

A modified high-fat diet induces insulin resistance in rat skeletal muscle but not adipocytes

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Wilkes, Jason J., Arend Bonen, and Rhonda C. Bell. A modified high-fat diet induces insulin resistance in rat skeletal muscle but not adipocytes. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E679–E686, 1998.—We hypothesized that variation in dietary fatty acid composition in rats fed a high-fat diet had tissue-specific effects on glucose uptake sufficient to maintain normal glucose tolerance. Rats were fed one of three diets for 3 wk. The isocaloric high-fat-mixed oil (HF-mixed) diet and the high-fat-safflower oil (HF-saff) diet both provided 60% kcal fat, but fat composition differed [HF-mixed = saturated, polyunsaturated (n-3 and n-6), and monounsaturated fatty acids; HF-saff = polyunsaturated fatty acids (mainly n-6)]. The control diet was high carbohydrate (HCHO, 10% kcal fat). Insulin-stimulated 3-*O*-methylglucose uptake into perfused hindlimb muscles was reduced in rats fed HF-saff and HF-mixed diets compared with those fed HCHO diet ($P < 0.02$). Basal uptake increased in HF-saff- and HF-mixed-fed rats vs. HCHO-fed rats ($P < 0.04$). In adipocytes, HF-saff feeding decreased 2-deoxyglucose uptake vs. HF-mixed feeding and HCHO feeding ($P < 0.05$), but 2-deoxyglucose uptake in HF-mixed-fed rats did not differ from that in HCHO-fed rats ($P > 0.05$). Glucose tolerance was significantly reduced in HF-saff-fed rats but was unaffected by the HF-mixed diet. Therefore, in skeletal muscle of rats, 1) feeding a diet high in fat induces a reduction in insulin-stimulated glucose uptake but 2) provides an increase in basal glucose uptake. In contrast, 3) in adipocytes, insulin-stimulated glucose transport is reduced only when the high-fat diet is high in n-6 polyunsaturated fatty acids but not when fat comes from these mixed sources. Glucose intolerance becomes evident when insulin resistance is seen in multiple tissues.

high-fat feeding; fatty acids; rats

IN RODENTS AND HUMANS, dietary intake of high amounts of fat has been shown to have adverse effects on insulin sensitivity and to contribute to the development of glucose intolerance and overt diabetes [recently reviewed by Storlien et al. (32)]. Much of the work reported in the literature has used a model in which the absolute amount of dietary fat is considered to be the central variable affecting insulin sensitivity. Fat intakes ranging from ~40 to 75% of total kilocalories, usually in the form of saturated fat, safflower oil, or corn oil (both high in n-6 polyunsaturated fatty acids), have been reported to reduce whole body insulin-stimulated glucose uptake (6, 10, 14, 15, 17, 19, 23, 27, 34, 35). This is accompanied by reductions in insulin-

stimulated glucose uptake in individual skeletal muscles (6, 11, 14, 15, 17, 19, 23, 33, 35) and adipose tissue (6, 8, 35) along with enhanced hepatic glucose production (10, 14, 34). Thus high dietary fat intake will alter the regulation of glucose transport in insulin-sensitive tissues, leading to changes in the whole body.

In addition to the amount of dietary fat being an important determinant of insulin sensitivity, there is also evidence that variation in fatty acid composition may independently affect insulin action and alter insulin sensitivity. Storlien et al. (34) demonstrated that when a portion of the fat from a high polyunsaturated fat diet (60% total kcal as fat) was replaced with fish oil (6% kcal of total kcal) containing long-chain n-3 fatty acids, glucose uptake in skeletal muscle and hepatic glucose output were restored to levels observed in chow-fed rats. Saturated fat combined with these large amounts of linolenic acid (C18:3, n-3) similarly alleviated insulin resistance in muscle and in the whole body (33). Monounsaturated fatty acids, when fed at high levels, may not lead to the reductions in insulin sensitivity observed with the high polyunsaturated fat diets (4, 26) and may improve indexes of glucose tolerance in non-insulin-dependent diabetes mellitus patients (18). Thus different dietary fatty acids appear to have varying effects on insulin-sensitive tissues, and this may affect the expression of glucose tolerance.

The purpose of this study was to examine direct measures of insulin resistance in several insulin-dependent tissues in high-fat-fed rats consuming diets of 60% kcal from fat but with different fatty acid combinations and to relate these measures to whole body glucose tolerance. Initial studies in our laboratory used a modified high-fat version (60% kcal fat) of the American Institute of Nutrition 1993 (high-fat-mixed oil, HF-mixed) diet as a model of insulin resistance. We predicted that 3 wk of feeding this diet would produce a similar degree of insulin resistance and glucose intolerance as had been observed with a safflower oil-based high-fat diet (33, 34) because the HF-mixed diet contained a high degree of polyunsaturated and saturated fatty acids and a relatively low amount of n-3 fatty acids, in addition to a large proportion of calories as fat. In fact, in preliminary studies, we observed no difference in glucose tolerance in rats fed the HF-mixed diet vs. those fed a control, 60% carbohydrate diet (1). This prompted us to consider the possibility that this combination of fatty acids in the HF-mixed diet may lead to tissue-specific changes in glucose uptake. We hypothesized that normal glucose tolerance observed after feeding the HF-mixed diet may reflect reciprocal changes in either the presence or degree of insulin resistance in insulin-sensitive tissues.

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MATERIALS AND METHODS

Animals and diets. Male Sprague-Dawley rats weighing ~200 g were individually housed, with a 12:12-h light-dark cycle, and were randomly assigned to one of three dietary groups (Dyets, Bethlehem, PA): high carbohydrate (HCHO: 20% kcal protein, 10% fat, 70% carbohydrate), high fat-safflower oil (HF-saff, 20% kcal protein, 59% fat, 20% carbohydrate), or HF-mixed (20% kcal protein, 59% fat, 20% carbohydrate). The source of fat in the HCHO and HF-saff diets was safflower oil, whereas the HF-mixed diet contained a mixture of soybean oil (12% of kcal), hydrogenated coconut oil (24% of kcal), and high-oleic sunflower oil (24% of kcal). Diets used in this study are outlined in Table 1.

Before the start of the experimental diets, food intake (g/day) on standard rat chow (lab chow, PMI Feeds, St. Louis, MO) was determined for 3 days. Rats were fed their respective experimental diets at 98% of ad libitum chow energy intake daily; access to water was unlimited at all times. Food was generally consumed in its entirety by all animals each day. Rats were weighed a minimum of three times weekly to ensure that growth rates in all dietary groups were similar. After 3 wk of feeding, rats underwent an intravenous glucose tolerance test (details below). Rats recovered from this procedure for 5 days before glucose transport was directly assessed in skeletal muscle and adipose tissue. All procedures used in this study were approved by the Animal Care Committee at the University of Waterloo and followed the Guidelines of the Canadian Council on Animal Care.

Intravenous glucose tolerance tests. One subgroup of rats ($n = 10$ rats/dietary group) underwent the intravenous glucose tolerance tests (IVGTTs) in the anesthetized state, whereas a second subgroup of rats ($n = 5$ rats/dietary group) were conscious during their IVGTTs. All rats were fasted overnight before the IVGTT. For the anesthetized group, rats were anesthetized with a tail vein injection of pentobarbital sodium (65 mg/kg body wt, less 10%), and buprenorphine

(Temgesic, 0.003 mg/100 g body wt) was administered subcutaneously as an analgesic. Animals were kept on a heating blanket during both the surgery and experiment to prevent hypothermia during anesthesia (16). The jugular vein was fitted with a catheter, and animals were allowed to rest for 30 min. Two basal blood samples were drawn at 5-min intervals, and then a bolus of glucose (300 mg/kg, 10% solution) was injected into the catheter and immediately rinsed with heparinized saline (16 units/ml) to prevent clotting. Blood samples (~0.2 ml) were collected into NaF/heparinized microcentrifuge tubes at 1, 2.5, 3.5, 5, 6.5, 8, 12, and 18 min post-glucose dose and placed on ice. Samples were centrifuged (Eppendorf microcentrifuge, 12,000 rpm, 4 min), and plasma was separated from red cells. Plasma for glucose and insulin determinations was stored frozen at -20°C until use. Glucose was determined by a spectrophotometric method (glucose Trinder method, no. 315; Sigma, St. Louis, MO), and insulin was determined by RIA using a rat-specific antibody (Linco, St. Charles, MO). After the IVGTT, the jugular catheters were tied off, and rats were allowed to recover under a heating lamp and were returned to their cages later the same day. Rats ate normal quantities of food 24–48 h after surgery, and body weights were back to preoperative levels by 2–3 days after surgery.

For the conscious group, rats were anesthetized under halothane, given buprenorphine, fitted with the jugular catheter, and then allowed to regain consciousness. Rats rested quietly in the experimental cage for 30 min before the basal blood samples were drawn. Glucose administration, timing of samples after the glucose bolus, sample separation and storage, and animal recovery were identical to those in the protocol outlined for the anesthetized rats.

Glucose tolerance (K_g) was determined as the slope of the log of blood glucose vs. time, using the samples obtained from the 2.5- to 12-min period after glucose injection. In both the conscious and anesthetized groups, K_g showed significant effects of diet treatment ($P < 0.001$), but the effects of anesthesia were not statistically different ($P > 0.05$); therefore, values from the anesthetized and conscious groups were combined. The acute insulin response to glucose was calculated as the area under the insulin curve from 1 to 6.5 min after glucose injection and was corrected for the prestimulus baseline levels. Plasma from basal (fasting) samples was also used for determination of triglycerides (Sigma, no. 339) and free fatty acids (Waco Chemicals, Richmond, VA).

3-O-methylglucose uptake into perfused hindlimbs. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt), and the epididymal fat pads were quickly removed for assessment of glucose uptake into isolated adipocytes. Rats were surgically prepared for hindlimb perfusion as described by Ruderman et al. (29), with modifications previously made in our lab (7, 20, 21). Briefly, the gastrointestinal tract was removed, and all major blood vessels within the abdominal cavity were tightly sutured. Heparin (500 mU/ml) was injected into the vena cava of each rat (0.5 ml) before cannulation to inhibit clotting. The descending aorta was cannulated (20-gauge angiocath) to deliver perfusate to the hindlimb, and the inferior vena cava was cannulated (14-gauge angiocath) to remove the effluent to complete a one-pass perfusion system. A cell-free perfusate containing Krebs-Henseleit buffer with 4% BSA (Sigma) under constant gassing (95% O_2 -5% CO_2) was connected to the indwelling cannulas via Tygon tubing, which was connected to a pump while the rat was layed in a warm chamber (30°C). Over the first 20 min of the perfusion protocol, the flow rate was gradually increased to 20 ml/min once flow back pressure stabilized between -80 and -100 mmHg. This flow

Table 1. Compositions of diets used in this study

	HCHO Diet	HF-Saff Diet	HF-Mixed Diet
	<i>g/kg Diet</i>		
Casein	188	254	200
Cornstarch	438	169	227
Sucrose/maltose dextrin*	219	85	132
Wheat bran/cellulose*	38	51	50
Safflower oil	41	339	
Soybean oil			70
High-oleic sunflower oil			135
Hydrogenated coconut oil			135
Gelatin	14	19	
Salt mix†	50	67	35
Vitamin mix‡	9.7	13	10
Vitamin E acetate (500 IU/g)		0.31	
DL-Methionine/L-cystine*	2.3	3	3
Choline bitartrate			2.5
	<i>%kcal</i>		
CHO	69	20	27
Fat	10	59	59
Protein	21	21	15
Caloric value (kcal/g)	3.82	5.20	5.16

HCHO, high carbohydrate (CHO); HF-saff, high fat-safflower oil; HF-mixed, high fat-mixed oils. *Ingredient in HF-mixed-based diet. †Salt mix for HCHO and HF-saff diets is no. 200301 and for HF-mixed diet is no. 210025. ‡Vitamin mix for HCHO and HF-saff diets is no. 310027 and for HF-mixed diet is no. 310025.

rate is sufficient to perfuse and oxygenate the entire hindlimb (7). After 20 min, 30 mM 3-*O*-methylglucose containing 5 mCi 3-*O*-[methyl-³H]glucose and 2.5 mCi L-[¹⁴C]glucose were added to the perfusate, and the hindlimb was perfused for an additional 5 min. Addition of L-[¹⁴C]glucose to the perfusate allowed us to account for the nontransported diffusion component of glucose uptake into skeletal muscle. To assess insulin-stimulated glucose uptake, insulin (40 nM, porcine insulin, Iletin II regular; Eli Lilly, Indianapolis, IN) was added to the buffer at the start of the perfusion and was maintained at this concentration for the duration of the experiment. This concentration of insulin has been previously shown by our laboratory and others to stimulate glucose uptake maximally in skeletal muscle of normal, chow-fed rats with the use of this perfusion system (7). Muscles were categorized on the basis of their oxidative potential, and results were pooled accordingly (22).

2-Deoxyglucose uptake into adipocytes. At the time of the hindlimb perfusion experiment, epididymal fat pads were rapidly excised, weighed, and minced, and adipocytes were isolated by collagenase digestion according to the method of Rodbell (28), with modifications by Wilkes et al. (37). After collagenase digestion (which contained 100 nM *N*⁶-phenylisopropyl adenosine), isolated adipocytes were washed 3 times with PBS with 1 mM MgCl₂ and 0.68 mM CaCl₂, 1 mg/ml BSA, and 1 mM pyruvic acid, pH 7.4. Adipocytes were counted in a hemacytometer and resuspended to 5 × 10⁵ cells/ml. Adenosine deaminase (Boehringer Mannheim, Doval, PQ, Canada) was added to cell suspensions in all treatments to remove endogenous adenosine. Adipocytes were treated in round-bottom test tubes with insulin (Iletin II regular, Eli Lilly), ranging from 0 (basal) to 10 nM (final concentrations), for 30 min in a shaking water bath (37°C, 100 oscillations/min). Nonspecific uptake was measured in cells preincubated with phloretin (0.3 mM) after 25 min of incubation, and radioactivity from this condition was subtracted from all other treatments to obtain specific uptake; nonspecific uptake was confirmed using cytochalasin B (data not shown). In some incubations, the phosphatidylinositol (PI) 3-kinase-specific inhibitor LY-294002 (50 μM final concentration; Calbiochem, La Jolla, CA) was added at the start of the preincubation, and 4 nM insulin was added after 27 min of incubation to demonstrate inhibition of insulin-stimulated glucose uptake.

After the preincubation, 150-ml aliquots of adipocytes were layered over 100 ml of oil (light mineral oil, Fischer Scientific, Mississauga, ON, Canada; silicon oil, Aldrich Chemical, St. Louis, MO; 57:43) in 400 ml of polyethylene microcentrifuge tubes. Glucose (10 mM) containing 2-deoxy-[1,2-³H]glucose (50 μl, 0.5 μCi/tube) was added to the adipocytes, and uptake was allowed to occur for 3.5 min. Glucose uptake was stopped by centrifugation for 6 s at 14,000 rpm (Eppendorf microcentrifuge). The top layer containing the adipocytes was cut off, using a razor blade, into polypropylene scintillation vials, and radioactivity associated with adipocytes was quantified by liquid scintillation counting.

Determination of fatty acid composition of adipose tissue. Fatty acid composition was determined in samples of epididymal adipose tissue from a subset of rats (*n* = 5 rats/group) with the use of the method described by Wainwright et al. (36). Homogenized adipose tissue was extracted using chloroform-methanol (1:1, vol/vol) in the presence of 0.02% butylated hydroxytoluene (wt/vol). After separation, samples were dried under nitrogen, and the total lipids were fractionated by TLC with the use of silica gel plates (Analtech GF) and a chloroform-methanol-acetic acid-water (50:30:4:2) solvent system. The fatty acids of the resulting phospholipid fractions

were methylated with 14% boron trifluoride in methanol and analyzed by gas chromatography (Perkin-Elmer 8420, Norwalk, CT). Fatty acids were identified by comparison of their retention times with those of authentic standards.

Statistical analysis. Body weight, food intake, fat pad weight, and *K_g* data were assessed for differences among dietary groups using a one-way ANOVA (Statview, Abacus Concepts, Berkeley, CA). Differences in glucose uptake among different muscles and in adipocytes at the various concentrations of insulin were assessed using a repeated-measures one-way ANOVA, with muscle type and insulin concentration, respectively, as the repeated factors. Significant effects were further analyzed using Tukey's *t*-test; for all analyses, *P* < 0.05 was accepted as statistically significant.

RESULTS

Body weights of rats fed the HF-mixed, HF-saff, and HCHO diets are shown in Fig. 1. Body weights did not differ among any of the dietary groups at any time during the feeding protocol. Rats in all dietary groups consumed similar amounts of food (kcal/day) throughout the study. Mean intake for the different dietary groups was as follows: HCHO, 91 ± 0.77 kcal/day; HF-saff, 91 ± 1.56 kcal/day; and HF-mixed, 91 ± 1.04 kcal/day. Food intake did not differ significantly among dietary groups.

Final body weights; fat pad weights; fasting glucose, insulin, triglyceride, and free fatty acid concentrations; characteristics of *K_g*; and the acute insulin response to glucose are presented in Table 2. Final body weights measured on the day of the hindlimb perfusions did not differ significantly among the experimental treatment groups. Despite similar body weights and similar caloric intakes among dietary groups, fat pads of rats in the HF-mixed group weighed significantly more than fat pads from rats in the other two groups (*P* < 0.005). Fasting plasma levels of glucose, insulin, triglycerides, and free fatty acids did not differ significantly among experimental treatment groups.

K_g, calculated as the slope of the log of blood glucose concentrations over time for 2.5–12 min after the bolus glucose injection, showed a significant main effect of

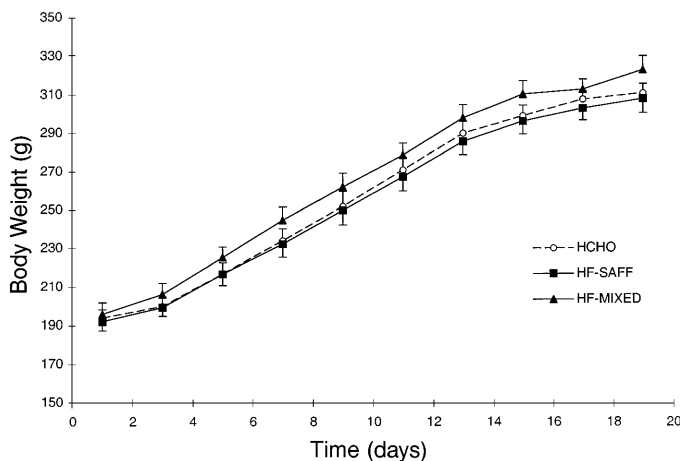


Fig. 1. Mean body weights (\pm SE) of groups of rats fed a high-carbohydrate (HCHO), a high-fat-safflower oil (HF-saff), or a high-fat-mixed oil (HF-mixed) diet for 3–4 wk. Body weights did not differ significantly among dietary groups at any time during study.

Table 2. Final body weights; fat pad weights; fasting plasma triglyceride, free fatty acid, and glucose concentrations; characteristics of glucose tolerance; and acute insulin response to glucose in rats after 3–4 wk of experimental diets

Measurement	HCHO Diet	HF-Saff Diet	HF-Mixed Diet
Final body wt, g	309 ± 4	317 ± 5	321 ± 4
Fat pad wt, g	3.49 ± 0.19*	3.55 ± 0.11*	4.65 ± 0.025†
Glucose, mM	7.1 ± 1.7	6.7 ± 0.2	6.5 ± 0.2
Insulin, ng/ml	0.68 ± 0.08	0.54 ± 0.12	0.58 ± 0.08
Triglycerides, mg/dl	17.1 ± 5.2	13.4 ± 3.9	16.9 ± 4.4
Free fatty acids, mmol/l	0.85 ± 0.26	0.78 ± 0.22	0.74 ± 0.19
K_g , %/min	3.70 ± 0.4*	2.70 ± 0.04†	3.90 ± 0.02*
Acute insulin response to glucose (AUC – basal)	11.5 ± 1.7	13.7 ± 2.6	17.8 ± 2.3

Values are means ± SE. AUC, area under the curve; K_g , glucose tolerance. For glucose, there was a significant main effect of anesthesia, $P < 0.001$. For K_g , there was a significant main effect of diet ($P < 0.02$) but no significant main effect of anesthesia and no significant interaction ($P > 0.05$). Values within a row with different symbols differ significantly ($P < 0.01$).

diet, such that K_g was significantly reduced in the HF-saff group relative to the HCHO-fed and HF-mixed-fed groups. K_g values in rats fed the HF-mixed diet did not differ from those in the HCHO group. Insulin secretion in response to intravenous glucose, measured as area under the curve minus basal, did not differ among rats from the three dietary groups.

The fatty acid composition of rat adipose tissue reflected the amount of those fatty acids found in the various diets (Table 3). In the HCHO and HF-saff dietary groups, fatty acid composition of adipose tissue was similar except for a significant absolute increase in the amount of C18:2 (n-6) fatty acids in the HF-saff-fed group, reflective of the approximately eightfold increase in n-6 polyunsaturated fatty acid found in the HF-saff diet. The absolute increase in C18:2 (n-6) in adipose tissue from HF-saff-fed rats resulted in a dramatic increase in the n-6-to-n-3 ratio observed in fat tissue from rats in the HF-saff group relative to the HCHO-fed rats. In adipose tissue from rats fed the HF-mixed diet, fatty acids of the C10, C14, and C18:1 (n-9) families were significantly elevated compared

Table 3. Fatty acid composition of adipose tissue from rats fed experimental diets for 3–4 wk

Fatty Acid	HCHO Diet	HF-Saff Diet	HF-Mixed Diet
C10	0.3 ± 0.04*	0.3 ± 0.02*	10.4 ± 0.7†
C12	1.7 ± 0.10*	2.0 ± 0.30*	2.2 ± 0.17*
C14	2.2 ± 0.05*	1.7 ± 0.80*	5.1 ± 0.5†
C18:1 (n-9)	26.5 ± 5.9*	22.7 ± 5.0*	40.4 ± 1.7†
C18:2 (n-6)	31.8 ± 4.0*	51.0 ± 7.3†	18.0 ± 1.8‡
C20: (n-6)	0.6 ± 17.0	0.7 ± 0.12	0.3 ± 0.06
C18:3 (n-3)	0.8 ± 0.10*	0.7 ± 0.14*	1.8 ± 0.2†
C22:6 (n-3)	0.1 ± 0.01*	0.11*	0.2 ± 0.03†
Ratio of n-6 to n-3	36.8*	72.8†	9.1‡

Values are means ± SE. Fatty acid composition from epididymal fat pad is expressed as a relative percentage. Ratio of n-6 to n-3 was determined from fatty acids reported here. Values with different symbols are significantly different from each other ($P < 0.05$).

with adipose tissue from the other dietary groups, whereas C18:2 (n-6) and the C20: (n-6) families were relatively reduced ($P < 0.05$). Fatty acids in the n-3 family [C18:3 (n-3) and C22:6 (n-3)] were approximately twice as abundant in adipocytes from HF-mixed-fed rats compared with cells from rats in the HF-saff group, despite a similar total quantity of energy from fat; this is therefore reflective of the quantity of n-3 fatty acids from the soybean oil and not from de novo synthesis (HF-mixed significantly different from HCHO and HF-saff, $P < 0.05$). This elevation in n-3 content in tissue from rats fed the HF-mixed-based diet decreased the n-6-to-n-3 ratio relative to rats in the other two dietary groups.

Figure 2 shows that the capacity of skeletal muscle for glucose uptake under both insulin-stimulating and -nonstimulating conditions (basal) was altered by a feeding of a high absolute quantity of energy as fat. Basal glucose uptake was significantly increased in oxidative (soleus, red gastrocnemius, red tibialis anterior) and mixed fiber-type muscles (extensor digitorum longus and plantaris) from HF-mixed and HF-saff groups relative to rats fed the HCHO diet ($P < 0.03$). In glycolytic muscle (white tibialis anterior and white gastrocnemius), basal glucose uptake was elevated in rats fed the HF-saff diet relative to rats in the HCHO diet group ($P < 0.04$), and glucose uptake in glycolytic muscles from the HF-mixed-fed rats was intermediate between the groups fed the HF-saff and HCHO diets and did not differ significantly from either of the other two dietary groups.

Insulin-stimulated glucose uptake, which was quantified as the amount of glucose uptake above basal in the presence of a maximally stimulating concentration of insulin, was reduced in both the HF-mixed- and HF-saff-fed groups relative to the HCHO group ($P < 0.04$). Insulin-stimulated glucose uptake was similar across muscle types of HF-mixed-fed rats, yet, within each muscle group, it was reduced compared with HF-saff-fed rats. Total glucose uptake (basal plus insulin-stimulated uptake) was significantly reduced in oxidative, mixed-fiber, and glycolytic muscles of all rats fed the HF-mixed-based diet relative to those fed the HCHO diet ($P < 0.03$).

Insulin-stimulated glucose uptake into adipocytes is shown in Fig. 3. Glucose uptake by isolated adipocytes did not differ among dietary groups after pretreatments without insulin (0) and at lower concentrations of insulin (1 nM and 2 nM). At higher concentrations of insulin, adipocytes from rats fed the HF-mixed and HCHO diets had significantly higher rates of glucose transport compared with adipocytes isolated from rats fed the HF-saff diet ($P < 0.001$). Insulin-stimulated glucose uptake was completely blocked in the presence of LY-294002, but the reduction was similar across all dietary treatments.

DISCUSSION

Results from this study demonstrate that the total fat content of the diet was not the sole factor affecting glucose tolerance and that the combination of dietary

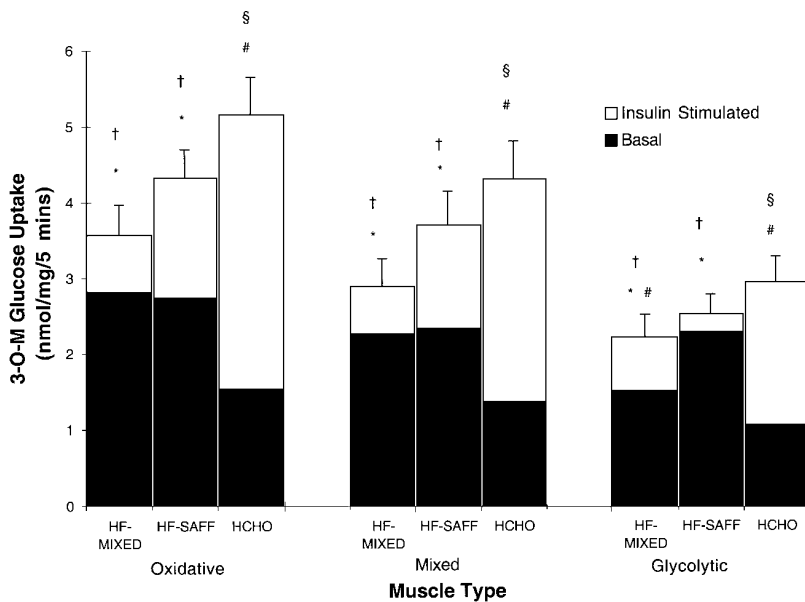


Fig. 2. Mean 3-O-methylglucose (3-O-M glucose) uptake (\pm SE), measured in absence of insulin (basal uptake, solid bars) and in presence of 40 nM insulin (insulin stimulated, open bars), into hindlimb muscles of rats fed experimental diets for approximately 3–4 wk. Muscles were grouped as follows: oxidative muscles are soleus, red gastrocnemius, and red tibialis anterior; mixed muscles are extensor digitorum longus and plantaris; glycolytic muscles are white gastrocnemius and white tibialis anterior. Basal uptake: * or #. Bars within muscle types with different symbols differ significantly from each other, $P < 0.04$. Insulin-stimulated uptake: † or §. Bars within muscle types with different symbols differ significantly from each other, $P < 0.02$. L-[14 C]glucose was used to determine extracellular water space and passive diffusion: dpm/mg was not different between dietary groups, $P = 0.6$.

fatty acids plays a significant role in affecting this measure. Varying the fatty acid composition of a high-fat diet from a single source of predominantly n-6 polyunsaturated fatty acids to include a large portion of oleic acid and short-chain saturated fatty acids and to improve the n-6-to-n-3 ratio allowed normal glucose tolerance to be maintained in the presence of insulin resistance in skeletal muscle. This supports the hypothesis that the HF-mixed diet has differential effects on insulin resistance in the insulin-sensitive tissues, skeletal muscle and adipose tissue. Varying the fatty acid composition in this way had a tissue-specific effect on insulin-stimulated glucose uptake in muscle and fat cells, with insulin resistance being observed only in muscle but not in adipocytes. Moreover, basal glucose uptake in skeletal muscle increased. This suggests that in certain tissues, insulin resistance may be accompanied by compensatory changes in an attempt to offset diminished glucose uptake. Together, these observations suggest that in the rat, high-fat feeding induces

changes in both skeletal muscle and adipose tissues but that the variation in dietary fat composition contributes significantly to the overall ability of the animal to maintain glucose homeostasis.

A large proportion of a glucose load is disposed of in insulin-sensitive tissues, with muscle accounting for >80% of the insulin-mediated glucose disposal in healthy chow-fed rodents (2). It is not known whether the different dietary interventions may affect the relative contribution of muscle, fat, and other insulin-sensitive tissues to insulin-mediated glucose disposal. Our data provide some insight into how diet may affect the partitioning of glucose uptake and the physiological significance of these changes. In our study, glucose intolerance was observed only when insulin resistance was present in both skeletal muscle and adipose tissue (after HF-saff feeding) but not when insulin resistance was observed in only skeletal muscle (i.e., after HF-mixed feeding). Thus glucose tolerance was preserved in rats with uncompromised glucose uptake into adipose

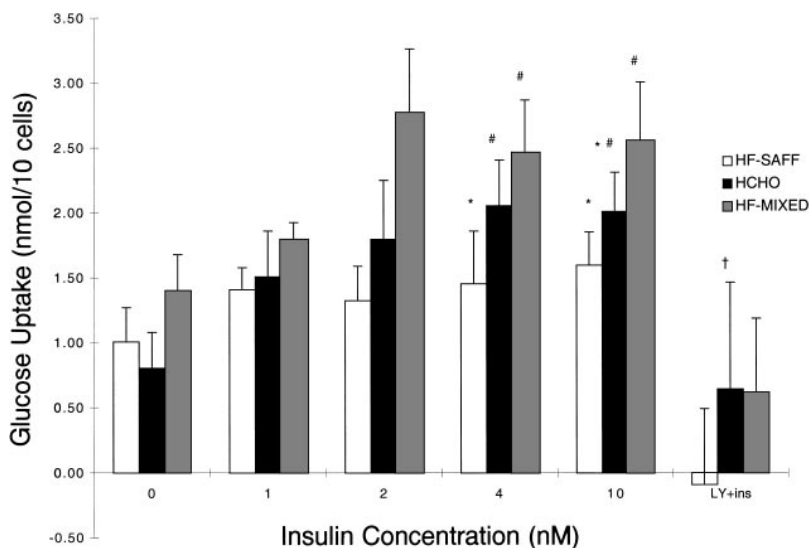


Fig. 3. Mean 2-deoxyglucose uptake (\pm SE) into adipocytes after preincubation of cells with various concentrations of insulin. Adipocytes were isolated from epididymal fat pads of animals fed experimental diets for 3–4 wk. In some incubations, LY-294002 (LY) and 4 nM insulin (ins) were added during preincubation, as described in MATERIALS AND METHODS. Within each concentration of insulin tested, data points with different symbols are significantly different from each other, $P < 0.05$. LY+ins resulted in a significant reduction in glucose uptake relative to all other conditions tested (main effect of LY+ins, $P < 0.05$) but did not differ by dietary group.

cytes. The epididymal fat pad mass was increased in the HF-mixed group relative to the other two dietary groups; thus the larger fat mass in these animals may account for an absolute increase in the amount of glucose disposed and may help to preserve glucose tolerance. Because adipocyte cell size and the absolute number of adipocytes per fat pad were not assessed in this study, it is not clear whether the increased fat pad mass reflects an increased cell number, cell size, or both. Results from this study suggest that glucose uptake per adipocyte was increased in the cells from rats fed the HF-mixed diet relative to those fed the HF-saff diet, although, in the absence of cell volume measurements, this cannot be confirmed in this study. Other investigators have reported that the rate of glucose uptake into isolated adipocytes is similar regardless of whether results are expressed per cell or per unit cell volume (3).

The recent suggestion that particular fatty acids may act as natural ligands for peroxisome proliferator-activated receptor- γ (PPAR γ) (13, 30) points to another possible mechanism through which different dietary fats may contribute to maintenance of normal glucose tolerance. PPAR γ , when activated, induces adipogenesis and also improves insulin sensitivity (30). It is unclear whether PPAR γ activation is able to reverse insulin resistance induced by high-fat feeding (10, 31), although newly formed, smaller adipocytes are more sensitive to the effects of insulin (5). Thus it is conceivable that if PPAR γ were differentially activated by the two high-fat diets used in this study because of differing proportions of available fatty acids, then glucose tolerance may be preserved in the face of increased fat mass. This may be an area for future research.

Another alternative may be that insulin sensitivity must be reduced in all or some critical number of insulin-sensitive tissues before glucose tolerance is disturbed. Others have shown that with high-fat feeding, hepatic insulin resistance develops more rapidly than insulin resistance in skeletal muscle (14); therefore, we assume that this is also present in our study. Hepatic insulin sensitivity was not assessed in this study, and, therefore, the effects of the HF-mixed diet on the liver remain unknown.

Feeding of either high-fat diet (HF-mixed or HF-saff) to rats resulted in a reduction in insulin-stimulated glucose uptake in all muscle groups relative to HCHO-fed rats. This effect is well documented in animals consuming diets with a fat content >50% of total calories (6, 10, 14, 15, 17, 19, 23, 27, 33, 34) and may be related to the large amount of stored lipids that accumulates within the muscle cells (24, 25, 33). In fact, both the high polyunsaturated fat diet (HF-saff) and the monounsaturated/short-chain fatty acid soybean oil-based diet (HF-mixed) similarly reduced insulin sensitivity in skeletal muscle in these rats. Using a clamp technique, Storlien et al. (33) observed reversal of insulin resistance in skeletal muscle after including 6% of kilocalories from long-chain n-3 fatty acids in a high polyunsaturated fat diet or after including 6% of kilocalories from short-chain n-3 fatty acids along with a

high saturated fat diet. The HF-mixed-based diet used in this study contained ~10 times less (0.8% kcal) n-3 fatty acid (linolenic acid) than the high fish oil diet used by Storlien et al. (33). This suggests that the level of n-3 fatty acids in the HF-mixed diet was not sufficient to alleviate insulin resistance in muscle when found in combination with the high saturated short-chain and monounsaturated fatty acids.

It is unknown whether changes in the muscle cell membrane fluidity and/or composition under these varied dietary conditions could have modulated insulin-stimulated glucose transport through changes in cell content of phospholipid signaling molecules. It is evident from studies by others that high-fat feeding changes insulin-signaling patterns. Feeding a high-fat beef tallow-based diet with ratio of saturated to monounsaturated fat similar to that in the HF-mixed diet results in a reduction in the gene expression of the insulin receptor substrate-1 and PI 3-kinase in rat skeletal muscle compared with muscle from rats fed a high safflower oil diet (similar to the HF-saff diet) (12), contributing to glucose intolerance in these animals. Furthermore, both the expression of the insulin-regulatable glucose transporter GLUT-4 (mRNA and protein) and the ability of insulin-stimulated PI 3-kinase activity to increase GLUT-4 translocation are dramatically reduced in skeletal muscle after feeding a high-lard-based diet to mice (9, 38). These studies may suggest that our findings of reduced insulin sensitivity in muscle of high-fat-fed groups could in part be due to changes in insulin signaling, which may ultimately produce a lower glucose uptake.

In skeletal muscle, when insulin was not present in the perfusate, an increased glucose uptake was observed in all the muscle groups (oxidative, mixed fiber, and glycolytic muscles) from HF-saff-fed rats and in two muscle groups (oxidative and mixed muscle) from HF-mixed-fed rats relative to HCHO-fed rats. In both high-fat groups, the elevation in basal glucose uptake was most apparent in oxidative muscle. Thus both groups of high-fat-fed rats were able to achieve a higher glucose uptake during the 5-min perfusion period than would have been possible with a normal basal uptake component coupled with the compromised insulin-stimulated uptake. This could be an adaptive response to the development of insulin resistance in this tissue, since oxidative muscles as a group have the highest insulin sensitivity in normal, healthy rats (22). The upregulation of basal uptake in oxidative muscle may result from alteration of the diet fatty acid composition, since Storlien et al. (35) saw elevated basal uptake in oxidative muscle after supplementing a high saturated fat diet with n-3 fatty acids. The weak positive relationship observed between non-insulin-stimulated glucose uptake and oxidative capacity in muscle from HCHO-fed rats is also present in muscle from rats fed the HF-mixed diet, albeit the level of basal uptake is higher in this latter group. In contrast, the HF-saff-fed rats showed a fairly constant increased basal level of uptake, and this was irrespective of the fiber composition of these muscles. Subtle differences between the effects

of the HF-saff and HF-mixed diet may be due to the nature of the cellular mechanisms responsible for up-regulating basal glucose transport. Kahn and Pedersen (9) observed reduced muscle GLUT-1 mRNA after 7 wk of feeding an 80% fat diet to rats, whereas, in rats fed a high safflower oil diet (60% kcal) for 4 wk, Stevenson et al. (31) reported no change in the amount of GLUT-1. In a different model of insulin resistance, the denervated rat, we have observed that increases in basal glucose uptake are accompanied by increases in GLUT-1 expression (7). The cellular mechanisms and/or transporter proteins involved in the upregulation of basal transport remain to be determined in this particular high-fat feeding model. Interestingly, as in the denervated rat model, the increase in basal uptake after high-fat feeding does not fully restore the muscle's total capacity for glucose transport when insulin is available. This suggests that the basal compensatory mechanisms are only partially capable of offsetting the reduction in glucose uptake when insulin is present. This may help account for the normal fasting glucose concentrations in the same animals that expressed glucose intolerance after a glucose challenge.

In contrast to its effects in skeletal muscle, the HF-mixed diet had no adverse effect on insulin-stimulated glucose uptake into isolated adipocytes, whereas HF-saff feeding reduced insulin-stimulated glucose transport in isolated adipocytes relative to HCHO-fed rats. The absolute multiple of increase in glucose uptake after insulin stimulation compared with basal levels of uptake was approximately twofold and most likely reflects the combination of the specific incubation conditions used in this study along with properties inherent to cells from animals of this particular size and age fed purified diets. Still, the relative impairment in glucose uptake by adipocytes after HF-saff feeding but not HF-mixed feeding suggests that in adipose tissue, unlike skeletal muscle, the total amount of dietary fat was not the important factor affecting glucose uptake, but the fatty acid composition of the diet had an important impact on this process. Our results suggest that, in the presence of large amounts of dietary fat, glucose uptake can proceed normally in adipocytes if fatty acid profiles allow and points to an interactive effect between fatty acid classes and amount of dietary fatty acid as determinants of glucose uptake in fat cells.

Membrane fatty acid composition of adipocytes reflected dietary fatty acid intake after feeding of the experimental diets. Whether changes in fatty acid profiles in adipocytes from rats fed the HF-mixed diet containing the mixture of saturated, n-6 and n-3 polyunsaturated, and monounsaturated fatty acids lead to changes in numbers of glucose transporters or restore some aspect of the insulin signal transduction-glucose transporter translocation pathway (8) is not known. If the proportion of membrane n-3 fatty acids is important in determining glucose uptake in adipocytes, then this study suggests that in adipocytes, unlike muscle, only a small amount of dietary n-3 fatty acid is required to remedy the impaired glucose uptake induced by a

high-fat diet. Understanding these observations as well as their application to humans will require more detailed understanding of the relationships among dietary availability of particular fatty acids, responsiveness of membrane composition to diet, and biochemical or molecular mechanisms controlling incorporation of specific fatty acids into membranes.

In summary, this study demonstrates that dietary fatty acid composition of a high-fat diet is critical in determining the effects on tissue-specific insulin resistance and whole body glucose tolerance in rats. When the fatty acid composition of a high-fat diet is changed from a single source of predominantly n-6 polyunsaturated fatty acids to include a large portion of oleic acid and short-chain saturated fatty acids and an increased n-3-to-n-6 ratio, normal glucose tolerance is maintained despite the presence of insulin resistance in skeletal muscle. It is conceivable then that overall glucose tolerance in rats may be sustained when one of the compartments mediating glucose homeostasis becomes less responsive to insulin. Varying the fatty acid composition to the HF-mixed diet appears to have tissue-specific effects on glucose uptake such that although hormonally regulated glucose uptake in skeletal muscle is adversely affected, basal glucose uptake in skeletal muscle is increased and glucose uptake into adipose tissue is apparently spared. These results underline the importance of examining dietary effects in multiple tissues and at both the cellular and whole body levels to gain insight into how diet affects the interaction among tissues to achieve glucose homeostasis. The extent to which our observations are specifically related to a small amount of n-3 fatty acids or other fatty acids such as monounsaturates or some combination remains to be determined but may be important for understanding the development and reversal of insulin resistance, glucose intolerance, and impairment of glucose homeostasis in animal models and in humans.

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