Effects of dietary fatty acids on the activity of glucose transport in adipocytes

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Lipid structure of plasma membrane plays an important role in regulating D-glucose transport in adipocytes. To investigate the effects of diet-induced alteration in cellular fatty acids on the activity of D-glucose transport in adipocytes, we fed rats iso-nutrient diets with high (20 energy% safflower oil) or low (2 energy% safflower oil plus 18 energy% beef tallow) safflower oil. After 31 days, adipocytes were isolated from epididymal fat pads using collagenase and incubated at 310°K (37°C) in KRB buffer containing 3% bovine serum albumin. Two-deoxyglucose transport into adipocytes was measured using an oil centrifugation method at 2, 5, and 10 minutes of incubation. The composition of fatty acids was analyzed in the fat pad and adipocyte incubation mixture by using gas chromatography. In the fat pad, fatty acids were composed of 61% linoleic, 18% each of palmitic and oleic, and 0.7% arachidonic acids in the high safflower oil group, and 56% oleic, 31% palmitic, 8% linoleic acids in the low safflower oil group. The compositions of dietary fatty acids were reflected directly in the composition of fatty acids in the fat pads and in the compositions of fatty acids released from isolated adipocytes. The high safflower oil diet significantly increased D-glucose transport in adipocytes compared with the low safflower oil diet group (8.66 vs. 6.31 nmol 2-deoxyglucose transport per 10^6 cells at 5-minute incubation). The results suggest that lipid composition in the tissue may have a regulatory effect on adipocyte glucose transport. Diet-induced high polyunsaturated fatty acids in the tissue facilitates carrier-mediated D-glucose transport in adipocytes.

Keywords: glucose transport; fatty acids, adipocytes

Introduction

D-glucose is transported across cell membranes by carrier-mediated, facilitated diffusion. This transport process involves a specific interaction between glucose and a membrane component, glucose transporter, which has been found in a variety of tissues including rat adipocytes[1] and human erythrocytes.[2] The transporter is a membrane-spanning glycoprotein[3]; its motion, and therefore its rate of operation, may be affected by the physical and/or chemical microenvironment of the membrane lipid bilayer. Isolated adipocytes have been used extensively to study the mechanism of facilitated glucose transport into cells. Several studies employing solubilized and reconstituted glucose transporter into phospholipid bilayer vesicles demonstrated that lipid structure of plasma membranes plays an important role in regulating the activity of D-glucose transport in adipocytes.[4-6] A positive correlation between membrane fluidity, induced by temperature[5] or induced by altered fatty acid composition,[4] and D-glucose transport in adipocyte membranes has been reported. Cholesterol-induced, altered membrane microviscosity also markedly influenced carrier-mediated D-glucose transport in various cell membranes.[4,5,7,8] The nonmetabolizable D-glucose analog, 2-deoxy-D-glucose (2-DG) is transported across cell membranes by the D-glucose transport system, phosphorylated by hexokinase, and accumulated intracellularly. The amount of 2-DG in the cell is the result of both transport and phosphorylation. The measurement of 2-deoxy-D-glucose transport (2-DG transport) provides an accurate assessment

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of glucose uptake provided that the rate of phosphorylation is rapid enough to prevent any accumulation of non-phosphorylated 2-DG (e.g., so that the transport itself is rate-limiting). Such conditions have been established for isolated adipocytes from rats[9,10] and have been used in the present experiment. The aim of this study was to investigate the effects of diet-induced alteration in cellular fatty acids on the basal rate of D-glucose transport into isolated fat cells harvested from rats. The results demonstrate a direct stimulatory effect on dietary polyunsaturated fatty acids on the D-glucose transport system in rat adipocytes.

Methods and materials

Animals and care

One-month-old male, non-obese Zucker rats were caged individually and maintained at 297°K (24°C) and 50% relative humidity. Twenty rats were distributed into two experimental diet groups to give close mean values for initial range of body weights between groups. Rats were fed either high safflower oil diet (HSO) containing 20% of total calories (en%) from safflower oil as the only fat source or low safflower oil diet (LSO) containing 2 en% from safflower oil and 18 en% from beef tallow for 31 days (*Table 1*). Diets were provided fresh twice-weekly ad libitum, and tap water was provided fresh daily ad libitum. Animals were weighed weekly, and food intakes were recorded.

Cell isolation

Epididymal fat pads from 5 rats per diet group were removed and pooled under ether anesthesia. The mean weights (n = 40) of pooled fat pads were 10.4 g in the HSO group and 11.2 g in the LSO group. The difference was not statistically significant. A sample of the pooled fat pad was stored at 253°K (−20°C) for later fatty acid analysis; the remainder of the pooled fat pad was subjected to an immediate isolation of adipocytes using collagenase, following the method of Rodbell.[11] Briefly, the tissue was incubated with collagenase (Sigma, St. Louis, MO, 2 mg/g tissue) in Krebs-Ringer Bicarbonate buffer (KRB, pH 7.4) containing 3% bovine serum albumin (KRB-BSA), at 310°K (37°C) for 1 hour, and gassed with 95% O_2-5% CO_2 (Cooks Inc., Algona, IA). The digestion mixture was filtered through a 250 μm nylon screen (Tetko, Elmford, NY) with KRB rinses. The crude cell suspension was centrifuged (50g for 1 minute) and the isolated cells were resuspended in KRB-BSA. The number of isolated cells was counted microscopically using a hemocytometer and the viability of the cells was determined by the trypan blue exclusion test. In all cases, the cell viability was greater than 95%. Sizes of cells were determined using a Coulter counter.[12] The mean cell size in μm diameter was 74.6 in the HSO group and 75.9 in the LSO group after 31 days' feeding. The difference was not statistically significant.

Table 1 Composition of diets containing high (HSO) or low (LSO) concentrations of linoleic acid from safflower oil

Ingredient	HSO	LSO
	(g/100 g)	
Casein[a]	16.8	16.8
l-Methionine[a]	0.2	0.2
Corn starch[b]	32.6	32.6
Sucrose[c]	32.6	32.6
Cellulose[d]	2.2	2.2
Salt mix[e]	4.4	4.4
Vitamin mix[f]	1.0	1.0
Safflower oil[g]	9.3	1.1
Beef tallow[h]	0.0	8.2
	(mg/100 g)	
PABA[i]	83.8	83.8
Inositol[i]	165.0	165.0
Choline chloride[i]	331.8	331.8
a-Tocopherol[i]	212.8	212.8
Energy distribution		
	(percent)	
Casein	16.3	16.3
Corn starch	31.7	31.7
Sucrose	31.7	31.7
Safflower oil	20.3	2.4
Beef tallow	0.0	17.9

[a] United States Biochemical Co., Cleveland, OH.
[b] Argo, Best Foods CPC International Inc., Englewood Cliffs, NJ.
[c] California and Hawaiian Sugar Co., San Francisco, CA.
[d] Teklad, Madison, WI.
[e] ICN Nutritional Biochemicals, Cleveland, OH, AIN Mineral Mixture 76.
[f] ICN Nutritional Biochemicals, Cleveland, OH, AIN Vitamin Mixture 76.
[g] Hollywood Health Foods, Los Angeles, CA.
[h] Meat Lab, Dept. of Animal Sciences, Iowa State Univ., Ames, IA.
[i] Nutritional Biochemicals, Cleveland, OH.
[j] Sigma, St. Louis, MO.

Two-deoxy glucose transport

Optimum experimental conditions to test the rate of D-glucose transport, affected by the composition of dietary fatty acids, were determined as follows. Four hundred μL of prepared cell suspension, containing 4 × 10^4 to 6.2 × 10^5 cells per mL, was preincubated for 30 minutes and incubated with test mixtures for 5 to 30 minutes at 310°K (37°C) in a metabolic shaker with 400 μL of KRB-BSA, and gassed with 95% O_2-5% CO_2. The measurement of 2-DG transport was initiated by adding a test mixture containing 2-deoxy-D-[1-^{3}H]-glucose at 2 μCi, inulin-[^{14}C]Carboxylic acid at 0.5 μCi (Amersham, Arlington Heights, IL), and 2-deoxy-D-glucose (Sigma, St. Louis, MO) at 0.1 mmol/L in KRB-BSA. At intervals, the reaction was terminated by transferring 200 μL of the incubation mixture into a 1 mL silicone oil (d = 0.943, Milwaukee, WI) layered tube and centrifuging it at 3,000g for 1 minute.[13] Adipocytes were removed from atop the oil with a plastic-tipped automatic pipet, and the radioactivity was determined by using a liquid scintillation counter (Tri-Carb Liquid Scintillation Spectrometer C2425, Packard Instrument Co., Downers Grove, IL) with 15 mL of scintillation solution (Scin-

tiverse Bio-HP, Fisher, Springfield, NJ). Extracellular space was calculated using ^{14}C-inulin as the marker and that value was used in subtracting 2-DG that was not transported into cells but trapped extracellularly. The mean trapped 2-DG in the extracellular space was 15.9 ± 8.9% (mean ± SEM) of the total amount of the 2-DG found in the packed cell at the 10-minute incubation time. Based on the results of these pilot tests, the protocol of 2-DG transport into adipocytes was determined; 400 μL of prepared cell suspension containing 4×10^4 to 4×10^5 cells per mL of incubation mixture was preincubated for 30 minutes and 2-DG transport was tested during a 10-minute incubation. Reaction rates were plotted as nmol 2-DG transport per 10^6 cells versus incubation time.

Fatty acid analysis

Fifty mg epididymal adipose tissue was saponified in 2 mL, 10% KOH/Ethanol at 333°K (60°C). After the saponification, 2 mL H_2O was added, and non-saponifiable lipids were extracted three times with 4 mL portions of petroleum ether. The aqueous phase was then acidified, and fatty acids were extracted three times with petroleum-ether. In the adipocyte incubation mixture, free fatty acids were extracted using Dole reagent[14] and quantified in the adipocyte incubation mixture using heptadecanoic acid (Sigma, St. Louis, MO) as an internal standard. The fatty acid extracts were combined and evaporated to dryness under nitrogen and methylated by reacting with boron tri-fluoride in methanol (14% w/v, Alltech, Deerfield, IL).[15] The methylated fatty acids were extracted and the composition of fatty acid methyl esters was examined using a gas chromatograph (Beckman GC 72-5) equipped with a CRS-208 Columbia Scientific integrator, a Model 20 Texas Instrument's recorder, and a stainless steel column packed with Alltech 10% CS-10; column temperature 453°K (180°C), injection port and line temperature 493°K (220°C), detector temperature 298°K (250°C).

Statistical analysis

Data were analyzed statistically using Analysis of Variance and General Linear Model's Procedure.[16] Group means were compared by Least Significant Difference at a 5% level of probability and reported with the standard error of the mean (SEM) if the variance between treatment groups was homogeneous by Bartlett's test.[17] Correlation coefficients were determined between percentage composition of fatty acids in the tissue and fatty acids released into the incubation mixture and were deemed significant if the $r > 0.5$ and $P \leq 0.05$.

Results

Food intakes and body weight changes

The mean daily food intake was 14.23 ± 0.36 versus 13.09 ± 1.09 g/d (LSO vs. HSO, mean ± SEM, $n = 40$); however, the difference was not statistically significant. The mean growth rate was 5.52 ± 0.11 g/d in the HSO group, and 5.57 ± 0.18 g/d in the LSO group.

Table 2 Effects of diets different in fat qualities on the fatty acid composition of epididymal fat pads of rats[a]

	Diet	
Fatty Acid	HSO	LSO
	(percent)	
14:0[b]	0.70 ± 0.05	2.15 ± 0.03[c]
16:0	18.05 ± 0.28	31.29 ± 0.81[c]
18:0	1.50 ± 0.06	2.32 ± 0.82[c]
18:1	18.14 ± 0.36	55.67 ± 0.59[c]
18:2	60.77 ± 0.49	8.05 ± 0.62[c]
18:3	0.16 ± 0.04	0.48 ± 0.04[c]
20:4	0.71 ± 0.03	0.05 ± 0.01[c]

[a] Mean + SEM. $n = 8$. HSO diet contained 9.3% safflower oil and LSO diet contained 1.1% safflower oil and 8.2% beef tallow by weight.
[b] Number of carbons:number of double bonds.
[c] Values were signficantly different from HSO ($P \leq 0.05$).

Fatty acid composition

Dietary treatment markedly changed the composition of fatty acids in the epididymal fat pads (*Table 2*). Fat pads harvested from rats fed the HSO diet contained significantly higher linoleic acid (14.2-times the LSO), compared with the LSO group, at the expense of other fatty acids (oleic acid; 0.3-times the LSO, palmitic acid; 0.6-times the LSO, stearic acid, myristic acid, and linolenic acid). All the observed differences in the composition of fatty acids in the epididymal fat pads between the two diet groups were statistically significant.

Adipocytes harvested from rats fed HSO diet released significantly greater proportions of linoleic acid (6-times the LSO) and lesser proportions of palmitic, oleic, and myristic acids (0.7-times, 0.3-times, and 0.3-times the LSO, respectively) during the 15-minute incubation (*Table 3*). The significant correlation coefficients between percentage composition of each fatty acid in the tissue and that released from isolated adipocytes during the 15-minute incubation were + 0.76, + 0.92, + 0.56, and + 0.99, and + 0.78 for palmitic, stearic, oleic, linoleic, and linolenic acids, respectively. Adipocytes in the HSO group released twice ($P \leq 0.05$) the total free fatty acids as the LSO group during the 15-minute incubation. This difference was mainly caused by the elevated release of linoleic acid in the HSO group (*Table 3*).

Two-deoxy glucose transport

The amount of 2-DG transported was increased linearly with incubation time for a period of 15 minutes. After 15 minutes, the rate slowed, and the reaction curve became curvilinear, suggesting that some efflux of 2-DG was occurring. Based on the result of this

Table 3 Effects of diets different in fat qualities on the concentration and composition of free fatty acids released from adipocytes[a]

Diet Fatty Acid	HSO	LSO	HSO	LSO
	(μmol/10^6 cells)		(percent)	
14:0[b]	0.07 ± 0.00	0.11 ± 0.01	0.85 ± 0.08	2.54 ± 0.21[c]
16:0	1.45 ± 0.00	1.02 ± 0.19	18.50 ± 1.08	27.25 ± 1.24[c]
18:0	0.15 ± 0.02	0.09 ± 0.02[c]	2.21 ± 0.51	2.66 ± 0.51
18:1	1.11 ± 0.00	1.89 ± 0.21[c]	15.63 ± 0.66	55.75 ± 0.63[c]
18:2	4.40 ± 0.75	0.36 ± 0.05[c]	61.96 ± 1.21	10.72 ± 1.33[c]
18:3	0.02 ± 0.00	0.02 ± 0.01	0.27 ± 0.12	0.65 ± 0.15
20:4	0.04 ± 0.02	0.01 ± 0.00	0.58 ± 0.13	0.43 ± 0.31
Total	7.24 ± 1.39	3.50 ± 0.44[c]	100.00 ± 0.54	100.00 ± 0.63

[a] Mean ± SEM. n = 8. HSO diet contained 9% safflower oil and LSO diet contained 1.1% safflower oil plus 8.2% beef tallow by weight. Adipocytes were isolated from epididymal fat pads, preincubated 30 minutes, and incubated for 15 minutes at 310°K (37°C).
[b] Number of carbons:number of double bonds.
[c] Values were significantly different from HSO ($P \leq 0.05$).

pilot study, cells were incubated for 10 minutes in the subsequent experiments. The amount of 2-DG transported was increased linearly with the increase of the number of fat cells up to 4.2 × 10^5 cells per mL of incubation mixture. More than 4.2 × 10^5 cells per mL caused a curvilinear response. Based on this result, incubation mixtures containing 4 × 10^4 to 4 × 10^5 cells per mL incubation mixture were used in the subsequent experiments. *Figure 1* shows the effect of diets on 2-DG transport into isolated adipocytes. Adipocytes from rats fed the HSO diet had greater 2-DG transport than the LSO group throughout the 10-minute incubation. The difference was statistically significant at the 2-, and 5-minute incubation times. Nanomoles of 2-DG transport into 10^6 adipocytes of HSO and LSO groups were 4.48 ± 0.43 and 2.96 ± 0.19 at 2 minutes, 8.66 ± 0.79 and 6.31 ± 0.54 at 5 minutes, and 12.28 ± 1.18 and 10.22 ± 1.17 at 10 minutes incubation, respectively.

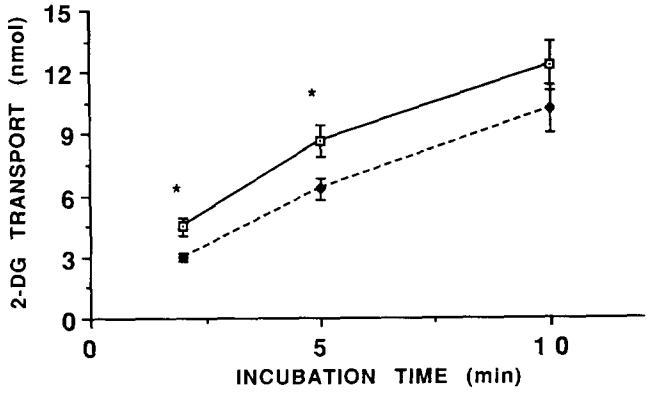

Figure 1 Effects of diets on adipocyte 2-deoxyglucose transport. Mean ± SEM, n = 40. Nanomoles 2-deoxyglucose transport into 10^6 cells during a 10-minute incubation. Rats were fed diets with 20 energy% safflower oil (HSO) or 2 energy% safflower oil plus 18 energy% beef tallow (LSO) for 31 d. Adipocytes were isolated from epididymal fat pads, preincubated 30 minutes, and incubated for 10 minutes at 310°K (37°C). Values were significantly different between HSO and LSO: *, $P \leq 0.05$; —— HSO; ---- LSO.

Discussion

Safflower oil contains approximately 74% linoleic acid, 13% oleic acid, and 7% palmitic acid. Beef tallow contains 29% palmitic acid, 48% oleic acid, and 15% stearic acid,[18] and small amounts of linoleic acid. The observed compositions of fatty acids in the epididymal fat pads in this study show that the composition of fatty acids in the dietary fats were directly reflected in the composition of fatty acids in the adipose tissue, and the composition of fatty acids in the tissue was also directly reflected in the compositions of fatty acids released from isolated adipocytes. This result is consistent with previous studies on depot fat and erythrocytes.[19] Adipocytes in the HSO group released 2 times the amount of total free fatty acids compared to the LSO group during the 15-minute incubation. The mass of the free fatty acids, released during 15-minute incubation, was 7.2 μmol in the HSO group and 3.5 μmol in the LSO group. The fact that the difference was due mainly to the elevated release of linoleic acid in the HSO group, suggests that linoleic acid may be a preferable substrate for activities of lipolytic enzymes, and that the turnover rate of linoleic acid may be faster than other fatty acids in adipocytes. Linoleic acid is the most important dietary precursor of eicosanoids which evoke a wide spectrum of biological reactions. Eicosanoids are not stored within the cell, and one regulatory step in eicosanoid synthesis is the precursor availability.[20] Precursors are located in the phospholipids of membanes which also house glucose transporter. Reports about the effects of eicosanoids on carrier-mediated, D-glucose transport are equivocal.[21-26] However, many papers report that eicosanoids, particularly PGE_1[21,22,26] have an insulin-like effect on D-glucose uptake in adipocytes. According to the literature,[27] two-deoxyglucose uptake is saturable at 5 mmol/L and shows Km at 1-1.3 mmol/L in isolated rat adipocytes. In the present study, 2-deoxyglucose transport was measured at submaximal, 0.1 mmol/L D-glucose concentrations; therefore, we do

not know if the observed increment in D-glucose transport in the HSO group was due to a decreased Km or an increased Vm, or both. That remains for future elucidation through kinetic studies on the effects of dietary fatty acids on the D-glucose transport system in adipocytes.

Our results are consistent with the hypothesis[4-6,28] that the physical and/or chemical microenvironment of membrane lipid domains associated with the transporter may be an important factor in regulating carrier-mediated glucose transport. Mechanisms postulated for this hypothesis include the following: A). Changes in the structure of membrane microenvironment may alter the conformation of quarternary structure of the transporter, thereby enhancing or reducing the accessibility of their substrate binding sites leading to changes in the activity.[26] An interconversion of glucose transporter between two conformations (one with an inward-facing substrate binding site and one with an outward-facing substrate binding site) have been reported for human erythrocytes.[2] The presence of glucose induces this interconversion. It is not known if an altered microenvironment of lipid bilayer would affect this interconversion of conformations in glucose transporter. B). Phase transition of membrane phospholipids may alter the partition of existing glucose transporter between fluid and ordered lipid phases[5] or may affect penetration of glucose transporter into the membrane from an intracellular pool, altering the number of transporters available.[28] There are several reports about a carrier-mediated glucose transport system which are consistent with this notion. Altered composition of fatty acids in reconstituted phospholipid vesicles affects glucose transport activity; unsaturated fatty acids (oleic and vaccenic acids) stimulated and saturated fatty acids (stearic and palmitic acids) inhibited glucose transport in reconstituted liposomes.[4] The maximum D-glucose transport activity was achieved when the membrane bilayer fluidity was maximal in reconstituted liposomes with adipocyte intrinsic membrane proteins.[6] C). The transporter may require specific lipids or a specific organization of membrane lipids for its maximal function.[29] Hutchinson et al.[30] reported that alcohols (methanol to octanol) increased membrane fluidity but inhibited 2-deoxyglucose transport in isolated adipocytes at molar potencies comparable to the membrane disordering, measured by 5-nitroxide stearate spin probe. This observation suggests that glucose transport is inhibited if the hydrophobic regions of the transporter-containing membranes are perturbed even though the membrane is in a fluid state. Strålfers reported that fatty acid composition in 1,2-diacylglycerols affected D-glucose transport into isolated rat adipocytes when 1,2-diacylglycerols were added to the incubation mixture along with the emulsifier, sodium taurocholate. Myristic acid, palmitoleic acid, and oleic acid showed most prominent stimulatory effects on basal D-glucose transport in adipocytes.[31]

The results of the present study indicate that dietary fatty acids affect fatty acid composition in fat tissue, and tissue lipid composition affects glucose transport in adipocytes. A high-polyunsaturated fatty acid diet increases carrier-mediated glucose transport in adipocytes. The enhanced, intracellular glucose may be utilized as energy or may facilitate the synthesis of triglyceride and membrane phospholipids by providing glycerol backbones and acetyl-CoA building blocks.

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