Published in final edited form as:

Lipids. 2013 September ; 48(9): 939–948. doi:10.1007/s11745-013-3817-z.

STREPTOZOTOCIN-INDUCED DIABETES PARTIALLY ATTENUATES THE EFFECTS OF A HIGH-FAT DIET ON LIVER AND BRAIN FATTY ACID COMPOSITION IN MICE

Beth Levant^{1,3,*}, Marlies K. Ozias^{1,3}, Brianne L. Guilford^{2,3}, and Douglas E. Wright^{2,3}

¹Departments of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center Kansas City, KS 66160 USA

²Departments of Anatomy and Cell Biology, University of Kansas Medical Center Kansas City, KS 66160 USA

³Departments of Kansas Intellectual and Developmental Disabilities Research Center, University of Kansas Medical Center Kansas City, KS 66160 USA

Abstract

The current study addresses the effects of a high-fat diet on liver and brain fatty acid compositions and the interaction of that diet with diabetes in a type 1 mouse model. Adult, male, normal and streptozotocin-induced diabetic C57BL/6 mice were fed standard (14% kcals from fat) or high-fat (54% kcals from fat, hydrogenated vegetable shortening and corn oil) diets for 8 weeks. Liver and whole brain total phospholipid fatty acid compositions were then determined by TLC/GC. In the liver of non-diabetic mice, the high-fat diet increased the percentages of 18:1n-9, 20:4n-6, and 22:5n-6 and decreased 18:2n-6 and 22:6n-3. Diabetes increased 16:0 in liver, and decreased 18:1n-7 and 20:4n-6. The effects of the high-fat diet on liver phospholipids in diabetic mice were similar to those in non-diabetic mice, or were of smaller magnitude. In the brain, the high-fat diet increased 18:0 and 20:4n-6 of non-diabetic, but not diabetic mice. Brain 22:5n-6 acid was increased by the high-fat diet in both non-diabetic and diabetic mice, but this increase was smaller in diabetic mice. Diabetes alone did not alter the percentage of any individual fatty acid in brain. This indicates that the effects of a high-fat diet on liver and brain phospholipid fatty acid compositions are mitigated by concomitant hyperglycemia with hypoinsulinemia.

Keywords

High-fat diet; diabetes; streptozotocin; liver; brain; phospholipid

Introduction

A high-fat diet is associated with a variety of significant health concerns. Most notably, obesity and insulin resistance, key factors in metabolic syndrome, are associated with high intake of fats, particularly saturated fats, and the consequent high caloric intake [1-4]. Heart disease, kidney disease, and cancer are also associated with high dietary fat content [5-7]. In addition, epidemiologic evidence suggests diet may also affect Alzheimer's risk, with the Mediterranean diet conferring lower risk than the higher fat Western diet [8]. There is also a high comorbidity of depression with metabolic syndrome [9]. Thus, the effects of high

*To whom correspondence should be addressed. Department of Pharmacology, University of Kansas Medical Center, Mail Stop 1018, 3901 Rainbow Blvd., Kansas City, KS 66160, USA Phone: (913) 588-7527, FAX: (913) 588-7501, blevant@kumc.edu.

dietary fat intake have potentially serious health consequences for many organ systems including the liver and brain.

Diabetes, whether type 1 or type 2, is associated with variety of complications related to poor glycemic control including effects on the nervous system and liver. At least some of these complications, such as peripheral vascular disease and neuropathy, appear to be augmented by high dietary fat intake [10, 11]. Liver disease also appears to be a complication of both type 1 and type 2 diabetes [12-14]. In addition, the pathogenesis of Alzheimer's disease is associated with altered glucose utilization and insulin resistance in the brain [15]. In fact, metabolic syndrome and type 2 diabetes may represent independent risk factors for Alzheimer's disease [16]. Although type I diabetes is not as clearly associated with Alzheimer's disease, suboptimal cognitive function, including lower verbal and full scale IQ scores, is reported [17-19]. Decreases in white and gray matter are noted as early as 12 years after diagnosis [18]. Long-term consequences include type 1 diabetic encephalopathy, which includes loss of white and gray matter, temporal lobe sclerosis, synaptic alterations, increased microvascular permeability, neuron loss, and impaired cognitive function [20, 21]. In view of these effects of diabetes on liver and brain function, it is important to examine the interaction of a high-fat diet and diabetes on these organs.

Phospholipids form the lipid bilayer of cell membranes. The fatty acid composition of membrane phospholipids determines the physicochemical properties for the membrane. This influences cell function in variety ways including forming the microenvironment around membrane-bound proteins and altering the composition of lipid rafts, thus affecting their function [22, 23]. Some fatty acids, notably polyunsaturated fatty acids (PUFA) are cleaved from the membrane by phospholipases and act as signaling molecules, initiating gene transcription through nuclear receptors such as PPAR, RXR, and LXR [24]. In addition, PUFA such as arachidonic acid (ARA; 20:4n-6), eicosapentaenoic acid (EPA; 20:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) are precursors for a variety of pro- or anti-inflammatory mediators such as prostaglandins, leukotrienes, resolvins, protectins, and maresins [22, 25-30]. The fatty acid composition of membrane phospholipids varies depending on diet and physiologic state (e.g., pregnancy), and is also altered in disease states [31-36]. Accordingly, diet, physiological state, and disease may alter cellular function in numerous ways as a result of secondary changes in membrane fatty acid composition.

This study determined the effects of a high-fat diet and the interaction of that diet with increased blood glucose levels on liver and brain phospholipid fatty acid composition. Liver is examined because of its central role in lipid and glucose homeostasis and because of its relatively high rate of phospholipid turnover, makes it highly responsive to dietary and other manipulations [37-39]. Brain is examined because of its essential role in organismal function and because brain fatty acid composition is altered in neuropsychiatric disorders such as Alzheimer's disease and depression [34-36]. Hyperglycemia is examined independent of increased insulin levels using a mouse model of type 1 diabetes. It was hypothesized that a high fat diet would exacerbate the effect of diabetes. Contrary to our hypothesis we will show that the effects of a high-fat diet on liver and brain phospholipid fatty acid compositions are mitigated by concomitant hyperglycemia with hypoinsulinemia.

Methods

All experiments were performed in accordance the NIH Guide for the Care and Use of Laboratory Animals [40] and were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee (protocol #2010-1873).

Animals

Male C57Bl/6 mice (7 weeks old, n = 8 per group; Charles River, Wilmington, MA) were housed in a temperature- and humidity-controlled animal facility with a 12-hour dark-light cycle (on at 06:00), given food and water *ad libitum*. Mice were allowed to acclimate for 1 week prior to the experimental treatments.

Diets and diet treatment

The Control diet was a standard laboratory chow (Teklad 8604, Teklad, Madison, WI), which had 4.7% fat by weight and 14% kcal from fat, 32% kcal from protein, and 54% kcal from carbohydrate. The High-fat diet was Teklad TD.07011, which had 29% fat by weight and 54% kcal from fat (comprised of hydrogenated vegetable shortening and corn oil), 21% kcal from protein, and 24% kcal from carbohydrate). The fatty acid composition of the diets is presented in Table 1.

All mice were fed the Control diet during the acclimation week. Mice on the High-fat groups were fed the High-fat diet beginning 3 hrs after administration of streptozotocin (STZ). Mice in the Control diet groups continued to be fed the Control diet for the duration of the study.

Diabetes induction

Diabetes was induced by a single injection of STZ (180 mg/kg, ip, in 10 mM sodium citrate, pH 4.5, Sigma-Aldrich, St. Louis, MO). Mice in the non-diabetic groups were injected with vehicle (10 mM sodium citrate, pH 4.5, ip, 400 μ l). Blood glucose and body weight were monitored weekly beginning 1 week after STZ treatment (glucose diagnostic reagents, Sigma-Aldrich). Mice with blood glucose levels >300 mg/dL were considered diabetic. STZ-treated mice with blood glucose levels <300 mg/dL were excluded from the study.

Sample collection

At 8 weeks after STZ treatment, mice were deeply anesthetized with Avertin (1.25% v/v, ip, Sigma-Aldrich) and decapitated. Blood was collected into Eppendorf tubes for preparation of serum. Serum insulin and lipids were determined using commercial kits (Wako Diagnostics, Cayman Chemical, and Alpco). Liver samples (~100 mg) and brains were collected and frozen on dry ice for determine of fatty acid composition. Tissue samples were stored at -80° C until used.

Fatty acid analysis

Total phospholipid fatty acid composition was determined in liver samples and the left hemisphere of each brain as previously described [41]. Phospholipids were extracted and isolated by thin layer chromatography. The band containing total phospholipids was removed and transmethylated with boron trifluoride methanol (Sigma, St. Louis, MO) to yield fatty acid methyl esters. Individual fatty acid methyl esters were separated on a Varian 3400 gas chromatograph with an SP-2330 capillary column (30 m, Supelco, Inc., Belfonte, PA), with helium used as the carrier gas. The resulting peaks were identified and quantified on the basis of peak area by comparison to authentic standards (PUFA 1 and 2 and Supelco 37 FAME mix, Supelco, Inc. and 22:5n-6, Nu-Chek Prep, Elysian, MN), corrected for response factor, and expressed as %mol of total fatty acids.

Data Analysis

Results are presented as the mean $\pm$ SEM. Data are reported for the individual fatty acids detected in greater than trace amounts or are otherwise of interest. Data for classes of fatty acids include those detected in trace amounts. Data were analyzed for statistically significant

effects on each fatty acid measured by two-way ANOVA, with factors of diet (Control or High-fat) and Diabetes status (non-diabetic or diabetic). A significant ANOVA was followed by Tukey's test comparing all four groups (Systat, v12). An outlier identified by Systat (1 liver sample in the diabetic-Control diet group) was eliminated from the final analysis. Significance was assumed at $P < 0.05$.

Results

Effects of the diet and STZ treatments on body weight, and serum glucose, insulin, and lipids

Detailed data on the effects of these treatments across the 8-week observation period were previously reported [42], and are thus summarized briefly here. At the end of the 8 week treatment period, body weights were significantly different between all groups ($P < 0.05$), with non-diabetic mice fed the High-fat diet weighing 29% more than non-diabetic mice fed the Control diet. Diabetic mice fed the High-fat diet and diabetic mice fed the Control diet weighed 9% and 25% less than non-diabetic mice fed the Control diet. Serum insulin levels were decreased 90% and 71%, and serum glucose levels were increased 317% and 243%, respectively, compared to non-diabetic mice fed the Control diet. Non-diabetic mice fed the High-fat diet had increases in serum insulin and glucose concentration of 62% and 65%, respectively compared to non-diabetic mice fed the Control diet. Minor differences in total cholesterol and LDL-cholesterol were observed between groups; however, serum triacylglycerol was 400% higher in diabetic mice fed the High-fat diet than in non-diabetic mice fed the High-fat diet.

Effects of the diet and STZ treatments on liver phospholipid fatty acid composition

In non-diabetic mice, the High-fat diet increased the percentage of 18:1n-9 by 196% ($P < 0.001$), contributing to an increase in total monounsaturated fatty acids (MUFA) of 89% compared to those fed the Control diet ($P < 0.001$) (Table 2). The percentage of 20:4n-6 and 22:5n-6 also increased by 24% ($P < 0.001$) and 1005% ($P < 0.001$), respectively. Decreases were observed for 18:2n-6 (25%, $P < 0.001$), and 22:6n-3 (67%, $P < 0.001$), which contributed to a 69% decrease in total n-3 PUFA content ($P < 0.01$) and decreases in the DHA:ARA and n-3:n-6 ratios of 72-74% ($P < 0.001$). Total PUFA content was also decreased 17% ($P < 0.001$). In addition, the ratio of 18:3n-6 to 18:2n-6 was decreased 67% ($P < 0.001$). The ratio of 18:1n-9 to 18:0 was increased 174% ($P < 0.001$).

Diabetic mice fed the Control diet exhibited an 86% increase in the percentage of 16:0 ($P < 0.01$) and decreases in 18:1n-7 (64%, $P < 0.05$) and 20:4n-6 (18%, $P < 0.01$) compared to non-diabetic mice fed the Control diet. Together, these diabetes-induced changes in fatty acid composition contributed to increased total saturated fatty acids (SFA) (29%, $P < 0.01$), and decreases in total n-6 PUFA (14%, $P < 0.01$), total PUFA (11%, $P < 0.01$), and the PUFA:SFA ratio (32%, $P < 0.01$).

In diabetic mice fed the High-fat diet, the percentage of 20:4n-6 was increased 44% ($P < 0.001$) and 18% ($P < 0.01$) compared to diabetic and non-diabetic mice fed the Control diet, respectively, and was similar to that of non-diabetic mice fed the High-fat diet. The percentage of 22:5n-6 was 50% lower than in non-diabetic mice fed the High-fat diet ($P < 0.001$); however, the magnitude of the increase induced by the High-fat diet compared to diabetic mice fed the Control diet (970%, $P < 0.001$) was similar to the 10-fold increase produced by the High-fat diet in non-diabetic mice. In addition, the percentages of 18:1n-9 and total MUFA were increased by the High-fat diet in diabetic mice (87% and 68%, respectively, $P < 0.001$) compared to diabetic mice fed the control diet, but the magnitude of the increases produced by the High-fat diet were smaller than in non-diabetic mice fed the

High-fat diet (both $P < 0.01$). These changes in individual fatty acids resulted in an increase in total MUFA, and decreases in total n-3 PUFA, total PUFA, and the DHA:ARA and n-3:n-6 ratios; however, the magnitude of the changes in these classes of fatty acids were smaller than those produced by the High-fat diet in non-diabetic mice. Furthermore diabetic mice fed the High-fat diet had percentages of 16:0, total SFA, 18:1n-7, 18:2n-6, total n-6 PUFA, and PUFA:SFA ratio that were not different from non-diabetic mice fed the Control diet.

Compared to diabetic mice fed the Control diet, the ratio of 18:3n-6 to 18:2n-6 in diabetic mice fed the High-fat was decreased 50% ($P < 0.01$), similar to the effects of the High-fat diet in non-diabetic mice. The ratio of 20:4n-6 to 20:3n-6 was increased 63% ($P < 0.001$), again similar to the effect of the High-fat diet in non-diabetic mice. The ratio of 18:1n-9 to 18:0 was increased 56% ($P < 0.01$). This increase in the ratio of 18:1n-9 to 18:0 was significantly smaller than that produced by the High-fat diet in non-diabetic mice ($P < 0.0001$).

Effects of the diet and STZ treatments on brain phospholipid fatty acid composition

In non-diabetic mice, the High-fat diet increased 18:0 by 8% ($P < 0.05$) compared to those fed the Control diet (Table 3). The percentages of 20:4n-6 and 22:5n-6 increased 18% ($P < 0.01$) and 645% ($P < 0.001$), respectively, contributing to an increase in total n-6 PUFA content of 20% ($P > 0.001$). A significant main effect diet was also observed for 16:0, but post-hoc comparisons were not significant.

Diabetic mice fed the Control diet exhibited no changes in any of the major individual fatty acids in brain; however, these mice did exhibit a 12% increase in the percentage of total n-6 PUFA ($P < 0.05$) compared to non-diabetic mice fed the Control diet.

In diabetic mice fed the High-fat diet, 22:5n6 was increased roughly 250% compared to either diabetic or non-diabetic mice fed the Control diet ($P < 0.001$). This increase in 22:5n-6 was roughly half that produced by the High-fat diet in non-diabetic mice ($P < 0.001$). In contrast to its effects in non-diabetic mice, the High-fat diet produced no change in 18:0 compared to diabetic mice fed the Control diet. The percentage total n-6 PUFA was increased 12% ($P < 0.05$) compared to normal mice fed the Control diet; however, they were not different from those of diabetic mice fed the High-fat diet.

Discussion

The effects of a high-fat diet and its interactions with STZ-induced hyperglycemia with hypoinsulinemia on liver and brain total phospholipid fatty acid composition were examined in mice. The High-fat diet or diabetes produced a numerous changes in liver fatty acid composition and fewer alterations of smaller magnitude in brain fatty acid composition. Hyperglycemia with hypoinsulinemia partially attenuated the effects of a high-fat diet in both tissues.

Effects in the liver

In non-diabetic mice, the high-fat diet resulted in a number of alterations in hepatic phospholipid fatty acid composition (Table 2). Of perhaps greatest consequence were changes in PUFAs with known functional activities including a modest increase in ARA (21%) and a substantial decrease in DHA (68%). Docosapentaenoic acid (DPA n-6, 22:5n-6) was also dramatically increased (985%), most likely as a compensatory mechanism resulting from the change in DHA [43]. The net effect of these changes in individual fatty acids was significant changes in the n-3:n-6 and DHA:ARA ratios which have been previously shown to affect hepatic function (see below). The High-fat diet also decreased the ratio of 18:3n-3 to 18:2n-6, suggesting a decrease in $\Delta 6$ -desaturase activity. Insulin stimulates the activities

of both $\Delta 5$ - and $\Delta 6$ -desaturases by inducing expression of FADS1 and FADS2 [44, 45], suggesting that some insulin-resistance may be occurring in the mice fed the High-fat diet. In addition, the High-fat diet increased the ratio of 18:1n-9 to 18:0, consistent with increased stearoyl-CoA desaturase-1 activity. Expression of stearoyl-CoA desaturase-1 is stimulated by insulin, and increased expression and activity of the enzyme are also associated with insulin resistance [45-47]. These findings have both similarities and differences with other studies of the effects of high-fat diets in rodents. In a study in which pregnant and nursing rat dams were fed a high-fat diet (33% by weight lard-based), similar changes in DHA and ARA were observed in the pups at postnatal day 15. The dams also exhibited increases in the percentages of ARA and DPAn-6, however, hepatic DHA content was not decreased [48]. Another study in rats fed a linseed oil-based high-fat diet (13% by weight) for 20 days found decreases in both ARA and DHA in male rats compared to controls, whereas female rats fed the high-fat diet exhibited only a decrease in hepatic ARA content [49]. In contrast, yet another study in which rats were fed a 30% by weight coconut oil-based diet for 20 weeks resulted in decreased 16:0, 18:1 and ARA and increased 18:0, 18:2n-6, and DHA [50]. These findings suggest sex and species differences in the regulation of hepatic fatty acid composition, as well as differential effects of specific diets.

Streptozotocin-induced diabetes resulted in an increase in the percentage of 16:0 and a decrease in ARA in hepatic phospholipids. Diabetes also produced a main effect of increasing the ratio of 20:4n-6 to 20:3n-6, suggesting an increase in $\Delta 5$ -desaturase activity. Although the effects of STZ-induced diabetes on liver fatty acid composition varies between studies, decreased ARA content is highly consistent between studies and has also been reported in the Zucker rats and *ob/ob* mouse models of type 2 diabetes [48, 51-57]. Several of these studies with STZ-treated rats or *ob/ob* mice also found increases in the percentage of 18:0 and/or 18:1 and DHA [48, 51-53, 57], which were not observed in this study and may reflect species differences and differences between type 1 and type 2 diabetes models. Likewise, other studies indicate lower $\Delta 5$ - and $\Delta 6$ -desaturases and stearoyl-CoA desaturase-1 activities with reduced insulin levels [44-46], which were not observed in this study.

Feeding the high-fat diet in STZ-induced diabetic mice resulted in changes in hepatic fatty acid composition that were qualitatively similar to those observed in non-diabetic mice fed the high-diet; however, the magnitude of the changes was smaller for all affected fatty acids except ARA, as well as on the ratio of fatty acids indicative of stearoyl-CoA desaturase-1 activity. This suggests that in this model, the physiological effects of hypoinsulinemia, which results in effects such as excess glucagon secretion and increased production of glucose and lipid-derived ketones by the liver [58], attenuates at least some of the hepatic effects of the high-fat diet.

Variation in hepatic phospholipid composition affects liver function. In hepatocytes, changes in the ratio of ARA:DHA altered expression of genes encoding a variety of proteins including several cytochromes P-450 [59-61]. *In vivo*, altered relationship between ARA and DHA, induced by dietary manipulations, resulted in changes in hepatic expression of genes encoding numerous proteins such as cytochromes P-450 and peroxidases, and proteins involved in heme synthesis and utilization, bile acid metabolism, lipid synthesis and transport, and lipoprotein metabolism [62-65]. These changes in gene expression likely contribute to the changes in lipid metabolism and glucose regulation associated with consumption of a high-fat diet and/or diabetes. In addition, manipulation of total fat and n-3:n-6 ratios resulted in altered levels of a variety of eicosanoids including a variety of leukotrienes and prostacyclins [66]. Consistent with these observations, high dietary fat content was associated with hepatic inflammation in non-alcoholic fatty liver disease [67].

Thus, changes in liver fatty acid composition resulting most notably from consuming a high-fat diet likely affects numerous aspects of hepatic function.

Effects in the brain

Compared to the liver, there has been relatively limited investigation of the effects of a high-fat diet or diabetes on the fatty acid composition of the brain. Consistent with the slow response of the brain to dietary manipulations, which likely reflects the slower turnover of phospholipids in that tissue [39], the magnitude of the changes in fatty acid composition in brain were smaller than those observed in the liver after 8 weeks of dietary manipulation and/or experimentally-induced diabetes. Accordingly, future studies must determine whether additional changes in brain fatty acid composition or changes of greater magnitude might be observed with a longer duration of treatment.

In mice fed the high-fat diet, the percentages of ARA and DPAn-6 were increased, similar to the effect in liver (Table 3). In contrast to the liver, the percentage of DHA was not altered, resulting in no significant difference in n-3:n-6 ratio. Also in contrast with the liver, the high-fat diet resulted in an increase in 18:0 (8%) in the brain. Interestingly, these findings differ from those produced by a lard-based high-fat diet (19% by weight) fed from conception through 9 weeks of age in female C57BL/6j mice, which resulted in a decrease in brain DHA of roughly 50% and increases in 16:0 and 18:0 [68]. Thus, the effects of a high saturated fat diet may have different effects in the developing and mature brain.

In contrast to effects of the high-fat diet, STZ-induced diabetes did not alter the percentage of any of the abundant species of individual fatty acids in brain, though a small increase in total n-6 PUFA was detected (12%). A similar lack of effect has been reported in STZ-treated rats [55]. Likewise, no changes in brain fatty acid composition were observed in female Zucker rats [56]. However, other studies with STZ-induced diabetes in rats reported decreases in ARA [51-53], and one also observed a decrease in DHA [51]. Thus, as with the liver, these differences likely reflect species variation, as well as differences between diabetes models.

Also consistent with the effects observed in the liver, STZ-induced diabetes partially attenuated the effects of the High-fat diet on brain fatty acid composition. Notably, the only individual fatty acid that was significantly different between diabetic mice fed the Control and High-fat diets was DPAn-6. DPAn-6 was increased 273% in the diabetic mice fed the High-fat diet; however, this increase was significantly smaller than the 645% increase produced the High-fat diet in non-diabetic mice.

The changes in brain fatty acid composition induced by the high-fat diet have several potential functional consequences. Metabolic syndrome has been shown to have significant comorbidity with depression [9]. Consistent with the increased percentage of brain ARA observed in this study, high-fat diets have been shown to increase indices of inflammation in brain, an emerging component in the pathophysiology of depression [69], such as lipid peroxidation [70]. High-fat diets have also been shown to induce depression-like behavior in mice [71], and to impair hippocampal neurogenesis through a mechanism involving decreased hippocampal brain-derived neurotrophic factor [70, 72], both of which are observed in depressed patients [73]. Alterations in brain phospholipid fatty acid composition, notably decreased DHA, are also reported in depression [36, 74]. Decreases in tissue DHA content are typically associated with a concomitant increase in DPAn-6, which is thought to result from the compensatory substitution of 22-carbon n-6 PUFA for the 22-carbon n-3 PUFA [41, 43]. Interestingly, in this study, a dramatic increase in DPAn-6 content was observed in the absence of a change in DHA, raising the possibility that incorporation of DHA and DPAn-6 into phospholipids may be differentially regulated.

High-fat diets and insulin resistance are also associated with Alzheimer's disease [16]. In animal studies, high-fat diets have been shown to result in impaired cognitive function and decreased hippocampal plasticity [75-77]. Alzheimer's disease is also associated with altered brain fatty acid composition, most consistently decreased ARA (20:4n-6) and stearic acid (18:0) [34, 35]. The brain fatty acid changes observed in mice fed a high-fat diet and/or with STZ-induced diabetes did not recapitulate the changes observed in Alzheimer's disease. This suggests that the metabolic pathology in Alzheimer's disease cannot be attributed simply to high dietary fat intake, high blood glucose with hypoinsulinemia, or the interaction of these factors.

Conclusion—A high-fat diet or STZ-induced diabetes produced substantial changes in liver phospholipid fatty acid composition and smaller and more limited changes in brain fatty acid composition. These changes include increases in ARA, and may thus result in increased inflammatory tone. Interestingly, the effects of the high-fat diet were of smaller magnitude in diabetic mice compared to non-diabetic mice, suggesting that the apparently additive effects of a high-fat diet and diabetes on diabetic complications such as peripheral vascular disease and neuropathy, may not result from augmented changes in tissue fatty acid composition. Furthermore, the observed changes in brain do not recapitulate fatty acid alterations observed in depression or Alzheimer's disease.

Acknowledgments

The authors thank Janelle M. Ryals and Heather Spalding for expert technical and editorial assistance. Supported by the Juvenile Diabetes Research Foundation (DEW), and National Institutes of Health R01 NS43314 (DEW), P30 HD002528 (BL, DEW), P20 GM103418 from the Idea Network for Biomedical Research Excellence (INBRE) of the National Institute of General Medical Sciences (BL, DEW), and T32 HD057850 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (BLG).

Abbreviations

ANOVA	analysis of variance
ARA	arachidonic acid (20:4n-6)
Db	diabetes
DHA	docosahexaenoic acid (22:6n-3)
DPAn-6	docosapentaenoic acid (22:5n-6)
EPA	eicosapentaenoic acid (20:5n-3)
MUFA	monounsaturated fatty acid
NS	not significant
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
SEM	standard error of the mean
STZ	streptozotocin

References

1. Bremer AA, Mietus-Snyder M, Lustig RH. Toward a unifying hypothesis of metabolic syndrome. *Pediatrics*. 2012; 129:557–70. [PubMed: 22351884]

2. Thayer KA, Heindel JJ, Bucher JR, Gallo MA. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. *Environ Health Perspect.* 2012; 120:779–89. [PubMed: 22296744]
3. Kalupahana NS, Claycombe KJ, Moustaid-Moussa N. (n-3) Fatty acids alleviate adipose tissue inflammation and insulin resistance: mechanistic insights. *Adv Nutr.* 2011; 2:304–16. [PubMed: 22332072]
4. Coelho DF, Pereira-Lancha LO, Chaves DS, Diwan D, Ferraz R, Campos-Ferraz PL, et al. Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters. *Braz J Med Biol Res.* 2011; 44:966–72. [PubMed: 21876873]
5. Baum SJ, Kris-Etherton PM, Willett WC, Lichtenstein AH, Rudel LL, Maki KC, et al. Fatty acids in cardiovascular health and disease: a comprehensive update. *J Clin Lipidol.* 2012; 6:216–34. [PubMed: 22658146]
6. Odermatt A. The Western-style diet: a major risk factor for impaired kidney function and chronic kidney disease. *Am J Physiol Renal Physiol.* 2011; 301:F919–31. [PubMed: 21880837]
7. Davies NJ, Batehup L, Thomas R. The role of diet and physical activity in breast, colorectal, and prostate cancer survivorship: a review of the literature. *Br J Cancer.* 2011; 105(Suppl 1):S52–73. [PubMed: 22048034]
8. Solfrizzi V, Panza F, Frisardi V, Seripa D, Logroscino G, Imbimbo BP, et al. Diet and Alzheimer's disease risk factors or prevention: the current evidence. *Expert Rev Neurother.* 2011; 11:677–708. [PubMed: 21539488]
9. Koponen H, Jokelainen J, Keinänen-Kiukaanniemi S, Kumpusalo E, Vanhala M. Metabolic syndrome predisposes to depressive symptoms: a population-based 7-year follow-up study. *J Clin Psychiatry.* 2008; 69:178–82. [PubMed: 18232723]
10. Vincent AM, Hinder LM, Pop-Busui R, Feldman EL. Hyperlipidemia: a new therapeutic target for diabetic neuropathy. *J Peripher Nerv Syst.* 2009; 14:257–67. [PubMed: 20021567]
11. Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, et al. Vascular risk factors and diabetic neuropathy. *N Engl J Med.* 2005; 352:341–50. [PubMed: 15673800]
12. Amarapurkar D, Das HS. Chronic liver disease in diabetes mellitus. *Trop Gastroenterol.* 2002; 23:3–5. [PubMed: 12170918]
13. Bell DS, Allbright E. The multifaceted associations of hepatobiliary disease and diabetes. *Endocr Pract.* 2007; 13:300–12. [PubMed: 17599864]
14. Smith BW, Adams LA. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. *Nat Rev Endocrinol.* 2011; 7:456–65. [PubMed: 21556019]
15. de la Monte SM. Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. *Curr Alzheimer Res.* 2012; 9:35–66. [PubMed: 22329651]
16. Accardi G, Caruso C, Colonna-Romano G, Camarda C, Monastero R, Candore G. Can Alzheimer disease be a form of type 3 diabetes? *Rejuvenation Res.* 2012; 15:217–21. [PubMed: 22533436]
17. Roriz-Filho JS, Sa-Roriz TM, Rosset I, Camozzato AL, Santos AC, Chaves ML, et al. (Pre)diabetes, brain aging, and cognition. *Biochim Biophys Acta.* 2009; 1792:432–43. [PubMed: 19135149]
18. Northam EA, Rankins D, Lin A, Wellard RM, Pell GS, Finch SJ, et al. Central nervous system function in youth with type 1 diabetes 12 years after disease onset. *Diabetes Care.* 2009; 32:445–50. [PubMed: 19151204]
19. Kodl CT, Seaquist ER. Cognitive dysfunction and diabetes mellitus. *Endocr Rev.* 2008; 29:494–511. [PubMed: 18436709]
20. Sima AA. Encephalopathies: the emerging diabetic complications. *Acta Diabetol.* 2010; 47:279–93. [PubMed: 20798963]
21. Ahmadpour, S. CNS complications of diabetes mellitus type 1 (type 1 diabetic encephalopathy). In: Oguntibeju, OO., editor. *Pathophysiology and Complication of Diabetes Mellitus.* InTech; 2012. p. 1-18. <http://dx.doi.org/10.5772/49990> [On-line, Accessed 5/30/2013]
22. Salem N Jr, Litman B, Kim H-Y, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids.* 2001; 36:945–60. [PubMed: 11724467]
23. Stillwell W, Shaikh SR, Zerouga M, Siddiqui R, Wassall SR. Docosahexaenoic acid affects cell signaling by altering lipid rafts. *Reprod Nutr Dev.* 2005; 45:559–79. [PubMed: 16188208]

24. Khan SA, Vanden Heuvel JP. Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (review). *J Nutr Biochem*. 2003; 14:554–67. [PubMed: 14559106]
25. Horrocks LA, Farooqui AA. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. *Prostaglandins Leukot Essent Fatty Acids*. 2004; 70:361–72. [PubMed: 15041028]
26. Bazan NG. Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. *Brain Pathol*. 2005; 15:159–66. [PubMed: 15912889]
27. Bannenberg G, Arita M, Serhan CN. Endogenous receptor agonists: resolving inflammation. *ScientificWorldJournal*. 2007; 7:1440–62. [PubMed: 17767360]
28. de Urquiza AM, Liu S, Sjöberg M, Zetterstrom RH, Griffiths W, Sjövall J, et al. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. *Science*. 2000; 290:2140–4. [PubMed: 11118147]
29. Clarke SD, Thuillier P, Baillie RA, Sha X. Peroxisome proliferator-activated receptors: a family of lipid-activated transcription factors. *Am J Clin Nutr*. 1999; 70:566–71. [PubMed: 10500027]
30. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, et al. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med*. 2009; 206:15–23. [PubMed: 19103881]
31. Bourre JN, Pascal G, Durand G, Masson M, Dumont O, Piciotti M. Alterations in the fatty acid composition of rat brain cells (neurons, astrocytes, and oligodendrocytes) and of subcellular fractions (myelin and synaptosomes) induced by a diet devoid of n-3 fatty acids. *J Neurochem*. 1984; 43:342–8. [PubMed: 6736955]
32. Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity interact to alter maternal rat brain phospholipid fatty acid composition. *J Nutr*. 2006; 136:2236–42. [PubMed: 16857847]
33. Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity affect liver and erythrocyte phospholipid fatty acid composition in female rats. *J Nutr*. 2007; 137:2425–30. [PubMed: 17951480]
34. Prasad MR, Lovell MA, Yatin M, Dhillon H, Markesbery WR. Regional membrane phospholipid alterations in Alzheimer's disease. *Neurochem Res*. 1998; 23:81–8. [PubMed: 9482271]
35. Fraser T, Tayler H, Love S. Fatty acid composition of frontal, temporal and parietal neocortex in the normal human brain and in Alzheimer's disease. *Neurochem Res*. 2010; 35:503–13. [PubMed: 19904605]
36. McNamara RK, Hahn CG, Jandacek R, Rider T, Tso P, Stanford KE, et al. Selective deficits in the omega-3 fatty acid docosahexaenoic acid in the postmortem orbitofrontal cortex of patients with major depressive disorder. *Biol Psychiatry*. 2007; 62:17–24. [PubMed: 17188654]
37. Roden M, Bernroider E. Hepatic glucose metabolism in humans—its role in health and disease. *Best Pract Res Clin Endocrinol Metab*. 2003; 17:365–83. [PubMed: 12962691]
38. Gangong, WF. Review of Medical Physiology. 20th. McGraw-Hill; New York: 1999.
39. Bourre JM, Dumont OS, Piciotti MJ, Pascal GA, Durand GA. Dietary alpha-linolenic acid deficiency in adult rats for 7 months does not alter brain docosahexaenoic acid content, in contrast to liver, heart and testes. *Biochim Biophys Acta*. 1992; 1124:119–22. [PubMed: 1347458]
40. National RC. Guide for the Care and Use of Laboratory Animals. National Academy Press; Washington, D.C.: 1996.
41. Levant B, Ozias MK, Jones KA, Carlson SE. Differential effects of modulation of docosahexaenoic acid content during development in specific regions of rat brain. *Lipids*. 2006; 41:407–14. [PubMed: 16933785]
42. Guilford BL, Ryals JM, Wright DE. Phenotypic changes in diabetic neuropathy induced by a high-fat diet in diabetic C57BL/6 mice. *Exp Diabetes Res*. 2011; 2011:848307. [PubMed: 22144990]
43. Galli C, Trzeciak HI, Paoletti R. Effects of dietary fatty acids on the fatty acid composition of brain ethanolamine phosphoglyceride: reciprocal replacement of n-6 and n-3 polyunsaturated fatty acids. *Biochim Biophys Acta*. 1971; 248:449–54.
44. Brenner RR. Hormonal modulation of delta6 and delta5 desaturases: case of diabetes. *Prostaglandins Leukot Essent Fatty Acids*. 2003; 68:151–62. [PubMed: 12538079]

45. Arbo I, Halle C, Malik D, Brattbakk HR, Johansen B. Insulin induces fatty acid desaturase expression in human monocytes. *Scand J Clin Lab Invest.* 2011; 71:330–9. [PubMed: 21413848]
46. Mauvoisin D, Mounier C. Hormonal and nutritional regulation of SCD1 gene expression. *Biochimie.* 2011; 93:78–86. [PubMed: 20713121]
47. Bjermo H, Riserus U. Role of hepatic desaturases in obesity-related metabolic disorders. *Curr Opin Clin Nutr Metab Care.* 2010; 13:703–8. [PubMed: 20823776]
48. Ghebremeskel K, Bitsanis D, Koukkou E, Lowy C, Poston L, Crawford MA. Maternal diet high in fat reduces docosahexaenoic acid in liver lipids of newborn and sucking rat pups. *Br J Nutr.* 1999; 81:395–404. [PubMed: 10615212]
49. Childs CE, Romeu-Nadal M, Burdge GC, Calder PC. The polyunsaturated fatty acid composition of hepatic and plasma lipids differ by both sex and dietary fat intake in rats. *J Nutr.* 2010; 140:245–50. [PubMed: 20007335]
50. Mlekusch W, Celedin C, Aloia RC, Moller R. Effect of a high fat diet on phospholipid class distribution and fatty acid composition in rat liver. *Int J Biochem.* 1993; 25:1539–47. [PubMed: 8288021]
51. Celik S, Baydas G, Yilmaz O. Influence of vitamin E on the levels of fatty acids and MDA in some tissues of diabetic rats. *Cell Biochem Funct.* 2002; 20:67–71. [PubMed: 11835272]
52. Pari L, Venkateswaran S. Protective role of *Phaseolus vulgaris* on changes in the fatty acid composition in experimental diabetes. *J Med Food.* 2004; 7:204–9. [PubMed: 15298769]
53. Kumar G, Banu S, Murugesan AG. Influence of *Helicteres isora* administration for diabetes mellitus: the effect on changes in tissue fatty acid composition. *Food Chem Toxicol.* 2009; 47:1826–30. [PubMed: 19410628]
54. Lin CJ, Peterson R, Eichberg J. The fatty acid composition of glycerolipids in nerve, brain, and other tissues of the streptozotocin diabetic rat. *Neurochem Res.* 1985; 10:1453–65. [PubMed: 2935746]
55. Hu Q, Ishii E, Nakagawa Y. Differential changes in relative levels of arachidonic acid in major phospholipids from rat tissues during the progression of diabetes. *J Biochem.* 1994; 115:405–8. [PubMed: 8056750]
56. Guesnet P, Bourre JM, Guerre-Millo M, Pascal G, Durand G. Tissue phospholipid fatty acid composition in genetically lean (Fa/-) or obese (fa/fa) Zucker female rats on the same diet. *Lipids.* 1990; 25:517–22. [PubMed: 2250587]
57. Sena A, Rebel G, Bieth R, Hubert P, Waksman A. Lipid composition in liver and brain of genetically obese (ob/ob), heterozygote (ob/+) and normal (+/+) mice. *Biochim Biophys Acta.* 1982; 710:290–6. [PubMed: 7074117]
58. Ramnanan CJ, Edgerton DS, Kraft G, Cherrington AD. Physiologic action of glucagon on liver glucose metabolism. *Diabetes Obes Metab.* 2011; 13(Suppl 1):118–25. [PubMed: 21824265]
59. Li CC, Lii CK, Liu KL, Yang JJ, Chen HW. n-6 and n-3 polyunsaturated fatty acids down-regulate cytochrome P-450 2B1 gene expression induced by phenobarbital in primary rat hepatocytes. *J Nutr Biochem.* 2006; 17:707–15. [PubMed: 16517146]
60. Sung M, Kim I, Park M, Whang Y, Lee M. Differential effects of dietary fatty acids on the regulation of CYP2E1 and protein kinase C in human hepatoma HepG2 cells. *J Med Food.* 2004; 7:197–203. [PubMed: 15298768]
61. Jump DB, Thelen A, Mater M. Dietary polyunsaturated fatty acids and hepatic gene expression. *Lipids.* 1999; (34 Suppl):S209–12. [PubMed: 10419152]
62. Berger A, Roberts MA, Hoff B. How dietary arachidonic- and docosahexaenoic-acid rich oils differentially affect the murine hepatic transcriptome. *Lipids Health Dis.* 2006; 5:10. [PubMed: 16623957]
63. Polozova A, Gionfriddo E, Salem N Jr. Effect of docosahexaenoic acid on tissue targeting and metabolism of plasma lipoproteins. *Prostaglandins Leukot Essent Fatty Acids.* 2006; 75:183–90. [PubMed: 16837179]
64. Igarashi M, DeMar JC Jr, Ma K, Chang L, Bell JM, Rapoport SI. Upregulated liver conversion of alpha-linolenic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. *J Lipid Res.* 2007; 48:152–64. [PubMed: 17050905]

65. Igarashi M, Ma K, Chang L, Bell JM, Rapoport SI, DeMar JC Jr. Low liver conversion rate of alpha-linolenic to docosahexaenoic acid in awake rats on a high-docosahexaenoate-containing diet. *J Lipid Res.* 2006; 47:1812–22. [PubMed: 16687661]
66. Broughton KS, Wade JW. Total fat and (n-3):(n-6) fat ratios influence eicosanoid production in mice. *J Nutr.* 2002; 132:88–94. [PubMed: 11773513]
67. Zelber-Sagi S, Ratzin V, Oren R. Nutrition and physical activity in NAFLD: an overview of the epidemiological evidence. *World J Gastroenterol.* 2011; 17:3377–89. [PubMed: 21876630]
68. Yu H, Bi Y, Ma W, He L, Yuan L, Feng J, et al. Long-term effects of high lipid and high energy diet on serum lipid, brain fatty acid composition, and memory and learning ability in mice. *Int J Dev Neurosci.* 2010; 28:271–6. [PubMed: 20015474]
69. Krishnadas R, Cavanagh J. Depression: an inflammatory illness? *J Neurol Neurosurg Psychiatry.* 2012; 83:495–502. [PubMed: 22423117]
70. Park HR, Park M, Choi J, Park KY, Chung HY, Lee J. A high-fat diet impairs neurogenesis: involvement of lipid peroxidation and brain-derived neurotrophic factor. *Neurosci Lett.* 2010; 482:235–9. [PubMed: 20670674]
71. Sharma S, Fulton S. Diet-induced obesity promotes depressive-like behaviour that is associated with neural adaptations in brain reward circuitry. *Int J Obes (Lond).* 2012
72. Lindqvist A, Mohapel P, Bouter B, Frielingsdorf H, Pizzo D, Brundin P, et al. High-fat diet impairs hippocampal neurogenesis in male rats. *Eur J Neurol.* 2006; 13:1385–8. [PubMed: 17116226]
73. Schmidt H, Shelton R, Duman R. Functional biomarkers of depression: diagnosis, treatment, and pathophysiology. *Neuropsychopharmacology.* 2011; 36:2375–94. [PubMed: 21814182]
74. Conklin SM, Manuck SB, Yao JK, Flory JD, Hibbeln JR, Muldoon MF. High omega-6 and low omega-3 fatty acids are associated with depressive symptoms and neuroticism. *Psychosom Med.* 2007; 69:932–4. [PubMed: 17991818]
75. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. *Hippocampus.* 2008; 18:1085–8. [PubMed: 18651634]
76. McNay EC, Ong CT, McCrimmon RJ, Cresswell J, Bogan JS, Sherwin RS. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. *Neurobiol Learn Mem.* 2010; 93:546–53. [PubMed: 20176121]
77. Cui Y, Shu Y, Zhu Y, Shi Y, Le G. High-fat diets impair spatial learning of mice in the Y-maze paradigm: ameliorative potential of alpha-lipoic acid. *J Med Food.* 2012; 15:713–7. [PubMed: 22784184]

Table 1

Fatty acid composition of the Control and High-fat diets

Fatty Acid	<u>Content in diet (g/kg)</u>	
	Control	High-fat
14:0	0.59	0.73
16:0	5.80	46.35
18:0	1.15	35.48
16:1	0.80	0.21
18:1n-9	8.68	157.90
18:2n-6	19.67	32.87
18:3n-3	1.75	0.76
20:4n-6	0.16	ND
20:5n-3	1.08	ND
22:5n-3	0.22	ND
22:6n-3	0.79	ND

The control diet was Teklad 8604. The fatty acid composition for 8604 was supplied by Harlan Teklad. The High-fat diet was Teklad TD.07011 and was analyzed quantitatively using Supleco 37 Component FAME mix. Trace amounts of other fatty acids detected in these formulations are not presented. ND, not detected

Table 2

Effects of a high-fat diet and diabetes on liver phospholipid fatty acid composition.

Fatty Acid/Diet	Percent of Total Fatty Acids (%mol)				P		
	Non-diabetic		Diabetic				
	Control	High-fat	Control	High-fat	Diet	Db	Diet x Db
16:0	9.11 ± 1.56	8.18 ± 1.51	16.97 ± 0.66 ^{*†}	8.31 ± 1.65 [‡]	<0.01	<0.01	<0.05
18:0	17.54 ± 0.43	19.02 ± 0.95	18.23 ± 1.90	20.23 ± 0.43	NS	NS	NS
18:1n-9	4.73 ± 0.41	14.02 ± 0.77 [*]	5.73 ± 0.29 [†]	10.72 ± 0.45 ^{*†‡}	<0.001	<0.05	<0.001
18:1n-7	3.82 ± 0.76	2.98 ± 0.27	1.37 ± 0.11 [*]	2.65 ± 0.74	NS	<0.05	NS
18:2n-6	17.68 ± 0.62	13.22 ± 0.70 [*]	16.92 ± 0.53 [†]	16.24 ± 0.35 [†]	<0.001	NS	<0.01
20:4n-6	22.98 ± 0.73	28.41 ± 0.74 [*]	18.82 ± 1.03 ^{*†}	27.19 ± 0.66 ^{*‡}	<0.001	<0.01	NS
22:5n-6	0.21 ± 0.03	2.32 ± 0.10 [*]	0.10 ± 0.01 [†]	1.07 ± 0.23 ^{*†‡}	<0.001	<0.001	<0.001
22:6n-3	16.24 ± 0.47	5.36 ± 0.15 [*]	15.34 ± 0.29 [†]	9.33 ± 0.89 ^{*†‡}	<0.001	<0.01	<0.001
ΣSFA	27.80 ± 1.48	28.37 ± 1.74	35.99 ± 1.49 ^{*†}	29.41 ± 1.63 [‡]	NS	<0.01	NS
ΣMUFA	9.47 ± 0.73	17.90 ± 0.89 [*]	8.20 ± 0.52 [†]	13.76 ± 0.86 ^{*†‡}	<0.001	<0.01	NS
Σn-6	44.25 ± 1.38	48.09 ± 1.35	38.17 ± 1.25 ^{*†}	47.19 ± 0.79 [‡]	<0.001	<0.01	<0.05
Σn-3	18.49 ± 0.54	5.64 ± 0.17 [*]	17.64 ± 0.25 [†]	9.65 ± 0.93 ^{*†‡}	<0.001	<0.05	<0.001
ΣPUFA	61.34 ± 1.53	53.51 ± 1.47 [*]	54.38 ± 1.18 [*]	56.70 ± 1.05	NS	NS	<0.01
DHA:ARA	0.71 ± 0.03	0.19 ± 0.003 [*]	0.83 ± 0.06 [†]	0.34 ± 0.03 ^{*†‡}	<0.001	<0.01	NS
n-3:n-6	0.42 ± 0.02	0.12 ± 0.002 [*]	0.47 ± 0.02 [†]	0.21 ± 0.02 ^{*†‡}	<0.001	<0.001	NS
PUFA:SFA	2.26 ± 0.14	1.96 ± 0.16	1.54 ± 0.10 [*]	1.98 ± 0.13	NS	<0.05	<0.05
18:3n-6:18:2n-6	0.014 ± 0.0010	0.0046 ± 0.0011	0.014 ± 0.0022	0.0070 ± 0.0013	<0.001	NS	NS
20:4n-6:20:3n-6	8.54 ± 0.39	7.59 ± 0.24	11.08 ± 0.83	12.44 ± 1.25	NS	<0.001	NS
18:1n-9:18:0	0.27 ± 0.026	0.74 ± 0.040 [*]	0.34 ± 0.041	0.53 ± 0.028 ^{*†‡}	<0.001	NS	<0.001

Data are the mean ± SEM (n = 8 per group, except for Diabetic-Control diet, where n = 7). Fatty acids present only in trace amounts are not present individually, but are included in the summative data for each class of fatty acids.

ΣSFA includes 16:0, 17:0, 18:0, 20:0, 21:0, 22:0 and 24:0.

ΣMUFA includes 16:1, 18:1n-9, 18:1n-7, 20:1n-9, and 24:1.

Σn-6 includes 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, and 22:5n-6.

Σn-3 includes 18:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

ΣPUFA includes all n-3 and n-6.

NS – not significant.

^{*} Different from Control diet, non-diabetic (P<0.05).

[†] Different from High-fat diet, non-diabetic (P<0.05).

[‡] Different for Control diet, diabetic (P<0.05) by two-way ANOVA followed by Tukey's test comparing all four groups.

Table 3

Effects of a high-fat diet and diabetes on brain phospholipid fatty acid composition.

Fatty Acid/Diet	Percent of Total Fatty Acids (%mol)				P		
	Non-diabetic		Diabetic		Diet	Db	Diet x Db
	Control	High-fat	Control	High-fat			
16:0	25.91 ± 0.80	22.36 ± 0.90	23.10 ± 1.12	23.97 ± 1.01	NS	NS	<0.05
18:0	19.51 ± 0.21	21.11 ± 0.48 *	20.17 ± 0.51	19.69 ± 0.23	NS	NS	<0.05
18:1n-9	18.47 ± 0.48	18.51 ± 0.32	18.30 ± 0.37	19.18 ± 0.37	NS	NS	NS
18:1n-7	5.55 ± 0.21	4.89 ± 0.69	5.34 ± 0.20	5.35 ± 0.16	NS	NS	NS
20:1n-9	2.01 ± 0.15	2.20 ± 0.14	2.19 ± 0.21	2.20 ± 0.23	NS	NS	NS
20:4n-6	9.32 ± 0.35	11.02 ± 0.33 *	10.29 ± 0.39	10.64 ± 0.30	<0.01	NS	NS
22:5n-6	0.11 ± 0.02	0.82 ± 0.04 *	0.12 ± 0.01 †	0.41 ± 0.06 *†‡	<0.001	<0.001	<0.001
22:6n-3	15.30 ± 0.95	16.29 ± 0.73	16.80 ± 0.78	15.66 ± 0.45	NS	NS	NS
ΣSFA	46.91 ± 0.76	44.75 ± 0.59	44.65 ± 0.89	44.88 ± 0.85	NS	NS	NS
ΣMUFA	27.07 ± 0.82	26.19 ± 0.84	26.51 ± 0.72	27.21 ± 0.65	NS	NS	NS
Σn-6	10.58 ± 0.34	12.70 ± 0.34 *	11.87 ± 0.48 *	12.17 ± 0.34 *	<0.01	NS	<0.05
Σn-3	15.45 ± 0.96	16.35 ± 0.74	16.96 ± 0.79	15.74 ± 0.45	NS	NS	NS
ΣPUFA	26.02 ± 1.25	29.05 ± 0.99	28.83 ± 1.11	27.91 ± 0.76	NS	NS	NS
DHA:ARA	1.64 ± 0.06	1.48 ± 0.05	1.63 ± 0.06	1.47 ± 0.02	<0.01	<NS	NS
n-3:n-6	1.45 ± 0.06	1.29 ± 0.04	1.43 ± 0.06	1.30 ± 0.02	<0.01	NS	NS
PUFA:SFA	0.56 ± 0.04	0.65 ± 0.03	0.65 ± 0.03	0.62 ± 0.03	NS	NS	NS

Data are the mean ± SEM (n = 8 per group). Fatty acids present only in trace amounts are not present individually, but are included in the summative data for each class of fatty acids. ΣSFA includes 14:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0. ΣMUFA includes 16:1, 18:1n-9, 18:1n-7, 20:1n-9, and 24:1. Σn-6 includes 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, and 22:5n-6. Σn-3 includes 22:5n-3, and 22:6n-3. ΣPUFA includes all n-3 and n-6.

NS – not significant.

* Different from Control diet, non-diabetic (P<0.05).

† Different from High-fat diet, non-diabetic (P<0.05).

‡ Different for Control diet, diabetic (P<0.05) by two-way ANOVA followed by Tukey's test comparing all four groups.