EFFECTS OF LONG-TERM DIETARY ZINC MANIPULATION ON DIABETIC INDICES AND FATTY ACID COMPOSITION OF ADIPOSE TISSUE PHOSPHOLIPID AND TRIGLYCERIDE OF DIET-INDUCED OBESE C57BL/6J MICE

By

Diana L. Tallman

A thesis submitted to the Department of Foods and Nutrition
In partial fulfillment of the requirements for the degree of
Master of Science

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BY

Diana L. Tallman

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

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ABSTRACT

Effects of Long-Term Dietary Zinc Manipulation on Diabetic Indices and Fatty Acid Composition of Adipose Tissue Phospholipid and Triglyceride of Diet-Induced Obese C57BL/6J Mice

D.L. Tallman, MSc. Thesis, Department of Foods and Nutrition

Obesity is powerful predictor of insulin resistance and a major risk factor for Diabetes Mellitus Type 2 (DM2) in susceptible humans and rodent models. Dietary zinc deficiency has several characteristics common to DM2, but no information exists identifying the impact of altered dietary zinc status on the development of specific traits in diet-induced obesity and diabetes. Therefore, the objective of this experiment was to investigate the effects of zinc deficiency and supplementation on diabetic indicators and adipose tissue fatty acid composition using a diet–induced model of obesity and DM2 in C57BL/6J mice. Weanling C57BL/6J mice were randomized to diets containing 16% calories as soybean oil (SO) or 55% calories as mixed fat (MF) for 16 weeks. The SO and MF groups were further randomized to either a marginally zinc deficient (ZD, 3 ppm Zn), zinc control (ZC, 30 ppm Zn), or zinc supplemented (ZS, 150 ppm Zn) diet. Mice receiving the MF diet had greater fat pad weights, higher caloric intake and lower adipose zinc concentrations than the SO-fed mice. When the mice were dichotomized into low weight (LWT, < 39.5 g) and high weight (HWT, ≥ 39.6 g) groups, the HWT mice had greater body mass index (BMI), fat pad weights, caloric intakes, serum glucose and serum leptin, and lower adipose zinc and pancreatic zinc concentrations, compared to LWT mice. MF-fed mice stored a

lower percentage of total PUFA, n-6 and n-3 fatty acids in adipose triglyceride than SO-fed mice, a fatty acid profile that generally reflected the diet. The ZD and ZS groups had a reduced content of fatty acids in adipose triglyceride compared to the ZC group, suggesting that zinc status may influence fatty acid accumulation in adipose tissue. MF-fed mice had a higher percentage of C20:4 n-6 and ratio of n-6/n-3, and a lower ratio of PUFA/SAT and percentage of total n-3 fatty acids in adipose phospholipid compared to SO-fed mice. Adipose phospholipid of HWT mice had a reduced percentage of total n-3 fatty acids with a higher ratio of n-6/n-3 compared to LWT mice. Previous studies have associated these differences in phospholipid fatty acid composition of MF-fed and HWT mice with obesity-induced risks for insulin resistance and impaired glucose transport. In summary, the study was unable to induce obesity and diabetes in C57BL/6J mice by alteration of the dietary fatty acid composition and fat content, but it did identify obesity-prone mice fed the SO and MF diets. Dietary fat, but not dietary zinc, altered the fatty acid composition of adipose tissue phospholipid and triglyceride.

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LIST OF ABREVIATIONS

Arachidonic acid	AA
Body Mass Index	BMI
Diabetes Mellitus Type 2	DM2
Diet-Induced Obesity	DIO
Dihomo-δ-linolenic Acid	DGLA
Docosahexaenoic Acid	DHA
Docosapentaenoic Acid	DPA
Eicosapentaenoic Acid	EPA
High Body Weight	HWT
Linoleic Acid	LA
Linolenic Acid	LNA
Long Chain Polyunsaturated Fatty Acids	LC PUFA
Low Body Weight	LWT
Mixed Fat	MF
Monounsaturated Fatty Acids	MUFA
Phospholipid	PL
Polyunsaturated Fatty Acids	PUFA
Saturated Fatty Acids	SAT
Soybean Oil	SO
Zinc Deficient	ZD
Zinc Control	ZC
Zinc Supplemented	ZS

I. Literature Review

Introduction

Hormones such as insulin and leptin appear to interact in a central-peripheral regulatory loop that may be critical to homeostatic control in zinc and adipose tissue metabolism. Dysregulation of these interactions may contribute to metabolic disturbances found in obesity and diabetes. Despite current knowledge, no information exists identifying the impact of altered dietary zinc status on the development of specific traits in diet-induced obesity and diabetes. Therefore, the purpose of this literature review is to explore potential relationships among obesity, diabetes and zinc metabolism with a focus on hormones and fatty acid composition.

Obesity, Insulin Resistance and Diabetes

Obesity is a major risk factor for DM2. Within the past 15 years, the prevalence of obesity-induced DM2 has taken a giant leap in developed countries worldwide and includes up to 90% of diabetics in the Western world (Home 1997). Hypertension, coronary artery disease, and stroke are complications arising from obesity and DM2 that place heavy cost burdens on healthcare systems and reduce the quality of life for millions of people (Frankish et al. 1994; Ferrani 1997; Home 1997). The World Health Organization (WHO) defines DM2 on the basis of a raised plasma glucose concentration (fasting glucose >7.0 mmol/L) that can be controlled without exogenous insulin, although insulin may be required in some individuals for

adequate glycemic control (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, Gavin et al. 1997). DM2 is believed to be the result of the combined effects of environmental factors (physical activity and diet) and a genetic predisposition for the disease (Figure 1). Studies showing a high concordance rate among monozygotic twins as compared to dizygotic twins provide support for genetic predetermination playing a role in DM2 (Bogardus and Lillioja, 1992).

DM2 is characterized by insulin resistance, impaired glucose tolerance, pancreatic β-cell dysfunction, and increased hepatic glucose output leading to hyperglycemia (Figure 1) (Bogardus and Lilloja 1992). Insulin resistance renders insulin sensitive cells such as muscle and adipose tissues less effective in glucose uptake, glycogen biosynthesis, and glucose oxidation (McGarry 1992). Glucose tolerance tests can be used to determine glucose disposal within insulin sensitive tissues in response to oral or I.V. administration of glucose (Ferrannini 1997). During the development of impaired glucose tolerance, rising glucose concentrations are indicative of the first signs of insulin resistance and are known to correlate with insulin secreted from pancreatic β-cells (Ferrannini 1997). To fully compensate for impaired insulin action, β-cells enlarge and secrete more insulin until a plateau is reached and the β-cells cannot respond further (Ferrannini 1997). As well, plasma free fatty acids are elevated in most obese subjects and have been found to inhibit insulin-stimulated peripheral glucose uptake and to induce insulin secretion (Boden 1997). In obese subjects, normal responses to insulin action are blunted. As a result, hormone-sensitive lipase continues to mobilize fat from adipose tissue stores while clearance of triglycerides from the bloodstream by

lipoprotein lipase is reduced. Chronically elevated plasma free fatty acids in combination with hyperglycemia may produce peripheral and hepatic insulin resistance, which contribute to increased hepatic glucose production, frank hyperglycemia, and poor glycemic control (Boden 1997). With increasing adiposity, lipids may accumulate in pancreatic islets and become toxic to the β-cells, ultimately resulting in β-cell destruction and reduced insulin secretion (Unger 1995).

Leptin and Adiposity

Leptin is a hormone comprised of a 16Kda protein that is produced by the obesity (ob) gene found specifically in adipocytes (Zhang et al. 1994). The leptin protein is secreted in amounts that have been shown to correlate with measures of body fat (Klein et al. 1996) and body mass index (BMI), and to have the tightest correlation with percentage body fat (Considine et al. 1996). Obese humans are reported to have elevated leptin expression in adipose tissue (Considine et al. 1995; Lonnqvist et al. 1995) and serum concentrations (Considine et al. 1995; Maffei et al. 1995). Subcutaneous fat depots are deemed to be an important determinant of serum leptin concentrations, where the majority of fat cells reside and leptin mRNA is expressed to a greater degree than abdominal fat (Montague et al. 1997; Lefebvre et al. 1998). However, in DM2, there is a strong correlation with android (intra-abdominal) obesity and as opposed to gynecoid (peripheral or subcutaneous) obesity (Arner 1997). In DM2 subjects, serum leptin concentrations are dependent on body fat mass, not body fat distribution, and are not influenced by diabetes per se (Kennedy et al. 1997; Tasaka at al. 1997; Bertin et al. 1997). Sinha et al. (1996)

found DM2 subjects had higher leptin concentrations than lean subjects, but lower leptin concentrations than equally obese subjects without DM2. Fasting and loss of fat tissue result in decreased circulating leptin and ob gene expression (Maffei et al. 1995; Trayburn et al. 1995). Because leptin is known to suppress appetite and decrease adipogenesis (Pellymounter et al. 1995; Halaas et al. 1995; Campfield et al. 1995), evidence of elevated leptin concentrations in many obese subjects (Haffner et al. 1996), implies an impaired response or resistance to leptin, possibly by reduced transport into the brain through a saturable process (Banks et al. 1996; Kolacznski et al. 1996). Leptin expression and serum concentrations are known to be higher in female mice and humans than in males at equal levels of adiposity, suggesting gender variation in leptin production (Frederich et al. 1995; Lonnqvist et al. 1995). Obesity incurred by overfeeding rats until they were 130% overweight led to augmented leptin mRNA levels (Harris et al. 1996). In addition, a meal-feeding pattern produced by controlled tube feeding in rats over 2 days without an increase in calories or weight resulted in elevated leptin mRNA levels (Harris et al. 1996). The authors concluded that leptin expression appears to be controlled by level of adiposity as well as immediate changes in nutritional status.

Serum Leptin and Serum Insulin Interactions

Like leptin, insulin concentrations vary in proportion to fat mass (Bagdade et al. 1967). Mohamed and associates (1997) found that plasma leptin concentrations correlate with plasma insulin concentrations but not with insulin

resistance or impaired glucose disposal in subjects with DM2 (Mohamed-Ali et al. 1997), suggesting that insulin may modulate leptin secretion. Carrontoni et al. (1998) replicated these findings in females having a normal glucose tolerance and added that elevated plasma leptin concentrations do not play an important role in insulin-mediated glucose disposal or insulin secretion. Furthermore, Haffner and associates (1998) reported that serum leptin concentrations were positively correlated with fasting insulin and proinsulin concentrations, and inversely correlated with the proinsulin/insulin ratio. These observations suggest that elevated leptin levels are not associated with impairment in insulin secretion.

Carantoni and colleagues (1998) found elevated insulin concentrations to correlate with the degree of insulin resistance. Chronic hyperinsulinemia in insulin-resistant individuals may stimulate leptin production and secretion, leading to elevated leptin concentrations (Carontoni et al. 1998). This view is supported by evidence provided in several studies. It has been shown that exogenous insulin administration increases plasma leptin concentrations in normal and DM2 subjects (Malmstrom et al. 1996), consistent with previous findings in animal studies (Saladin et al. 1995). In addition, other studies in humans have demonstrated that insulin administration does not stimulate leptin secretion acutely but does elevate plasma leptin concentrations during chronic administration lasting several hours (Malmstrom et al. 1995; Kolacznski et al. 1996). However, a recent study has shown that physiological concentrations of insulin regulate plasma leptin acutely and dose dependently, within 30-60 minutes (Saad et al. 1998), corroborating the earlier results in rodents (Saladin et al. 1995). Thus, several in vivo studies suggest a

positive correlation between plasma insulin and plasma leptin concentrations, and that elevated plasma insulin may stimulate leptin production and secretion, but not vice versa (i.e., elevated plasma leptin concentrations do not stimulate insulin secretion).

Insulin and leptin appear to be involved in regulatory feedback loops involving adipose tissue, pancreas, and other organs. Insulin stimulates leptin production and secretion by adipocytes (Wabitsch et al. 1996; Barr et al. 1997) via a mechanism that requires glucose uptake and metabolism (Mueller et al. 1998). Indices of decreased glucose availability and increased lipolysis have been correlated with decreases in plasma leptin during negative energy balance in humans (Dubuc et al. 1998). These studies suggest that leptin may be regulated by insulin-stimulated substrate availability. Furthermore, there appears to be adaptive tissue-specific differences. Injecting mice with leptin before an oral glucose tolerance test impairs whole-body glucose uptake presumably by inhibition of insulin action (Harris 1998). However, leptin infusion for 7 days results in normal oral glucose tolerance, despite inhibition of insulin release, and enhanced insulin-stimulated glucose utilization in muscle but diminished insulin responsiveness in adipose tissue (Harris 1998). This would have the effect of diverting nutrients from adipose to muscle and liver tissue, and may explain some findings of in vitro studies.

Muller et al. (1997) found that leptin treatment in vitro impaired the metabolic actions of insulin in isolated rat adipocytes by impeding glucose transport, glycogenesis, and lipogenesis and activating lipolysis. Cohen and associates

(1996) reported that leptin modulates insulin action on liver cells by attenuating insulin-induced signaling. Others have not found an effect of insulin action in adipose or muscle cells (Berti et al. 1997; Ranganathan et al. 1998). However, the study by Berti and colleagues (1997) does provide evidence for positive cross-talk between insulin receptor and leptin receptor transduction pathways via action of phosphatidyl inosital 3-kinase in muscle cells (Berti et al. 1997). They found that leptin by itself was able to mimic approximately 80-90% of the insulin effect on glucose uptake and glycogen synthesis in C_2 C_{12} myotubues, a cell type with predominantly GLUT1 (Berti et al. 1997). Thus, leptin could be a modulator of basal glucose uptake in skeletal muscle (Berti et al. 1997).

Another peripheral effect of leptin is inhibition of insulin and proinsulin mRNA expression by pancreatic islets (Emilsson et al. 1997; Seufert et al. 1999) mediated in part by persistantly depolarizing β-cells (Keiffer et al. 1997). Thus, it has been proposed that insulin and leptin interact along an adipoinsular axis (Seufert et al. 1999) whereby insulin stimulates adipogenesis and leptin production, and leptin inhibits pancreatic insulin secretion. If pancreatic β-cells become desensitized to leptin, then dysregulation of the adipoinsular axis would lead to further hyperinsulinemia, adipogenesis and hyperleptinemia (Seufert et al. 1999).

Leptin & Insulin Interactions with Central & Peripheral Networks

Leptin appears to be an important feedback hormone acting between central networks and the periphery by gauging adiposity for the central nervous system (Figure 2) (Halaas et al. 1995). Leptin receptors are densely located in the

hypothalamus that regulates eating behaviour and the hippocampus that is associated with emotions (Tartaglia et al. 1995; Huang et al. 1996). Insulin receptors are also found throughout the brain and heavily concentrated in the hypothalamus (Baskin et al. 1988). Both leptin and insulin enter the brain by a saturable system that crosses the blood brain barrier but their entry is independent of the other (Banks et al. 1996; Baura et al. 1993). Peripheral receptors can be found for both insulin and leptin in many body tissues (Tartaglia et al. 1995; Emilsson et al. 1997). Continuous infusion of insulin to normal rodents causes increased adiposity and weight gain (Cusin et al. 1990), however, prolonged insulin administration into the brain will reduce appetite and weight in a dose-dependent fashion (Woods et al. 1979).

Leptin responses have been studied in various mouse models of obesity and diabetes. *Ob/ob* mice do not produce functional leptin (Zhang et al. 1994), but do possess upregulated leptin receptors (Huang et al. 1997) that are sensitive to the hormone. Peripheral and central leptin treatment in *ob/ob* mice results in increased thermogenesis, dramatic reductions of food intake and body weight with normalization of plasma insulin concentrations and glucose tolerance (Pelleymounter et al. 1995; Halaas et al. 1995; Campfield et al. 1995). Leptin treatment in *ob/ob* mice causes a reduction in hyperinsulinemia that does not correspond to variations in food intake or body weight (Pelleymounter et al. 1995; Stephens et al. 1995), indicating that other factors may be involved. *Db/db* mice have a mutation in the gene for the leptin receptor (Lee et al. 1996). Thus, *db/db* mice do not have operational leptin receptors (Lee et al. 1996) but overexpress the

ob gene and display high concentrations of plasma leptin (Murakami and Shima 1995). As a result of a lack of functional leptin receptors, *db/db* mice show resistance to leptin administration (Pelleymounter et al. 1995; Halaas et al. 1995; Campfield et al. 1995). Specific strains of mice demonstrate an increased propensity to develop diet-induced obesity (DIO) when ingesting an energy dense diet. DIO mice develop insulin resistance in conjunction with elevated serum leptin concentrations that are correlated with increased fat mass (Surwit et al. 1997). In addition, DIO mice manifest peripheral resistance to subcutaneous injections of leptin but remain sensitive to central administration of leptin (Van Heek et al. 1997). These findings raise questions as to whether hormonal dysfunction involving leptin may play an etiological role in DM2.

Rodent Models of Obesity

Several animal models of obesity exist that enable researchers to develop a broader understanding and more comprehensive framework within which to investigate successful strategies for prevention and treatment of obesity and diabetes. The ideal model would develop diabetogenic traits by administering a high fat diet over a prolonged period of time to mimic the development of obesity-induced diabetes in humans. As well, the ideal model would become obese, hyperinsulinemic, hyperglycemic and provide enough tissue to determine required measurements. This section explores examples of rodent models currently available.

The Zucker fatty (*fa/fa*) rat becomes obese, insulin resistant, and

hyperglycemic but remains euglycemic and, therefore, non-diabetic (White and Dean 1998). The *fa* mutation has been determined to be a mutation in the extracellular domain of the leptin receptor exhibiting functional defects that result in reduced signal transduction (Iido et al. 1996; Yamashita et al. 1997; da-Silva et al. 1998). The diabetic Zucker rat (ZDF/Gmi-fa/fa), derived from inbreeding for a diabetic trait, becomes suitably hyperglycemic, and this strain is obviously an established genetic model of DM2 (Zhou and Dolan, 1997).

Three other rat models are the Sprague-Dawley, Koletsky and Wistar rats. On a refined high energy diet, approximately 50% male Sprague-Dawley rats develop diet-induced obesity (DIO), insulin resistance, and hyperinsulinemia without developing hyperglycemia (Levin and Routh, 1996). The Koletsky (cp/cp corpulent) rat was originally derived by crossbreeding a Wistar-derived hypertensive female with a normotensive male Sprague-Dawley rat (Wu-Peng et al. 1997; Russell et al. 1997). The Koletsky rat has a similar mutation to the obese Zucker rat that is located in the leptin receptor and, as a result, becomes grossly obese (Wu-Peng et al. 1997). Although the obesity is associated with hyperinsulinemia, the Koletsky rat genetic mutation may not mimic DM2 in humans. Finally, the last rat model identified is the Wistar rat that does not develop diabetic signs other than insulin resistance. Based on these observations, no ideal rat model exists that represents human obesity and diabetes. Although mice produce less tissue samples, a number of mouse models exist that may more closely resemble human DM2.

Two well-studied models are the *ob/ob* and *db/db* mice that manifest identical phenotypes including early diabetes, severe obesity, and insulin resistance (Wu-

Peng et al. 1997). Recent discoveries have identified these mice as having genetic mutations in the leptin gene and leptin receptor, respectively. Other models include the AKR and A/J strains of mice that become obese but not diabetic when fed a diet high in fat (West et al. 1992; Rebuffe-Scrive et al. 1993; Van Heek et al. 1997; Surwit et al. 1997).

The majority of human obesity cannot be attributed to defects in leptin or its receptor (Van Heek et al. 1997). Unlike obesity caused by a single-gene inheritance, human obesity appears to be due to a polygenetic inheritance that is exacerbated by environmental factors such as diet, similar to the DIO and diet-resistant phenotypes in rodents (Levin and Routh 1996). C57BL/J mice develop both obesity and diabetes within 8 to 12 weeks when exposed to a diet high in fat (58% of total calories) and appear to be excellent examples of interactions of genotype with the environment (Surwit et al. 1997; Levin and Routh 1996).

Diet-Induced Changes in Adipose Fatty Acid Composition

Obesity or excess body fat is a powerful predictor of insulin resistance and a major risk factor for DM2 and cardiovascular disease (Pan and Storlien 1993) in susceptible humans and animal models. Obesity is associated with hypertrophy (enlarged cells) and/or hyperplasia (increased number of cells) of adipose tissue. Adipose cells are primarily composed of triglycerides and water (Lehninger et al. 1993). Phospholipids comprise the structural component of adipose membrane and form a lipid bilayer that renders the lipid and protein molecules associated with the bilayer in a fluid state. Changes in membrane fluidity can culminate in restricted

movement of lipids and proteins and altered cell communication leading to insulin resistance and impaired glucose transport (Clandinin et al. 1985). Fat is a crucial constituent of the North American diet. Changing the type of fat consumed alters the lipid environment by modifying the cell fatty acid composition and influencing the fatty acids available to the body and the function of proteins associated with the membrane (Field et al. 1985).

To elaborate on this concept, when C57BL/6J male mice were fed a highly saturated diet composed of 58% of calories from coconut oil, the mice became obese, hyperglycemic, and hyperinsulinemic after 14 weeks of dietary intervention compared to A/J mice fed the same diet (Surwit et al. 1997). The glucose-to-insulin ratio was used to determine the presence of insulin resistance in the C57BL/6J strain (Surwit et al. 1997). These observations highlight the influence of environment (diet) on a susceptible genetic background resulting in abnormal homeostatic mechanisms.

Zsigmond and associates (1990) demonstrated that sunflower-oil versus lard feeding for about 21 days in 7–8 week old male Wistar rats significantly elevated the polyunsaturated fatty acid composition of membrane phospholipids from epididymal fat pads. Accompanying these alterations in membrane lipid composition, the sunflower-oil diet was significantly associated with greater binding of the high-density-lipoprotein to adipocyte membranes as compared to the lard diet. KhuuThi-Dinh and colleagues (1990) demonstrated that the type and amount of dietary fat significantly altered both the plasma membrane phospholipid content and size of epididymal adipocytes in male weanling Wistar rats. The sunflower oil

diet (approximately 20% of total calories) administered over an 8 week period resulted in a lower total phospholipid content and more polyunsaturated fatty acids in the phosphatidylcholine class of phospholipids (Khuu Thi-Dinh et al. 1990) as compared to a mixed fat diet (approximately 20% of calories from cocoa butter and <5% of calories from sunflower oil). The control diet containing <5% of calories from sunflower oil produced a total phospholipid content and polyunsaturated fatty acid content in the phosphatidylcholine component of the adipose tissue equal to that of the mixed fat diet. In addition, a greater rate of 5'-nucleotidase enzyme activity in the adipose plasma membrane was seen in rats fed the control diet as compared to rats fed the other diets. Observations from these studies show that the nature and amount of dietary fat induces changes in the adipose membrane composition and in protein function related to the membrane component.

A study by Field et al. (1988) found that weanling Sprague-Dawley rats fed a diet containing a polyunsaturated/saturated fatty acid ratio of 1.0 (high P/S) for 28 - 42 days had a higher content of polyunsaturated fatty acids in adipose membrane phospholipids that paralleled greater insulin binding than rats fed a diet containing a polyunsaturated/saturated fatty acid ratio of 0.25 (low P/S). Streptozotocin-induced diabetic rats demonstrated a reduction in the polyunsaturated fatty acid content of membrane phospholipids (Field et al. 1988) when fed either diet. At high insulin concentrations, in vitro, adipocytes from diabetic rats bound less insulin than adipocytes from non-diabetic rats fed the high P/S diet, but bound equal amounts of insulin as adipocytes from non-diabetic animals fed the low P/S diet (Field et al. 1988). These observations suggest that the type of dietary fat and the diabetic

state alters fatty acid composition of the adipocyte membrane phospholipid that may impact on insulin binding to its receptor in the adipose membrane.

Dietary Fat and Body Fat Distribution

Amount and type of dietary fat provided in the diet can influence both body weight and body fat distribution. Several studies have demonstrated the effects of an energy dense diet containing greater proportions of dietary fat on positive weight gain in rodents (Surwit et al. 1997; Surwit et al. 1988; Rebuffe-Scrive et al. 1993; Van Heek et al. 1997). Hill et al. (1993) fed adult rats diets consisting of 45% calories from fat (corn oil, fish-oil or lard) over a period of 6 months. The rats fed the fish-oil diet consumed less energy than rats in the other groups but gained the same amount of weight. However, body fat distribution and insulin resistance were both modified by the dietary fatty acid composition. Fish oil-fed rats had less total body fat, less intra-abdominal fat, and less insulin resistance. The lard-fed rats showed a consistently greater degree of central adiposity (total intra-abdominal fat divided by total body fat) over the 6 month feeding trial compared to the other groups of rats. Adipocyte size and cell number were significantly greater in the rats fed diets containing corn-oil and lard compared to fish-oil. Adipocyte phospholipid and triglyceride fatty acid composition approximately resembled the diet administered to each group. Insulin resistance was 3-fold greater in the lard-fed compared to the fish-oil-fed group as measured by the euglycemic insulin clamp. Corn oil fed rats showed less insulin resistance than lard-fed rats. The authors felt these observations demonstrated a relationship between body fat distribution and

metabolic function (Hill et al. 1993).

A previous study (Parrish et al. 1990) found that adipose depots (epididymal and perirenal) from fish oil-fed 7 – 8 week old male Wistar rats weighed less and contained smaller cells than lard-fed rats after 4 weeks of feeding, although there was no difference in cell number. The length of the feeding trial may contribute to the difference observed in body weight and cell number between these 2 studies. Interestingly, a Japanese study (Ikemoto et al. 1996) examining the effects of several oils (60% of calories) in weanling C57BL/6J mice found after 19 weeks of dietary intervention that soybean oil, palm oil, and lard induced the greatest body weights compared to rapeseed oil, safflower oil, and perilla oil (contains high levels of linolenic acid). Fish-oil induced the least body weight compared to the other diets. Glucose concentrations following a glucose load were highest for the safflower oil-fed mice, modest for rapeseed oil-, soybean oil-, and lard-fed mice, and mild for perilla oil-, fish oil- and palm oil-fed mice. Only palm oil produced fasting hyperinsulinemia.

In a study by Okuno et al. (1997), 4 week old rats fed perilla oil (26% of calories) for 4 months had smaller epididymal fat pads compared to rats fed diets containing 26% of calories from beef tallow or olive oil. The authors speculated that this involved down-regulating adipocyte differentiation with perilla oil feeding (Okuno et al. 1997). In general, these studies show that the amount and type of fat in the diet influences body fat distribution and body metabolism.

Zinc and Zinc Functions

The following sections will provide background on zinc metabolism and its potential roles in insulin metabolism and DM2. The current state of knowledge regarding zinc and leptin, adipose tissue and fatty acid profiles will all be discussed.

Zinc Involvement with Homeostasis

Zinc is the most abundant intracellular trace mineral, ubiquitously distributed throughout the body, and intricately involved in regulation of homoeostatic mechanisms or internal equilibrium of the body (Cousins 1997). Zinc has been found to play important roles in structural and biochemical function involving metalloemzymes, transcription factors, and signal transduction factors (Cousins 1997). Zinc concentrations within the body are dependent on adequate nutritional intakes. Humans and animals fed zinc-inadequate diets experience poor growth, anorexia, skin lesions, depressed appetite, skeletal abnormalities, impaired reproductive functions, and blunted immune responses (Cousins 1997). Zinc deficiency in animals results in anorexia and reduced food consumption with low serum zinc concentrations evident after about 2 weeks (O'Dell 1989). Zinc-deficient humans suffer from diminished sensitivity of taste or hypogeusia that is thought to be responsible for their loss of appetite (Aitkin-Thor et al. 1978). When zinc supplementation has been administered, taste sensation, appetite and normal intake are restored (Aitkin-Thor et al. 1978), highlighting the potential for zinc supplementation in regulating homeostasis and well-being.

Zinc, Insulin Metabolism, and Diabetes Mellitus Type 2

Zinc is directly involved in the metabolism of insulin, an important anabolic hormone produced by the pancreas, and zinc may play a role in the etiology of DM2 (Kinlaw 1983). Insulin is stored in pancreatic β-cells within secretory vesicles in an orderly crystalline structure associated with a variable number of zinc atoms (Brody 1994). This insoluble complex is thought to control insulin release into the bloodstream (Brody 1994). Absence of this complex due to impoverished levels of zinc within the pancreas may result in exaggerated insulin responses to glucose (Chen 1993) and insulin resistance.

Indeed, zinc-deficient animals appear less sensitive to insulin, exhibiting impaired glucose tolerance (Huber 1973) and degranulated islets of Langerhans (Boquist and Lernmark 1969) within the β-cells of pancreatic tissue. When glucose is administered by oral, intraperitoneal or intravenous route (Quarterman et al. 1966; Hendricks and Mahoney 1972; Roth and Kirchgessner 1981), zinc-deficient rats display impaired glucose tolerance curves compared with pair-fed controls. Peripheral insulin resistance is deemed responsible for the glucose intolerance in zinc deficiency by some investigators. Park and colleagues (1986) showed that force-fed zinc-deficient rats have impaired glucose tolerance curves, in spite of elevated blood insulin and normal glucagon concentrations, and normal microscopical anatomy of the pancreas. These observations suggest that pancreatic insulin secretion was not a limiting factor. In addition, exogenous injections of insulin resulted in a significantly prolonged period prior to onset of coma in zinc-deficient rats as compared to pair-fed control rats (Quarterman et al.

1966). Therefore, effects of dietary zinc are not limited to the pancreas, and zinc deficiency has several characteristics in common with DM2 (Table 1) that include insulin resistance and glucose intolerance.

Effects of dietary zinc on insulin also have been investigated in both the *db/db* and *ob/ob* mice, genetic animal models of obesity-induced diabetes. Zinc-deficient *db/db* mice have elevated serum glucose (Levine et al. 1983) and liver glycogen concentrations compared to their non-diabetic heterozygous littermates (Southern et al. 1988). Alternately, zinc supplementation (1000 ppm zinc) for 4 weeks (Begin-Heick et al. 1985) has been shown to improve glycemic control by attenuating exaggerated insulin secretion to glucose stimulation from pancreatic islets and significantly reducing fasting plasma glucose in male 9-12 week old *ob/ob* mice.

Diabetic mice have tissue zinc concentrations that are significantly higher in liver, muscle and adipose tissues, but lower in pancreas and bone (Southern et al. 1988; Levine et al. 1983; Begin-Heick et al. 1985). Hyperzincuria was noted in both diabetic mice (Levine et al. 1983) and humans (Begin-Heick et al. 1985). Hyperzincuria along with low zinc serum concentrations in humans with DM2 (Kinlaw et al. 1983) suggests reduced zinc reserves and altered metabolic function. Recent studies in human subjects have implicated moderate zinc deficiency with DM2 (Blostein-Fuji et al. 1997). However, more studies are required to clarify the role of zinc in preventing and ameliorating diabetic complications.

The insulin signaling pathway is a critical component of tissue response to activities of insulin. Not only do insulin receptor activities require zinc as a co-factor

for phosphorylation reactions (Klein et al. 1993) in signal transduction, but recent studies have revealed that both insulin and zinc interact at the cell membrane and nucleus (Radulescu 1995) to influence gene regulation and cell growth. For example, transcription factors that promote adipogenesis require zinc fingers for DNA binding in the nucleus (Speigelman and Flier 1996). Zinc has been shown to enhance insulin effects on lipogenesis and glucose transport in rat adipocytes during in vitro studies, as well as augment insulin binding in hepatocytes (Coulston and Dandona 1980; May and Contoreggi 1982; Ezaki 1989). Zinc-finger proteins are also located among signal transduction components such as protein kinases and may be redistributed during signal transduction (Cousins 1997). Therefore, zinc status could influence intracellular responses to insulin such as receptor binding, cell signaling, secretion, β-oxidation and cell growth, as well as communication among other intercellular messengers connecting different regions of the body such as the leptin hormone.

Zinc, Leptin and Adipose Tissue

Leptin is a relative newcomer and, to date, few studies have addressed the effects of zinc status on plasma leptin concentrations. As previously indicated, low leptin concentrations are strongly associated with weight loss and reduced body mass (Maffei et al. 1995; Trayhurn et al. 1995). Zinc deficiency results in decreased growth, total carcass lipid content and plasma leptin concentrations in weanling male Sprague-Dawley rats fed a zinc deficient diet (1 ppm) for 27 days compared to ad libitum fed control rats (30 ppm zinc) (Mangian et al. 1998). These

results suggest that leptin is responding normally by signaling low body fat levels. However, when zinc-deficient rats were compared to pair-fed controls, this was not the case. The zinc-deficient and pair-fed rats had similar total carcass lipid content but plasma leptin concentration and body weight was lower in the zinc-deficient rats (Mangian et al. 1998). In addition, plasma leptin concentrations were similar in the zinc adequate pair-fed and ad libitum groups, despite the 1.6 fold greater body weight in the ad libitum group. A recent study in humans demonstrated that zinc depletion reduced leptin concentrations that were restored to normal values by zinc repletion (Mantzoros et al. 1998). These changes in plasma leptin and zinc status occurred even though body weight and body fat remained constant throughout the study. In addition, tumor necrosis factor-α and interleukin-2 production were found to be decreased during zinc depletion and increased during zinc repletion (Mantzoros et al. 1998). These cytokines appear to be involved in neuroendocrine control of appetite regulation by stimulating leptin mRNA expression (Grunfeld et al. 1996). The leptin receptor itself belongs to the class 1 cytokine family (Huang et al. 1996). Further studies on interactions among leptin, cytokines, appetite, zinc and adipose tissue may prove quite interesting.

Like insulin and leptin, there appears to be a strong relationship between zinc and fat stores. Weanling *ob/ob* mice and DIO mice fed a zinc-deficient diet (4-6 ppm zinc) for 6 weeks displayed higher body fat content than their lean counterparts (Chen et al. 1996). Zinc supplementation increased body fat deposition in both groups of obese mice indicating that zinc may be associated with impaired energy balance in obesity. Another study by the same authors found that brown adipose

tissue thermogenesis was greatly attenuated by zinc supplementation implicating zinc in abnormal energy metabolism in *ob/ob* mice (Chen et al. 1997) or suppression of some other problem. In *ob/ob* mice, leptin treatment is known to increase energy expenditure and promote fat oxidation and weight loss (Hwa et al. 1997). An interaction between zinc and fat stores has also been demonstrated in metallothionein (MT)-null mice. Zinc is a necessary co-factor for MT that detoxifies heavy metals and scavenges free radicals (Beattie et al. 1998). MT-null mice become obese and manifest higher concentrations of plasma leptin and upregulated leptin mRNA expression compared to age-matched controls. These results implicate MT and its association with zinc in the regulation of energy balance (Beattie et al. 1998) and deposition of adipose tissue.

Other types of cellular studies also support an interaction among zinc, adipose metabolism and energy balance. Zinc added in vitro has been found to increase the insulin-stimulated conversion of glucose into lipids in adipocytes from rats or *ob/ob* and lean mice (Shisheva et al. 1992, Chen et al. 1998), while zinc deficiency in rats reduced (by 75%) [^{14}C]glucose incorporation into fatty acids of epididymal fat pads and increased [^{14}C]glucose incorporation into liver glycogen (Reeves and O'Dell 1983). Decreased insulin receptor binding has been observed in adipocytes of rats fed a zinc-deficient diet (Gomot et al. 1992). These authors proposed that modification of cell membrane fatty acid composition in zinc deficiency could affect insulin receptor binding by altering membrane fluidity and receptor translocation (Gomot et al. 1992). Furthermore, zinc deficiency has been shown to alter metabolism of the essential fatty acids linoleate (18:2 n-6, LA) and

α-linolenate (18:3 n-3, LNA) towards increased β-oxidation and utilization of linoleate in de novo lipid synthesis (Cunnane and Yang 1995), and this may affect the amount and distribution of fatty acids in fat stores. For example, zinc-deficient rats still maintain some adipose stores in the inguinal region while their pair-fed energy restricted controls become completely depleted (Prescod 1997).

Zinc and Fatty Acid Profiles

A marginal zinc deficiency in animal models has been associated with suppressed formation of arachidonic acid (20:4 n-6, AA) in the phospholipid component of plasma and liver membranes (Cunnane et al. 1984) without effecting the triglyceride content of (Cunnane 1987). In vitro studies (Clejan et al. 1982; Tsai et al. 1983; Ayala and Brenner 1983) have linked these findings to impaired synthesis of AA that is controlled by Δ6 and Δ5 desaturase activity, enzymes involved in the desaturase-elongation pathway (Figure 3). Conversely, AA content in the plasma membrane of the liver was highest in the zinc-supplemented group as compared to the control and zinc-deficient groups (Cunnane 1987). In addition, following a zinc-supplemented diet, the plasma and liver phospholipids were associated with a lower ratio of LA/AA acid compared to rats fed the control and zinc-deficient diets (Cunnane 1987). Alternately, the plasma and liver phospholipids after a zinc-deficient diet were associated with a higher ratio of LA/AA compared to the zinc-supplemented diet implicating impaired enzyme activity in fatty acid metabolism (Cunnane 1987).

Eder and Kirchgessner (1994a) discovered that the dietary fatty acid

composition influenced the incorporation of polyunsaturated fatty acids (PUFA) into the phosphatidylcholine component of liver phospholipids during the development of a severe zinc-deficiency achieved by force-feeding 5–6 week old Sprague-Dawley rats 18% calories as fat for 10 days. The authors found that the low levels of LA and AA in the phospholipid component of membrane tissue of zinc-deficient rats were replaced by the long chain fatty acid, docosapentaenoic (22:5 n-6, DPA) and docosahexaenoic (22:6 n-3, DHA), when the diet contained coconut oil. Eicosapentaenoic acid (20:5 n-3, EPA) replaced the low levels of hepatic membrane phospholipid LA and AA in the rats fed the fish oil (Eder and Kirchgessner 1994a) and linseed oil (Eder and Kirchgessner 1994b). The production of n-3 long chain (LC) PUFA (EPA, DPA and DHA) suggests that the synthesis of eicosanoids that inhibit blood coagulation and thrombocyte aggregation may be altered (Eder and Kirchgessner 1994a). These studies demonstrate unequivocally, both dietary fat and zinc status affect the fatty acid composition of the hepatic cell membrane phospholipid that, in turn, could impact on cell health and well-being of the organism. However, a lack of research on dietary zinc and adipose fatty acid composition limits knowledge in this area. Certainly, more research is required to further investigate the impact of diet on zinc status and elucidate mechanisms involved.

Summary

Thus, there appear to be complex interactions among zinc status, fat metabolism, leptin function and insulin action within the body. Sometimes the

emerging information is contradictory, but this may be due in part to differences between the genetic diabetic obese mouse models and DM-2 in humans. In humans and normal rodents, plasma insulin and leptin are positively correlated with measures of body fat (Bagdade et al. 1967; Klein et al. 1996; Considine et al. 1996, Surwit et al. 1997). In the genetically obese mouse models, obesity in db/db is associated with hyperinsulinemia and hyperleptinemia (due to a mutation in the leptin receptor gene)(Lee et al. 1996, Murakami and Shima 1995). However, obesity in *ob/ob* mice is associated with hyperinsulinemia in the absence of circulating leptin (due to a mutation in the leptin gene) (Zhang et al. 1994).

Interestingly, zinc supplementation attenuates hyperglycemia and hyperinsulinemia in both the db/db and ob/ob mouse models (Simon and Taylor 2000; Begin-Heick et al. 1985; Chen et al. 1998), regardless of differences in presence or absence of plasma leptin. There may also be differences in body fat accumulation as zinc supplementation increased total carcass body fat in the *ob/ob* mice (Chen et al. 1996), but tended to lower fat pad weight in zinc-supplemented db/db mice (Simon and Taylor 2000). Although zinc depletion appears to lower leptin in normal rodents and humans (Mangian et al. 1998, Mantzoros et al. 1998), there do not currently appear to be any experimental data on effects of zinc supplementation on leptin and body fat metabolism in non-diabetic or DM2 models.

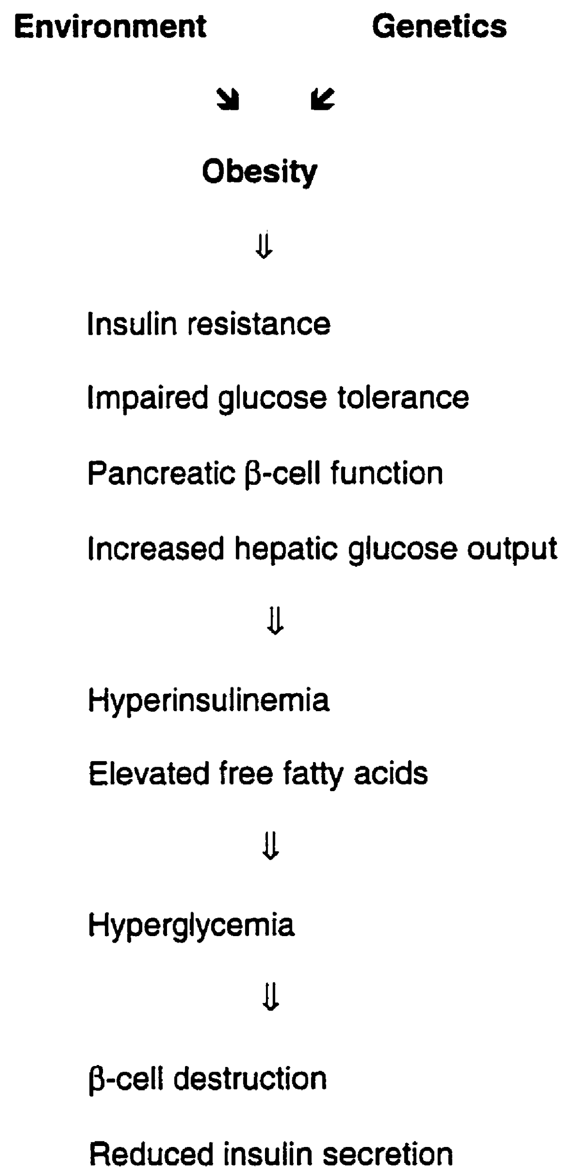


Figure 1. Metabolic changes in the progression of obesity-induced diabetes or DM2.

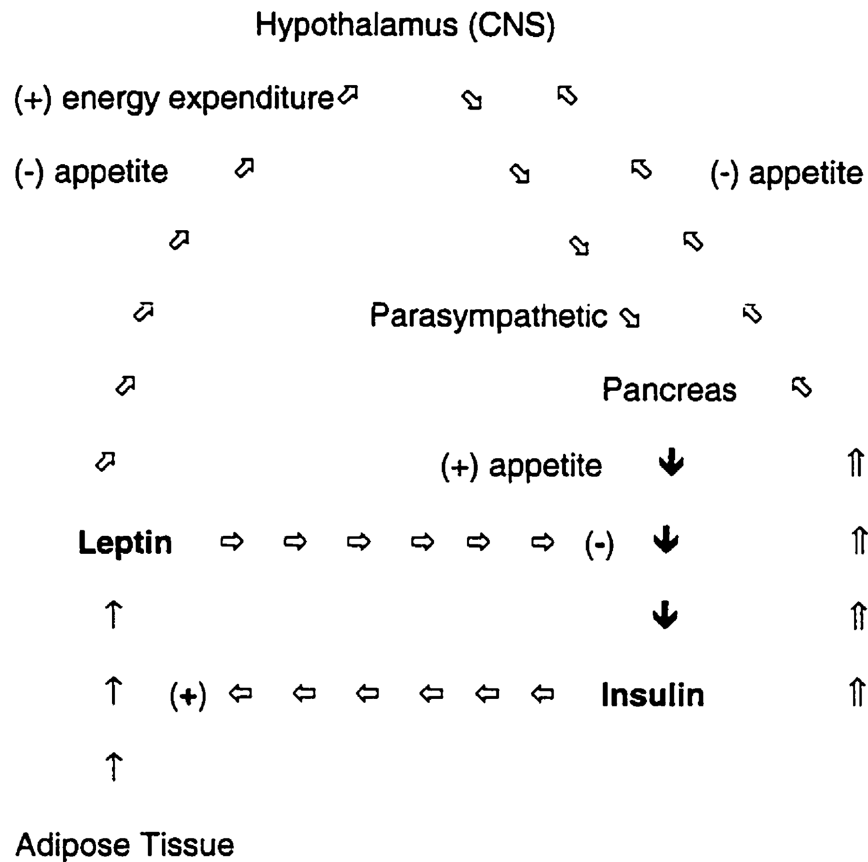
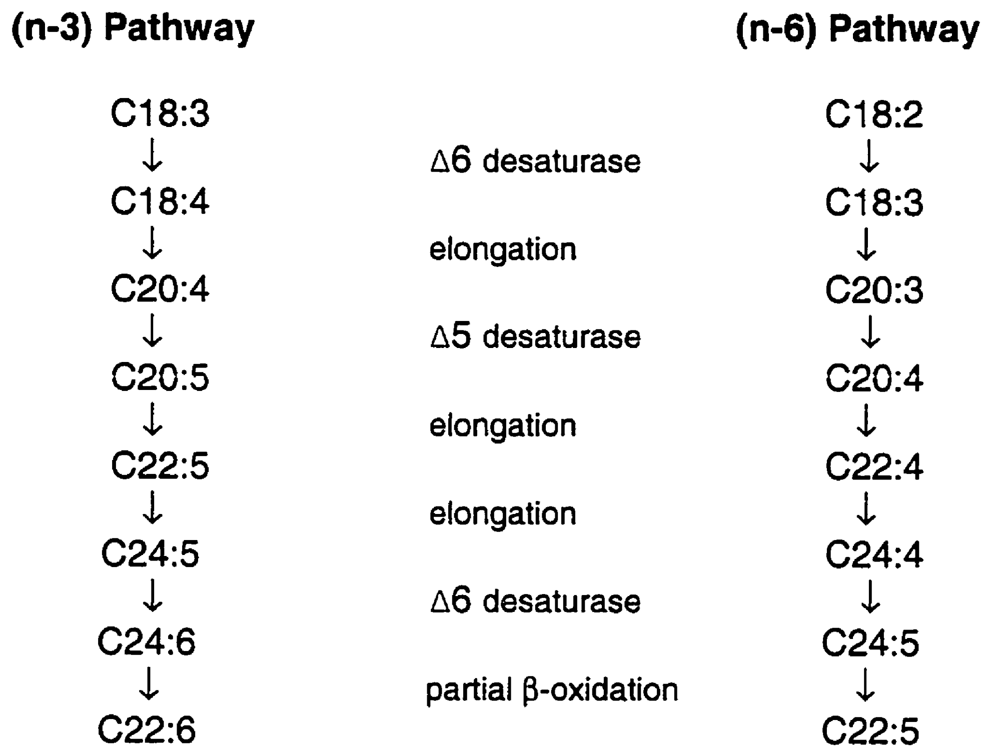


Figure 2. Proposed counterregulatory interactions between insulin and leptin in the central nervous system (CNS) and peripheral tissues (body). Centrally, insulin and leptin receptors are located in the hypothalamus that controls appetite, energy expenditure, and stimulation of pancreatic insulin production via the parasympathetic nervous system. Centrally, insulin depresses appetite, whereas leptin depresses appetite but stimulates energy expenditure. Peripherally, insulin stimulates appetite and leptin secretion. In turn, leptin inhibits production of insulin.



(adapted from Sprecher 1999)

Figure 3. Proposed desaturation-elongation pathway for n-3 and n-6 fatty acid endogenous production of long chain polyunsaturated fatty acids (LC PUFA). Linolenic acid (LNA, C18:3 n-3) and linoleic acid (LN, C18:2 n-6) are precursors for the desaturation-elongation pathway that culminates in LC PUFA such as docosahexaenoic acid (DHA, C22:6 n-3) and docosapentaenoic acid (DPA, C22:5 n-6). The pathway involves desaturase enzymes that add double bonds to fatty acids and elongase enzymes that lengthen fatty acid chains. The final step in producing DHA and DPA involves enzymes regulating partial β-oxidation. PUFA are commonly partially degraded to manufacture

chain-shortened metabolites for anabolic purposes. LNA and LA can be incorporated into triglycerides or phospholipids or oxidized for energy. Eicosapentaenoic acid (EPA, C20:5 n-3) and arachidonic acid (AA, C20:4 n-3) are precursors for eicosanoid formation that are potent metabolites modulating functions in many cells. LC PUFA that are produced as a result of the n-3 and n-6 pathway contain several double bonds. When enzymatically esterified to phospholipids, these LC PUFA enhance membrane fluidity and protein mobility within the lipid bilayer.

Table 1. Characteristics common to dietary zinc deficiency and diabetes mellitus type 2.

Characteristics
Insulin resistance (impaired glucose disposal)
Impaired glucose tolerance test
Insulin secretory defects
Hyperglycemia
Hyperzincuria
Reduced serum zinc concentration

II. Study Rationale

Obesity and insulin resistance appear to be key predictors of DM2 that may be responsible for β-cell incompetence and reduced secretory function. Research indicates that zinc may play a critical role in the development of DM2. Zinc deficiency manifests in diabetogenic traits that include insulin resistance, impaired glucose tolerance and degranulated islets of Langerhans (insulin-producing cells of the pancreas) (Boquist and Lernmark 1969). Zinc has been shown to be involved in the structure, storage and release of insulin from pancreatic β-cells (Boquist and Lernmark 1969). Diabetic humans present with reduced serum zinc concentrations and hyperzincuria that may result in zinc depletion within body reserves (Kinlaw et al. 1983). Studies have demonstrated that zinc supplementation has improved glycemic control, enhanced insulin effects, and enhanced glucose transport (Begin-Heick et al. 1985).

Zinc has been found to increase insulin binding to plasma membrane of liver cells (Aquilla et al. 1978). Fat cells require insulin for the uptake of glucose. In fact, zinc supplementation has been implicated with stimulation of lipogenesis (Chen 1998). Reeves and O'Dell (1982) found that zinc deficiency reduced incorporation of fatty acids into epididymal fat pads and liver. Other studies have demonstrated that dietary zinc levels influence fatty acid composition within the phospholipid component of liver and plasma (Cunnane 1984; Cunnane 1988; Eder and Kirchgessner 1994). A change within the membrane structure may alter membrane performance and fluidity, thus affecting proteins embedded within the phospholipid

layers associated with hormone reception and sensitivity (for example, insulin, leptin and membrane bound enzymes).

Dietary fatty acid composition can also modify phospholipid fatty acid composition within the stored adipose tissue and structural lipids (Thi-Dinh 1990; Clandinin et al. 1985). In addition, the diabetic state is associated with a decreased content of long chain polyunsaturated fatty acids, particularly arachidonic acid, in adipocyte membrane phospholipids (Field et al. 1988). Thus, **it is hypothesized that dietary zinc will influence the development of obesity and DM2 and alter the fatty acid composition of adipose tissue triglycerides and phospholipids in DIO mice. Furthermore, zinc supplementation will prevent the onset of DM2 in a diet–induced model of obesity and DM2.**

The objective of this experiment was to investigate the effects of dietary zinc deficiency and supplementation on diabetic indicators and adipose fatty acid composition using a diet–induced model of obesity and DM2 (C57BL/6J mice) by modifying the dietary fatty acid composition and fat content over a 16 week period. Analysis of zinc concentration in the femur, pancreas, liver and adipose tissue was performed to determine the effects of dietary zinc treatment. Serum insulin, glucose, zinc and leptin concentrations and urine glucose, zinc and creatinine concentrations were measured to verify diabetic characteristics. Adipose tissue fatty acid composition was determined in 2 components: triglycerides and phospholipid.

To date, no other investigations in diet-induced obesity and DM2 in the C57BL/6J mice have involved the effects of zinc deficiency and supplementation on

the fatty acid composition of adipose tissue triglyceride (TG) and phospholipid (PL). The literature contains some evidence for relationships between zinc status and diabetes, zinc status and essential fatty acid composition, as well as dietary fatty acid composition and membrane fatty acid composition. However, few studies have addressed the interaction of dietary zinc and dietary fat on adipose fatty acid composition in obesity and diabetes. An exploration of the effects of dietary zinc and high levels of dietary fat on adipocyte membrane PL composition in any animal model is lacking in the literature.

Diet Rationale

The intent of the study was to induce a marginal zinc deficiency using a 3 ppm zinc diet that has demonstrated efficacy in lean and *db/db mice* (Simon and Taylor 2000). The zinc-supplemented diet contained 150 ppm zinc which is 5 times the recommended dietary amount for mice (30 ppm zinc, Reeves et al. 1993). The goal was to prevent DM2 without incurring a zinc toxicity and copper insufficiency. The copper status of rodents has been shown to be impaired by the addition of excessive amounts of zinc (L'Abbe & Fisher 1984).

A critical part of the current study was to induce obesity in C57BL/6J mice that are prone to develop adipogenic diabetes when placed on a diet high in fat. A balanced diet is required that adequately addresses the need for physiological growth in weanling mice and simultaneously contains sufficient quantities of fat to encourage obesity-induced DM2. As a result, a diet containing 55% calories from soybean oil and lard was selected and based on the nutrient recommendations of

the American Institute of Nutrition, AIN-93G diets for growth (G), pregnancy, and lactation (Reeves at al. 1993).

Recent animal studies inducing diabetes in C57BL/J mice have done so with diets containing 45% to 58% calories from fat (Surwit 1988, 1991, 1995, 1997; Rebuffe-Scrive 1993). Surwit et al. (1988, 1991, 1995, 1997) used a combination of 58% calories from hydrogenated coconut oil and sunflower seed oil over a period of 14 weeks. Rebuffe-Scrive et al. (1993) used 45% calories from lard over 20 weeks. One study induced obesity and peripheral leptin resistance after 56 days by administering a diet containing 45% calories from lard (Van Heek 1997). However, the study did not examine measurements pertaining to diabetes such as glucose and insulin concentrations. A study examining glucose transport in skeletal muscle used a diet containing 55% calories from fat to demonstrate impaired insulin-stimulated glucose transport associated with diabetes (Zierath 1996). Based on these findings, the decision was made to use a diet with 55% calories obtained from fat (39% calories from lard and 16% calories from soybean oil) over a period of 16 weeks to ensure the development of diabetogenic traits.

The prevailing AIN-93G recommendations suggest a fat composition of 7% by weight or 70 g per 1000 g diet during the rapid growth stage of rodents (Reeves et al. 1993). This is based on reaching maximal concentrations of fatty acids in neural tissues of the growing rat. The guidelines recommend 50 g to 60 g soybean oil to provide an adequate amount of linoleic acid (LA) and linolenic acid (LNA) and to sustain growth. Fifty grams of soybean oil will provide about 25.5 g (51%) LA and 3.5 g (7%) LNA in the diet with an n-6/n-3 ratio of 7 and a polyunsaturate/saturate

ratio of 4 (Reeves at al. 1993). This amount of soybean oil will reach maximal concentrations of essential fatty acids in many tissues of growing rodents (Reeves et al. 1993). In addition, a 15% margin of safety has been included above the highest recommendation of 60 g bringing the total to 70 g soybean oil per 1000 g diet (Reeves et al. 1993). The intent of the study was to meet requirements for physiological growth.

The study was designed to produce two distinctly different groups (diabetic and non-diabetic) through dietary manipulation and this required considerable differences in type and amount of dietary fat. Therefore, the decision was made to use 70 g soybean oil (SO) per 1000 g diet, providing 16% of calories from fat, and compare this diet to a mixed fat diet (MF) consisting of a proportional amount of soybean oil (88 g) (Appendix 1) plus lard (209 g) (Appendix 1), providing 55% of calories from fat. The SO diet produced a total calorie count of 3856 calories as fat per 1000 g with about 15% saturated fatty acids (SAT), 61% polyunsaturated fatty acids (PUFA) with a PUFA/SAT ratio of 4, 54% n-6, 7% n-3 and a n-6/n-3 ratio of 8 (Appendix 2). The MF diet containing lard and soybean oil produced a calorie count of 4860 calories as fat per 1000 g with about 31% SAT, 27% PUFA with a PUFA/SAT ratio of 0.9, 24% n-6, and 2.3% n-3 with an n-6/n-3 ratio of 11 (Appendix 2). In summary, the SO and MF diets were formulated to attain obesity and diabetes in a susceptible rodent model and to sustain physiological growth.

III. Material and Methods

Animals and Diet

Weanling C57BL/6J mice can be induced to obesity and diabetes by ingesting an energy dense diet over a period of 12-16 weeks. Only male mice were used to avoid profound gender differences in circulating hormones such as leptin concentrations. Initially, the mice were placed in individual stainless steel hanging cages and fed a nutritionally complete diet containing soybean oil (16% calories from fat and 30 ppm zinc, Table 2) and based on the AIN-93 diet recommendations for rodent growth (Reeves et al. 1993) for a 7 day adaptive period. The diet was offered in the form of a paste consistency.

After one week, half of the mice were randomized to the SO diet that contained 7% by weight from fat (Table 2). The other half of the mice were randomized to a more energy dense diet. To increase energy density, the diet was combined with an additional amount of lard to render a MF diet of 29.7% by weight from fat. On a caloric basis, the MF diet containing lard consisted of 16.6% protein, 28.3% carbohydrate, and 55.1% fat (total 4.86 kcal/g), and the SO diet consisted of 16.6% protein, 67.1% carbohydrate and 16.3% fat (3.86 kcal/g) (Table 2). Mice fed the SO and MF diets were then randomly assigned to one of 6 treatment groups and fed *ad libitum* diets composed of 3 ppm (zinc-deficient, ZD), 30 ppm (Zinc-control, ZC) or 150 ppm (Zinc-supplemented, ZS) zinc (Tables 2 and 3). Initially, the study design contained a group designated for pair-feeding. However, the feed intake of the ZD and ZS mice did not differ from the ZC groups. Thus, the mice

designated for pair-feeding were fed ad libitum throughout the study and are included as ZC mice in the analysis. The experimental design has 6 dietary treatment groups: SO-ZD, SO-ZC, SO-ZS, MF-ZD, MF-ZC, and MF-ZS. Throughout the study period, the mice were maintained in a controlled environment of 55% humidity, 21-23^0 C and a 14 hour light, 10 hour dark cycle. During the feeding trial, the animals were fed *ad libitum* zinc-free double deionized water available in polypropylene bottles with stainless steel sipper tubes to prevent zinc contamination. Feed intake was measured over the last 8 weeks. At months 0, 1, 2, 3, and 4 of the trial, the mice were weighed and fasted overnight in polycarbonate metabolic cages (Nalgene, Fisher Scientific) to obtain urine specimens that were not contaminated by zinc from the feed nor environment. During the fast, animals had free access to water. The mice were terminated at weeks after an overnight fast in the metabolic cages. The study was approved by the University of Manitoba, Fort Garry Campus Protocol Management and Review Committee.

Tissue Collection

Mice were placed in metabolic cages and fasted overnight at 0, 1, 2, 3 and 4 months. Urine specimens were collected in 10 ml clean plastic tubes, transferred into 5 ml preweighed and capped containers, weighed and stored at -80°C to be assayed for glucose, zinc and creatinine concentration.

At 4 months, mice were terminated by CO_2 asphyxiation and cervical dislocation following Canadian Council on Animal Care Guidelines (1993). The length of the prone mouse body (nose tip to anus) was measured using a ruler.

Trunk blood was collected in 1.0 ml microcentrifuge tubes, stored on ice for 20 minutes and centrifuged at 1290xg for 15 minutes in a Beckman TJ-6 centrifuge to obtain serum samples. Serum was separated into individual tubes and stored at -80°C to be analyzed for glucose, zinc, insulin and leptin concentration. The liver, pancreas, kidney, epididymal fat pad, and lower extremities were excised, frozen in liquid nitrogen and stored at -80°C for future analysis.

Glucose Assay

An enzymatic colorimetric kit developed for the quantitative determination of glucose in biological fluids (Procedure #315, Sigma Chem., St. Louis, MO) and based on the method of Trinder (1969) was used to assess serum and urine glucose.

A standard curve using a dilution series of glucose standard (3 mg/dl) showed a linear response to a glucose concentration of 750 mg/dl. The glucose assay involves the conversion of glucose to a quinoneimine dye that has an absorbance maximum of 505 nm. The concentration of glucose in the sample is directly proportional to the intensity of the colour produced by the glucose reaction.

Reagents:

Trinder reagent:

4-Aminoantipyrine	0.5 mmol/L
p-Hydoxybenzene Sulfonate	20 mmol/L
Glucose Oxidase (Asperigillus niger)	15 000 U/L
Peroxidase (Horseradish)	10 000 U/L

Buffer pH 7.0 ± 0.1

Stabilizers and fillers

Urine control, Level 2 (Sigma Chem. Co.)

Glucose Standard (300 mg/dl)

Procedure:

Blood was collected without anticoagulant and centrifuged promptly after clot formation to prevent glucose metabolism by red blood cells (about 5% of the serum glucose content during each hour is consumed by red blood cell glycolysis). Serum was stored at – 80°C until analysis. One ml of Trinder reagent was pipetted into labeled 10 mm disposable cuvets and allowed to warm to room temperature. At 30 second intervals, 5 µl volumes of distilled water (assay blank), glucose standard, urine control, and serum or urine samples were pipetted in duplicate into each cuvet. Each cuvet was covered with parafilm, gently inverted 3 times to ensure a homogeneous mixture and allowed to incubate at room temperature for 18 minutes. Each sample was read at 30 second intervals at 505 nm with a Milton Roy Spectronic spectrophotometer (Fisher Scientific, Nepean, ON). In order to verify the linearity of the procedure, a set of glucose standards in the range of 0-750 mg/dl was used. Distilled water (1.0 ml volume) was used as a reference.

Calculation:

$$\text{Glucose (mg/dl)} = \frac{(\text{[A] sample} - \text{[A] blank} \times \text{dilution factor}}{\text{[A] standard} - \text{[A] blank}} \times \text{Conc. of Std.}$$

where [A] = absorbance

Glucose (mg/dl) x 0.0555 = glucose (mmol/L)

Insulin Assay

Serum insulin was assayed using a sensitive rat insulin radioimmunoassay kit (#SRI-13K, Linco Research Inc., St. Charles, MO). The radioimmunoassay procedure is based on competitive binding between radioactive and nonradioactive antigen for a fixed number of antigen sites. The amount of labeled insulin (^{125}I-insulin) bound is inversely proportional to the concentration of unlabeled insulin present in the serum.

Reagents:

Assay Buffer: 0.05 M Phosphosaline, pH 7.4, containing: 0.025M EDTA, 0.08% sodium azide, and 1% RIA grade BSA

^{125}I-Insulin label (< 3 uCi)

Label hydrating buffer

Insulin standards (0.02 – 1.0 ng/ml)

Quality controls

Precipitating reagent

Procedure:

On day 1, assay buffer was pipetted into Borosilicate glass tubes (12 x 75 mm): 300 µl into 2 non-specific binding (NSB) tubes, 200 µl each into 2 total binding reference tubes, and 100 µl into tubes for standards, controls and unknowns. One hundred µl of standards and quality controls were pipetted in duplicate into appropriate tubes. Serum samples were pipetted in duplicate and diluted in assay buffer by 2.5 to 10 fold to attain a total volume of 100 µl. Sensitive rat insulin antibody (100 µl) was pipetted into all the tubes except total count tubes and NSB

tubes. The tubes with the insulin antibody were vortexed, covered with parafilm, and incubated overnight (20-24 hours) at $4^{0}C$ in a sealed plastic container. The next day, 100 µl of ^{125}I-Insulin was pipetted into all tubes. These tubes were vortexed, covered with parafilm and incubated overnight at 4^{0} C in a sealed plastic container. On the third day, 1.0 ml of precipitating reagent was added to all tubes except the total count tubes. Tubes with the precipitating reagent were vortexed, incubated 20 minutes at $4^{0}C$, and centrifuged for 40 minutes at ~ 2000 x g to obtain a firm pellet. The supernate was immediately decanted from all tubes except the total count tubes by inverting tubes for 60 seconds and blotting excess liquid from the lip of each tube. The pellets were counted for ^{125}I-Insulin in a gamma counter (Beckman Gamma 8000, Scientific Instruments, Irvine, CA).

Calculation:

Definitions

Total count = total ^{125}I-Insulin alone

Non-specific binding = tube with no antibody reagents except antibody and sample or standard

Total binding reference tube = all reagents except standard or sample.

1. Non-specific binding count was subtracted from all tubes except total counts.
2. Percentage of maximum binding ($\%B/B_o$) = $\frac{\text{sample or standard cpm}}{\text{total binding reference cpm}} \times 100$

A log-log plot was developed for $\%B/B_o$ using the Microsoft Excel program with standard cpm on the y-axis versus the known concentration of the particular standard on the x-axis. A reference curve was created by joining points with a

smooth curve. Insulin concentrations of unknown samples were then determined by interpolation of the reference curve.

Leptin Assay

Serum leptin was assayed using a mouse leptin radioimmunoassay kit (#ML-82K, Linco Research Inc., St. Charles, MO). The radioimmunoassay procedure is based on competitive binding between radioactive and nonradioactive antigen for a fixed number of antigen sites. The amount of labeled leptin (^{125}I-leptin) bound is inversely proportional to the concentration of unlabeled leptin present in the serum.

Reagents:

Assay Buffer: 0.05 M Phosphosaline, pH 7.4, containing: 0.025M EDTA, 0.08% sodium azide, 0.05% triton X-100, and 1% RIA grade bovine serum albumin (BSA)

^{125}I-leptin label (< 3 uCi, <111kBq) per 27 ml of label hydrating buffer

Leptin Standards (0.2 – 20.0 ng/ml)

Quality controls

Precipitating reagent

Procedure:

On day 1, assay buffer was pipetted into Borosilicate glass tubes (12 x 75 mm): 300 µl into 2 non-specific binding (NSB) tubes, 200 µl each into 2 total binding reference tubes, and 100 µl into tubes for standards, controls and unknowns. One hundred µl of standards and quality controls were pipetted in duplicate into appropriate tubes. Serum samples were pipetted in duplicate and diluted in assay

buffer by 2 to 13.3 fold to attain a total volume of 100 µl. Mouse leptin antibody (100 µl) was pipetted into all the tubes except total count tubes and NSB tubes. The tubes with leptin antibody were vortexed, covered with parafilm, and incubated overnight (20-24 hours) at 4^0 C in a sealed plastic container. The next day, 100 µl of ^{125}I-leptin was pipetted into all tubes. The tubes were vortexed, covered with parafilm, and incubated overnight at 4^0 C in a sealed plastic container. On the third day, 1.0 ml of precipitating reagent was added to all tubes except the total count tubes. Tubes with the precipitating reagent were vortexed, incubated 20 minutes at 4^0C, and centrifuged for 40 minutes at ~ 2000 x g to obtain a firm pellet. The supernate was immediately decanted from all tubes except the total count tubes by inverting the tubes for 60 seconds and blotting the excess liquid from the lip of each tube. The pellets were counted for ^{125}I-leptin in a gamma counter (Beckman Gamma 8000, Scientific Instruments, Irvine, CA).

Calculations:

Definitions

Total count = total ^{125}I-leptin alone

Non-specific binding = tube with no antibody reagents except antibody and sample or standard

Total binding reference tube = all reagents except standard or sample.

1. Non-specific binding count was subtracted from all tubes except total counts.
2. Percentage of maximum binding ($\%B/B_o$) $= \frac{\text{sample or standard cpm}}{\text{total binding reference}} \times 100$

A log-log plot was developed for $\%B/B_o$ using the Microsoft Excel program

with standard cpm on the y-axis versus the known concentration of the particular standard on the x-axis. A reference curve was created by joining points with a smooth curve. Leptin concentrations of unknown samples were then determined by interpolation of the reference curve.

Zinc Determinations

Femur, pancreas, liver, serum, urine, and diets were analyzed for zinc concentration by atomic absorption spectrophotometry. Serum and urine samples (100 µl) were diluted 10 fold in deionized water and subjected to a direct analysis, while tissues, bone and diet required acid digestion.

Procedure:

The following technique of digesting tissue is based on the method of Clegg et al. (1981). Skin and soft tissue were scraped from one femur per animal. Portions of tissues (approximately 0.15 g pancreas, 0.2 g liver, 0.5 g adipose tissue) were randomly sampled from each organ. After obtaining wet weights, the organs were loosely wrapped in foil for drying. The tissues were placed in a preheated drying oven at 85^{0}C for 2 days, removed, immediately weighed again to determine dry weights, and stored in the refrigerator. Pyrex test tubes and glass beads were acid-washed (30% nitric acid) and rinsed with double de-ionized (Millipore) water. Dried sample tissues were removed from the foil and placed into the labeled acid-washed test tubes containing 1 ml 70% nitric acid (trace metal grade, Fisher Scientific, Napean, Ontario). For the treatment diets, 1 ml 70% nitric acid was added to 0.5 g diet. A bovine liver reference (0.1g/10ml; ref.#1577b, U.S.

Department of Commerce, National Institute of Standards and Technology, Gainsburg, MD) was digested and used as a quality control. Each test tube was covered with an acid-washed glass bead and left at room temperature for at least 1 hour. Dry bath heaters were preheated to 85°C and test tubes were placed in the dry bath heater for 24 to 48 hours to allow digestion to take place. Once the test tubes were cooled, tissue samples were diluted with double deionized water (to a volume of 5 ml for the zinc deficient pancreas and 10 ml for other pancreas, tissue and diet samples). Samples were analyzed using an atomic absorption spectrophotometer (Varian Spectra AA-30 Spectrophotometer, Georgetown, ON). Zinc standards (0.1-1 ppm) were prepared from atomic absorption standard (1000 ppm, #H595-01 Mallinckrodt, Paris, Kentucky).

Calculations:

1. For femur, pancreas, liver, & diet:

$$\text{Zinc } (\mu g/g \text{ dry weight}) = \frac{\text{Sample zinc concentration X dilution factor}}{\text{Dry weight of sample}}$$

2. For serum & urine samples:

$$\text{Zinc } (\mu g/ml) = \text{Sample zinc concentration X dilution factor}$$

Creatinine Assay

Creatinine is the final product of breakdown product of phosphocreatine metabolism, an important energy reservoir in skeletal muscle. Creatinine is excreted in the urine in proportion to muscle mass (Bowers & Wong, 1980) in relatively constant amounts and was used as a basis for calculating urinary zinc and glucose concentrations. The colourmetric creatinine assay (Procedure #555, Sigma

Chem. Co., St. Louis, MO) is based on the work of Slot (1965) and Theinegard and Tiderstrom (1973) that measures colour intensity before and after acidification to determine creatinine content. Creatinine forms a yellow/orange colour upon contact with alkaline picrate that is destroyed at acid pH. The difference in colour intensity is proportional to creatinine concentration.

Reagents:

Creatinine colour reagent, 0.6% picric acid, sodium borate and surfactant

Acid reagent, sulfuric and acetic acid mixture

Creatinine standard, 3.0 and 15 mg/dl creatinine, in 0.02 N hydrochloric acid

Sodium hydroxide solution, 1.0 N

Alkaline picrate solution, 5 parts colour reagent to 1 part NaOH (1.0 N)

Assay procedure:

Duplicate samples of 100 µl of distilled water (blank), 3 mg/dl creatinine standard, urine control or sample were measured into 10 mm disposable cuvets. A 1.0 ml volume of alkaline picrate solution was pipetted into all cuvets, inverted 3 times and allowed to stand at room temperature for 10 minutes. All cuvets were then read at 500 nm (Initial [A]) using a Milton Roy Spectronic 3000 Spectrophotometer (Fisher Scientific, Nepean, On). After the reading, acid reagent (33.3 µl) was pipetted into all cuvets, inverted 3 times and allowed to stand at room temperature for 5 minutes. All cuvets were then read at 500 nm (Final [A]). Distilled water (1.0 ml) was used as a reference for the spectrophotometer during the assay. The procedure required a calibration curve.

Calculation:

Following subtraction of Blank [A] from standard and unknown samples, creatinine was calculated as follows:

$$\text{Creatinine} = \frac{(\text{Initial [A] test} - \text{Final [A] test}) \times \text{dilution factor}}{\text{Initial [A] standard} - \text{Final [A] standard}} \times \text{Conc. of Std. (mg/dl)}$$

Thin Layer Chromatography

Membrane lipids are effectively extracted by polar organic solvents that reduce hydrophobic interactions among lipid molecules (Lehninger et al. 1993). These solvents also weaken hydrophobic bonds and electrostatic interactions that bind membrane lipids to membrane proteins. The membrane lipids can be further separated using chromatographic procedures based on the different polarities of each type of lipid. The lipids interact with the silica gel (silicic acid) and, depending on their charge, bind tightly and remain stationary or have less tendency to bind and move farther (Lehninger et al. 1993).

Epididymal Adipose Tissue Extraction

Epididymal adipose lipids were extracted by modified Bligh and Dyer extraction procedures (Bligh and Dyer, 1959). The antioxidant, butylated hydroxytoluene (0.005% concentration), (Sigma-Aldrich, Oakville, ON) was added to the solvents. Ten ml of 2 parts optima grade chloroform (Fisher Scientific, Nepean, Ontario) to one part pesticide grade methanol (Fisher Scientific, Nepean, ON) (2:1) was pipetted into chloroform-rinsed tubes and approximately 0.25 grams of adipose tissue was added to each tube. The tissues were homogenized with a

Polytron Homogenizer for about 45 seconds. The solution was filtered through Whatman # 4 filter paper and washed with 2.3 ml 0.73% sodium chloride solution. The tubes were vortexed and centrifuged at 1500 RPM for 10 minutes using a GS-6 centrifuge (Beckman Instruments, Fullerton, CA). The top layers were removed and discarded. The bottom layers were rinsed twice with 1-2 ml of theoretical upper phase (chloroform:methanol:water, 3:48:47, v:v:v) then transferred to clean tubes and evaporated to dryness under nitrogen in a 30°C water bath. The sides of the tubes were washed with 2 ml chloroform. The lipid extracts were flushed with nitrogen and stored in the freezer in 4 ml screw top vials at –20°C for future use.

Thin Layer Chromatography Procedure

Whatman K8 Silica Gel 80A plates (Fisher Scientific, Nepean, ON) were activated by heating in a preheated 120°C oven for 30 minutes and cooled in a dessicator for 10-15 minutes prior to use. Thin layer chambers were lined with Whatman Chromatography filter paper and allowed to equilibrate for 30 minutes using a solution of 80 ml optima grade petroleum ether (Fisher Scientific, Nepean, ON), 20 ml anhydrous certified A.C.S. ethyl ether (Fisher Scientific, Nepean, ON) and 1 ml glacial acetic acid (Fisher Scientific, Nepean, Ontario) as the mobile phase (Gasbarro 1972). For triglycerides, the internal standard, triheptadecanoin (Nu-Check, Elysian, MN) (100 µl of 20 mg/ml chloroform) was combined with 100 µl of extracted lipid. Of this mixture, 10 µl was spotted in duplicate under nitrogen onto the prepared plates with 9 sample spots and 1 standard (triheptadecanoin) spot per plate. For phospholipids, the internal standard, 1,2-dipentadecanoyl-sn-glycero-3-

phosphocholine (Avanti, Alabaster, AL) (50 µl of 0.7mg/ml toluene) was combined with 1 ml lipid extract. The standard and lipid extract were then dried under nitrogen in a water bath (Organomation Associates, Berlin, MA) and washed down with 100 µl chloroform. The mixture was spotted in duplicate (100 µl per band) under nitrogen onto the prepared plates with 3 sample bands and 1 standard (phosphotidylcholine) spot per plate. Once spots were dry, the plates were transferred to the chambers. When the solvent front had advanced to within 1 cm of the top of the of the plate, the plate was removed and dried at room temperature for 15-20 minutes. The plates were sprayed with 0.1% 8-anilino-1-napthalene-sulfonic acid (Sigma Chemical Co., St. Louis, MO) and the lipids identified under a UV light. The triglyceride spots or phospholipid bands were removed, transferred to a tube (15 x 100 mm) and covered with 1 ml optima grade toluene (Fisher Scientific, Nepean, ON). The lipids were methylated with 1.2 ml 3N-methanolic hydrochloric acid (Supelco, Bellefonte, PA) and vortexed for 30 seconds prior to being placed in a preheated 80^{0}C oven for 3 hours (triglycerides) or 12 hours (phospholipids). After cooling, the solution was washed with 1 ml deionized water and vortexed. The top layer was transferred to a clean tube. The bottom layer was washed with petroleum ether (1 ml), vortexed and centrifuged. The resulting top layer was transferred to the previous top layer and washed with 2 ml deionized water. The tubes were vortexed and centrifuged. The top layer was transferred to a gas chromatography (GC) vial and evaporated under nitrogen in a tissue float bath (Lab Line Instruments, Melrose Park, ILL) heated to 30°C. Once the solvent had evaporated, 50 ul hexane was added to each vial in preparation for GC analysis

using the "fatacid" method. The fatty acids are volatized by heat and emerge from the column depending on the boiling point of the components of the lipid mixture and the nature of the solid adsorbant in the column. Fatty acid esters were separated on a DB-225 capillary column (30 m x 0.25 mm I.D. with 0.25 µm film thickness) using a Varian Star 3400 Gas Chromatography System (Georgetown, ON) with a flame ionization detector. The GC was set for a split ratio of 10 with injection volumes of 1.5 ul for triglycerides and 5 ul for phospholipids.

Statistical Analysis

Differences between dietary treatment groups were analyzed using ANOVA (SAS 6.04, SAS Institute, Cary, NC). For the initial analysis, the two-way ANOVA, the main effects were dietary zinc, dietary fat, and dietary zinc x dietary fat interaction. Duncan's multiple range test was used to determine significant differences between means. Pearson's Correlation Coefficient was used for correlation analysis of serum leptin and serum concentrations of glucose, insulin and zinc, tissue zinc concentrations of pancreas, adipose and femur, and final body weight and body weight gain over 4 months. Sample size for fatty acid composition was based on previous studies that have found significant differences among groups using 5 to 7 rodents. Sample size for zinc determinations was based on previous studies using 5 to 10 rodents per group. Originally, the animals were randomized into groups of 12 animals to compensate for possible losses that can occur in a long-term zinc study. Discrepancies in numbers of mice per group arose as a result of premature termination from the experiment or limited quantities of

serum for analysis. Significant differences were accepted at $P \leq 0.05$. For many of the parameters, there was a significant main effect of dietary fat, but not dietary zinc. Thus, ANOVA was determined for SO vs MF mice.

Post Hoc Analysis

One of the observations made during data collection were differences between 2 groups of mice across diets – low body weight (LWT) and high body weight (HWT) mice. For purposes of data analysis, the mice were dichotomized by median body weight into 2 distinct groups. The first group of mice weighed < 39.6 g and the second group of mice weighed $\geq$ 39.6 g. Statistical analysis revealed significant differences between LWT and HWT mice. We felt this was an opportunity to examine the variation in metabolic characteristics between the two groups of mice.

Table 2. Diet Formulation[1]

Ingredients (g/kg)	Soybean Oil 16% of calories 3 ppm Zinc SO-ZD	Soybean Oil 16% of calories 30 ppm Zinc SO-ZC	Soybean Oil 16% of calories 150 ppm Zinc SO-ZS	Mixed Fat 55% of calories 3 ppm Zinc MF-ZD	Mixed Fat 55% of calories 30 ppm Zinc MF-ZC	Mixed Fat 55% of calories 150 ppm Zinc MF-ZS
Soybean Oil[2]	70	70	70	88	88	88
Lard				209	209	209
Egg White	200	200	200	252	252	252
Cornstarch	397	397	397	29	29	29
Maltodextrin	120	120	120	151	151	151
Sucrose	100	100	100	126	126	126
Mineral Mix	35	35	35	44	44	44
Vitamin Mix	10	10	10	13	13	13
Cellulose	50	50	50	63	63	63
Choline	2.5	2.5	2.5	3.0	3.0	3.0
KPO4	5.4	5.4	5.4	7.0	7.0	7.0
Zinc Premix[3]	1.0	10	50	1.3	13	65
Biotin Premix[4]	2.0	2.0	2.0	2.6	2.6	2.6
Total calories/g	3.86 calories/g	3.86 calories/g	3.86 calories/g	4.87 calories/g	4.87 calories/g	4.87 calories/g

[1] Diet ingredients were purchased from Harlan Teklad (Madison, WI) unless otherwise indicated. [2] Soybean oil was purchased from Vita Health (Winnipeg,MB). [3] Zinc premix (5.775 g Zinc carbonate/1000 g dextrose). [4] Biotin Premix (20 mg biotin/10 g maltodextrin).

Table 3. Experimental Design[1,2,3]

Diet	Soybean Oil (SO) (16% of total calories)	Mixed Fat (MF) (55% of total calories)
Zinc Deficient (ZD. 3 ppm Zinc)	SO-ZD (n = 12)	MF-ZD (n = 12)
Zinc Control (ZC, 30 ppm Zinc)	SO-ZC (n = 12)	MF-ZC (n = 12)
Zinc Supplemented (ZS, 150 ppm Zinc)	SO-ZS (n = 12)	MF-ZS (n = 12)
Pair-Fed (ZC, 30 ppm Zinc)	SO-ZC (n = 12)	MF-ZC (n = 12)

[1] Experimental diets are SO-ZD = soybean oil zinc deficient; SO-ZC = soybean oil zinc control; SO-ZS = soybean oil zinc supplemented; MF-ZD = mixed fat zinc deficient; MF-ZC = mixed fat zinc control; MF-ZS = mixed fat zinc supplemented
[2] ppm = parts per million for zinc concentrations
[3] Pair-fed groups not required and added to ZC groups

IV. RESULTS

Observations from this study arise from manipulations of dietary zinc content and dietary fat in a rodent model susceptible to diet-induced obesity and diabetes. Since many of the changes were due to a significant effect of dietary fat and not dietary zinc, it was of interest to compare mice fed SO versus MF diet. Furthermore, a number of mice developed overt signs and metabolic abnormalities characteristic of the obese, prediabetic state that were independent of experimental diet. Characteristics that determined obesity included greater body weights and intra-abdominal accumulation of body fat. The mice were dichotomized into LWT and HWT groups based on median weight, and these groups were used for further analysis. The HWT mice were considered to be obese. Thus, in the Results section, the data are presented in three sets of Tables for each parameter:

(1) Tables for the Experimental Diets present the main effects for dietary fat [SO diet, MF diet], dietary zinc [ZD=3ppm zinc, ZC=30 ppm zinc, and ZS=150 ppm zinc], and the interaction between dietary fat x dietary zinc, and the means ± standard error of the mean (SEM) for the six experimental diets [SO-ZD, SO-ZC, SO-ZS, MF-ZD, MF-ZC, MF-ZS],

(2) Tables for the Mice fed SO or MF diet represent the F-values and means ± SEM for the SO and MF groups, and

(3) Tables for the LWT or HWT mice present the F-values and means ± SEM for the LWT and HWT groups. For some of the fatty acid data (Table 23 and Table 28), main effects for dietary fat [SO, MF], body weight [LWT,

HWT] and the interaction of dietary fat x body weight, and the means ± SEM for the SO-LWT, MF-LWT, SO-HWT and MF-HWT groups are presented.

Characteristics of Animals

After 16 weeks of dietary treatment, the body weights among the experimental groups of mice were similar, regardless of dietary zinc level or dietary fatty acid composition. Mice fed the MF diets had significantly greater epididymal fat pad weights (intra-abdominal fat), ratios of fat pad weight to body weight and caloric intakes than mice fed the SO diets (Tables 4 and 5).

When the mice were dichotomized into groups based on body weight, the HWT group had significantly greater body weight, BMI, epididymal fat pad weights, ratios of fat pad to body weight and caloric intake compared to the LWT group (Table 6). Fifty-six percent of mice fed the MF diet and 44% of mice fed the SO diet were in the HWT group.

Serum Glucose, Insulin, Leptin, and Zinc Concentrations

There were no significant differences due to dietary zinc or dietary fat for serum glucose, serum insulin, serum leptin, and serum zinc concentrations among the experimental groups of mice (Tables 7 and 8). Although serum insulin and serum zinc concentrations were comparable, serum glucose and serum leptin concentrations were 1.2 fold and 2.4 fold, respectively, higher in the HWT compared to the LWT mice (Table 9).

Tissue Zinc Concentrations

There was a significant main effect of dietary zinc on femur zinc concentration (Table 10) and a significant main effect of dietary fat on adipose zinc concentration (Table 10 and 11). Experimental groups had similar zinc concentrations and similar weights of both the liver and the pancreas (Tables 10 and 11). Femur zinc concentration was significantly higher in the ZS group compared to ZC group and significantly higher in the ZC group compared to the ZD group (220 ± 6 µg/g vs 197 ± 2 µg/g vs 173 ± 4 µg/g, respectively). Adipose zinc concentrations were significantly lower in mice fed the MF diet than mice fed the SO diet (Table 11). Although femur zinc concentrations and liver zinc concentrations were similar for LWT and HWT mice, adipose and pancreatic zinc concentrations were 1.5 fold and 1.2 fold lower in the HWT compared to the LWT mice (Table 13). As well, liver and pancreas weights were significantly greater in the HWT compared to the LWT mice (Table 14).

Leptin Correlations

Serum leptin correlated positively with final body weight, BMI (kg/m^2), weight gain over 16 weeks, epididymal fat pad weight, and serum glucose, and negatively with adipose zinc concentration. No correlations were found between serum leptin and serum insulin, serum zinc or pancreatic zinc concentrations (Table 15).

Urinary Glucose, Creatinine and Zinc Concentrations and Volume

No significant differences were found in urinary glucose, zinc, and creatinine

concentrations among experimental groups (Table 16) or between LWT and HWT mice (Tables 18). Urine excretion was significantly lower in the MF-fed mice compared to the SO-fed mice (Table 17).

Fatty Acid Profiles of Triglycerides in Epididymal Adipose Tissue

All fatty acid data will be presented as percentage composition of fatty acids. There was a significant main effect of dietary fat, but not dietary zinc, on triglyceride (TG) fatty acid composition of epididymal fat pads in C57BL/6J mice among the experimental groups (Tables 19 and 20). However, dietary zinc did have a main effect on the milligrams fatty acid per gram adipose tissue (mg/g) (605 ± 37 and 602 ± 28 mg/g for ZD and ZS vs 728 ± 28 mg/g for ZC group) (Table 19). Although the percentage of palmitic acid (C16:0) was higher in adipose TG from mice on the SO diet, stearic acid (C18:0) and oleic acid (C18:1 n-9) percentages were higher in adipose TG from mice on the MF diet (Table 21). The SO diet also resulted in significantly elevated percentages of adipose TG LA, LNA and the n-3 and n-6 LC PUFA (≥ 20 carbons) compared to the MF diet. No change was seen in total fatty acid (mg/g) of adipose TG in SO vs MF groups. The percentages of total saturated fatty acids (SAT), total monounsaturated fatty acids (MUFA) and the ratio of n-6/n-3 were higher in adipose TG from mice on the MF diet, whereas, percentages of total PUFA, total n-6, total n-3 and the ratio of PUFA/SAT ratio were higher in adipose TG from mice on the SO diet (Table 21).

Fatty acid triglyceride profiles were similar in the LWT and HWT groups of mice, although the HWT mice had greater amounts of stored fatty acid (mg/g)

compared to the LWT group (Table 22). The LWT and HWT groups were further subdivided into groups consuming the SO or MF diets (Table 23). The SO-fed LWT and SO-fed-HWT groups had elevated percentages of total PUFA, total n-6, total n-3, n-6/n-3 ratio and PUFA/SAT ratio, and depressed percentages of total SAT and total MUFA compared to the MF-fed LWT and MF-fed HWT groups (Table 23). There was a significant main effect of body weight on total MUFA and total n-6. In addition, there was a significant interaction of dietary fat x body weight, such that the SO-HWT mice had significantly lower percentages of total PUFA and n-6 than the SO-LWT mice (Table 23).

Fatty Acid Profiles of Phospholipid in Epididymal Adipose Tissue

There was a significant main effect of dietary fat, but not dietary zinc, on the fatty acid composition of adipose PL (Table 26). There were elevations in the percentages of palmitic acid, LA, LNA, docosahexaenoic acid (DHA, C22:6 n-3), total PUFA, total n-3 and total DHA + eicosapentaenoic acid (EPA, C20:5 n-3), whereas there were reductions in the percentages of stearic acid, oleic acid, arachidonic acid (AA, C20:4 n-6), and total MUFA in the SO-fed mice compared to the MF-fed mice (Table 26). However, quantities of total fatty acids in the PL component were similar in the SO and MF groups (Table 26). Ratios of PUFA/SAT and DHA/docosapentaenoic acid (DPA, C22:5 n-3) were elevated in mice fed the SO diet, and ratios of n-6/n-3 and AA/dihomo-δ-linolenic acid (DGLA, C20:3 n-6) were elevated in mice fed the MF diet (Table 26). There were no changes in percentages of EPA, docosapentanoic acid (DPA, C22:5 n-3), total SAT, and total

n-6 nor in the ratio of DGLA/LA.

The LWT mice had lower percentages of oleic acid and DPA, and higher percentages of DHA, total n-3 and total EPA + DHA compared to the HWT mice (Table 27). The ratio of n-6/n-3 was lower, whereas the ratio of DHA/DPA was higher in the LWT compared to the HWT group. The SO-fed LWT and SO-fed HWT groups had depressed total MUFA, but elevated total PUFA, total n-3 and total EPA + DHA percentages compared to the MF-fed groups (Table 28). No changes were seen in percentages for total SAT and total n-6, or in ratios for PUFA/SAT and DGLA/LA. The ratios of n-6/n-3 and AA/DGLA were lower in the LWT groups compared to the HWT groups. In addition, the MF-HWT mice had a lower ratio of DHA/DPA compared to the MF-LWT mice and SO-fed mice. No changes were seen for total fatty acids (mg/g) among the SO- and MF-fed LWT and the SO- and MF-fed HWT groups of mice.

Table 4. Characteristics of C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

Characteristics	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[3]		
							FAT	ZN[4]	FAT x ZN
Body Weight (g)	38.4 ± 1.1 (n=11)	39.3 ± 1.0 (n=16)	39.0 ± 1.4 (n=12)	39.8 ± 1.7 (n=11)	40.8 ± 1.1 (n=23)	39.2 ± 2.1 (n=11)	NS	NS	NS
BMI (kg/m^2)	4.36 ± 0.09 (n=11)	4.45 ± 0.11 (n=16)	4.34 ± 0.14 (n=12)	4.47 ± 0.15 (n=11)	4.52 ± 0.09 (n=23)	4.53 ± 0.22 (n=10)	NS	NS	NS
Epididymal Fat Pad Weight (g)	1.74 ± 0.1 (n=11)	1.88 ± 0.1 (n=16)	1.75 ± 0.2 (n=12)	2.11 ± 0.2 (n=11)	2.08 ± 0.1 (n=23)	1.97 ± 0.2 (n=11)	0.0148	NS	NS
Epididymal Fat Pad to Body Weight Ratio	4.5 ± 0.2[ab] (n=11)	4.8 ± 0.2[ab] (n=16)	4.4 ± 0.3[b] (n=12)	5.2 ± 0.2[a] (n=11)	5.1 ± 0.2[ab] (n=23)	4.9 ± 0.3[ab] (n=11)	0.0066	NS	NS
Caloric Intake (Kcal/d)	16.7 ± 0.3[b] (n=8)	15.9 ± 0.3[b] (n=15)	16.0 ± 0.4[b] (n=8)	16.7 ± 0.5[b] (n=8)	17.9 ± 0.3[a] (n=16)	18.4 ± 0.5[a] (n=7)	0.0001	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, HF-ZS = 55% of total calories from fat-zinc supplemented.
[3] F-values for main effects determined by two-way ANOVA. [4] ZN = zinc.

Table 5. Characteristics of C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1]

Characteristics	Soybean Oil (16% of Calories)	Mixed Fat[2] (55% of Calories)	F-Values[3]
Body Weight (g)	38.9 ± 0.7 (n=39)	40.2 ± 0.8 (n=45)	NS
BMI (kg/m²)	4.39 ± 0.07 (n=39)	4.51 ± 0.08 (n=44)	NS
Epididymal Fat Pad Weight (g)	1.80 ± 0.07 (n=39)	2.06 ± 0.07 (n=45)	0.0148
Epididymal Fat Pad to Body Weight Ratio	4.57 ± 0.13 (n=39)	5.08 ± 0.13 (n=45)	0.0066
Caloric Intake (Kcal/d)	16.2 ± 0.2 (n=40)	17.7 ± 0.2 (n=45)	0.0001

[1] Values are means ± SEM.
[2] Mixed Fat Diet = 16% of total calories from soybean oil and 39% of total calories from lard.
[3] F-values determined by ANOVA.

Table 6. Characteristics of Low and High Body Weight C57BL/6J Mice[1,2]

Characteristics	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[3]
Body Weight (g)	35.6 ± 0.5 (n=42)	43.7 ± 0.4 (n=42)	0.0001
BMI (kg/m^2)	4.15 ± 0.05 (n=42)	4.76 ± 0.06 (n=41)	0.0001
Epididymal Fat Pad Weight (g)	1.63 ± 0.07 (n=42)	2.25 ± 0.04 (n=42)	0.0001
Epididymal Fat Pad to Body Weight Ratio	4.5 ± 0.2 (n=42)	5.2 ± 0.1 (n=42)	0.0006
Caloric Intake (Kcal/d)	16.2 ± 0.2 (n=31)	17.6 ± 0.2 (n=31)	0.0001

[1] Values are means ± SEM.
[2] Low body weight mice weigh < 39.6 grams, high body weight mice weigh ≥ 39.6 grams.
[3] F-values determined by ANOVA.

Table 7. Serum Glucose, Insulin, Leptin and Zinc Concentrations of C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

Serum Concentrations	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[3] FAT	ZN[4]	FAT x ZN
Glucose (mmol/L)	8.67 ± 0.83 (n=10)	7.94 ± 0.82 (n=15)	9.71 ± 5.6 (n=12)	9.71 ± 0.49 (n=11)	9.26 ± 0.46 (n=22)	10.3 ± 0.81 (n=10)	NS	NS	NS
Insulin (ng/mL)	0.53 ± 0.12 (n=8)	0.96 ± 0.40 (n=11)	2.82 ± 2.34 (n=8)	1.92 ± 0.59 (n=8)	1.68 ± 0.87 (n=13)	0.78 ± 0.32 (n=7)	NS	NS	NS
Leptin (ng/mL)	20.0 ± 4.4 (n=9)	40.3 ± 14.1 (n=9)	27.9 ± 9.6 (n=8)	31.1 ± 10.7 (n=9)	42.7 ± 7.9 (n=8)	31.3 ± 10.3 (n=8)	NS	NS	NS
Zinc (µg/mL)	1.08 ± 0.09 (n=6)	1.14 ± 0.15 (n=10)	1.23 ± 0.20 (n=5)	1.04 ± 0.11 (n=8)	1.00 ± 0.09 (n=12)	1.18 ± 0.02 (n=6)	NS	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, HF-ZS = 55% of total calories from fat-zinc supplemented.
[3] F-values for main effects determined by two-way ANOVA.
[4] ZN = zinc.

Table 8. Serum Glucose, Insulin, Leptin and Zinc Concentrations of C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1]

Serum Concentrations	Soybean Oil (16% of Calories)	Mixed Fat[2] (55% of Calories)	F-Values[3]
Glucose (mmol/L)	8.71 ± 0.45 (n=37)	9.63 ± 0.33 (n=43)	NS
Insulin (ng/mL)	1.38 ± 0.71 (n=27)	1.52 ± 0.44 (n=28)	NS
Leptin (ng/mL)	29.5 ± 5.9 (n=26)	34.9 ± 5.6 (n=25)	NS
Zinc (μg/mL)	1.14 ± 0.08 (n=21)	1.05 ± 0.05 (n=26)	NS

[1] Values are means ± SEM.
[2] Mixed Fat Diet = 16% of total calories from soybean oil and 39% of total calories from lard.
[3] F-values determined by ANOVA.

Table 9. Serum Glucose, Insulin, Leptin and Zinc Concentrations of Low and High Body Weight C57BL/6J Mice[1,2]

Serum Concentrations	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[3]
Glucose (mmol/L)	8.48 ± 0.38 (n=39)	9.89 ± 0.37 (n=41)	0.0087
Insulin (ng/mL)	1.32 ± 0.627 (n=31)	1.62 ± 0.49 (n=24)	NS
Leptin (ng/mL)	18.1 ± 4.0 (n=23)	43.6 ± 5.8 (n=28)	0.0011
Zinc (μg/mL)	1.10 ± 0.09 (n=21)	1.09 ± 0.05 (n=26)	NS

[1] Values are means ± SEM.
[2] Low body weight mice weigh < 39.6 grams, high body weight mice weigh ≥ 39.6 grams.
[3] F-values determined by ANOVA.

Table 10. Tissue Zinc Concentrations in C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

Tissue Zinc Concentrations[5]	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[3]		
							FAT	ZN[4]	FAT x ZN
Femur (µg/g)	177 ± 6 ^{c} (n=11)	195 ± 4 ^{b} (n=16)	214 ± 4 ^{a} (n=11)	168 ± 4 ^{c} (n=11)	198 ± 3 ^{b} (n=22)	227 ± 11 ^{a} (n=10)	NS	0.0001	NS
Adipose (µg/g)	19.6 ± 2.1 ^{a} (n=9)	14.1 ± 1.3 ^{ab} (n=9)	13.9 ± 2.4 ^{ab} (n=9)	13.3 ± 2.6 ^{b} (n=9)	12.0 ± 1.8 ^{b} (n=9)	12.4 ± 1.1 ^{b} (n=9)	0.0456	NS	NS
Liver (µg/g)	92.0 ± 2.4 (n=11)	90.1 ± 2.5 (n=11)	101 ± 8 (n=11)	93.7 ± 3.8 (n=9)	94.5 ± 4.2 (n=10)	94.7 ± 4.0 (n=11)	NS	NS	NS
Pancreas (µg/g)	144 ± 8 (n=10)	143 ± 8 (n=10)	148 ± 10 (n=10)	132 ± 7 (n=10)	134 ± 7 (n=10)	150 ± 13 (n=10)	NS	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, HF-ZS = 55% of total calories from fat-zinc supplemented.
[3] F-values for main effects determined by ANOVA.
[4] ZN = zinc.
[5] Zinc concentrations based on dry weight of tissue.

Table 11. Tissue Zinc Concentrations in C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1]

Tissue Zinc Concentrations[2]	Soybean Oil (16% of Calories)	Mixed Fat[3] (55% of Calories)	F-Values[4]
Femur (μg/g)	195 ± 4 (n=38)	196 ± 4 (n=43)	NS
Adipose (μg/g)	15.8 ± 1.2 (n=27)	12.6 ± 1.1 (n=27)	0.0456
Liver (μg/g)	94.5 ± 2.9 (n=33)	94.4 ± 2.2 (n=30)	NS
Pancreas (μg/g)	145 ± 5 (n=30)	139 ± 6 (n=30)	NS

[1] Values are means ± SEM.
[2] Zinc concentrations based on dry weight of tissue.
[3] Mixed Fat Diet = 16% of total calories from soybean oil and 39% of total calories from lard.
[4] F-values determined by ANOVA.

Table 12. Liver and Pancreas Weights in C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

Organ Wet Weight	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[3]		
							FAT	ZN[4]	FAT x ZN
Liver (g)	1.36 ± 0.05 (n=11)	1.31 ± 0.06 (n=16)	1.40 ± 0.06 (n=12)	1.36 ± 0.07 (n=11)	1.43 ± 0.08 (n=23)	1.36 ± 0.07 (n=11)	NS	NS	NS
Pancreas (g)	0.16 ± 0.01 (n=11)	0.15 ± 0.01 (n=16)	0.14 ± 0.01 (n=12)	0.16 ± 0.01 (n=11)	0.17 ± 0.01 (n=23)	0.14 ± 0.01 (n=10)	NS	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, HF-ZS = 55% of total calories from fat-zinc supplemented.
[3] F-values for main effects determined by two-way ANOVA.
[4] ZN = zinc.

Table 13. Tissue Zinc Concentrations in Low and High Body Weight C57BL/6J Mice[1,2]

Tissue Zinc Concentrations[3]	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[4]
Femur (μg/g)	197 ± 4 (n=39)	196 ± 4 (n=41)	NS
Adipose (μg/g)	17.7 ± 1.5 (n=31)	11.8 ± 0.7 (n=24)	0.0003
Liver (μg/g)	97.9 ± 2.9 (n=23)	90.9 ± 2.1 (n=28)	NS
Pancreas (μg/g)	150 ± 5 (n=21)	133 ± 5 (n=26)	0.0200

[1] Values are means ± SEM.
[2] Low body weight mice weigh < 39.6 grams, high body weight mice weigh ≥ 39.6 grams.
[3] Zinc concentrations based on dry weight of tissue.
[4] F-values determined by ANOVA.

Table 14. Liver and Pancreas Weights in Low and High Body Weight C57BL/6J Mice[1,2]

Organ Wet Weight	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[3]
Liver (g)	1.20 ± 0.02 (n=42)	1.55 ± 0.04 (n=42)	0.0001
Pancreas (g)	0.14 ± 0.00 (n=42)	0.17 ± 0.01 (n=26)	0.0074

[1] Values are means ± SEM.
[2] Low body weight mice weigh < 39.6 grams, high body weight mice weigh ≥ 39.6 grams.
[3] F-values determined by ANOVA.

Table 15. Correlations Between Serum Leptin Concentrations and Other Parameters

Serum Leptin Concentration Versus	r value	P value
Final Body Weight (g, n=51)	0.62	0.0001
Body Weight Gain Over 16 Weeks (g, n=51)	0.59	0.0001
BMI (kg/m^2, n=49)	0.49	0.003
Epididymal Fat Pad Weight (g, n=51)	0.48	0.0004
Serum Glucose Concentration (mmol/L, n=51)	0.39	0.005
Serum Insulin Concentration (ng/ml, n=36)	0.07	NS
Serum Zinc Concentration (µg/ml, n=36)	-0.10	NS
Pancreatic Zinc Concentration (µg/g, n=40)	-0.13	NS
Adipose Zinc Concentration (µg/g, n=36)	-0.45	0.0056
Femur Zinc Concentration (µg/g, n=49)	0.11	NS

Table 16. Urinary Creatinine, Glucose and Zinc Concentrations, and Urine Volume of C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

Urinary Concentrations	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[3] FAT	ZN[4]	FAT x ZN
Creatinine (mg/12 h)	0.59 ± 0.09 (n=7)	0.48 ± 0.09 (n=15)	0.50 ± 0.09 (n=7)	0.47 ± 0.13 (n=6)	0.52 ± 0.06 (n=16)	0.37 ± 0.13 (n=8)	NS	NS	NS
Glucose (mg/12 h)	0.63 ± 0.13 (n=5)	0.53 ± 0.06 (n=11)	0.70 ± 0.11 (n=6)	0.56 ± 0.09 (n=6)	0.58 ± 0.08 (n=12)	0.78 ± 0.30 (n=6)	NS	NS	NS
Glucose/Creatinine (mg/mg)	0.95 ± 0.25 (n=5)	1.58 ± 0.54 (n=11)	1.33 ± 0.31 (n=5)	1.51 ± 0.31 (n=6)	1.05 ± 0.16 (n=11)	0.95 ± 0.64 (n=3)	NS	NS	NS
Zinc (μg/12 h)	0.74 ± 0.29 (n=7)	5.78 ± 3.47 (n=15)	1.53 ± 0.59 (n=7)	0.72 ± 0.22 (n=6)	3.52 ± 2.16 (n=17)	1.51 ± 1.04 (n=9)	NS	NS	NS
Zinc/Creatinine (μg/mg)	1.67 ± 0.75 (n=6)	9.32 ± 5.76 (n=15)	3.82 ± 2.19 (n=6)	1.70 ± 0.47 (n=6)	5.29 ± 3.26 (n=16)	1.57 ± 0.59 (n=7)	NS	NS	NS
Urine Volume (mL)	2.70 ± 0.56 [a] (n=6)	1.79 ± 0.21 [b] (n=15)	1.95 ± 0.13 [ab] (n=6)	1.58 ± 0.30 [b] (n=6)	1.87 ± 0.18 [ab] (n=16)	1.12 ± 0.21 [b] (n=7)	0.0103	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, HF-ZS = 55% of total calories from fat-zinc supplemented.
[3] F-values of main effects from diet means as determined obtained by ANOVA.
[4] ZN = zinc

Table 17. Urinary Creatinine, Glucose and Zinc Concentrations, and Urine Volume of C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1]

Urinary Zinc Concentrations	Soybean Oil (16% of Calories)	Mixed Fat[2] (55% of Calories)	F-Values[3]
Creatinine (mg/12 h)	0.51 ± 0.05 (n=29)	0.47 ± 0.05 (n=30)	NS
Glucose (mg/12 h)	0.60 ± 0.05 (n=22)	0.62 ± 0.08 (n=24)	NS
Glucose/Creatinine (mg/mg)	1.37 ± 0.30 (n=21)	1.18 ± 0.16 (n=20)	NS
Zinc (µg/12 h)	3.61 ± 1.89 (n=28)	2.43 ± 1.18 (n=32)	NS
Zinc/Creatinine (µg/mg)	6.40 ± 3.25 (n=27)	3.65 ± 1.81 (n=29)	NS
Urine Volume (mL)	2.03 ± 0.18 (n=27)	1.63 ± 0.14 (n=29)	0.0103

[1] Values are means ± SEM.
[2] Mixed Fat = 16% of total calories from soybean oil and 39% of total calories from lard.
[3] F-values determined by ANOVA.

Table 18. Urinary Creatinine, Glucose and Zinc Concentrations, and Urine Volume of Low and High Body Weight C57BL/6J Mice[1,2]

Urinary Zinc Concentrations	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[3]
Creatinine (mg/12 h)	0.47 ± 0.05 (n=29)	0.51 ± 0.05 (n=30)	NS
Glucose (mg/12 h)	0.55 ± 0.07 (n=20)	0.66 ± 0.07 (n=26)	NS
Glucose/Creatinine (mg/mg)	1.28 ± 0.34 (n=19)	1.27 ± 0.12 (n=22)	NS
Zinc (µg/12h)	3.78 ± 1.9 (n=28)	2.29 ± 1.16 (n=32)	NS
Zinc/Creatinine (µg/mg)	8.04 ± 3.68 (n=27)	2.12 ± 0.50 (n=29)	NS
Urine Volume (mL)	1.83 ± 0.21 (n=27)	1.81 ± 0.12 (n=27)	NS

[1] Values are means ± SEM.
[2] Low body weight mice weigh $<$ 39.6 grams, high body weight mice weigh $\geq$ 39.6 grams.
[3] F-values determined by ANOVA.

Table 19. Fatty Acid Profiles (%) of Epididymal Adipose Tissue Triglycerides for C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

FATTY ACID[3]	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[4] FAT	ZN[5]	FAT x ZN
Percentages:									
C16:0	17.4 ± 0.3 [a]	17.1 ± 0.2 [ab]	17.0 ± 0.3 [ab]	16.6 ± 0.2 [ab]	17.0 ± 0.4 [ab]	16.4 ± 0.2 [b]	0.0391	NS	NS
C18:0	1.50 ± 0.03 [c]	1.59 ± 0.03 [c]	1.53 ± 0.05 [c]	2.80 ± 0.16 [b]	3.11 ± 0.14 [a]	3.14 ± 0.09 [a]	0.0001	NS	NS
C18:1 n-9	30.9 ± 0.6 [b]	31.3 ± 0.3 [b]	31.0 ± 0.4 [b]	44.3 ± 0.3 [a]	44.3 ± 0.5 [a]	44.4 ± 0.2 [a]	0.0001	NS	NS
C18:2 n-6	33.2 ± 0.4 [a]	34.0 ± 0.5 [a]	34.0 ± 0.5 [a]	23.0 ± 0.2 [b]	22.9 ± 0.3 [b]	23.3 ± 0.2 [b]	0.0001	NS	NS
C18:3 n-3	2.75 ± 0.10 [a]	2.66 ± 0.04 [a]	2.69 ± 0.07 [a]	1.26 ± 0.05 [b]	1.26 ± 0.07 [b]	1.33 ± 0.03 [b]	0.0001	NS	NS
C20:3 n-6	0.23 ± 0.00 [a]	0.23 ± 0.01 [a]	0.22 ± 0.01 [a]	0.13 ± 0.00 [b]	0.14 ± 0.01 [b]	0.13 ± 0.01 [b]	0.0001	NS	NS
C20:4 n-6	0.35 ± 0.02 [a]	0.32 ± 0.01 [a]	0.32 ± 0.02 [a]	0.24 ± 0.02 [b]	0.24 ± 0.02 [b]	0.24 ± 0.01 [b]	0.0001	NS	NS
C22:5 n-3	0.09 ± 0.00 [a]	0.09 ± 0.00 [a]	0.09 ± 0.00 [a]	0.04 ± 0.01 [b]	0.04 ± 0.01 [b]	0.04 ± 0.01 [b]	0.0001	NS	NS
C22:6 n-3	0.19 ± 0.01 [a]	0.25 ± 0.07 [a]	0.18 ± 0.01 [a]	0.06 ± 0.02 [b]	0.07 ± 0.01 [b]	0.09 ± 0.01 [b]	0.0001	NS	NS
mg FA/g	602 ± 37	728 ± 23	623 ± 43	607 ± 67	729 ± 53	582 ± 38	NS	0.0123	NS

[1] Values are means ± SEM, n=7 per group. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, MF-ZS = 55% of total calories from fat-zinc supplemented.
[3] Carbon chain length : number of double bonds, n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g).
[4] F-values for main effects determined by ANOVA. [5] ZN = zinc.

Table 20. **Fatty Acid Profiles (% and Ratios) of Epididymal Adipose Tissue Triglycerides for C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]**

FATTY ACID[3]	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[4]		
							FAT	ZN[5]	FAT x ZN
Percentages:									
Total SAT	20.2 ± 0.3 [b]	20.0 ± 0.2 [b]	19.8 ± 0.4 [b]	20.7 ± 0.3 [a]	21.3 ± 0.5 [ab]	20.6 ± 0.3 [ab]	0.0041	NS	NS
Total MUFA	41.8 ± 0.6 [b]	41.3 ± 0.4 [b]	41.6 ± 0.6 [b]	53.6 ± 0.3 [a]	53.1 ± 0.4 [a]	53.1 ± 0.3 [a]	0.0001	NS	NS
Total PUFA	37.1 ± 0.5 [a]	37.8 ± 0.5 [a]	37.8 ± 0.4 [a]	25.1 ± 0.2 [b]	25.0 ± 0.4 [b]	25.4 ± 0.2 [b]	0.0001	NS	NS
Total n-6	34.0± 0.5 [a]	34.8 ± 0.5 [a]	23.8 ± 0.2 [a]	23.7 ± 0.3 [b]	23.6 ± 0.3 [b]	24.0 ± 0.2 [b]	0.0001	NS	NS
Total n-3	3.03 ± 0.11 [a]	3.00 ± 0.06 [a]	2.97± 0.07 [a]	1.37 ± 0.05 [b]	1.37 ± 0.08 [b]	1.45 ± 0.04 [b]	0.0001	NS	NS
Ratios:									
PUFA/SAT	1.84 ± 0.04 [a]	1.89 ± 0.04 [a]	1.91 ± 0.05 [a]	1.22 ± 0.03 [b]	1.18 ± 0.04 [b]	1.24 ± 0.02 [b]	0.0001	NS	NS
n-6/n-3	11.3 ± 0.4 [b]	11.6 ± 0.3 [b]	11.8 ± 0.3 [b]	17.6 ± 0.7 [a]	17.7 ± 1.3 [a]	16.6 ± 0.5 [a]	0.0001	NS	NS

[1] Values are means ± SEM, n=7 per group. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, MF-ZS = 55% of total calories from fat-zinc supplemented.
[3] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat; n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except for the ratios.
[4] F-values for main effects determined by ANOVA.
[5] ZN = zinc

Table 21. Fatty Acid Profiles (% and Ratios) of Epididymal Adipose Tissue Triglycerides for C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks

FATTY ACID[1,2]	Soybean Oil (16% of Calories)	Mixed Fat[3] (55% of Calories)	F-Values[4]
	(n=21)	(n=21)	
Percentages:			
C16:0	17.2 ± 0.2	16.7 ± 0.2	0.0391
C18:0	1.54 ± 0.02	3.02 ± 0.08	0.0001
C18:1 n-9	31.0 ± 0.2	44.3 ± 0.0	0.0001
C18:2 n-6	33.7 ± 0.3	23.1 ± 0.1	0.0001
C18:3 n-3	2.69 ± 0.04	1.28 ± 0.03	0.0001
C20:3 n-6	0.23 ± 0.00	0.14 ± 0.00	0.0001
C20:4 n-6	0.33 ± 0.01	0.24 ± 0.01	0.0001
C20:5 n-3	Not detectable	Not detectable	
C22:5 n-3	0.09 ± 0.00	0.04 ± 0.00	0.0001
C22:6 n-3	0.21 ± 0.02	0.07 ± 0.01	0.0001
mg FA/g	651 ± 22	639 ± 33	NS
Total SAT	20.0 ± 0.2	20.9 ± 0.2	0.0041
Total MUFA	41.6 ± 0.3	53.3 ± 0.2	0.0001
Total PUFA	37.5 ± 0.3	25.2 ± 0.2	0.0001
Total n-6	34.5 ± 0.3	23.8 ± 0.1	0.0001
Total n-3	3.00 ± 0.05	1.40 ± 0.04	0.0001
Ratios:			
PUFA/SAT	1.88 ± 0.02	1.21 ± 0.02	0.0001
n-6/n-3	11.6 ± 0.2	17.3 ± 0.5	0.0001

[1] Values are means ± SEM.
[2] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond; SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat. Fatty acids are expressed as % composition except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[3] Mixed Fat Diet = 16% of total calories from soybean oil and 39% total calories from lard.
[4] F-values determined by ANOVA.

Table 22. Fatty Acid Profiles (% and Ratios) of Epididymal Adipose Tissue Triglycerides for Low Body Weight Mice and High Body Weight Mice[1]

FATTY ACID[2,3]	Low Body Weight (LWT) Mice	High Body Weight (HWT) Mice	F-Values[4]
	(n=21)	(n=21)	
Percentages:			
C16:0	17.0 ± 0.2	16.9 ± 0.1	NS
C18:0	2.21 ± 0.19	2.35 ± 0.16	NS
C18:1 n-9	35.8 ± 1.4	39.5 ± 1.4	NS
C18:2 n-6	29.9 ± 1.2	26.9 ± 1.1	NS
C18:3 n-3	2.17 ± 0.15	1.82 ± 0.16	NS
C20:3 n-6	0.19 ± 0.01	0.17 ± 0.00	NS
C20:4 n-6	0.30 ± 0.01	0.28 ± 0.01	NS
C20:5 n-3	Not detectable	Not detectable	
C22:5 n-3	0.07 ± 0.01	0.06 ± 0.01	NS
C22:6 n-3	0.15 ± 0.01	0.13 ± 0.03	NS
mg FA/g	585 ± 26	704 ± 24	0.0018
Total SAT	20.4 ± 0.3	20.4 ± 0.2	NS
Total MUFA	45.6 ± 1.3	49.2 ± 1.3	NS
Total PUFA	33.1 ± 1.4	29.6 ± 1.3	NS
Total n-6	30.7 ± 1.3	27.6 ± 1.1	NS
Total n-3	2.39 ± 0.17	2.01 ± 0.19	NS
Ratios:			
PUFA:SAT	1.64 ± 0.08	1.46 ± 0.07	NS
n-6/n-3	13.6 ± 0.6	15.2 ± 0.9	NS

[1] Low body weight mice weigh < 39.6 grams, high body weight mice weigh ≥ 39.6 grams.
[2] Values are means ± SEM.
[3] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond; SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat. Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[4] F-values determined by ANOVA.

Table 23. Fatty Acid Profiles (% and Ratios) of Epididymal Adipose Tissue Triglycerides for Low Body Weight and High Body Weight Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1,2]

FATTY ACID[3,4]	SO-LWT	SO-HWT	MF-LWT	MF-HWT	F Values[5]		
					FAT	BWT[6]	FAT x BWT
	(n=13)	(n=8)	(n=8)	(n=13)			
Percentages:							
Total SAT	19.9 ± 0.2 ^{b}	20.2 ± 0.2 ^{b}	21.3 ± 0.5 ^{a}	20.6 ± 0.2 ^{ab}	0.0024	NS	NS
Total MUFA	41.2 ± 0.3 ^{b}	42.1 ± 0.5 ^{b}	52.8 ± 0.3 ^{a}	53.5 ± 0.3 ^{a}	0.0001	0.0193	NS
Total PUFA	38.0 ± 0.3 ^{a}	36.8 ± 0.3 ^{b}	25.2 ± 0.2 ^{c}	25.2 ± 0.2 ^{c}	0.0001	NS	0.0351
Total n-6	35.0 ± 0.3 ^{a}	33.7 ± 0.3 ^{b}	23.7 ± 0.2 ^{c}	23.8 ± 0.2 ^{c}	0.0001	0.0322	0.0110
Total n-3	2.98 ± 0.06 ^{a}	3.04 ± 0.08 ^{a}	1.44 ± 0.04 ^{b}	1.37 ± 0.05 ^{b}	0.0001	NS	NS
Ratios:							
PUFA/SAT	1.92 ± 0.03 ^{a}	1.82 ± 0.02 ^{b}	1.19 ± 0.03 ^{c}	1.23 ± 0.02 ^{c}	0.0001	NS	0.0251
n-6/n-3	11.8 ± 0.3 ^{b}	11.1 ± 0.2 ^{b}	16.6 ± 0.5 ^{a}	17.7 ± 0.8 ^{a}	0.0001	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] SO = soybean oil (16% of total calories) diet; MF = mixed fat (55% of total calories) diet; LWT = low weight mice (< 39.6 g); HWT = high weight mice (≥ 39.6 g).
[3] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat.
[4] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[5] F-values for main effects determined by ANOVA.
[6] BWT = body weight.

Table 24. Fatty Acid Profiles (%) of Phospholipid in Epididymal Adipose Tissue for C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

FATTY ACID[3]	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[4]		
							FAT	ZN[5]	FAT x ZN
C16:0	16.5 ± 0.4 [a]	16.6 ± 0.3 [a]	15.7 ± 0.3 [a]	13.7 ± 0.4 [b]	13.3 ± 0.3 [ab]	12.9 ± 0.2 [b]	0.0001	NS	NS
C18:0	17.3 ± 0.5 [c]	18.9 ± 0.5 [b]	17.9 ± 0.5 [bc]	21.2 ± 0.5 [a]	21.6 ± 0.4 [a]	22.7 ± 0.3 [a]	0.0001	NS	NS
C18:1 n-9	9.42 ± 0.33 [b]	8.88 ± 0.44 [b]	9.49 ± 0.42 [b]	12.4 ± 0.4 [a]	13.0 ± 0.4 [a]	12.8 ± 0.6 [a]	0.0001	NS	NS
C18:2 n-6	18.4 ± 0.5 [ab]	18.8 ± 0.6 [a]	19.4 ± 1.1 [a]	16.1 ± 0.9 [c]	16.4 ± 0.5 [bc]	18.0 ± 0.3 [abc]	0.0009	NS	NS
C18:3 n-3	0.41 ± 0.10 [a]	0.40 ± 0.10 [a]	0.47 ± 0.08 [a]	0.13 ± 0.02 [b]	0.13 ± 0.04 [b]	0.25 ± 0.06 [ab]	0.0001	NS	NS
C20:3 n-6	0.71 ± 0.02 [a]	0.69 ± 0.03 [ab]	0.68 ± 0.04 [ab]	0.62 ± 0.02 [ab]	0.63 ± 0.03 [ab]	0.60 ± 0.04 [b]	0.0038	NS	NS
C20:4 n-6	10.2 ± 0.3 [b]	9.7 ± 0.4 [b]	9.8 ± 0.8 [b]	13.0 ± 0.8 [a]	12.2 ± 0.6 [a]	11.1 ± 0.8 [ab]	0.0002	NS	NS
C20:5 n-3	0.31 ± 0.06	0.24 ± 0.04	0.37 ± 0.07	0.30 ± 0.04	0.25 ± 0.07	0.17 ± 0.03	NS	NS	NS
C22:5 n-3	0.51 ± 0.02	0.47 ± 0.03	0.51 ± 0.04	0.51 ± 0.04	0.49 ± 0.02	0.43 0.09	NS	NS	NS
C22:6 n-3	4.66 ± 0.16 [ab]	4.77 ± 0.16 [a]	4.88 ± 0.27 [a]	3.90 ± 0.47 [bc]	3.71 ± 0.24 [c]	3.32 ± 0.22 [c]	0.0001	NS	NS
mg FA/g	0.83 ± 0.04	1.01 ± 0.15	1.03 ± 0.11	1.21 ± 0.08	1.03 ± 0.14	1.13 ± 0.15	NS	NS	NS

[1] Values are means ± SEM, n=7 per group. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).

[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, MF-ZS = 55% of total calories from fat-zinc supplemented.

[3] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g).

[4] F-values for main effects determined by ANOVA.

[5] ZN = zinc

Table 25. Fatty Acid Profiles (% and Ratios) of Phospholipid in Epididymal Adipose Tissue for C57BL/6J Mice Fed Experimental Diets for 16 Weeks[1,2]

FATTY ACID[3]	SO-ZD	SO-ZC	SO-ZS	MF-ZD	MF-ZC	MF-ZS	F-Values[4]		
							FAT	ZN[5]	FAT x ZN
Percentages:									
Total SAT	39.3 ± 0.6	41.0 ± 0.6	39.4 ± 0.7	39.3 ± 0.5	39.7 ± 0.5	40.6 ± 0.5	NS	NS	NS
Total MUFA	16.3 ± 0.2 ^{b}	15.3 ± 0.5 ^{b}	16.1 ± 0.6 ^{b}	18.3 ± 0.5 ^{a}	18.7 ± 0.5 ^{a}	18.2 ± 0.7 ^{a}	0.0001	NS	NS
Total PUFA	36.9 ± 0.5 ^{ab}	36.8 ± 0.8 ^{ab}	37.9 ± 0.7 ^{a}	36.0 ± 0.6 ^{ab}	35.6 ± 0.9 ^{b}	35.2 ± 0.9 ^{b}	0.0010	NS	NS
Total n-6	30.4 ± 0.5	30.2 ± 0.7	31.0 ± 0.7	30.4 ± 0.5	30.2 ± 0.9	30.5 ± 0.7	NS	NS	NS
Total n-3	6.56 0.18 ^{a}	6.57 ± 0.17 ^{a}	6.97 ± 0.19 ^{a}	5.59 ± 0.39 ^{b}	5.37 ± 0.28 ^{bc}	4.72 ± 0.21 ^{c}	0.0001	NS	0.0341
ΣC22:6+C20:5	5.16 ± 0.17 ^{ab}	5.23 ± 0.19 ^{ab}	5.38 ± 0.28 ^{a}	4.41 ± 0.47 ^{bc}	4.19 ± 0.23 ^{c}	3.75 ± 0.25 ^{c}	0.0001	NS	NS
Ratios:									
PUFA/SAT	0.94 ± 0.02 ^{ab}	0.90 ± 0.03 ^{ab}	0.96 ± 0.03 ^{a}	0.92 ± 0.02 ^{ab}	0.90 ± 0.03 ^{ab}	0.87 ± 0.02 ^{b}	0.0467	NS	NS
n-6/n-3	4.65 ± 0.15 ^{c}	4.60 ± 0.12 ^{c}	4.47 ± 0.18 ^{c}	5.57 ± 0.33 ^{b}	5.75 ± 0.40 ^{b}	6.52 ± 0.26 ^{a}	0.0001	NS	NS
C20:3/C18:2	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	NS	NS	NS
C20:4/C20:3	14.4 ± 0.6 ^{b}	14.2 ± 0.5 ^{b}	14.4 ± 0.9 ^{b}	20.8 ± 1.3 ^{a}	19.8 ± 1.5 ^{a}	18.5 ± 1.0 ^{a}	0.0001	NS	NS
C22:6/C22:5	9.27 ± 0.46 ^{ab}	10.5 ± 0.5 ^{a}	9.90 ± 0.88 ^{ab}	7.95 ± 1.14 ^{b}	7.72 ± 0.73 ^{b}	7.95 ± 0.61 ^{b}	0.0028	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] Experimental diets were SO-ZD = 16% of total calories from soybean oil-zinc deficient, SO-ZC = 16% of total calories from soybean oil-zinc control, SO-ZS = 16% of calories from soybean oil-zinc supplemented, MF-ZD = 55% of total calories from mixed fat-zinc deficient, MF-ZC = 55% of total calories from mixed fat-zinc control, MF-ZS = 55% of total calories from fat-zinc supplemented; n=7 per group.
[3] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat; Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except ratios.
[4] F-values for main effects determined by ANOVA. [5] ZN = zinc

Table 26. Fatty Acid Profiles (% and Ratios) of Phospholipid in Epididymal Adipose Tissue for C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks

FATTY ACID[1,2,3]	Soybean Oil (16% of Calories)	Mixed Fat[4] (55% of Calories)	F-Values[5]
	(n=21)	(n=21)	
Percentages:			
C16:0	16.2 ± 0.2	13.3 ± 0.4	0.0001
C18:0	17.9 ± 0.3	21.8 ± 0.2	0.0001
C18:1 n-9	9.26 ± 0.5	12.7 ± 0.3	0.0001
C18:2 n-6	18.7 ± 0.6	16.8 ± 0.4	0.0009
C18:3 n-3	0.43 ± 0.05	0.17 ± 0.03	0.0001
C20:3 n-6	0.69 ± 0.04	0.62 ± 0.03	0.0038
C20:4 n-6	9.91 ± 0.3	12.1 ± 0.4	0.0002
C20:5 n-3	0.31 ± 0.03	0.24 ± 0.03	NS
C22:5 n-3	0.49 ± 0.02	0.48 ± 0.02	NS
C22:6 n-3	4.77 ± 0.110	3.64 ± 0.19	0.0001
mg FA/g	0.97 ± 0.37	1.12 ± 0.07	NS
Total SAT	39.9 ± 0.4	39.9 ± 0.3	NS
Total MUFA	15.9 ± 0.3	18.4 ± 0.3	0.0001
Total PUFA	37.2 ± 0.4	35.6 ± 0.4	0.0110
Total n-6	30.5 ± 0.4	30.4 ± 0.4	NS
Total n-3	6.70 ± 0.11	5.23 ± 0.19	0.0001
ΣC20:5 + C22:6	5.26 ± 0.12	4.12 ± 0.19	0.0001
Ratios:			
PUFA/SAT	0.94 ± 0.02	0.89 ± 0.01	0.0467
n-6/n-3	4.57 ± 0.09	5.59 ± 0.21	0.0001
C20:3/C18:2	0.04 ± 0.00	0.04 ± 0.00	NS
C20:4/20:3	14.4 ± 0.4	19.7 ± 0.7	0.0001
C22:6/C22:5	9.87 ± 0.37	7.87 ± 0.47	0.0028

[1] Values are means ± SEM.
[2] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond; SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat.
[3] Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[4] Mixed Fat Diet = 16% total calories from soybean oil and 39% of total calories from lard.
[5] F-values determined by ANOVA.

Table 27. Fatty Acid Profiles (% and Ratios) of Phospholipid in Epididymal Adipose Tissue for Low Body Weight Mice and High Body Weight C57BL/6J Mice[1,2]

FATTY ACID[3,4]	Low Body Weight (LWT) Mice	High Body Weight (HBT) Mice	F-Values[5]
	(n=21)	(n=21)	
Percentages:			
C16:0	14.8 ± 0.4	14.8 ± 0.4	NS
C18:0	19.6 ± 0.6	20.3 ± 0.5	NS
C18:1 n-9	10.3 ± 0.5	11.7 ± 0.4	0.0379
C18:2 n-6	18.1 ± 0.6	17.6 ± 0.3	NS
C18:3 n-3	0.29 ± 0.05	0.31 ± 0.06	NS
C20:3 n-6	0.64 ± 0.01	0.67 ± 0.02	NS
C20:4 n-6	10.5 ± 0.5	11.5 ± 0.3	NS
C20:5 n-3	0.30 ± 0.03	0.24 ± 0.03	NS
C22:5 n-3	0.46 ± 0.02	0.51 ± 0.02	0.0491
C22:6 n-3	4.53 ± 0.20	3.88 ± 0.18	0.0194
mg FA/g	1.11 ± 0.08	0.97 ± 0.06	NS
Total SAT	39.8 ± 0.4	39.9 ± 0.3	NS
Total MUFA	16.7 ± 0.4	17.7 ± 0.4	NS
Total PUFA	36.6 ± 0.5	36.3 ± 0.4	NS
Total n-6	30.2 ± 0.4	30.7 ± 0.4	NS
Total n-3	6.34 ± 0.22	5.59 ± 0.19	0.0139
ΣC20:5 + C22:6	4.98 ± 0.21	4.39 ± 0.18	0.0387
Ratios:			
PUFA/SAT	0.92 ± 0.02	0.91 ± 0.01	NS
n-6/n-3	4.89 ± 0.19	5.63 ± 0.22	0.0146
C20:3/C18:2	0.04 ± 0.00	0.04 ± 0.00	NS
C20:4/20:3	16.4 ± 0.79	17.6 ± 0.84	NS
C22:6/C22:5	10.0 ± 0.4	7.72 ± 0.4	0.0003

[1] Values are means ±SEM.
[2] Low body weight mice weigh < 39.6 grams; high body weight mice weigh ≥ 39.6 grams.
[3] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond.
[4] Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[5] F-values determined by ANOVA.

Table 28. Fatty Acid Profiles (% and Ratios) of Phospholipid in Epididymal Adipose Tissue for Low and High Body Weight C57BL/6J Mice Fed Soybean Oil (SO) or Mixed Fat (MF) Diets for 16 Weeks[1,2]

FATTY ACID[3,4]	SO-LWT	SO-HWT	MF-LWT	MF-HWT	F-Values[5]		
					FAT	BWT[6]	FAT x BWT
	(n=13)	(n=8)	(n=8)	(n=13)			
Percentages:							
Total Sat	39.5 ± 0.5	40.5 ± 0.5	40.3 ± 0.5	39.6 ± 0.4	NS	NS	NS
Total MUFA	15.8 ± 0.3^{b}	16.1 ± 0.5^{b}	18.1 ± 0.5^{a}	18.5 ± 0.4^{a}	0.0001	NS	NS
Total PUFA	37.3 ± 0.5	37.1 ± 0.5	35.4 ± 0.8	35.7 ± 0.6	0.0120	NS	NS
Total n-6	30.4 ± 0.5	30.7 ± 0.4	29.9 ± 0.5	30.7 ± 0.6	NS	NS	NS
Total n-3	6.87 ± 0.13^{a}	6.42 ± 0.13^{a}	5.48 ± 0.40^{b}	5.07 ± 0.18^{b}	0.0001	NS	NS
C20:5 + C22:6	5.32 ± 0.16^{a}	5.16 ± 0.20^{a}	4.44 ± 0.43^{b}	3.92 ± 0.17^{b}	0.0001	NS	NS
mg FA/g	1.01 ± 0.09	0.87 ± 0.08	1.27 ± 0.12	1.03 ± 0.08	NS	NS	NS
Ratios:							
PUFA/Sat	0.95 ± 0.02	0.92 ± 0.01	0.88 ± 0.02	0.90 ± 0.02	NS	NS	NS
n-6/n-3	4.44 ± 0.12^{b}	4.79 ± 0.09^{b}	5.62 ± 0.32^{a}	6.15 ± 0.26^{a}	0.0001	NS	NS
C20:3/C18:2	0.03 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.00	NS	NS	0.0482
C20:4/C20:3	14.7 ± 0.5^{b}	13.7 ± 0.4^{b}	19.2 ± 1.5^{a}	20.0 ± 0.8^{a}	0.0001	NS	NS
C22:6/22:5	10.4 ± 0.5^{a}	9.01 ± 0.45^{a}	9.41 ± 0.66^{a}	6.93 ± 0.49^{b}	0.0001	NS	NS

[1] Values are means ± SEM. Values with different superscript letters differ significantly as determined by Duncan's multiple range test ($P < 0.05$).
[2] SO = soybean oil (16% of total calories) diet; MF = mixed fat (55% of total calories) diet; LWT = low weight mice (< 39.6 g); HWT = high weight mice (≥ 39.6 g).
[3] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat.
[4] Carbon chain length : number of double bonds; n = number of carbons from the methyl end of the carbon chain to the first double bond. Fatty acids are expressed as % composition of fatty acids except for total milligrams of fatty acid per gram (mg FA/g) and the ratios.
[5] F-values for main effects determined by ANOVA.
[6] BWT = body weight.

V. Discussion

Overview of Study Observations

The study began with the intent of inducing obesity and diabetes in weanling C57BL/6J mice by altering the fatty acid composition and percentage of energy as fat in the diet. In addition, dietary intervention was initiated at an early age to determine if a marginal zinc deficiency exacerbates the diabetic condition and if dietary zinc supplementation prevents the onset of diabetes. Mice receiving the zinc deficient diets showed a reduction in femur zinc, a long-term indicator of dietary zinc status (Table 4). Mice receiving the zinc-supplemented diet had elevated femur zinc concentrations (Table 4). Otherwise, the different dietary zinc levels had no significant effects on body characteristics (Table 4), diabetic parameters (Tables 7 and 16) or fatty acid composition of adipose TG and PL (Tables 19, 20, 24 and 25).

Mice fed the MF diets had significantly greater epididymal fat pad weights, ratios of fat pad weight to body weight and caloric intakes compared to mice fed the SO diets (Table 5). Diabetic indices and tissue zinc concentrations were not different except for a lower urine excretion (Table 17) and a lower adipose zinc concentration (Table 11) in MF groups. The adipose tissue fatty acid composition for TG and, to a lesser extent, the PL, generally resembled the fatty acid composition of the diet for the SO and MF groups (Tables 21 and 26). Interesting differences between SO and MF groups were apparent in the adipose PL. Mice fed the SO diet had higher PL ratios of PUFA/SAT and DHA/DPA, while mice fed the

MF diet had higher PL ratios of n-6/n-3 and AA/DGLA (Table 26).

When the mice were dichotomized into LWT and HWT groups based on the median body weight (39.6 g), approximately half of the LWT and HWT mice had consumed the SO diet and the other half had consumed the MF diet. In addition, the HWT group had a higher body weight, BMI, fat pad weight, caloric intake (Table 6), elevated serum glucose and serum leptin concentrations (Table 9), and lower adipose and pancreatic zinc concentrations (Table 13) compared to the LWT group. The PL composition of adipose tissue from the HWT mice had a greater percentage of C18:1, DPA and ratio of n-6/n-3 with lower percentages of DHA, total n-3, total EPA + DHA and ratio of DHA/DPA compared to the PL composition of adipose tissue from the HWT mice (Table 27). The following sections will elaborate on these points.

Diet-Induced Obesity and Diabetes in C57BL/6J Mice

C57BL/6J mice are genetically predisposed to the development of diet-induced obesity and diabetes (West et al. 1992; Surwit et al. 1997; Rebuffe-Scrive et al. 1993; Van Heek et al. 1997). Forty-four percent of weanling C57BL/6J mice fed the SO diet containing 16% of calories from fat and 56% of weanling C57BL/6J mice fed the MF diet containing 55% of calories from fat became obese (body weight > 39.6 g and greater epididymal fat pad weights than mice in the SO diet), but did not become hyperglycemic (serum glucose > 12.5 mmol/L) (Surwit et al. 1997; Ikemoto et al. 1996; Luo et al. 1998). These diets were administered over a

period of 16 weeks. Previous studies have shown that C57BL/6J mice become obese and diabetic when fed diets containing 45% or 58% calories from fat are fed over a period of 14 to 20 weeks (Surwit et al. 1997; Surwit et al. 1995; Rebuffe-Scrive et al. 1993; Van Heek et al. 1997). Ikemoto et al. (1996) induced C57BL/6J mice to obesity and diabetes using several types of dietary fats at 60% calories as fat over a period of 19 weeks. The discrepancies in outcomes among these studies and the current study may arise from differences in experimental design.

In the current study, weanling C57BL/6J mice fed 16% and 55% calories as fat over 16 weeks had final body weights of 38.9 g and 40.2 g, and serum glucose concentrations of 8.71 mmol/L and 9.63 mmol/L, respectively (Table 5). Surwit et al. (1997) found that weanling mice on the diet containing 58% calories from fat weighed about 48 g compared to 30 g for the mice on the diet containing 10% calories from fat. These researchers used a more saturated fat (hydrogenated coconut oil and soybean oil containing about 85% SAT, 7% MUFA and 3% PUFA) that contributed to a considerably lower PUFA/SAT ratio compared to the MF diet in the current study (lard plus soybean oil containing 31% SAT, 37% MUFA and 27% PUFA) (Appendix 2). Several studies have associated diets high in saturated fatty acids with obesity and insulin resistance (elevated glucose and insulin responses after glucose tolerance tests) (Takeuchi et al. 1995; Young-Bum et al. 1996; Storlien et al. 1991).

In the study by Surwit et al. (1997), mice fed the diet with 58% calories as fat became hyperglycemic as evidenced by fasting plasma glucose concentrations well above 12.5 mmol/L. Coconut oil contains no n-3 fatty acids and is about 90%

saturated fatty acids (Grundy 1998). Hence, the coconut and soybean oil diet resulted in a considerably lower percentage of n-3 fatty acids and lower ratio of PUFA/SAT ratio compared to a lard and soybean oil diet (Appendix 2). Reductions in n-3 fatty acids and low PUFA/SAT ratios have been associated with weight gain, insulin resistance, and hyperglycemia in rodents (Hill et al. 1993; Field et al. 1989; Ikemoto et al. 1996). The differences in dietary fatty acid composition in the present study compared to Surwit et al. (1997) may be responsible for the dissimilarity in outcomes as compared to the current study.

Rebuffe-Scrive et al. (1993) fed weanling C57BL6J mice a diet with 45% calories as fat, primarily in the form of lard, and compared mice fed the lard diet to mice fed a chow diet with 10% kcal as fat. After 20 weeks, lard-fed mice weighed about 48 g compared to 28 g for chow-fed mice. Obesity is associated with accumulation of intra-abdominal fat in rodents (Hill et al. 1993). Visceral (intra-abdominal) fat pad to body weight ratio was greater in the lard-fed group compared to the chow-fed group (about 7.5 vs 2.1, respectively) and this finding paralleled observations in the current study (Tables 4 and 5). Plasma glucose concentrations rose to approximately 14 mmol/L in lard-fed mice compared to 10 mmol/L in chow-fed mice. The study was limited by the fact that the researchers did not clarify whether or not these were fasting or random values. However, the current study using 55% calories as fat was limited to 16 weeks, as opposed to 20 weeks in the study by Rebuffe-Scrive et al. (1993) using 45% calories as fat. It is possible that length of time on the diet is more important than total fat percentage when administering feeding regimes contributing these levels of dietary fat.

However, the type of fat is known to influence body weight, body fat distribution and diabetic indices (Hill at al. 1993; Field et al. 1989; Ikemoto et al. 1996). The addition of 16% calories as soybean oil to the MF diet containing 39% calories as lard may have modified the deleterious effects of the lard. For example, the n-6/n-3 and PUFA/SAT ratios for lard alone were 28 and 0.3 (Appendix 1) compared to 11 and 0.9, respectively, for the MF diet (Appendix 2). The lard diet used in the Rebuffe-Scrive study (1993) resulted in a higher n-6/n-3 ratio of 30 with a lower PUFA/SAT ratio of 0.23 compared to the MF diet. In addition, lard has a high proportion of MUFA (Appendix 1) that has been associated with inducing obesity and diabetes in C57BL/6J mice (Tsunoda at al. 1998). These differences in dietary fatty acid composition provided over a longer time frame may have more of an impact on the desired outcome than the total amount of fat provided in the diet over a shorter time frame. Therefore, a greater percentage of lard (45% as calories) in the Rebuffe-Scrive study (1993) compared to the percentage contained in the current study (39% as calories) may be more successful in promoting an obese state and diabetic traits.

In spite of the fact that caloric intakes were significantly higher in mice fed the MF diet (Table 5), only 56% of mice in the MF group became obese (>39.6 g, greater epididymal fat pad weights). Chang et al. (1990) have shown that there is considerable variability in weight gain of rats within the same strain when offered a diet high in fat content. Regardless, 44% of mice in the SO group ingesting a lower total caloric intake also became obese. These observations imply a strong genetic predisposition to obesity in half of the mice that was not identified in any of the

previous studies. Preliminary mapping studies have identified polygenic aberrations in the C57BL/6J strain that control body fat and make the strain sensitive to dietary obesity (West and York 1998). Therefore, it may be possible to induce obesity in C57BL/6J mice by offering a diet containing only 16% calories as fat. Hyperphagia is a common response to diets with a high fat content that produce a consistent increase in body fat content over time (West and York 1998). Sweet-tasting hydrated diets (such as a gel, paste or solution) promote hyperphagia during the first 4 to 5 weeks of ingestion compared to diets in powdered or pelleted form (West and York 1998). Although the percentage of sucrose was kept constant in the SO and MF diets (Table 2), this effect is reduced in diets containing larger amounts of fat (West and York 1998). Moreover, the study was limited by the fact that intake was calculated over the last 8 weeks and not over the entire current study period, which may have influenced the final estimated amount.

On the other hand, Ahren et al. (1997) fed female weanling C57BL/6J mice diets containing 58% calories as fat and 11% calories as fat. These diets resulted in body weights of about 30 g and 25 g and plasma glucose concentrations of 8 mmol/L and 6.5 mmol/L over 10 months of feeding. In view of the length of the feeding trial, the difference in weight for mice fed diets with 58% calories as fat compared to mice fed diets with 11% calories as fat was surprisingly low (5 g). These observations cast doubt on the reliability of the strain in producing obesity and diabetes by diet over an extended time frame.

Serum leptin and serum insulin concentrations reached 29.5 ng/ml and 34.9 ng/ml, and 1.38 ng/ml and 1.52 ng/ml (Table 8) in male mice fed the diets with 16%

and 55% calories from fat, respectively, by 16 weeks in the current study. These serum leptin and insulin concentrations were not significantly different. Surwit et al. (1997) reported that male mice fed diets containing 10% and 58% calories as fat attained plasma leptin and plasma insulin concentrations of about 20 ng/ml and 40 ng/ml, and 1.75 ng/ml and 5.94 ng/ml, respectively, by 14 weeks of feeding. Alternatively, female mice fed diets with 11% and 58% calories as fat over 10 months had plasma leptin and plasma glucose concentrations of about 14 ng/ml and 18 ng/ml, and 0.93 ng/ml and 1.75 ng/ml, respectively (Ahren et al. 1997). Female mice and humans are reported to have higher leptin concentrations at any given level of adiposity (Frederich et al. 1995; Havel et al. 1996). These studies highlight the variability in results in the same strain over a prolonged feeding period.

The significantly reduced adipose zinc concentrations in tissues of MF-fed mice compared to tissues of SO-fed mice (Table 10 and 11) could have meaningful implications. However, this observation must be interpreted with caution as it is based on ug zinc/g dry weight of adipose tissue and it does not take into account potential differences in adipocyte size, lipid content, or lipid to protein ratio of adipose tissue from HWT mice. Unfortunately, there is little information available regarding zinc concentrations in adipose tissue following long-term ingestion of diets containing large percentages of fat. Whether or not type of fat or amount of fat in the diet influences zinc concentrations in adipose tissue remains to be determined. It is unknown whether intestinal zinc absorption is altered when diets contain a high percentage of calories from fat. It is interesting to note that epididymal fat pad weights were elevated while adipose zinc concentrations were reduced, implying

that tissue expansion during obesity may require greater amounts of dietary zinc for growth and maintenance.

Although urinary glucose, creatinine, glucose/creatinine, zinc and zinc/creatinine concentrations were not influenced by diet, MF-fed mice excreted a lower volume of urine compared to SO-fed mice (Table 17). To date, no information is available on these urinary concentrations during dietary fat and zinc manipulation. A greater urine volume observed for mice fed the SO diets may be due to greater glycogen stores and their metabolism during fasting that would result in greater urine production. It is possible that inaccuracies in measurement, spillage and evaporation during the 24 hour collection in metabolic cages may have contributed to this observation. Further exploration on the effects of dietary fatty acid composition on kidney function may provide a more complete understanding.

Leptin Correlations

The present study found that leptin correlated positively with final body weight (r=0.62), BMI (r=0.49), body weight gain over 4 months (r=0.59), epididymal fat pad weight (r=0.48), and serum glucose concentrations (r=0.39), and correlated negatively with adipose zinc concentrations (r=0.45) (Table 15). No correlation between serum leptin and serum insulin concentrations was found in the present study.

Ahren at al. (1997) demonstrated a positive correlation with plasma leptin and body weight over 10 months (r=0.84, $p<0.001$) and plasma insulin (r=0.33, $p<0.001$), as well as log plasma leptin with intra-abdominal fat (r=0.90, $p<0.001$)

and serum glucose (r=0.39, p<0.001). One study demonstrated that leptin is directly involved in glucose metabolism independent of plasma insulin concentrations (Kamohara 1997). An earlier study conducted by Frederich et al. (1995) demonstrated that obesity-prone mice fed diets comprising 17% and 41% calories as fat had serum leptin levels (as determined by Western blotting) dramatically correlating with total body lipid (r=0.95) as determined by carcass analysis. Consequently, leptin correlations with serum concentrations and body fat appear to be highly variable in strength, suggesting other factors may be involved in leptin regulation. Additionally, age, sex, duration of feeding, measurement technique or experimental design may contribute to the variability in leptin correlations observed among the studies (Havel et al. 1996; Ahren et al. 1997).

The fact that leptin correlates negatively with adipose zinc concentrations suggests the hormone may play a role in the regulation of adipose zinc or that zinc in adipose tissue may influence leptin production. Leptin is a member of the cytokine family, identified by its crystalline structure (Friedman 1998), and cytokines are known to influence zinc metabolism although mechanisms remain to be determined (Cousins 1996). The relationship between adipose zinc and leptin has not been reported in previous studies. Indeed, mechanisms involved in this regulation were not investigated in this study and will require future examination. Studies in animals and humans have found an association between leptin and serum zinc or plasma zinc concentrations. Reduced leptin concentrations are found during zinc deficiency and increased leptin concentrations during zinc repletion in the presence of weight loss and weight stability (Mangian et al. 1998; Mantzaros et

al. 1998). These observations suggest that zinc and leptin may operate as integral components in homeostatic control.

In summary, the experiment was unable to replicate the model of diet-induced obesity and diabetes in C57BL/6J mice reported in the literature (West et al. 1992; Surwit et al. 1997; Rebuffe-Scrive et al. 1993; Van Heek et al. 1997). This was probably not due to an inadequate sample size (n=39 and n=45 for SO- and MF-fed mice, respectively, for body weight). A limitation of the experimental design for diet-induced obesity and diabetes in C57BL/6J mice is that both fatty acid composition and energy density are different in the SO and MF diets. This factor must be considered when interpreting observations for SO and MF groups. However, a post hoc analysis revealed several interesting differences between the LWT and HWT groups that were derived from mice fed the SO and MF diets over a 4 month period. The following section will elaborate on these results.

Characteristics of Low Body Weight and High Body Weight C57BL/6J Mice

When the mice were dichotomized into LWT and HWT groups derived from SO and MF groups, several interesting differences began to appear. Urinary concentrations of glucose, creatinine and zinc were similar (Table 18), but body weight, BMI, epididymal fat pad weight, fat pad to body ratio, and serum leptin concentrations were significantly elevated in the HWT group compared to the LWT group (Tables 6 and 9). These responses to diet were representative of the obese

state. Higher caloric intakes in HWT mice implied these mice ingested more energy than LWT mice (Table 6). Elevated serum glucose concentrations in the HWT group (Table 9), although not greater than 12.5 mmol/L, suggested a trend towards insulin resistance, hyperglycemia, and diabetes that could evolve into frank diabetes over a longer time frame with exposure to a permissive environment (diet and reduced physical activity). C57BL/6J mice are the background strain for *ob/ob* (obese) and *db/db* (diabetic) mice and they may be more susceptible to a permissive environment than other mouse strains (Weigle and Kuijper 1996).

Although femur and liver concentrations of zinc were unaltered, adipose and pancreatic tissues of HWT mice contained lower zinc concentrations (Table 13) and weighed more than those of LWT mice (Table 14). Tissue concentrations of zinc in hair and serum have been reported to be lower in obesity (Chen et al. 1988; Di Martino et al. 1993). Obesity implies increased body fat mass and possibly a greater need for basic physiological zinc requirements to replace endogenous loss (turnover of tissue and intestinal, urinary and pancreatic secretions) and to supply tissues during growth and maintenance (Cousins 1996). The possibility exists that the lower zinc concentrations may be the result of a dilution effect of more fat mass with the same zinc content or reduced absorption of zinc into the adipose tissue. The lower zinc concentrations may suggest that higher levels of zinc may be required in the obese state. Chen and associates (1996) found lower zinc concentrations in the carcasses of genetically obese (ob/ob) and obesity-prone mice fed diets high in fat (80% calories as fat). However, the study was limited by the fact that the diet contained no carbohydrate required for physiological growth in rodents (Chen et al.

1996). Further investigation into mechanisms and zinc requirements appear warranted.

Simon and Taylor (2000) demonstrated that *db/db* mice have lower pancreatic zinc concentrations than their lean counterparts and that zinc supplementation of *db/db* mice elevated pancreatic zinc concentrations to those of lean mice (Simon and Taylor 2000). The study by Begin-Heick et al. (1985) found that the femur and pancreas of the *ob/ob* mice contained less zinc, whereas the liver contained more zinc than the lean mice. Another study reported that *ob/ob* mice had lower zinc concentrations in liver, pancreas, muscle and femur compared to their lean controls (Kennedy et al. 1986). However, adipose zinc concentrations were not reported. Further examination revealed that the *ob/ob* mice retained a greater amount of subcutaneously injected zinc in the liver and adipose tissue but a lesser amount in muscle and bone than lean mice (Kennedy et al. 1986). These results demonstrate that genetically obese mice may have altered zinc metabolism and distribution that may be paralleled in obesity-prone mice.

An obese state may have been attributed to an interaction of genetic predisposition and environment in obesity-prone C57BL/6J mice. The HWT group developed traits that were characteristic of the obese state. Conflicting results from the previous studies indicate a need for controlled studies in identifying and characterizing the mechanisms in operation to further our understanding of dietary fat, obesity and obesity-related diseases.

Zinc Status and the Obese State

The initial goal of the study was to determine if zinc deficiency exacerbates and zinc supplementation attenuates DM2. Although the model did not produce diabetes, the diet intervention was successful for altering zinc status. A marginal zinc deficiency was induced over the 4 months in the ZD groups that resulted in reduced femur zinc concentrations (Table 10), an indicator of zinc nutriture. Bobilya et al. (1994) demonstrated that bone acts as a labile source of zinc by diverting plasma zinc to other organs during zinc depletion rather than incorporating plasma zinc into bone. Regardless, most fluctuations in dietary zinc supply are met by decreasing muscle synthesis and using the reserves from muscle where > 50% of body zinc is located (Golden 1989; Cousins 1989). A low zinc intake tends to increase efficiency of zinc absorption by increasing the transfer rate through the small intestine where dietary zinc is absorbed (Hoadley et al. 1987; Menard and Cousins 1983). This adaptation to low zinc intakes makes a zinc deficient state difficult to attain when zinc is provided at marginal levels. However, recent research indicates that a mild zinc deficiency may be common throughout the world and that the Western diet may not provide zinc at recommended levels (Mantzoros et al. 1998).

As expected, the ZS mice had significantly elevated femur zinc concentrations compared to ZC and ZD mice (Table 10) indicating higher zinc stores. There were no differences in adipose, liver, or pancreatic zinc tissue concentrations, indicating that the levels of dietary zinc were not severe enough or restricted over a long enough time frame to affect the labile exchangeable zinc pool

and stored tissue reserves (Miller et al. 1994; King 1990). Of these tissues, the pancreas and liver are most sensitive to zinc status and represent the organs most likely to be affected by zinc intake (Cousins 1996).

The first body response to a primary nutritional deficiency of zinc is anorexia and reduced growth without any initial evidence of reduced tissue concentrations (Cousins 1996). In the present study, the dietary zinc interventions had no effect on body weight, BMI, epididymal fat pad weight, pad to body weight ratio and caloric intake (Table 4), and urinary concentrations of glucose, creatinine and zinc (Table 16), nor serum concentrations of glucose, insulin, leptin and zinc (Table 7). These observations may not be entirely unexpected given other outcomes in previous studies and the marginal zinc deficient diet in the present experiment. A previous study by the same laboratory found that the lean counterparts, unlike the *db/db* mice, had urinary glucose, creatinine and zinc concentrations and serum glucose and insulin concentrations that were unaffected by zinc depletion (3 ppm) or supplementation (300 ppm) for 6 weeks (Simon and Taylor 2000).

Plasma zinc concentrations are known to be an unreliable indicator of dietary zinc status and are influenced by other factors such as fasting and stress (Cousins 1996). Indeed, normal plasma or serum zinc concentrations could reflect transfer of zinc to other body tissues during moderate zinc depletion (King 1990). Dreosti et al. (1986) reported that pregnant C57BL/6J mice do not become anorexic when fed low zinc diets (5 ppm) but their offspring develop teratogenic complications. However, Lepage (1997) found that C57BL/6J mice became

anorexic and weighed less than control mice when fed a severely zinc restricted diet (1 ppm) for 4 weeks. This was not replicated in a later study using severe zinc restriction (Giesbrecht 2000). In these studies, serum zinc concentrations were lower in the ZD group but femur zinc concentrations may (Lepage 1997) or may not be lower (Giesbrecht 2000) compared to the ZC group. These observations suggest that C57BL/6J mice may be an unreliable model for zinc studies by producing an inconsistent tissue response to dietary intake of zinc.

Chen et al. (1996) fed weanling obesity-prone ICR mice diets with 10% calories as fat (control) or 80% calories as fat (HF) in combination with 3 levels of dietary zinc (4-6 ppm, zinc adequate, and 200 ppm) over 6 weeks. The HF mice fed a marginal zinc intake (4-6 ppm) had lower zinc concentrations in their serum and carcass, and higher body fat content than their respective lean controls receiving the same zinc treatment (Chen et al. 1996). The diet containing 80% fat did not include any carbohydrate in its composition and so did not meet requirements for the basic physiological needs of rodent growth. Unfortunately, an insufficient diet during early development could produce abnormal metabolism and confound results putting into question the observations found in this study.

Indeed, several studies have demonstrated a reduction in plasma zinc concentrations when zinc is severely (<1 ppm) but not moderately (3-6 ppm) restricted (Gordon et al. 1982; Cousins 1989; Kauwell et al. 1995). Nevertheless, Cunnane et al. (1988) restricted the zinc intake (3-4 ppm) of young Sprague-Dawley male rats for 10 weeks. The rats experienced reduced body weight, reduced weight gain and depressed plasma zinc concentrations compared to rats fed a zinc

adequate (34 ppm) or supplemented (411 ppm) diet. These findings indicate that a marginal zinc deficient diet administered to growing rats over a prolonged period could deplete mobile pools of body zinc and depress plasma zinc concentrations. However, this may not be the case with marginal zinc deficiency in mice as indicated by normal serum zinc but lower femur zinc concentrations (Tables 7 and 10; Simon and Taylor 2000). Moreover, other factors such as age may complicate outcomes.

Obesity and Fatty Acid Composition in Adipose Tissue

Fatty acid composition was determined in adipose tissue, not adipocytes, and thus, the observations represent the fatty acid composition of all cell types in adipose tissue, including the vascular system.

Triglyceride Fatty Acid Composition

As expected, the type and amount of fat in the diet had a major effect on the fatty acid composition in adipose tissue TG (Tables 19-23). Triglycerides are storage compartments for excess fatty acids, most of which are derived from the diet (Grundy 1997). In this study and others, adipose tissue composition basically reflects the fatty acid composition of the diet (Appendix 2) (Field et al. 1985; Field et al. 1989). These stored fatty acids can later be used as a fuel or for other cell functions, depending on the body's requirements (Grundy 1997).

The most notable exception to reflecting the fatty acid composition of the

diet was a greater percentage of stored palmitic acid (C16:0) in TG from mice fed the SO diet compared to mice fed the MF diet (Table 21), which did not mirror the percentage in the diets (Appendix 2). The greater percentage of TG palmitic acid in mice fed the SO diet likely represented the end product of endogenous de novo fatty acid production (Jones and Kubow 1998). By comparison, the lower percentage of stored palmitic acid in the MF-fed mice was likely the result of enhanced oxidation of palmitic acid or accelerated elongation of palmitic acid to stearic acid and desaturation of stearic acid to oleic acid (Leyton 1986; Brenner 1989). Desaturase-elongation fatty acid metabolism is known to lengthen and shorten carbon chains in the tissues of several living species (Sprecher 1999; Cho et al. 1998). Interestingly, the oleic acid was higher in adipose TG from mice fed the MF diet compared to mice fed the SO diet (Table 21), although oleic acid percentages were lower in the respective diets (Appendix 2). Once again, these observations probably reflected elongation-desaturase activity with subsequent esterification and storage into TG in both groups.

Likewise, differences in proportions of LA, LNA, and the LC PUFA between the adipose TG and the diet were in part due to the desaturase-elongation pathway (Figure 3). LA and LNA are precusors for LC PUFA of the desaturase-elongase pathway (Brenner 1989). These LC PUFA are then subject to chain-shortening by partial β-oxidation to their precursors as required by the body (Sprecher 1995). However, a reduction in esterification of these fatty acids into the TG component could produce changes in the adipose TG fatty acid composition. In addition, studies examining oxidation of fatty acids have demonstrated that LA and LNA

represent major sources of fatty acids for energy purposes (Leyton et al. 1986; Cunnane and Anderson 1997). Variation in these systems due to dissimilar rates of activity or competition for enzymes (Brenner 1989; Sprecher 1995) could contribute to differences in availability of LC PUFA for storage between the SO- and MF-fed groups. Otherwise, the higher percentages of total SAT, total MUFA and a higher ratio of n-6/n-3, but lower percentages of total PUFA, total n-6 and total n-3 in TG of MF-fed mice compared to SO-fed mice (Table 21) generally reflected the fatty acid composition of the diet (Appendix 2).

Stored fatty acids in adipose tissue TG of LWT and HWT mice were remarkably similar with the exception of total fatty acids that were higher in tissues of HWT mice (Table 22). When Chang et al. (1990) looked at metabolic differences between obesity-prone and obesity-resistant rats they discovered that rats prone to obesity had a reduced capacity for fatty acid oxidation and a greater capacity for fatty acid storage. It is possible that the HWT mice have a greater propensity for fat storage as opposed to fat oxidation when confronted with diets composed of high proportions of fat. As previously reported, epididymal adipose tissue weighed significantly more in the HWT mice compared to the LWT mice (Table 4).

Adipose TG fatty aid composition in SO-LWT, SO-HWT, MF-LWT and MF-HWT groups generally resembled the SO and MF diets (Appendix 2) (significant main effect of fat in Table 23). In addition, both diet and body weight interacted to impact on total percentages of PUFA and total n-6 as well as the PUFA/SAT ratio. Further examination revealed that the SO-HWT mice had a lower percentage of PUFA and n-6 and lower PUFA/SAT ratio compared to the SO-LWT mice. These

observations suggest an alteration in fatty acid metabolism and storage in obesity-prone mice as proposed by Chang et al. (1990). However, mechanisms responsible for these variations remain to be established.

Phospholipid Fatty Acid Composition

Dietary fatty acids can alter the fatty acid composition of adipose tissue membrane, but structural lipids appear to be more resistant to modification by diet-induced changes (Zsigmond et al. 1990; Field et al. 1985). The fatty acid composition of adipose PL resembled the fatty acid pattern of the SO and MF diets to a limited extent (Tables 24, 25 and 28). Adipose PL contained a greater percentage of LC PUFA (number of carbons $\geq$ 20 carbons) than adipose TG that most likely resulted from greater metabolic activity and endogenous regulation (Zsigmond et al. 1990).

The elevated percentages of palmitic acid in adipose PL from SO-fed mice compared to MF-fed mice (Table 26) were likely in part the end product of endogenous de novo fatty acid synthesis (Jones and Kubow 1998). Palmitic acid can then be desaturated to oleic acid (Brenner 1989). Substantially reduced percentages of palmitic acid were paralleled by elevated percentages of oleic acid in MF-fed mice compared to SO-fed mice suggesting accelerated desaturation and elongation activity (Cooke and Spence 1987; Guesnet et al. 1990). Other mechanisms such as reduced esterification to membrane PL, enhanced oxidation, and suppressed de novo synthesis may also have been involved (Leyton et al. 1986; Brenner 1989). Early research demonstrated that the majority of de novo

synthesis takes place in adipose tissue that can be suppressed by high levels of dietary fats (Clarke et al. 1977; Romsos et al. 1974). However, Nelson et al. (1987) demonstrated that de novo fatty acid synthesis still continues in animals consuming diets high in fat. Triscari et al. (1985) showed an elevation of fatty acid synthesis when rats were given a high fat diet. As well, high intakes of saturated fatty acids have been shown to induce desaturase activity in the adipose tissue of mice and elevate concentrations of oleic acid (Field et al. 1989).

LA and LNA are essential fatty acids that can be endogenously produced to a minimal extent and, therefore, are required in the diet to avoid a deficiency (Cunnane et al. 1995). It is interesting to note that both LA and LNA percentages (Table 26) are substantially lower than the percentage contributed by the diet (Appendix 2). These observations indicate that incorporation of LA and LNA may be reduced or that LA and LNA may be oxidized at a rapid rate for energy purposes (MacDonald and Sprecher 1991; Leyton et al. 1986). Moreover, LA and LNA are competitive substrates for the desaturation-elongation n-3 and n-6 fatty acid metabolic pathway (Figure 3), possibly explaining a large percentage of their disappearance into LC PUFA (Brenner 1989). In particular, these mechanisms probably explain the lower percentages of LNA in membrane PL compared to LA because LNA is oxidized at a higher rate than LA (Cunnane and Anderson 1997) and is the preferred substrate for the rate-limiting Δ6 desaturase enzyme (Brenner 1989).

The presence of a relatively abundant percentage of AA in MF-fed mice and DHA in SO-FED mice (Table 26) suggests an accelerated desaturase-elongase

pathway from LA and LNA (Figure 3) (Holman 1998; Cooke and Spence 1987). Insulin is known to enhance delta 6 desaturase activity (Innis 1996). Although insulin concentrations were not significantly different for the SO and MF groups, elevations of insulin in mice fed the MF diet may have contributed to the accelerated pathway. Compared to PL from mice fed the SO diet, percentages of LA, LNA, DHA, $\sum$EPA + DHA and total n-3 fatty acids were reduced in PL of mice fed the MF diet. No differences were detected in total n-6 fatty acids between the groups. Oxidation of LC PUFA is considerably slower than LA and LNA (Leyton et al. 1986). In addition, the desaturase-elongase pathway has an affinity for fatty acids containing a greater number of double bonds and, therefore, favors LNA (C18:3 n-3) > LA (C18:2 n-6) (Brenner and Peluffo 1966; Cooke and Spence 1987). The variability in observations likely resulted from competition that exists between the n-6 and n-3 fatty acids for the desaturase enzymes (Brenner 1974; Budowski and Crawford 1985). The reductions in LC PUFA of the n-3 family in adipose PL of mice fed the MF diet suggest that precursors for these fatty acids may be preferentially oxidized or that esterification into PL may be reduced.

The higher percentage of AA in PL of mice fed the MF diet are expected to be associated with eicosanoid formation promoting pro-inflammatory, prothrombotic, and vasoconstrictive properties in metabolic functions (Simpoulos 1999). However, LC PUFA of the n-3 family antagonize these effects and are capable of reducing or ameliorating obesity-induced complications occurring in the cardiovascular system such as hypertension and arteriosclerosis (Simopoulos 1999; Grundy 1998). An elevated ratio of AA/DGLA in MF-fed mice may reflect enhanced desaturase

enzyme activity governing the production of AA (Figure 3) (Cooke and Spence 1987; Guesnet et al. 1990). Evidence of an accelerated desaturase enzyme is paralleled in the PL of SO-fed mice by an elevated ratio of DHA/DPA yielding higher percentages of DHA (Cooke and Spence 1987). Unlike the n-6 fatty acids, those of the n-3 family are known to have a beneficial influence in preventing the development of insulin resistance in rodents, despite the fact that the relationship to insulin action is as yet unclear (Storlien et al. 1988; Parrish et al. 1990). In this regard, Storlien et al. (1988) demonstrated that substituting only 6% of n-6 fatty acids with n-3 fatty acids prevented the insulin resistance associated with diets high in fat content.

Another possible mechanism involved in promoting a beneficial environment is altered membrane fluidity (Peluffo et al. 1976; Karp 1996) that occurs as a result of competitive desaturase activity to produce LC PUFA (Figure 3). Indeed, this may prove to be the most important cellular process in operation. Membrane fluidity increases with the number of double bonds located in unsaturated fatty acids that are incorporated into the phospholipid component by membrane-bound enzymes (Pelufo et al. 1976; Karp 1996). Changes in membrane fluidity influence movement of proteins within the lipid layer and could facilitate insulin reception and glucose transport (MacDonald and Sprecher 1991; Clandinin et al. 1985; Storlien et al. 1988; Storlien et al. 1991).

Furthermore, evidence indicates that a diet rich in saturated fatty acids may affect physiological properties of adipose tissue (Field et al. 1985). For example, adipose membrane with a high content of saturated fatty acids had reduced glucose

uptake and utilization, reduced insulin binding and receptor dysfunction (Awad 1981; Field et al. 1988; Field et al. 1989). This is likely the result of a paucity of double bonds in the lipid bilayer, restricting protein movement and function within the membrane (Karp 1996). Indeed, the PUFA/SAT ratio in MF-fed mice was lower than in SO-fed mice (Table 26). Although the difference was significant, it was also small, possibly indicating a propensity towards the development of metabolic disturbances associated with more saturated membranes.

Upon further examination, adipose PL in HWT mice were found to have a higher percentage of DPA and lower percentages of DHA, total n-3 and total DHA + EPA with a higher ratio of n-6/n-3 and lower ratio of DHA/DPA than adipose PL in HWT mice (Table 27). Overproduction of long chain n-6 fatty acids has been associated with deleterious eicosanoid production involved with obesity and insulin resistance, although mechanisms remain elusive (Storlien et al. 1988; Cunnane 1985). As previously mentioned, several authors have reported improved insulin binding, receptor function and reduced insulin resistance with the addition of n-3 fatty acids to the diet (Garg et al. 1988; Storlien et al. 1988; Storlien et al. 1991).

The reduced amounts of total n-3 fatty acids in HWT mice with no change in total n-6 fatty acids compared to LWT mice suggest alterations in enzyme activities involving desaturation, oxidation or incorporation of membrane PL. Competitive inhibition by a higher proportion of n-6 fatty acids (Table 27) may contribute to the lower amount of DHA in HWT mice compared to LWT mice (Jones and Kubow 1998). Alternatively, the reduced percentages of DHA in the HWT mice may in part result from different rates and activities of enzymes during

retroconversion of DPA to DHA involving a delta 6 desaturase enzyme and partial β-oxidation (Sprecher et al. 1995; Marzo et al. 1996). Notwithstanding, this study is limited by the fact enzyme activity, expression, degradation and oxidation rates were not examined.

In summary, the reduced amounts of n-3 fatty acids, higher ratios of n-6/n-3 fatty acids, and lower ratios of DHA/DPA and PUFA/SAT in adipose PL of HWT mice may contribute to altered cellular functions. These changes may ultimately lead to obesity-induced risk factors for diabetes and cardiovascular disease.

Zinc Status and Adipose Fatty Acid Composition

Zinc is thought to play a critical role in stabilizing biomembranes by binding to sulphydryl groups, in addition to its catalytic and structural role in enzymes and nucleic acids associated with the membrane (Golden 1989; O'Dell et al. 1987). A deficiency of zinc nutrition may cause oxidative stress, structural tension, altered activities of membrane-bound enzymes and dysfunction in membrane receptors (Golden 1989; O'Dell et al. 1987; Dreosti 1987). Several studies have investigated the role of zinc restriction on fatty acid composition of phospholipid, neutral lipid and total lipid in several tissues (Ayala and Brenner 1975; Cunnane et al. 1988; Cunnane et al. 1995). Other studies have used different types of dietary fats when inducing zinc deficiency (Eder and Kirchgessner 1994a; Eder and Kirchgessner 1994b), while some studies have examined the effects of zinc deficiency and supplementation on fatty acid composition (Clejan et al. 1982; Cunnane 1988). The

studies have concluded that zinc appears to have an effect on the desaturase enzymes, incorporation of fatty acids into membrane lipids and oxidation of fatty acids.

Although these studies reported an influence of zinc status on lipid composition, the current study was unable to parallel these observations. However, zinc status did have an effect on total milligrams of fatty acids per gram of adipose TG. Zinc deficient and ZS groups had significantly lower fatty acid concentration (mg/g) than the ZC group (605 ± 37 and 602 ± 28 mg/g vs. 728 ± 28 mg/g, respectively) (Table 19), although body weights, BMI, epididymal fat pads weights and caloric intakes were similar (Table 4). In contrast to some investigations of zinc deficiency, this study did not have differences in body weight as a confounding factor.

These observations are comparable to those in a 10 week study reported by Cunnane (1988), in which zinc control rats had higher adipose TG (mg/g) compared to zinc deficient and zinc supplemented groups. However, body weights were lower by 20% in the zinc deficient group than the zinc control and zinc supplemented groups. This observation implies different mechanisms may be responsible for lower adipose TG fatty acid concentration found in zinc deficient rats in the Cunnane study (1988) compared to ZD mice in the current study. Animal and human studies have demonstrated that zinc supplementation increases lean body mass while fat mass remains stable or decreases, whereas zinc depletion reduces lean body mass while fat mass remains stable or decreases (Mantzoros et al. 1998; Simon and Taylor 2000; Prescod et al.1997). These conclusions suggest that zinc

has differing impacts on muscle and adipose tissue involving regulatory mechanisms, which are dependent on dietary zinc status. It is possible that zinc status could influence the partitioning of fatty acids into stored tissue and oxidative pathways, or it could be an indirect effect of zinc on energy partitioning and muscle metabolism. Cunnane et al. (1993) demonstrated that a marginal zinc intake reduced whole-body accumulation of LC PUFA without altering food intake or body weight.

To date, this is the only study known to the author to compare changes in adipose fatty acid composition by manipulating dietary zinc in combination with an alteration of the dietary fatty acid content to induce obesity and diabetes in a nonmutant genetically susceptible rodent model.

VI. CONCLUSIONS

General Conclusions

- A MF diet with 55% calories as fat was unable to induce diabetes in C57BL/6J mice over a time frame of 16 weeks compared to a SO diet with 16% calories as fat.
- Fifty-six percent of MF-fed mice and 44% of SO-fed mice developed an obese state.
- The MF diet resulted in elevations of epididymal fat pad weight, fat pad to body weight ratio, caloric intake, and reductions in adipose zinc concentrations and urine excretion.
- HWT mice had elevations of body weight, BMI, epididymal fat pad weight, fat pad to body weight ratio, caloric intake, serum glucose concentrations, serum leptin concentrations, and liver and pancreas weights, and reductions in adipose and pancreatic zinc concentrations.
- Dietary zinc restriction resulted in reduced femur concentrations and dietary zinc supplementation resulted in elevated femur zinc concentrations.
- Serum leptin concentrations correlated positively with body weight, BMI, weight gain, epididymal fat pad weight and serum glucose concentrations, and negatively with adipose zinc concentrations.

Conclusions Regarding Adipose Tissue Triglyceride Fatty acid Composition

- Dietary zinc manipulation did not alter TG fatty acid composition but did result in reduced concentrations of total fatty acids (mg/g adipose tissue) in both ZD and ZS groups compared to the ZC group.
- Dietary fat influenced the fatty acid composition of adipose TG, generally reflecting the fatty acid composition of the SO and MF diets.
- HWT mice had a greater concentration of total fatty acids in adipose TG.
- Adipose TG in SO-HWT mice contained lower percentages of total PUFA and total n-6 than SO-LWT mice.

Conclusions Regarding Adipose Tissue Phospholipid Fatty Acid Composition

- The fatty acid composition of adipose PL reflected the diets to a limited extent.
- LC PUFA were present in greater percentages in PL than TG indicating a greater incorporation of LC PUFA into adipose PL than TG.
- Adipose PL from MF-fed mice contained lower percentages of palmitic acid, LA, LNA, DHA, total n-3 fatty acids and ratios of PUFA/SAT and DHA/DPA, but higher percentages of MUFA and ratios of n-6/n-3 and AA/DGLA compared to SO-fed mice.

• HWT mice had higher percentages of MUFA, DPA and ratio of n-6/n-3, but lower percentages of DHA, total n-3 fatty acids and ratio of DHA/DPA in adipose PL than LWT mice.

FUTURE RESEARCH

• More research is required regarding the specific molecular mechanisms by which zinc deficiency affects adipose zinc concentrations and adipose TG fatty acid concentrations.

• More research is needed to investigate the genetic differences in C57BL/6J mice leading to metabolic differences of LWT and HWT mice.

• More research is needed to investigate the impact of altering dietary fatty acid composition on the underlying mechanisms involved in fatty acid and zinc metabolism in obesity-prone and obesity-resistant rodents.

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Appendix 1. Fatty Acid Composition (%) of Soybean Oil and Lard Fats

Fatty Acid[1,2]	Soybean Oil	Lard
<u>Percentages:</u>		
C16:0	10.3	24.5
C18:0	4.0	14.5
C18:1 n-9	23.8	40.0
C18:2 n-6	52.0	11.6
C18:3 n-3	7.1	0.4
Total SAT	15.1	41.0
Total MUFA	23.9	44.2
Total PUFA	59.2	12.0
Total n-6	52.0	11.6
Total n-3	6.7	0.42
<u>Ratios:</u>		
PUFA/SAT	4	0.3
n-6/n-3	8	28

[1] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat.
[2] Carbon chain length : number of double bonds, n = number of carbons from the methyl end of the carbon chain to the first double bond.

Appendix 2. Fatty Acid Composition (% and Ratios) of Soybean Oil and Mixed Fat Diets Fed to C57BL/6J Mice over 16 Weeks

Fatty Acid[1,2,3]	Soybean Oil Diet	Mixed Fat Diet
	(16% of total calories from soybean oil)	(55% of total calories from mixed fat)
Percentages:		
C16:0	10.2	19.5
C18:0	4.3	11.4
C18:1 n-9	21.0	34.7
C18:2 n-6	53.9	24.4
C18:3 n-3	6.7	2.3
Total SAT	14.8	31.3
Total MUFA	21.2	36.5
Total PUFA	60.7	26.8
Total n-6	54.0	24.4
Total n-3	6.7	2.3
Ratios:		
PUFA/SAT	4	0.9
n-6/n-3	8	11

[1] SAT = saturated fat; MUFA = monounsaturated fat; PUFA = polyunsaturated fat.
[2] Carbon chain length : number of double bonds, n = number of carbons from the methyl end of the carbon chain to the first double bond.
[3] The diet contained minute amounts of long chain PUFA that are not included in data.