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Effects of dietary fat and zinc on adiposity, serum leptin and adipose fatty acid composition in C57BL/6J mice

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Abstract

Zinc (Zn) has been implicated in altered adipose metabolism, insulin resistance and obesity. The objective of this study was to investigate the effects dietary Zn deficiency and supplementation on adiposity, serum leptin and fatty acid composition of adipose triglycerides and phospholipid in C57BL/6J mice fed low-fat (LF) or high-fat (HF) diets for a 16 week period. Weanling C57BL/6J mice were fed LF (16% kcal from soybean oil) or HF (39% kcal from lard and 16% kcal from soybean oil) diets containing 3, 30 or 150 mg Zn/kg diet (ZD = Zn-deficient, ZC = Zn control and ZS = Zn-supplemented, respectively). HF-fed mice had higher fat pad weights and lower adipose Zn concentrations than the LF-fed mice. The ZD and ZS groups had a reduced content of fatty acids in adipose triglycerides compared to the ZC group, suggesting that zinc status may influence fatty acid accumulation in adipose tissue. Serum leptin concentration was positively correlated with body weight and body fat, and negatively correlated with adipose Zn concentration. Dietary fat, but not dietary Zn, altered the fatty acid composition of adipose tissue phospholipid and triglyceride despite differences in Zn status assessed by femur Zn concentrations. The fatty acid profile of adipose triglycerides generally reflected the diets. HF-fed mice had a higher percentage of C20:4 n-6, elevated ratio of n-6/n-3, lower ratio of PUFA/SAT and reduced percentage of total n-3 fatty acids in adipose phospholipid, a fatty acid profile associated with obesity-induced risks for insulin resistance and impaired glucose transport. In summary, the reduced adipose Zn concentrations in HF-fed mice and the negative correlation between serum leptin and adipose Zn concentrations support an interrelationship among obesity, leptin and Zn metabolism. © 2003 Elsevier Science Inc. All rights reserved.

Keywords: Zinc; Leptin; Obesity; Fatty acids; Adipose

1. Introduction

Zinc (Zn) has been implicated in altered adipose metabolism, insulin resistance and obesity. Dietary Zn deficiency in rats reduces glucose incorporation into fatty acids of epididymal fat pads [1] while in vitro addition of Zn enhances insulin-stimulated conversion of glucose into lipids of adipocytes from rats, and ob/ob and lean mice [2,3]. At the whole body level, Zn-deficient rats have significantly reduced body weight and carcass fat, and lower circulating leptin concentrations compared to pair-fed controls [4]. Furthermore, Zn-deficient rodents have diabetogenic traits such as impaired glucose tolerance, insulin resistance and impaired insulin signaling [5]. On the other hand, Zn supplementation improves glucose control in db/db and ob/ob mice [6,7]. In terms of adipose metabolism, one study reports that Zn supplementation may increase total carcass body fat in ob/ob mice and mice fed a high fat diet (80% fat/20% protein) [8]. However, fat pad weight is not adversely affected in db/db and lean mice when Zn-supplementation is combined with a low fat diet [6]. An interaction of Zn and fat stores has also been implicated in metallothionein (MT)-null mice as they are obese and have elevated plasma leptin and adipose leptin mRNA expression compared to age-matched controls [9]. Taken together, these observations raise questions about potential interactions among dietary Zn, dietary fat, adipose metabolism, leptin and insulin.

Although the effects of dietary Zn and dietary fat are usually investigated in separate studies, both nutrients are known to alter membrane and tissue fatty acid composition [10–13]. Altered fatty acid composition may influence sev-

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eral processes related to insulin resistance and obesity e.g. hormone binding, signal transduction, and availability of precursor molecules for lipid synthetic and catabolic pathways. Reductions in insulin receptor binding in adipocytes have been attributed to modification of cell membrane fatty acid composition by Zn deficiency and dietary fat [14,15]. Insulin-resistant states have been associated with a reduction in long chain polyunsaturated fatty acids, particularly arachidonic acid, in adipocyte membrane phospholipids [15, 16], and both Zn deficiency and Zn supplementation alter arachidonic acid in phospholipids from various tissues [10, 11]. Furthermore, Zn deficiency has been shown to alter metabolism of essential fatty acids towards increased β-oxidation and greater utilization of linoleic acid in de novo lipid synthesis [17] and this may affect the amount and distribution of fatty acids in fat stores.

The objective of this study was to investigate the effects dietary Zn deficiency and supplementation on adiposity, serum leptin and fatty acid composition of adipose triglycerides and phospholipid in C57BL/6J mice fed low-fat (LF) or high-fat (HF) diets for a 16 week period. C57BL/6J mice are considered an obesity-prone strain when fed diets high in fat for several weeks [18] and obesity is characterized by elevated circulating leptin, a hormone produced by adipocytes [19–20]. For the HF diet, the dietary fat content (55% calories from fat) and fat composition (39% calories from lard and 16% calories from soybean oil) was based on studies of diet-induced obesity in C57BL/6J mice that have reported presence of obesity, leptin and insulin resistance [19–20]. The diets also varied in amount of Zn: 3 mg Zn/kg diet (ZD) to produce a marginal Zn deficiency [6], 30 mg Zn/kg diet (ZC) to meet the dietary recommendation for mice [21], and 150 mg Zn/kg diet (ZS) to supplement the diet without incurring Zn toxicity or copper insufficiency [22].

2. Methods and materials

2.1. Animals and diet

Weanling C57BL/6J male mice were housed in individual stainless steel hanging cages and maintained in a controlled environment (55% humidity, 21–23°C, 14 h light/10 h dark cycle). During the 7 day acclimatization period, mice were fed the control diet (LF-ZC; 70 g soybean oil/kg diet and 30 mg Zn/kg diet). Then, mice were randomly assigned to low fat (LF) or high fat (HF) diets containing 3 mg Zn/kg (Zn-deficient, ZD), 30 mg Zn/kg (Zn-control, ZC) or 150 mg Zn/kg (Zn-supplemented, ZS) (Table 1) and fed ad libitum for 16 weeks. The LF diet provided 16% of calories from fat (70 g soybean oil/kg diet) and was based on the AIN-93G formulation [21] while the more energy-dense HF diet contained a contained a proportional amount of soybean oil (88 g) plus lard (209 g) and provided 55% of calories from fat (Table 2). The diet was offered in a paste consistency to minimize feed spillage and Zn-free deionized water was provided in polypropylene bottles with stainless steel sipper tubes to prevent Zn contamination. All animal care procedures were approved by the University of Manitoba Protocol Management and Review Committee.

Table 1
Diet formulation

Ingredients[3] (g/kg)	LF[1]	HF[2]
Soybean oil[4]	70	88
Lard[5]	0	209
Egg white	200	252
Cornstarch[6]	395	29
Maltodextrin	120	151
Sucrose	100	126
Cellulose	50	63
AIN-93M zinc-free mineral mix	35	44
KH_4PO_4[7]	5.4	7.0
Zinc premix[8]	10	13
AIN-93-VX vitamin mix	10	13
Choline bitartrate	2.5	3.0
Biotin premix[9]	2.0	2.6

[1] LF = low fat diet with 16% of calories from fat (3.9 kcal/g), [2] HF = high fat diet with 55% of calories from fat (4.9 kcal/g), [3] Diet ingredients purchased from Harlan Teklad (Madison, WI) unless otherwise indicated, [4] Vita Health, Winnipeg, MB, [5] HMS Foods, Winnipeg, MB, [6] Best Foods, Etobicoke, ON, [7] Addition of KH_2PO_4 (Fisher Scientific, Fair Lawn, NJ) to make mineral mix equivalent to AIN-93G, [8] Addition of zinc premix (5.775 g zinc carbonate/kg maltodextrin) as 1, 10 and 50 g/kg diet for the 3 (ZD), 30 (ZC) and 150 (ZS) mg Zn/kg diets, respectively, with corresponding adjustment of maltodextrin, [9] Addition of biotin premix (20 mg biotin/10 g maltodextrin) for adequate biotin in an egg white-based diet.

After 16 weeks, the mice were weighed and fasted overnight in polycarbonate metabolic cages (Nalgene, Fisher Scientific) to obtain urine specimens that were not contaminated by Zn from the feed nor environment. The mice were asphyxiated with CO_2. The length of the prone mouse body (nose tip to anus) was measured using a ruler. Trunk blood

Table 2
Fatty acid composition (% and ratios) of LF and HF diets

Fatty acid[1,2]	LF diet	HF diet
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	0.9
n-6/n-3	8.0	11.0

[1] Fatty acids >2% are shown.
[2] SAT = saturated, MUFA = monounsaturated, PUFA = polyunsaturated.

Table 3
Characteristics of C57BL/6J mice fed experimental diets for 16 weeks[1,2]

	LF-ZD	LF-ZC	LF-ZS	HF-ZD	HF-ZC	HF-ZS	F-values[3]		
							FAT	Zn	FAT × Zn
Body weight (g)	38.4 ± 1.1	39.3 ± 1.4	38.6 ± 1.5	39.8 ± 1.7	42.1 ± 1.1	40.2 ± 2.1	NS	NS	NS
Body mass index (kg/m^2)	4.4 ± 0.1	4.4 ± 0.1	4.3 ± 0.2	4.5 ± 0.2	4.6 ± 0.1	4.5 ± 0.2	NS	NS	NS
Femur zinc (μmol/g)[4]	2.70 ± 0.09^c	2.98 ± 0.06^b	3.27 ± 0.06^a	2.56 ± 0.06^c	3.02 ± 0.05^b	3.47 ± 0.17^a	NS	0.0001	NS
Epididymal fat pad weight (g)	1.74 ± 0.09	1.88 ± 0.09	1.72 ± 0.17	2.11 ± 0.16	2.10 ± 0.09	2.05 ± 0.19	0.0073	NS	NS
Epididmal fat pad to body weight ratio ($\times 10^{-2}$)	4.5 ± 0.2^{ab}	4.8 ± 0.2^{ab}	4.4 ± 0.3^b	5.2 ± 0.2^a	5.1 ± 0.2^{ab}	5.0 ± 0.3^{ab}	0.0076	NS	NS
Epididmal fat pad zinc (nmol/g)[4]	299 ± 32^a	215 ± 20^{ab}	212 ± 37^{ab}	203 ± 40^b	183 ± 27^b	189 ± 17^b	0.0456	NS	NS

[1] Experimental diets were low fat (LF) or high fat (HF) containing 3 (ZD), 30 (ZC) or 150 (ZS) mg Zn/kg diet.
[2] Values are means ± SEM, n = 10 (HF-ZS, 11 (LF-ZD), LF-ZS, HF-ZD) or 12 (LF-ZC, HF-ZC) except n = 9/group for epididymal fat pad zinc. Values with different superscript letters are significantly different as determined by Duncan's multiple range test ($P < 0.05$).
[3] F-values for main effects determined by two-way ANOVA.
[4] Dry weight of tissue.

was collected, centrifuged to obtain serum and stored at −80°C. Tissues were weighed, frozen in liquid nitrogen and stored at −80°C.

2.2. Biochemical analyses

Glucose and creatinine were assayed using an enzymatic colorimetric methods (Procedure #315 and #555, respectively, Sigma Chem., St. Louis, MO). Serum insulin and leptin were determined using radioimmunoassay kits (#SRI-13K and #ML-82K, Linco Research Inc., St. Charles, MO). Zn was analyzed by atomic absorption spectrophotometry (Varian Spectra AA-30 Spectrophotometer, Georgetown, ON) after dilution of serum, urine and acid-digested tissue samples [23].

2.3. Lipid analyses

Epididymal adipose lipids were extracted [24] in chloroform:methanol (2:1) containing 0.005% butylated hydroxytoluene. Tissues were homogenized with a Polytron Homogenizer and the solution was filtered through Whatman # 4 filter paper and washed with 0.73% sodium chloride. After vortexing and centrifuging, the bottom layers were rinsed twice with 1–2 ml of theoretical upper phase (chloroform:methanol:water, 3:48:47) and evaporated to dryness under nitrogen in a 30°C water bath. The lipid extracts were flushed with nitrogen and stored at −20°C.

Triglycerides and phospholipids were separated by thin layer chromatography using Whatman K8 Silica Gel 80A plates (Fisher Scientific, Nepean, ON) and petroleum ether: ethyl ether:glacial acetic acid (80:20:1) as the mobile phase [25]. The internal standards were triheptadecanoin (Nu-Check, Elysian, MN) and 1,2-dipentadecanoyl-sn-glycero-3-phosphocholine (Avanti, Alabaster, AL) for triglycerides and phospholipids, respectively. The plates were sprayed with 0.1% 8-anilino-1-napthalene-sulfonic acid (Sigma Chemical Co., St. Louis, MO) to identify lipids under a UV light. The lipids were methylated with 3N-methanolic hydrochloric acid (Supelco, Bellefonte, PA) for 3 hr (triglycerides) or 12 hr (phospholipids) at 80°C. 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.

2.4. Statistical analysis

Data were analyzed by a two-way ANOVA (SAS 6.04, SAS Institute, Cary, NC) and Duncan's multiple range test was used to determine significant differences between means. Pearson's Correlation Coefficient was used for correlation analyses. Significant differences were accepted at $P < 0.05$.

3. Results

After 16 weeks of dietary treatment, the body weights and body mass index of C57BL/6J mice were similar, regardless of dietary zinc or dietary fat and fatty acid composition (Table 3). Dietary zinc had a significant main effect on femur zinc concentrations. Femur zinc concentrations were higher in ZS mice compared to ZC mice and higher in ZC mice compared to ZD mice (3.36 ± 0.09 vs 3.01 ± 0.03 vs 2.64 ± 0.06 μmol/g, respectively). Mice fed the HF diets had greater epididymal fat pad weights (intra-abdominal fat) and ratios of fat pad weight to body weight compared to mice fed the LF diet. However, adipose tissue zinc concentrations were significantly lower in HF mice compared to LF mice (192 ± 17 vs 241 ± 18 nmol/g, respectively).

Serum glucose, insulin and leptin concentrations were not altered by the dietary treatments (Table 4). However, serum leptin concentration correlated positively with final body weight (r = 0.62, $p = 0.0001$), body mass index (r =

Table 4
Serum glucose, insulin and leptin of C57BL/6J mice fed experimental diets for 16 weeks[1]

Serum concentrations	LF groups	HF groups
Glucose (mmol/L)	8.71 ± 0.45 (n = 37)	9.63 ± 0.33 (n = 43)
Insulin (ng/mL)	1.38 ± 0.71 (n = 27)	1.52 ± 0.44 (n = 28)
Leptin (ng/mL)	29.5 ± 5.9 (n = 26)	34.9 ± 5.6 (n = 25)

[1] Values are means ± SEM. There were no significant main effects for fat level, zinc level, or fat × zinc interaction, thus, only means for low fat (LF) and high fat (HF) groups are shown.

0.49, $p = 0.003$), epididymal fat pad weight (0.48, $p = 0.0004$) and serum glucose (r = 0.39, $p = 0.005$). Serum leptin concentration correlated negatively with adipose zinc concentration (r = -0.45, $p = 0.0056$) but not with femur zinc concentration ($p > 0.05$). There were no differences in urinary creatinine, glucose or zinc excretion (data not shown).

Fatty acid profiles of triglycerides in epididymal adipose tissue are shown in Table 5. There was a significant main effect of dietary zinc on the amount of fatty acids per gram of adipose tissue. ZD and ZS mice (605 ± 37 and 602 ± 28 mg/g, respectively) had a lower fatty acid concentration than ZC mice (728 ± 28 mg/g). Otherwise, triglyceride fatty acid profiles were affected by dietary fat. Mice fed the LF diet (soybean oil) had higher percentages of palmitic acid (C16:0), linoleic acid (LA, C18:2 n-6), α-linolenic acid (LNA, C18:3 n-3), n-3 and n-6 long chain polyunsaturated fatty acids (LC PUFA, ≥ 20 carbons), total PUFA, total n-6, total n-3 and PUFA/SAT ratio in adipose triglycerides than mice fed the HF diet. Mice fed the HF diet (mixture of soybean oil and lard) had higher percentages of stearic acid (C18:0), oleic acid (C18:1 n-9), total saturated fatty acids (SAT), total monounsaturated fatty acids (MUFA) and n-6/n-3 ratio in adipose triglyderides than mice fed the LF diet.

In adipose tissue phospholipids, there was a significant main effect of dietary fat, but not dietary zinc, on fatty acid composition (Table 6). Mice fed the LF diet had elevated percentages of palmitic acid, LA, LNA, docosahexaenoic acid (DHA, C22:6 n-3), total PUFA and total n-3, and lower percentages of stearic acid, oleic acid, arachidonic acid (AA, C20:4 n-6), and total MUFA compared to the HF-fed mice. Ratios of PUFA/SAT and DHA/docosapentaenoic acid (DPA, C22:5 n-3) were elevated in mice fed the LF 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 HF diet. There were no changes in percentages of eicosapentaenoic acid (EPA, C20:5 n-3), docosapentanoic acid (DPA, C22:5 n-3), total SAT, and total n-6 nor in the ratio of DGLA/LA. Quantities of total fatty acids in the phospholipid component were similar in the LF and HF groups.

Table 5
Fatty acid profiles (% and ratios) of triglycerides in epididymal adipose tissue for C57BL/6J mice fed experimental diets for 16 weeks[1,2]

Fatty acid[3]	LF-ZD	LF-ZC	LF-ZS	HF-ZD	HF-ZC	HF-ZS	F-values[4]		
							FAT	Zn	FAT × 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
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}	33.8 ± 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
Amount:									
mg FA/g	602 ± 37	728 ± 23	623 ± 43	607 ± 67	729 ± 53	582 ± 38	NS	0.0123	NS

[1] Experimental diets were low fat (LF) or high fat (HF) containing 3 (ZD), 30 (ZC) or 150 (ZS) mg Zn/kg diet.
[2] Values are means ± SEM, n = 7/group. Values with different superscript letters ae significantly different as determined by Duncan's multiple range test ($P < 0.05$).
[3] Fatty acids are expressed as % composition of fatty acids except for ratios and total milligrams of fatty acid per gram (mg FA/g).
[4] F-values for main effects determined by two-way ANOVA.

Table 6
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]	LF-ZD	LF-ZC	LF-ZS	HF-ZD	HF-ZC	HF-ZS	F-values[4]		
							FAT	Zn	FAT × Zn
Percentages:									
C16:0	16.5 ± 0.4^{a}	16.6 ± 0.3^{a}	15.7 ± 0.3^{a}	13.7 ± 0.4^{a}	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
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
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/b-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	0.40 ± 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
Amount:									
mg FA/g	0.83 ± 0.04	1.01 ± 0.15	103 ± 0.11	1.21 ± 0.08	1.03 ± 0.14	1.13 ± 0.15	NS	NS	NS

[1] Experimental diets were low fat (LF) or high fat (HF) containing 3 (ZD), 30 (ZC) or 150 (ZS) mg Zn/kg diet.
[2] Values are means ± SEM, n = 7/group. Values with different superscript letters ae significantly different as determined by Duncan's multiple range test ($P < 0.05$).
[3] Fatty acids are expressed as % composition of fatty acids except for ratios and total milligrams of fatty acid per gram (mg FA/g).
[4] F-values for main effects determined by two-way ANOVA.

4. Discussion

The novel findings in this study were reduced adipose Zn concentrations in epididymal fat pads from HF mice and a negative correlation between serum leptin and fat pad Zn concentration. These results support the proposed role of Zn in leptin production. It has been demonstrated that Zn-deficient rats have lower serum leptin concentrations and less leptin secretion in vitro from adipocytes versus pair-fed rats [26] but this model is complicated by reduced growth and body weight. The present study suggests an association of serum leptin with adipose Zn concentration independent of body weight. The adipose Zn concentration represents Zn in the various cell types of adipose tissue, including adipocytes and the stromal vascular cells, and Zn as a component of numerous metalloproteins, including MT. Trayhurn et al [27] have shown that MT is expressed in the adipocytes, but not stromal vascular cells, of white adipose tissue. In contrast to other tissues, adipose MT is unchanged by Zn administration [27]. Although we did not measure adipose MT, we found that adipose Zn was not responsive to dietary Zn intake and not associated with femur Zn concentrations. Both Zn and MT are implicated in regulation of energy metabolism and antioxidant function [9,28,29]. It has been proposed that MT in adipose tissue may be providing antioxidant protection and protecting fatty acids from peroxidation [27]. Furthermore, obesity, hyperleptinemia and elevated leptin expression in adipose tissue of MT-null mice implies a relationship of Zn with leptin and energy regulation [9].

We used C57BL/6J mice because they are an obesity-prone strain [18] that has been used in research on diet-induced obesity. However, mice are more resistant to development of dietary Zn deficiency compared to other rodents [30] and most research on dietary Zn deficiency utilizes acute periods coinciding with high physiological demands due to growth, pregnancy, etc. Over longer periods of time, adaptation to a low Zn intake increases the efficiency of intestinal Zn absorption [31]. Thus, the results of the present study are unique to combination of species, dietary Zn levels, age of diet initiation and duration of dietary intervention, and they may not be applicable to acute dietary interventions limited to the rapidly growing stage. Although mice in the present study were fed many weeks beyond the rapid growth phase (from 4 to 20 weeks of age), there were significant differences in femur Zn concentrations, albeit of smaller magnitude than acute studies lasting 4–6 weeks [6,32]. The ZD mice fed the marginally deficient diet (3 mg Zn/kg diet) had reduced femur Zn concentrations, but they were not Zn-deficient based on similar growth rates

(data not shown) and final body weights as ZC mice, and the absence of clinical signs of deficiency. However, the results do provide evidence that long term consumption of a marginally Zn-deficient diet influences Zn stores in femur.

Dietary Zn supplementation in combination with the HF diet in this study (55% kcal from fat) did not alter body weight. The elevation of carcass fat in Zn-supplemented mice reported by Chen (1996) may be due in part to the composition of the diet (80% fat/20% protein) [8]. Mice fed the HF diet in this study had greater body fat but not body weight compared to LF mice (Table 3). Others have used diets with 45–55% kcal from fat, and lard or hydrogenated coconut oil in combination with soybean or sunflower oil to induce obesity in C57BL/6J mice over a period of 14–20 weeks [19,20,33,34]. In those studies, the differences in body weight have been variable, and this could be due to diet composition and/or variability in weight gain when animals within the same strain are offered a diet high in fat content [35]. In the present study, it is possible that the addition of soybean oil (16% kcal) to lard (39% kcal) in the HF diet modified the deleterious effects of the lard-based diet (45% kcal) as reported by Rebuffe-Scrive et al [19]. However, high fat (60% kcal) diets containing either soybean oil or lard are reported to produce obesity and glucose intolerance in C57BL/6J mice, and the combination of obesity and higher intake of linoleic acid was associated with impaired glucose tolerance [36].

Fatty acid composition of adipose tissue triglycerides and phospholipids (Tables 5 and 6) was not modified by Zn status when body weight was constant across the dietary groups (Table 3). When short-term marginal Zn deficiency is investigated in experimental models without changes in feed intake and body weight, the alterations in fatty acid composition are limited and measures of fatty acid oxidation and accumulation are more informative [37,38]. In the present study, the triglyceride fraction from ZD and ZS mice had significantly less mg fatty acids per gram of tissue than ZC mice and perhaps this represents effects of Zn status on oxidation versus storage of fatty acids. Similarly, Cunnane (1988) reported that rats fed Zn-deficient or Zn-supplemented diet had less fatty acids (mg/g) in liver triglycerides than rats fed control diet [11]. Otherwise, the adipose tissue fatty acid composition for triglycerides and, to a lesser extent, the phospholipids, generally resembled the fatty acid composition of the diet for the LF and HF groups (Tables 2, 5, and 6). Others have reported that adipose tissue fatty acid composition reflects the fatty acid composition of the diet and that structural lipids appear to be more resistant to diet modification [39–41]. However, HF-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 phospholipids compared to LF-fed mice. This fatty acid profile has characteristics associated with obesity-induced risks for insulin resistance and impaired glucose transport [16,42].

In summary, dietary fat altered body fat deposition and adipose tissue fatty acid composition while dietary Zn affected femur Zn concentrations and amount of fatty acid (mg/g) in adipose tissue triglycerides of young mice fed the dietary treatments for 16 weeks. The reduced adipose Zn concentrations in HF-fed mice and the negative correlation between serum leptin and adipose Zn concentrations support an interrelationship among obesity, leptin and Zn metabolism that requires further investigation.

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