acid in the VLDL-TG pool that arises from *de novo* synthesis ($DNFA_n$) using the $DNFA_r$ value and an estimate of VLDL-TG pool size for each subject.

TABLE 6 EFFECT OF DIET TREATMENT ON PLASMA TG LEVELS, $DNFA_r$ AND $DNFA_n$ IN EXPERIMENT 2

Diet	Plasma TG (mmol/L)	$DNFA_r$ (%)	$DNFA_n$ (mg)
Low 16:0/Low 18:2	0.89 ± 0.05^{b}	4.4 ± 0.9	48.0 ± 10.9
Low 16:0/High 18:2	0.70 ± 0.05^{a}	5.0 ± 0.9	46.4 ± 10.9
High 16:0/Low 18:2	0.80 ± 0.04^{ab}	4.0 ± 0.7	49.3 ± 8.8
High 16:0/High 18:2	0.76 ± 0.05^{ab}	4.5 ± 0.9	37.7 ± 10.7

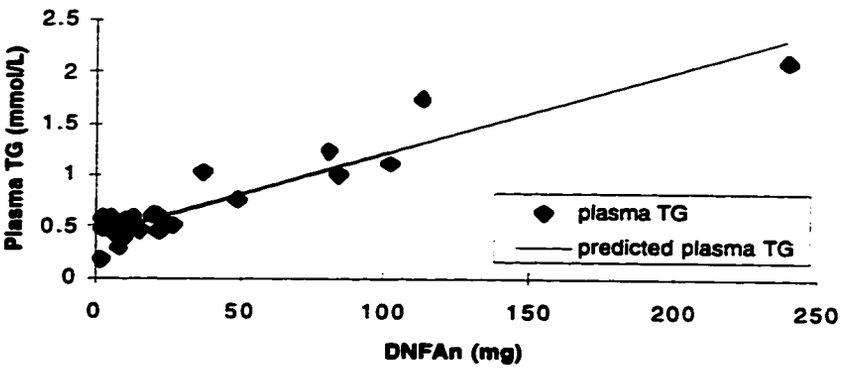
Values are lsmeans ± SEM (n= 6 for all diet treatments except high16:0/low 18:2 where n= 8). Diet treatment effects were determined by blocked two-way analysis of variance. Values in columns with different superscripts are significantly different ($p<0.05$). A significant effect of linoleic acid on plasma TG level was found ($p<0.05$), such that increasing linoleic acid decreased plasma TG level. When the subject found to be an outlier was removed from the statistical analyses, the effect of linoleic acid became non-significant, while the interaction effect shown above did not change. Plasma TG level was determined enzymatically. The ratio between the actual enrichment of VLDL-TG over 24 hours and the maximum enrichment possible, represents the relative amount of the VLDL-TG pool which is made up of *de novo* synthesized fatty acids ($DNFA_r$). An estimate was made of the net amount of fatty acid in the VLDL-TG pool that arises from *de novo* synthesis ($DNFA_n$) using the $DNFA_r$ value and an estimate of VLDL-TG pool size for each subject.

FIGURE 7 RELATIONSHIP BETWEEN $DNFA_r$ AND PLASMA TG LEVEL FOR EXPERIMENT 2



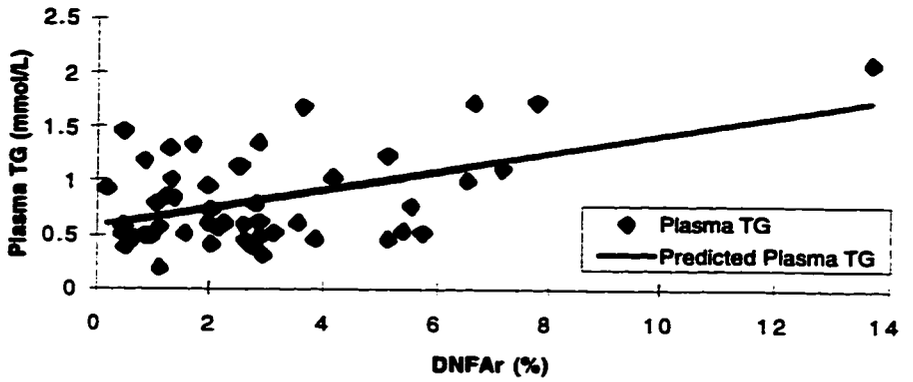
Linear Regression Analysis was performed on data from experiment 2. $DNFA_r$ and plasma TG level are correlated ($r = 0.8$, $p < 0.00001$). After removing the outlier, this relationship was still significant ($r = 0.7$, $p < 0.0002$).

FIGURE 8 RELATIONSHIP BETWEEN $DNFA_n$ AND PLASMA TG LEVEL FOR EXPERIMENT 2



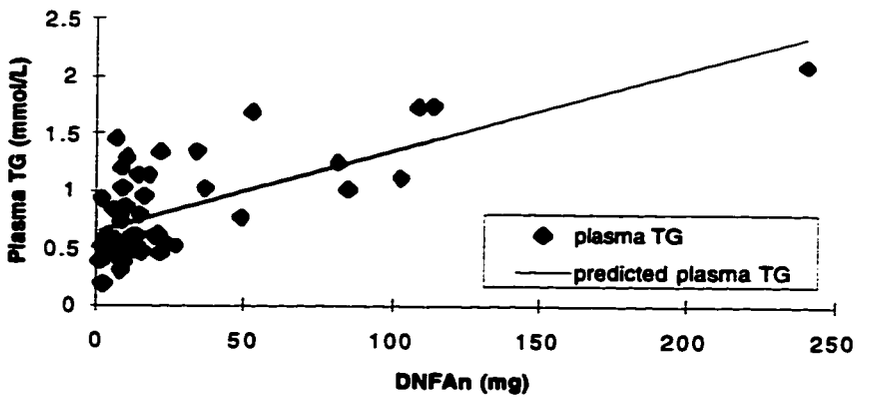
Linear Regression Analysis was performed on data from experiment 2. $DNFA_n$ and plasma TG level are correlated ($r = 0.9$, $p < 0.00001$). After removing the outlier, this relationship remained significant ($r = 0.9$, $p < 0.00001$).

FIGURE 9 RELATIONSHIP BETWEEN $DNFA_r$ AND PLASMA TG LEVEL USING DATA FROM BOTH EXPERIMENTS



Linear Regression Analysis was performed on data from both experiments. $DNFA_r$ and plasma TG level are correlated ($r = 0.5$, $p < 0.0004$). Removing the outliers resulted in a non-significant relationship.

FIGURE 10 RELATIONSHIP BETWEEN $DNFA_n$ AND PLASMA TG LEVEL USING DATA FROM BOTH EXPERIMENTS



Linear Regression Analysis was performed on data from both experiments. $DNFA_n$ and plasma TG level are correlated ($r = 0.7$, $p < 0.00001$). After removing the outliers, this relationship remained significant ($r = 0.5$, $p < 0.0002$).

CHAPTER 4

DISCUSSION

Previous studies suggest that when SFA, MUFA and PUFA replace CHO in the diet, plasma TG levels are reduced to about the same degree (Katan et al., 1994). Studies involving hypertriglyceridemic subjects found that linoleic acid reduced serum TG level compared to SFA (Grundy, 1975; Chait et al., 1974). In experiment 1 of this research a trend towards lower plasma TG level was found after consuming the diet lower in C16:0 and higher in C18:2. In experiment 2, increasing dietary C18:2 significantly decreased plasma TG level. This occured when C18:2 was increased at the expense of C18:1, and C16:0 level was held constant at < 4% of energy. When C16:0 was held constant at approximately 8-9% of energy and C18:1 at approximately 7-8% of energy, increasing C18:2 level had no effect on plasma TG level, nor were plasma TG levels higher than those found on the low C16:0/ high C18:2 diet.

Rate of hepatic VLDL-TG secretion and capacity to hydrolyze circulating TG are the two main factors influencing plasma TG level. Rate of *de novo* fatty acid synthesis influences rate of hepatic VLDL-TG production and was therefore studied as one mechanism by which dietary fatty acid composition may influence plasma TG level in humans. Deuterium incorporation was chosen to determine rate of hepatic lipogenesis, and this method has certain limitations. It is necessary to choose a ratio of deuterium to hydrogen incorporation in order to determine theoretical maximum TG enrichment. For this research, a value of 0.48 D/H was used, and this was calculated from a ratio of tritium to carbon incorporated into fatty acids synthesized in rat adipose tissue (Jungas, 1968). Fraction of deuterium incorporation could vary from that of tritium incorporation, and values obtained in rat adipose tissue may not be consistent with deuterium incorporation during human liver fatty acid synthesis. Estimations of net synthesis per day are presently limited by the need to estimate VLDL-TG production rates.

The $DNFA_r$ values obtained in experiments 1 and 2 ranged from 0.2 - 13.7 % and are comparable to those found by others (Leitch & Jones, 1991; Jones et al., 1992; Leitch & Jones, 1993). The hypothesis that a high dietary linoleic acid level suppresses lipogenic rate versus a low dietary linoleic acid level was not supported by data from either experiment. These results are in contrast to the results of research in animal models and in cells *in vitro* where linoleic acid has been shown to suppress hepatic lipogenesis through inhibition of lipogenic gene transcription (Clarke et al., 1990b). The second hypothesis, that a high dietary palmitic acid level does not suppress lipogenic rate versus a low dietary palmitic acid level was supported by the data from both experiments. These results are in agreement with research showing that SFA do not suppress lipogenesis compared to PUFA (Shillabeer et al., 1990). The hypothesis that a high dietary linoleic acid level suppresses lipogenic rate at both high and low levels of dietary palmitic acid was again not supported by this research.

We calculated the net amount of *de novo* fatty acid in the VLDL-TG pool at the time of blood sampling. $DNFA_n$ ranged from 1.1 - 240.2 mg, which is lower than the 2 g/day estimate made using VLDL-TG production rates (Leitch & Jones, 1993), as our values do not represent net per day values. Using our $DNFA_r$ values and the estimate of 300 mg/kg/day for VLDL-TG production used previously (Leitch & Jones, 1993), net synthesis values from this research range from 0.05 to 3 g/day.

While there were no significant effects of diet treatment on rate of lipogenesis as measured by deuterium incorporation, the effect of dietary fatty acid compostion in some individuals is worth noting. Figures 2 and 3 reveal a unique response to diet by subject 1 in experiment 1. Decreasing level of C18:2 and increasing level of C16:0 in the diet resulted in much higher $DNFA_r$ and $DNFA_n$ values than any other values in this experiment. Figures 5 and 6 reveal a similar response by subject 7 in experiment 2. $DNFA_r$ and $DNFA_n$ were both increased when C18:2 level in the diet was decreased and C16:0 level was maintained at a high level. The influence of decreasing C18:2 level at a

constant low level of C16:0 was not tested in this subject. $DNFA_r$ for subject 7 was 13.7% on the high 16:0/low 18:2 diet, a value which is similar to values found in E2-associated hyperlipidemic subjects by Jones et al. (1992). It is possible that certain populations are sensitive to the lipogenic suppressive effects of linoleic acid, while the majority of the healthy population studied in this research was not.

The remaining hypothesis to be tested was that changes in lipogenic rate are reflected in level of plasma TG. In the population studied in this research, diet did not influence rate of hepatic *de novo* fatty acid synthesis. However, a relationship between rate of hepatic *de novo* fatty acid synthesis and plasma TG level was found. Removing the outliers from experiment 1 data caused the relationship between $DNFA_r$ and plasma TG to become non-significant, but otherwise, the relationship was significant. Diet treatment influenced plasma TG level but not the rate of lipogenesis, and yet these parameters were correlated. This is probably due to the fact that differences in lipogenic rate due to subject were greater than differences due to diet. Higher rate of lipogenesis does contribute to higher plasma TG level, but other mechanisms are probably still involved. Lipogenic enzyme activity and plasma TG level were found to be directly correlated in rats (Geelen et al., 1995). Consumption of a medium chain TG oil diet, raised both plasma TG levels and the activities of hepatic lipogenic enzymes compared to a corn oil diet rich in linoleic acid. Consumption of a fish oil diet compared to a corn oil diet decreased both plasma TG levels and the activities of hepatic lipogenic enzymes in rats. The effects of medium chain TG and fish oils in the diet on rate of lipogenesis remain to be studied in humans.

This research involved normal healthy people consuming normal foods. Current recommendations suggest that the diet should include no more than 30% of energy as fat and no more than 10% of energy as saturated fat. The diet treatments in this research met these recommendations, altering the proportions of various fatty acids within these guidelines. Level of palmitic acid ranged from 2% to 9% of energy, while level of linoleic acid ranged from 2% to 10% of energy. Under these conditions, fatty acid composition

of the diet did not greatly affect plasma TG level and did not influence lipogenic rate. However, it appears that 2 subjects were more sensitive to the level of linoleic acid in the diet. The fact that some individuals may respond differently to fatty acid composition of the diet is an important concept. Deuterium incorporation methodology will be useful for future research in this area. The influence of dietary linoleic acid on plasma TG level and lipogenic rate needs to be studied in other metabolic states such as hyperlipidemia and obesity. The influence of fatty acids found in fish oils and medium chain fatty acids, both shown to have a greater influence on plasma TG and lipogenic rate in animal models than the fatty acids studied here, also needs to be studied in humans.

In conclusion, neither level of dietary linoleic acid nor palmitic acid influenced rate of lipogenesis in normal subjects consuming normal foods. However, it is possible that some individuals may be more responsive to changes in the dietary fatty acid composition. Higher rate of lipogenesis may contribute to higher plasma TG levels.

CHAPTER 5

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