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Feeding docosahexaenoic acid impairs hormonal control of glucose transport in rat adipocytes

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High fat diets decrease glucose tolerance and increase insulin resistance at peripheral tissues such as adipose and muscle. Fish oils, containing a high concentration of ω3 fatty acids, have been reported to counter the development of insulin resistance in rats in response to high fat diets. Because fish oils contain a number of long chain fatty acids that may be mediating this response, we investigated the specific effects of docosahexaenoic acid (DHA), which comprises approximately 10% of the fatty acids in fish oils, on the hormonal regulation of glucose uptake in isolated adipocytes. Weanling rats were fed diets containing 12% of calories as corn oil (LF-CO), or 26% of calories as safflower oil (ω6 rich, MF-SO) or DHASCO™ (44.6% DHA, MF-DHA). Feed consumption and growth did not differ between the dietary treatments. After 8 weeks of feeding, fasting serum glucose levels were higher in both the high fat diet groups compared to LF-CO. Basal uptake of 2.5 mM [^{3}H]-2-deoxyglucose (2DG) was reduced in MF-DHA compared to LF-CO. Insulin stimulated 2-DG uptake in all three diet groups. However, despite this stimulus, uptake was lower in MF-SO rats and further reduced in MF-DHA rats. Decreased insulin-stimulated uptake was associated with a reduction in total quantity of GLUT4 in MF-SO rats, but was independent of any change in GLUT4 in MF-DHA fed rats. The β-adrenergic agonist, isoproterenol, decreased 2DG uptake in insulin-stimulated adipocytes by 51% and 36%, respectively, in the LF-CO and MF-SO groups, but had no effect after MF-DHA feeding. This loss of β-adrenergic responsiveness was associated with a decrease in quantity of immunoreactive $G\alpha_s$ protein. These data indicate that long-term feeding of MF-DHA diets impaired basal glucose disposal and disrupted normal hormonal regulation of glucose uptake by the adipocyte. (J. Nutr. Biochem. 7:356–363, 1996.)

Keywords: insulin; β-adrenergic receptor; high fat diets; fish oil; diabetes; docosahexaenoic acid

Introduction

High fat diets have been associated with the development of non-insulin dependent diabetes (NIDDM) in a number of populations. However, the source of dietary fat may be a critical component in the etiology of the disease. For example, high intakes of fish among Greenland Eskimo have been implicated in the low incidence of NIDDM in this population.[1] Similarly, a longitudinal study of elderly men and women in the Netherlands found a negative association between fish consumption and glucose intolerance.[2] The active component in fish is attributed to the high content of ω3 fatty acids, particularly eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3). However, interventions that supplement the diet of individuals with NIDDM with fish oil do not always improve glucose homeostasis, despite a consistent improvement in blood lipid abnormalities.[3–6] Indeed, many intervention studies have reported increases in fasting glucose concentrations after fish oil supplementation,[4–7] whereas few have reported no change[8] or improved glucose tolerance.[3]

In animal models, high fat diets induce insulin resistance and impair glucose tolerance.[9–11] The inclusion of fish oil as part of the high fat diet has been reported to have variable effects on glucose tolerance; responses appear to depend on species, gender, length of supplementation, and tissue analyzed. Supplementation of high animal fat diets for 3 months with ω3 fatty acids improves glucose tolerance in

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male mice, but is ineffective in female mice,[12] or male Wistar rats.[13] Inclusion of fish oil in the diet has an ameliorating effect on high fat diet-induced insulin resistance in adipocytes over the short term in rats.[10] However, over longer periods of feeding, high levels of fish oil lead to insulin resistance in adipose tissue.[14] Responses in skeletal muscle may differ from adipose; insulin-stimulated glucose disposal in skeletal muscle was improved by addition of long chain ω-3 fatty acids to diets high in saturated, monounsaturated, and polyunsaturated fats (PUFAs) for 30 days.[11]

Glucose uptake into tissues is regulated by a complex interaction among a variety of hormonal signal transduction pathways that control expression, translocation, and function of glucose transporters. Glucose uptake is mediated by a family of equilibrative transporters (GLUT 1-7).[15] In adipocytes and muscle cells, insulin-stimulation of glucose uptake is mediated by increased translocation of GLUT 4 transporters to the plasma membrane, whereas GLUT 1 transporters primarily mediate basal or non-stimulated levels of glucose transport.[16] Abnormal expression of the GLUT 4 transporter has been implicated in the development of glucose intolerance associated with NIDDM, streptozotocin-induced diabetes, and aging.[17–20] High fat diets decrease the quantity of GLUT 1 and GLUT4 in isolated adipocytes[14] and GLUT4 mRNA and protein quantity in skeletal muscle.[21,22] Short-term supplementation with fish oils prevents high-fat-induced loss of GLUT4 transporter quantity in adipocytes.[14] In contrast, diets high in fish oils fed for 21 days are just as effective as non-fish oil diets in reducing GLUT4 mRNA and protein in rat skeletal muscle.[23] No information is available on the long-term effects of fish oil on glucose transporter quantity and function in adipocytes. Therefore, we have investigated the long-term effects of moderate fat diets containing safflower oil (ω6 rich) or DHA on glucose transporter function and quantity in isolated adipocytes.

Methods and materials

Materials

Male Fisher rats were purchased from Charles River (St. Constant, Quebec). Diet ingredients were purchased from the following sources: DHASCO™[1] (Martek Biosciences Corp, Columbia, MD USA), corn oil (Mazola, local distributor), safflower oil (PC, local distributor), minerals (BDH, Pool, England and Fisher, Fairlawn, NJ USA), ferric citrate (Sigma, St. Louis, MO USA), cornstarch (Casco, Etobicoke, Ontario), casein (ICN, Aurora, Ohio USA), and all other diet components (United States Biochemicals, Cleveland, OH USA). Light mineral oil and calcium chloride were purchased from Fisher Scientific (Unionville, Ontario). [^{125}I]-anti-rabbit IgG was purchased from ICN (St. Laurent, Quebec). Antibodies to the C-terminal decapeptide of $G\alpha_i$ (AS/7) and $G\alpha_s$ were purchased from New England Nuclear (Missisauga, Ontario) and Gramsch Laboratories (FRG), respectively. Polyclonal antibodies recognizing multiple epitopes in the C-terminus of GLUT4 and GLUT1 were purchased from Calbiochem (La Jolla, CA USA) and East Acres Biologicals (Southbridge, MA USA), respectively. Adenosine deaminase was purchased from Boehringer Mannheim (Dorval, Quebec). Silicone oil was obtained from Aldrich Chemical Company (St. Louis, MO USA). All other chemicals were acquired from Sigma (St. Louis, MO USA). Water used in experiments was filtered through the Milli-Q-Water system (Millipore, Missisauga, Ontario). Endotoxin concentrations in the water were less than 0.06 ng/mL as determined by an Endotect™ kit from ICN Biomedicals.

Animal care and feeding

Twenty-four 3-week-old male Fisher rats were received, weighed, and acclimated on Purina rat chow for 3 days. Animals were housed individually in wire-bottom cages and provided with 15 cm diameter plastic tubing for environmental enrichment. Animals were maintained in a climate-controlled animal facility with 12 hr light/12 hr dark cycle. Procedures were approved by the University of Guelph, Animal Welfare Committee in accordance with the Guidelines from the Canadian Council on Animal Care. On experimental day 0, animals were weighed and randomly assigned to dietary treatment groups (eight animals/group). Dietary treatment groups were designated: low fat corn oil (LF-CO), safflower oil rich (MF-SO), and docosahexaenoic acid rich (MF-DHA). The energy composition of the LF-CO, MF-SO and MF-DHA diets were 12%, 26%, and 26% of energy as fat, 68%, 54%, and 54% of energy as carbohydrate, respectively. All diets contained 20% of energy from protein with a total energy content of 16.2 MJ/kg diet. The complete composition of the diets is shown in *Table 1*. Diets were prepared, evacuated by vacuum, gassed with nitrogen, vacuum packed, and stored at −20°C, Animals were allowed free access to diets and intake and weight gain were measured daily over an 8-week period. At the end of the experimental period, animals were fasted for 12 hr, placed under metophane anesthetic for cardiac puncture and subsequent decapitation. An aliquot of the blood collected by cardiac puncture was frozen for future analysis or placed in tubes containing 0.129 M buffered sodium citrate (Vacutainer tubes, Becton Dickinson, No. 6419) and centrifuged to separate red cells and plasma and stored at −20°C. Plasma glucose concentration was measured by the glucose oxidase method.[24] Cholesterol and triglyceride were measured by enzymatically using Sigma procedures No. 352 and 339, respectively.

Isolation of adipocytes

Adipocytes were isolated from the epididymal fat pads by collagenase digestion as described by Rodbell.[25] Fat pads were weighed, minced with scissors, and incubated at 37°C in a shaking water bath (80 rpm) for 50 min in 12 mls Hanks balanced salt solution containing 5 mg collagenase, 40 mg/ml bovine serum albumin, and 25 mM HEPES. Adipocytes were then filtered through a 250-μm nylon mesh and washed twice with 8 mls phosphate buffered saline with 1 mM $MgCl_2$, 0.68 mM $CaCl_2$ (PBS) containing 1 mg/mL BSA, and 1 mM pyruvic acid, pH 7.4. Adi-

Table 1 Diet composition (g/kg diet)

	LF-CO	MF-SO	MF-DHA
casein	200	200	200
D,L-methionine	3	3	3
choline bitartrate	2	2	2
vitamin mix (AIN 76)	10	10	10
mineral mix (AIN 76)	35	35	35
fiber (cellufil)	50	125	125
sucrose	100	100	100
cornstarch	550	415	415
corn oil	50	10	10
safflower oil	—	100	—
DHASCO	—	—	100
P/S ratio	4.76	8.67	1.06

pocytes were counted in a hemocytometer and cell concentration adjusted to 5.0×10^5 cells/mL.

Uptake of [^{3}H] 2-Deoxyglucose

One U/mL of adenosine deaminase was added to the cell suspension as indicated in the figure legends to remove endogenous adenosine. Adipocytes were treated with and without 4 nM insulin for 30 min at 37°C in a shaking water bath (130 rpm). The stimulatory G-protein ($G\alpha_s$) was activated by addition of 1 μM isoproterenol with 1 mM ascorbate after 20 min incubation. Nonspecific uptake was measured in cells pretreated with 0.3 mM phloretin after 25 min of incubation. After treatment with hormones, 150 μL aliquots of cells were then layered over 50 μL of mineral:silicone oil (57:43) in 400 μL polyethylene microcentrifuge tubes. Fifty μL of 10 mM 2-deoxy[1,2-[^{3}H]glucose (2DG)(0.5 μCi/tube) was added to start uptake measurements. This gave a final 2-deoxyglucose concentration of 2.5 mM, which is just below the Km values for Glut 1 (6.9 mM) and Glut 4 (4.6 mM). Reactions were terminated after 3.5 min by centrifugation for 10 sec at 10,000 × g. The top layer, containing the adipocytes was cut off with a razor blade, and transferred to a 1.5 mL polypropylene microcentrifuge tubes containing 1 mL of scintillation cocktail. The quantity of adipocytes present in the counting vial were readily solubilized by the scintillation cocktail. Radioactivity associated with the adipocytes was determined by scintillation counting. [^{3}H] 2-deoxyglucose uptake was linear with respect to time and cell concentration under these conditions (data not shown).

Western blot analysis of G proteins and glucose transporters

After isolation, adipocytes were frozen at −80°C in PBS containing 1 ng/ml each of aprotinin, leupeptin and phenylmethylsulfonylfluoride (PMSF). Adipocytes were thawed, sonicated and centrifuged at 500 × g for 2 min at 4°C and fat cake aspirated. A portion of the defatted homogenate was centrifuged at 18,000 × g for 30 min to prepare a crude membrane fraction. The pellet was dilute to 0.5 mg protein/ml in Laemmli buffer. Membrane proteins were separated by SDS-PAGE[26] for immunoblot detection. After electrophoresis, proteins were electrophorectically transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat dry milk in 10 mM Tris-HCl, 0.9% NaCl and 0.02% Na Azide (Tris/azide), pH 7.0 buffer for 2 h, incubated with antisera to GLUT4 (1:1000), GLUT1 (1:500), inhibitory G-protein ($G\alpha_i$) (1:1000) and $G\alpha_s$ (1:75,000) in 5% non-fat dry milk overnight and washed four times with 20 ml Tris/azide. Membranes were probed with [^{125}I]-goat anti-rabbit IgG. Unbound [^{125}I]-goat anti-rabbit IgG was removed by washing, membranes dried and placed at −70°C for autoradiography. Bands were quantitated by scanning densitometry. Under these conditions, absorptivity of labelled bands was linear from 10-100 μg membrane protein for $G\alpha_i$, 25-150 μg membrane protein for $G\alpha_s$, and 20-80 μg cellular protein for GLUT1 and GLUT 4 (data not shown).

Fatty acid analysis in phospholipid and triglyceride

Frozen adipocytes were thawed and used for lipid analysis. Total lipid was extracted from adipocytes according to the method of Bligh and Dyer.[27] The phospholipid and triglyceride fractions were separated from other lipids by thin layer chromatography on silica 60 plates (Merck, Germany) in a solvent of heptane/isopropyl ether/acetic acid (60:40:3). The origin, containing phospholipids, and the triglyceride fraction (furthest from origin) were scraped after visualization with 0.1% aminonaptholsulfonic acid and fatty acids were methylated after adding 17:0 (3 μg) as an internal standard. Methylated fatty acids were analyzed by gas phase chromatography.[28]

Statistical analysis

Values reported are mean ± SEM. Data were analyzed by the general linear models program on the SAS statistical package for personal computer. Differences between groups were determined by Bonferonni's multiple comparison test and least square means, except plasma lipid values which were compared with student's Neuman-Keuls test.

Results

Rats fed either MF-SO- or MF-DHA-enriched diets had higher fasting glucose concentrations compared to LF-CO (*Table 2*). Plasma lipid concentrations (cholesterol and triglycerides) were decreased with feeding the ω-3 rich MF-SO diet. Plasma lipid profiles were further improved by inclusion of DHASCO™ in the diet as the major source of dietary fat (*Table 2*). Whereas body weight was not affected by diet, total weight of the epididymal fat pads and the number of adipocytes isolated was decreased in MF-DHA fed rats compared with both the MF-SO and LF-CO diets (*Table 2*). This reduction in tissue weight was associated with a decrease in adipocyte cell number in the MF-DHA group. Whereas fat pad weight was not reduced in MF-SO-fed rats, the number of adipocytes was 30% lower than LF-CO rats (*Table 2*). Finally, the number of adipocytes per gram of tissue, was substantially lower in the MF-DHA-fed animals compared to both other dietary groups. This lower number of adipocytes per gram of tissue suggests that adipocytes from DHA-fed animals were on average twice as big as adipocytes from the other two groups. We were not

Table 2 Characteristics of diet groups

	LF-CO	MF-SO	MF-DHA
Final body weight (g)	259 ± 5	259 ± 5	261 ± 3
Epidydymal fat pad weight (g)	5.7 ± 0.5*	5.1 ± 0.4[a]	3.9 ± 0.13[b]
Adipocyte number (10^6 cells)	12.6 ± 1.7[a]	8.8 ± 1.0[b]	3.1 ± 0.6[c]
Adipocyte number per g fat pad (10^6 cells)	2.4 ± 0.4[a]	1.7 ± 0.3[a]	0.8 ± 0.1[b]
Serum Glucose (fasting) (mg/dL)	162.2 ± 6.4[a]	254.3 ± 49.2[b]	222.9 ± 13.8[b]
Cholesterol (mg/dL)	96.7 ± 10.2[a]	76.8 ± 6.8[b]	44.1 ± 3.4[c]
Triglyceride	203 ± 21[a]	113 ± 15[b]	101 ± 11[c]

Values given are the averages ± the standard error of the mean, n = 5–8 animals per group. Values in a row not sharing a letter are significantly different from each other, $P < 0.05$.

able to define the exact size distribution of the adipocytes in our study.

In isolated adipocytes, dietary fat content and composition had independent effects on 2DG uptake. Basal uptake of 2DG was not detectable in MF-DHA fed rats and was significantly lower than basal uptake in LF-CO (*Figure 1*). Basal uptake in MF-SO was not different than the other two groups. Treatment of adipocytes with insulin increased 2DG uptake in all three diet groups. However, rates of insulin-stimulated 2DG uptake were reduced in rats fed MF-SO and further decreased in MF-DHA (*Figure 1*). Transport of glucose is mediated by glucose transporter proteins.[15] GLUT1 is primarily responsible for basal transport in adipocytes whereas GLUT4 mediates insulin-stimulated uptake. Changes in the availability of transport proteins can contribute to changes in transport capacity.[17–20] To determine if there was a relationship between decreased transporter proteins and the high fat diet-induced decreases in glucose uptake, total quantity of GLUT1 and GLUT4 proteins in isolated adipocytes was measured by Western blot. GLUT1 immunoreactive protein was decreased by both moderate fat diets, but was not specifically reduced by MF-DHA feeding (*Figure 2*). The reduced basal glucose transport after MF-DHA feeding can only be partially due to a decrease in GLUT1 protein, because the reduction in GLUT1 protein had a greater effect in reducing basal transport in MF-DHA than MF-SO under the conditions used in this assay. The reduction in GLUT1 protein in both moderate-fat groups is consistent with the increase in fasting blood glucose observed in these rats.

Quantity of immunoreactive GLUT4 was responsive to both the concentration and type of fat in the diet. MF-SO feeding reduced total GLUT4 content. However, substitution of DHASCO™ for SO prevented this decrease in GLUT4 (*Figure 2*). Thus, the reduction in insulin-stimulated glucose uptake in MF-SO was correlated with decreased total GLUT4 content. In contrast, total GLUT4 protein concentrations were maintained in MF-DHA fed rats, suggesting that the mechanism for the reduction in insulin-stimulated glucose transport in these rats was different from the mechanism in MF-SO fed rats.

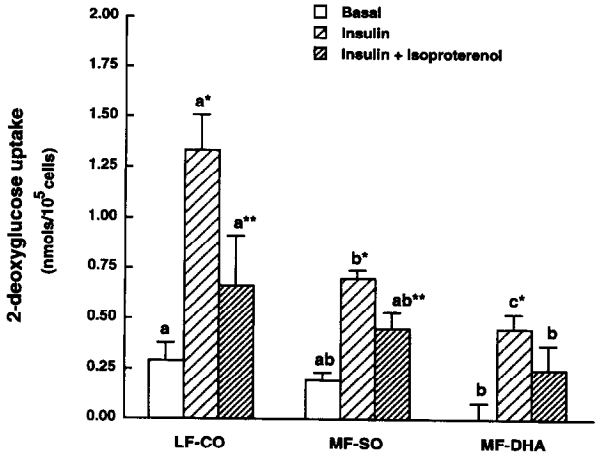

Figure 1 Uptake of [^{3}H]-2-deoxyglucose into isolated adipocytes. Adipocytes were isolated from the epididymal fat pads of LF-CO-, MF-SO-, and MF-DHA-fed rats and incubated with and without 4 nM insulin for 30 min. 1 μM isoproterenol was added during the last 10 min of the incubation. Uptake of 2.5 mM [^{3}H]-2-deoxyglucose was then measured over 3.5 min. Nonspecific uptake was determined in cells preincubated with 0.3 mM phloretin for 5 min. Values represent means ± SEM, n = 4. Values with different letters within a hormonal treatment are significantly different ($P < 0.05$). * indicate stimulation by insulin and ** indicate inhibition by isoproterenol within a dietary group ($P < 0.05$).

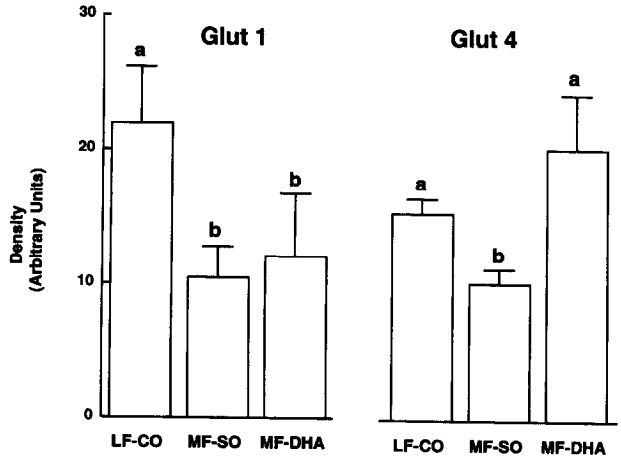

Figure 2 Quantity of GLUT1 and GLUT4 protein in isolated adipocytes. Crude membrane fractions were isolated from adipocytes from LF-CO-, MF-SO-, and MF-DHA-fed rats. Lysates were prepared and 50 μg of protein run in each lane on 10% PAGE. Western blots were carried out and the quantity of immunoreactive protein estimated by scanning densitometry. Values are mean ± SEM, n = 5–8 rats per group with each set done in duplicate. Values with different superscripts are significantly different ($P < 0.058$).

Whereas insulin is the primary stimulatory hormone for glucose transport in adipocytes, transport can be modulated by hormones which interact with guanine nucleotide regulatory proteins.[16] Treatment of insulin-stimulated adipocytes with 1 μM isoproterenol, a β-adrenergic agonist, inhibited 2-DG uptake in LF-CO and MF-SO fed rats (*Figure 1*). In contrast, isoproterenol did not decrease 2-DG uptake in MF-DHA fed rats (*Figure 1*). The lack of responsiveness to isoproterenol was associated with a 35% decrease in the quantity of $G\alpha_s$ in adipocytes from MF-DHA-fed rats compared to the other groups (*Figure 3*). No changes in the quantity of $G\alpha_i$ were observed between diet groups (*Figure 3*).

Dietary fat had a significant impact on the fatty acid composition of phospholipids and triglycerides in the adipocyte (*Table 3*). The proportion of specific fatty acids in phospholipids was affected by diet, with the ω-6/ω-3 ratio increased in the MF-SO diet and decreased in the MF-DHA diet. Particularly obvious were the substantially lower concentrations of 20:4ω-6 and higher concentrations of 22:6ω-3 in MF-DHA fed animals compared with the other two groups. Despite changes in specific fatty acids, dietary fat composition did not alter the total quantity of saturated, monounsaturated, and PUFAs in phospholipids. In stored triglycerides, however, the composition of saturated, monounsaturated, and PUFAs was more responsive to dietary fat composition. MF-SO diets decreased the concentrations of MUFA in triglycerides and increased PUFA concentrations. MF-DHA, in contrast, increased the concentration of

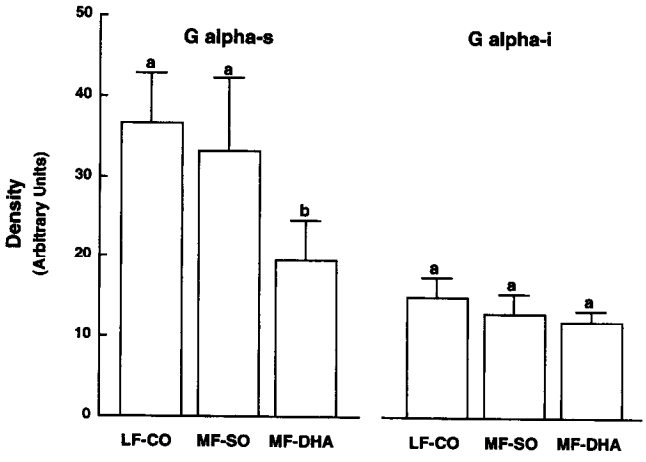

Figure 3 Quantity of $G\alpha_s$ and $G\alpha_i$ protein in isolated adipocytes. Crude membrane fractions were prepared from adipocytes isolated from LF-CO, MF-SO, and MF-DHA fed rats and Western blots were carried out. The quantity of immunoreactive protein estimated by scanning densitometry. Values are mean ± SEM, n = 5–6. Values with different superscripts are significantly different ($P < 0.05$).

saturates by 57% and decreased the MUFA concentration by 53% compared to LF-CO diets. The ratio of ω-6/ω-3 was decreased about 750 fold in the MF-DHA group compared to the other groups. Similarly, there were substantial differences in the unsaturation index in the triglyceride fraction, but no differences among diets in the phospholipid fraction. The LF-CO group had less than one double-bond per fatty acid in the triglyceride fraction, whereas the MF-CO and MF-DHA groups had 1.2 and 1.5 double bonds per fatty acid, respectively.

Discussion

High fat diets lead to the development of insulin resistance; however, the type of fat in the diet can also influence glucose tolerance. Population based investigations have implicated fish consumption in preventing glucose intolerance and NIDDM.[1,2] The mechanism for this apparent effect of fish consumption has been investigated in a number of studies, but has proven difficult to elucidate. The active component of fish is generally accepted to be the high concentration of ω3 fatty acids. However, supplementation of high fat diets with fish oils actually impairs glucose tolerance in a number of model systems.[4–7,12,13] Here we demonstrate that diets rich in DHA, one of the major forms of ω3 fatty acids in fish oil, impair glucose disposal after an 8-week feeding period. MF-DHA diets did not prevent the increase in fasting blood glucose concentrations induced by MF-SO diets. Moreover, insulin-stimulated glucose uptake in adipocytes was impaired to a greater extent in rats fed MF-DHA compared with both LF-CO and MF-SO diets. Given that the adipocytes from DHA fed animals appear, on average, to be twice as big as those from LF-CO and MF-SO animals, our estimate of the differences in uptake are likely to be underestimates, because our uptake data are expressed on a per cell basis. Thus moderate fat intake appears to severely compromise glucose homeostasis, despite the substantial hypolipidemic activity of MF-SO and the even more profound activity of MF-DHA diets on both serum triglyceride and cholesterol concentrations. These data indicate that DHA may be one of the components of fish that impair, rather than enhance, glucose tolerance after long periods of consumption. It is conceivable that responses in muscle may be different than the responses we have observed in adipose. Storlien et al.[11] reported that omega-3 fatty acid supplementation resulted in increased incorporation into muscle phospholipid and improved insulin action at this site. However, given that muscle is the major disposal site, this could not have been the case in our study. Because fasting plasma glucose levels were markedly elevated, this strongly suggests that glucose transport into the muscle was also impaired. Consistent with this suggestion. Dimitrakoudis and colleagues demonstrated that hyperglycemia decreases the number of glucose transporters in rat skeletal muscle.[29] Kim and coworkers[21] and Kahn and Pedersen[22] have shown that high fat diets impair GLUT4 mRNA and protein synthesis in muscle. In Kim's study this correlated with decreased transport of 2-deoxyglucose.[21] These results are similar to our own findings in adipocytes, although the level of regulation may be distinct in the two tissues.

The changes in basal and insulin-stimulated glucose uptake observed in response to different dietary fat sources were quite complex, involving responses that were both dependent and independent of changes in glucose transporter proteins. Moderate fat diets, independent of the source of fat, decreased the quantity of GLUT1 protein in adipocytes. In contrast, changes in GLUT4 were dependent on the composition of the fat in the diet; MF-SO diets decreased GLUT4 compared to LF-CO, whereas MF-DHA diets had no effect. However, the MF-DHA diet clearly impaired both basal and insulin-stimulated glucose uptake, suggesting that glucose transporter function was reduced in these rats. This loss of function could be due to a decrease in the catalytic capacity of the transporters and/or to an impairment of GLUT4 translocation to the plasma membrane after stimulation with insulin. GLUT4 translocation could be decreased due to a disruption in the insulin signalling cascade and/or to defects in the translocation process itself.

Membrane phospholipid concentration is sensitive to diet and influences the function of hormonal responses and transport function.[30] In cultured cells and adipocytes, increasing the concentration of PUFAs in the membrane is associated with increased responsiveness to insulin.[11,31–33] In contrast, saturated fatty acids are associated with impairment of insulin-mediated responses[31,34] and glucose transport in the small intestine.[35] Thus, specific changes in the composition of membrane phospholipids in adipocytes could contribute to altered basal and insulin-stimulated glucose transport. However, it is difficult to associate a particular change in function with a specific alteration in phospholipid composition because of the complexity of changes to the phospholipid fatty acid profiles. As well, we did not examine specific phospholipid subclasses that may have revealed specific differences that were masked by total phospholipid examination. It is conceivable that certain subclasses are more likely to be associated with transport sites and that their lipid composition may be more relevant than total fatty acid composition of the membrane. In addition to

Table 3 Fatty acid composition off adipocytes

Fatty acid	Phospholipids			Triglyceride		
	LF-CO	MF-SO	MF-DHA	LF-CO	MF-SO	MF-DHA
13:0				6.65 ± 0.49[a]	10.4 ± 2.2[a]	23.5 ± 4.8[b]
14:0	0.9 ± 0.09[a]	0.9 ± 0.13[a]	2.0 ± 0.12[b]	1.9 ± 0.09[a]	1.2 ± 0.08[a]	8.5 ± 0.87[b]
14:1	0.3 ± 0.08	0.3 ± 0.09	0.6 ± 0.34	0.2 ± 0.00[a]	0.1 ± 0.00[a]	0.2 ± 0.03[b]
15:0	0.4 ± 0.04	0.3 ± 0.04	0.5 ± .0.15	0.2 ± 0.00	0.1 ± 0.00	0.1 ± 0.00
16:0	21.4 ± 0.59[a]	19.7 ± 1.12[a]	26.3 ± 1.39[b]	24.4 ± 0.61[a]	17.5 ± 0.73[b]	21.7 ± 1.58[a]
16:1	2.7 ± 0.19	2.9 ± 0.75	3.7 ± 0.34	7.6 ± 0.80[a]	2.7 ± 0.29[b]	3.4 ± 0.39[b]
18:0	18.4 ± 1.62	17.8 ± 1.39	14.4 ± 1.08	1.9 ± 0.10	1.9 ± 0.03	1.9 ± 0.08
18:1	16.5 ± 0.50	14.8 ± 2.24	18.2 ± 1.54	28.0 ± 0.23[a]	17.9 ± 0.44	13.6 ± 0.68[c]
18:2n-6	17.5 ± 1.88[a]	21.9 ± 0.69[b]	18.8 ± 0.82[ab]	26.6 ± 0.95[a]	43.8 ± 0.95[a]	5.6 ± 0.29[c]
18:3n-6	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.03	0.4 ± 0.03[a]	0.2 ± 0.03[b]	0.2 ± 0.03[b]
18:3n-3	0.2 ± 0.07	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.03
18:4n-3	0.1 ± 0.03	0.1 ± 0.03	0.1 ± 0.00	ND	0.1 ± 0.00	ND
20:0	0.4 ± 0.05[a]	0.3 ± 0.03[ab]	0.2 ± 0.04[b]	0.1 ± 0.06	0.1 ± 0.03	ND
20:1	0.2 ± 0.06	0.2 ± 0.03	0.3 ± 0.05	0.3 ± 0.04	0.1 ± 0.04	0.6 ± 0.38
20:2n-6	0.2 ± 0.04[a]	0.2 ± 0.00[a]	0.1 ± 0.00[b]	0.3 ± 0.05	0.3 ± 0.03	ND
20:3n-6	0.3 ± 0.16	0.1 ± 0.03	0.2 ± 0.03	0.1 ± 0.00	0.2 ± 0.00	ND
20:4n-6	13.7 ± 0.44[a]	13.6 ± 1.90[a]	2.2 ± 0.09[b]	0.8 ± 0.04[a]	1.6 ± 0.05[b]	0.1 ± 0.00[c]
20:4n-3	0.0 ± 0.03	ND	0.1 ± 0.03	ND	ND	0.1 ± 0.00
20:5n-3	0.0 ± 0.08[a]	ND[a]	1.4 ± 0.17[b]	ND	ND	0.8 ± 0.06
22:0	0.8 ± 0.07[a]	1.0 ± 0.18[a]	0.4 ± 0.06[b]	ND	ND	ND
22:1	0.1 ± 0.03	ND	ND	ND	ND	ND
22:2n-6	0.1 ± 0.03	ND	ND	ND	ND	ND
22:4n-6	1.4 ± 0.21[a]	1.1 ± 0.07[a]	0.5 ± 0.05[b]	0.3 ± 0.03[a]	0.6 ± 0.03[b]	ND[c]
22:5n-6	1.1 ± 0.44[a]	1.3 ± 0.21[a]	ND[b]	0.2 ± 0.00[a]	0.3 ± 0.00	ND[c]
22:5n-3	0.3 ± 0.23	ND	ND	ND	ND	ND
22:6n-3	0.7 ± 0.09[a]	0.5 ± 0.09[a]	9.0 ± 1.23[a]	0.1 ± 0.03[a]	0.1 ± 0.05[a]	18.7 ± 1.19[b]
24:0	2.4 ± 0.26[a]	2.0 ± 0.41[a]	0.8 ± 0.09[b]	ND	ND	ND
24:1	0.7 ± 0.14	1.1 ± 0.30	0.5 ±0.13	ND	ND	ND
Saturates	44.5 ± 2.1	42.0 ± 2.1	44.6 ± 2.1	35.1 ± 0.3[a]	31.2 ± 1.7[a]	55.8 ± 2.5[b]
MUFA	20.4 ± 0.5	19.3 ± 2.8	23.2 ± 1.5	36.0 ± 1.0[a]	20.8 ± 0.7[b]	17.8 ± 0.8[c]
PUFAs	35.6 ± 2.0[ab]	38.8 ± 1.9[a]	32.0 ± 1.1[b]	28.9 ± 1.2[b]	47.0 ± 1.1[a]	25.4 ± 1.6[b]
P/S	0.8	0.9	0.7	0.8	1.5	0.46
n-6	34.2 ± 2.3[a]	38.1 ± 1.9[a]	21.4 ± 1.1[b]	28.8 ± 1.2[a]	46.8 ± 1.1[b]	5.9 ± 0.3[c]
n-3	1.4 ± 0.3[a]	0.65 ± 0.09[a]	10.6 ± 1.4[b]	0.15 ± 0.03[a]	0.28 ± 0.06[a]	19.6 ± 1.3[b]
n-6/n-3	28.3 ± 5.9[a]	62.3 ± 9.7[b]	2.1 ± 0.3[c]	215.5 ± 41.4[a]	223.5 ± 80.8[a]	0.3 ± 0.01[b]
Unsaturation index	1.29 ± 0.02	1.33 ± 0.02	1.34 ± 0.02	0.94 ± 0.01[a]	1.22 ± 0.01[b]	1.47 ± 0.02[c]

Values given are means ± standard error of the mean in mol % of total fatty acids. Values in a row, withing a lipid class, not sharing a letter are statistically different, $n = 4$, $P < 0.05$.

the large increase in DHA content in the phospholipid fraction in the MF-DHA fed rats, the concentration of a number of other fatty acids was changed, including increases in the content of shorter chain saturated fatty acids such as 14:0 and 16:0. Thus, it is likely that a number of individual changes in fatty acid composition worked in combination to create an environment that resulted in decreased glucose transport. One possible site of action in the insulin signalling/GLUT4 translocation cascade that may be affected by the fatty acid composition of the diet is the activity of phosphatidylinositol-3-kinase (PI-3-kinase). PI-3-kinase is activated in response to insulin receptor activation and is involved in the translocation of GLUT4 vesicles.[36] ω-3 fatty acids have been reported to decrease phosphoinositide phosphorylation by PI-4-kinase in platelets;[37] however, it is not known if ω-3 fatty acids alter the function of PI-3-kinase.

MF-DHA diets also resulted in a loss of inhibition of insulin-stimulated glucose uptake by the β-adrenergic agonist, isoproterenol. Activation of the β-adrenergic receptor leads to activation of $G\alpha_s$ and subsequent activation of adenylyl cylase. The decrease in $G\alpha_s$ protein expressed in MF-DHA fed rats could therefore contribute to the decreased response to isoproterenol. A number of previous investigations have demonstrated the influence of the fatty acid composition of the plasma membrane phospholipid on hormone stimulated adenylyl cyclase activity in liver.[30] Whereas no information is available as to the effects of fatty acid modifications on adenylyl cyclase in adipocytes, changes in the fatty acids in phospholipid resulting from MF-DHA feeding could also contribute to the loss of inhibition by isoproterenol.

The fatty acid composition of adipose membrane phospholipids are responsive to changes in dietary P/S ratio as well as the inclusion of specific fatty acids in the diet (*Table 3*).[30] The fatty acid composition of stored triglycerides was even more sensitive to dietary changes (*Table 3*). Stored triglycerides reflected the dietary source of fatty acids, i.e., increased concentrations of ω3 and saturated fatty acids in the MF-DHA fed rats. The unsaturation index, an estimate of the average number of double bonds per fatty acid, increased with MF-SO and MF-DHA feeding in the triglyceride fraction. In contrast, the unsaturation index in the

phospholipid fraction did not vary with diet. The increase of specific saturated fatty acids in the phospholipid fraction may have been part of an adaptive response by the organism to maintain a certain degree of membrane fluidity in the face of increased dietary polyunsaturated fatty acids.

A number of mechanisms have been proposed to account for changes in cellular metabolism resulting from changes in membrane fatty acid composition.[30] A favored hypothesis is that changes in membrane fluidity alter the interaction of different proteins within the plasma membrane and thus impact on their function. Alternatively, changes occurring within the microenvironment of a membrane protein might act more specifically to modify its function.[30] Another postulate is that changes in membrane composition influence the interaction of hormone binding to its receptor.[30] In adipose, diet-induced changes in the fatty acid composition of the membrane influence insulin receptor binding and function and, as a consequence, may modify the coupling of insulin receptor activation to glucose transport.[38,31]

Here we demonstrate that diet-induced changes in membrane phospholipid composition are also associated with changes in the quantity of specific membrane proteins involved in hormonal signal transduction and transport capacity. Not only was the total quantity of the glucose transporters (GLUT1 and GLUT4) regulated by changes in dietary fat content and composition, but the quantity of $G\alpha_s$, a protein involved in hormonal signal transduction, was also affected by dietary fat. These data suggest that, in addition to the dynamic responses of membrane phospholipid composition to diet, the quantity of specific membrane proteins is also regulated in response to long-term changes in the content and composition of dietary fat.

It is conceivable that some of the effects of DHA in the MF-DHA diet could be reversed by increasing the level of arachidonic acid (AA) available in the diet. Thus, the AA/DHA ratio may be an important consideration in addition to absolute PUFA levels. This needs to be addressed in additional experiments.

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