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Chronic High-Fat Feeding and Middle-Aging Reduce in an Additive Fashion Glut4 Expression in Skeletal Muscle and Adipose Tissue

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The interaction of middle-aging and chronic high-fat feeding on glucose transport in skeletal muscle and adipose tissue was examined. To this end, we studied the effects of 6 month treatment with a high-fat diet in 12-month old rats. Chronic high-fat feeding led to a substantial reduction in GLUT4 glucose transporter expression both in adipose tissue and in skeletal muscle, which was additive to the reduction in GLUT4 protein content detected in aged rats. In parallel, the high-fat diet led to a reduced insulin-stimulated glucose transport in the incubated soleus muscle and isolated adipocytes; insulin resistance induced by high-fat feeding was superimposed to the decreased insulin response detected in aged rats. Different mechanisms were responsible for GLUT4 repression in response to high-fat feeding or aging in skeletal muscles and adipose tissue.

Insulin stimulates glucose transport in muscle and adipose cells by promoting the translocation of GLUT4 glucose carriers from an intracellular site to the cell surface. There are several conditions in which insulin resistance develops, at least in part, as a consequence of alterations in the expression of GLUT4. Thus, aging is characterized by insulin resistance (1-3) and by low levels of GLUT4 in fat cells (4, 5) and in skeletal muscle (5, 6). Furthermore, diet also regulates insulin sensitivity and tissue GLUT4 expression. In this regard, high fat feeding is known to cause insulin resistance (7-10) and to reduce GLUT4 expression in adipose tissue and in skeletal muscle (11, 12). High-fat diet-induced hyperglycemia is prevented by expression of a GLUT4 minigene in transgenic mice (13) which indicates that GLUT4 repression is a mechanism, although perhaps not the only one, leading to insulin resistance after high-fat feeding.

In this study we have examined the interaction between middle-aging and chronic high-fat feeding on glucose transport and GLUT4 glucose transporter expression in muscle and adipose tissues.

MATERIALS AND METHODS

Materials. [^{125}I] Protein A, [^{14}C]-2-deoxyglucose, [^{3}H]mannitol and Hybond N were purchased from Amersham. 2-Deoxy[3H]glucose was from NEN. Immobilon was obtained from Millipore. Collagenase and adenosine deaminase were from Boehringer Mannheim. Molecular weight markers were obtained from BRL. Cytochalasin B, bovine serum albumin (fraction V), the kit for the determination of plasma glucose, electrophoresis reagents and most commonly used chemicals were from Sigma.

Animals. Between the age of 2 and 6 months, male Sprague-Dawley rats were fed with a control diet (A03, U.A.R.). At the age of 6 months, the rats were divided into 2 experimental groups: the high carbohydrate group (rats subjected to the high carbohydrate diet A03, U.A.R.) and high-fat group (rats subjected to a high-fat diet, A03, U.A.R. enriched with 31% corn oil and 4% safflower oil). Rats were subjected to both diets for 6 months. In some studies, 1.5 month- and 3 month-old male Sprague-Dawley rats that were fed the high carbohydrate diet were also studied. Rats were housed in animal quarters maintained at 22 °C with a 12-h light, 12-h dark cycle. Food and water intake was analyzed weekly.

On the day of the experiment, a sample of blood was taken from the tail and thereafter animals were anesthesized with sodium pentobarbital (5-7 mg/100 g body weight). Strips of soleus muscles were isolated and thereafter red and white skeletal muscle and epididymal fat pads were rapidly collected. Red muscle consisted of pooled red portions of the gastrocnemius and quadriceps muscles, and similarly, white portions of the gastrocnemius and quadriceps were pooled as the source of white muscle. After collection, tissues were rapidly frozen and kept in liquid nitrogen until analysis. Plasma samples were used for determination of insulin by a radioimmunoassay, and glucose was analyzed by a enzymatic method.

Incubated muscle studies. The dissection and isolation of strips of soleus muscle from rats were carried out under anaesthesia with pentobarbital (5-7 mg/100 g body weight, i.p.) by a modification of

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the method of Crettaz et al. (14). Such muscles are able to maintain normal ATP and creatine phosphate concentrations for 3 h incubation (15). Glucose transport by strips of soleus was assessed by using the non-metabolizable analogue 2-deoxy-D-glucose as previously reported (16).

Adipocyte isolation and 2-deoxyglucose transport determination. Epididymal adipose tissue was dissected out and digested in Krebs-Ringer bicarbonate buffer containing 12.5 mM Hepes, BSA (3.5% w/v), 2 mM pyruvate and 1.5 mg/ml collagenase as previously reported (17). Fat cell suspensions were incubated in plastic vials (final volume 400 μl) in which insulin was added before the beginning of the incubation of 4 μl portions to obtain the final concentrations. At the end of the incubation period, which lasted 45 min, a 200 μl sample of the cell suspension was further incubated in plastic tubes for 5 min in the presence of 100 μl of 2-deoxy-D-[3H]glucose (0.4 μCi) at a final concentration of 0.1 mM as reported (17).

Preparation of total membrane fractions from tissues and immunoblotting of membranes. Tissues were homogenized in 8 vols of ice-cold buffer containing 25 mM Hepes, 250 mM sucrose, 4 mM EDTA, 1 trypsin inhibitory unit/ml aprotinin, 25 mM benzamidine, 0.2 mM PMSF, 1 μM leupeptin and 1 μM pepstatin, pH 7.4 and processed as reported (18). The membrane pellets were resuspended in homogenization buffer before storage at −20°C. Proteins were measured by the method of Bradford (19) using gamma-globulin as a standard. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoreses and immunoblotting were performed on membrane protein as reported (18) using the polyclonal antibody OSCRX raised against the 15C-terminal peptide from GLUT-4.

RNA isolation and Northern blot analysis. Total RNA from tissues was extracted using the acid guanidinium thiocyanate/phenol/chloroform method as described by Chomczynski and Sacchi (20). All samples had a 260/280 absorbance ratio above 1.8. Northern blot analysis was performed as reported (18). The rat cDNA probe for GLUT-4 was a 2,470 base pair EcoRI fragment (obtained from Dr. Morris Birnbaum, University of Harvard).

RESULTS

At 6 months of age, male Sprague-Dawley rats were divided in 2 experimental groups, i.e., the control group subjected to a high carbohydrate diet and the high-fat group subjected to a high-fat diet (containing 31% corn oil and 4% safflower oil) for 6 months. Rats subjected to the high-fat diet showed a greater increase in body weight than rats fed the high carbohydrate diet (data not shown). In 6 months, control and high-fat groups gained 166 g and 277 g, respectively. Under these conditions, the high-fat group was only slightly hypercaloric (10% increase over control values). Plasma glucose was significantly increased (23%) in 12-month old rats (11.3 ± 0.8 mM) compared to 3-month old rats (9.2±0.6 mM) and no differences were detected in glycemia when comparing 12-month-old rats fed a high-carbohydrate diet and 12-month-old rats fed a high-fat diet (12.1±0.6 mM). Plasma insulin levels were also significantly higher in 12-month-old rats (170.7±46.5 μU/ml) than in the 3-month-old group (75.0±9.3 μU/ml) and high-fat feeding in 12-month-old rats caused a slight decrease in plasma insulin (123.9±14.4 μU/ml) although levels were still significantly greater than in the 3-month old group.

Strips of soleus muscles obtained from 1.5-month old rats, 3-month old rats, 12-month old rats and 12-month old rats fed high-fat diet were incubated and 2-deoxyglucose uptake was determined in the absence or in the presence of 100 nM insulin. Basal 2-deoxyglucose uptake did not show alterations with aging but high-fat feeding led to a significant reduction (Figure 1). Insulin-stimulated 2-deoxyglucose uptake was maximal in muscles from 1.5-month old rats and decreased in muscles from 3-month old and 12-month old rats (Figure 1). Furthermore, high-fat feeding led to a further decrease in insulin-stimulated 2-deoxyglucose uptake (Figure 1). As a result, the effect of insulin on glucose transport was maximal in muscles from 1.5-month old rats and minimal in 12-month old rats treated with high-fat (Figure 1).

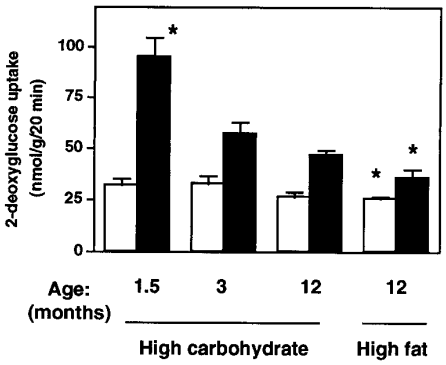

FIG. 1. Effect of middle-aging and long-term high-fat feeding on basal and insulin-stimulated 2-deoxyglucose uptake by strips of soleus muscle. Results are means+SE for 8-11 observations per group. Strips of soleus muscles obtained from 1.5 month old rats, 3-month old rats, 12-month old rats and 12-month old rats subjected to high-fat feeding were incubated for 50 min in the absence (open bars) or in the presence of 100 nM insulin (closed bars). 2-deoxyglucose uptake was determined during the last 20 min of incubation. *, indicates significant differences compared with the 12-month old group, at $P < 0.05$.

Isolated adipocytes from 1.5-month old rats displayed a nearly 9-fold stimulation of 2-deoxyglucose uptake in response to 100 nM insulin (data not shown). Aging caused a progressive reduction of insulin-stimulated glucose transport (insulin caused a 4.4-fold increase and 2.6-fold increase in 3-month old and 12-month old groups) and high-fat feeding caused a further reduction of insulin-stimulated glucose transport in adipocytes from 12-month old rats (insulin caused a 1.9-fold increase in glucose transport) (data not shown). Thus, in parallel to what was detected in muscle, high-fat feeding leads to insulin resistance in adipocytes and this effect is additive to the reduced insulin response already detected in adipocytes from aged, obese rats.

Aging from 3 to 12 months led to a variable pattern of changes in the expression of GLUT4 protein in skeletal muscle (Figure 2). Thus, whereas aging was associated with a 39% decrease in GLUT4 protein content in white skeletal muscle, no significant decrease in red skeletal

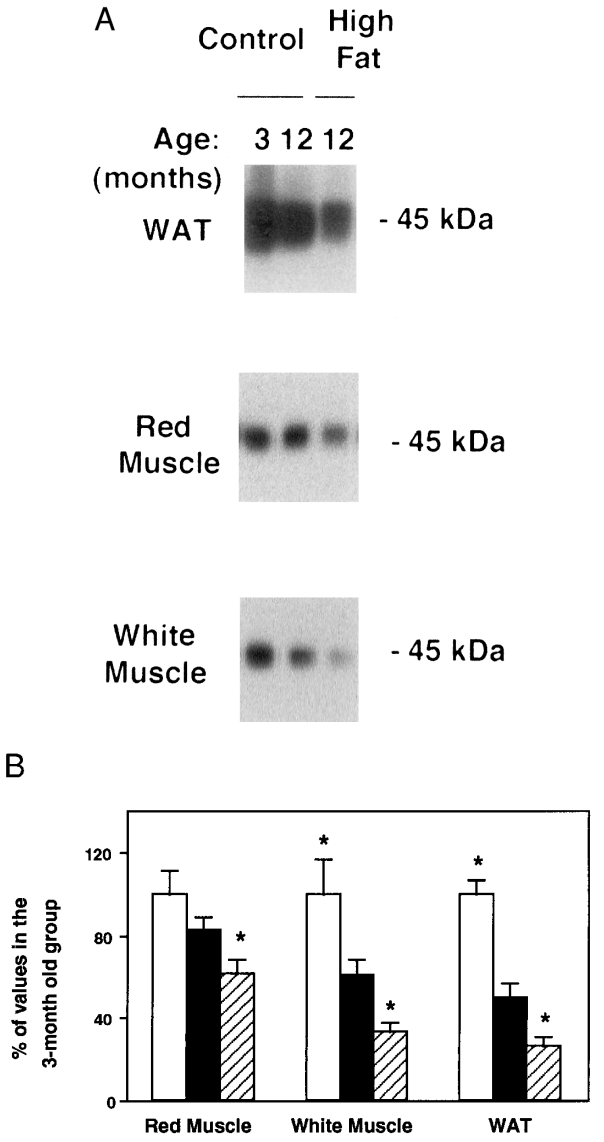

FIG. 2. Effect of long-term high-fat diet on GLUT-4 protein content in skeletal muscles and adipose tissue from middle-aged rats. Membrane proteins were purified from adipose tissue and red and white portions of skeletal muscle obtained from 3-month old rats (open bars), 12-month old rats (black bars) and 12-month old rats subjected to high-fat feeding (striped bars). 7 μg of membrane proteins was applied on gels and blotted with an antibody for GLUT-4. Representative autoradiograms are shown (panel A). Results are means ± SE of 8-11 observations per group. Data are expressed as a percentage of values in the 3-month old group. * indicates significant differences compared with the 12-month old group, at $P < 0.05$.

muscle was detected (Figure 2). Aging also caused a substantial decrease in the concentration of GLUT4 protein in epididymal adipose tissue (49% decrease) (Figure 2). Under these conditions, long-term treatment with a high-fat diet led to a generalized decrease in GLUT4 protein content both in red and white skeletal muscle (Figure 2); GLUT4 decreased to a greater extent in white skeletal muscle (46% decrease) than in red skeletal muscle (22% decrease) in response to high-fat feeding (Figure 2). High-fat feeding also caused a marked reduction in the concentration of GLUT4 protein (48% decrease) in white adipose tissue (Figure 2).

Tissue levels of GLUT4 mRNA were also determined. Aging from 3-month to 12-month did not significantly modify the levels of GLUT4 mRNA in red or white skeletal muscle fibers (Figure 3). However, aging led to a substantial decrease in the concentration of GLUT4 mRNA in epididymal adipose tissue (32% decrease) (Figure 3). Furthermore, high-fat feeding did not alter the concentration of GLUT4 mRNA in red or white fibers of skeletal muscle (Figure 3). In contrast, high-

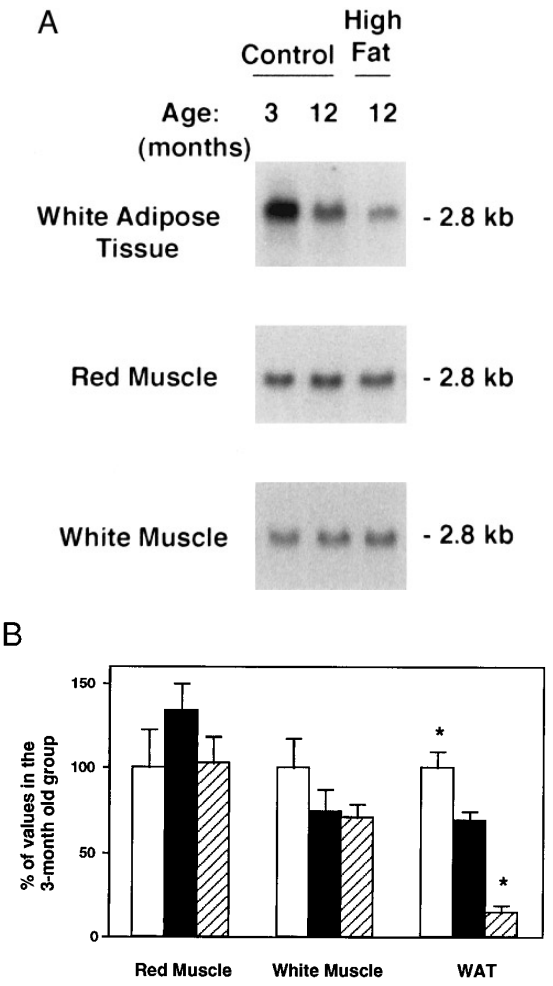

FIG. 3. Effect of long-term high-fat diet on GLUT-4 mRNA levels in skeletal muscle and adipose tissue from middle-aged rats. Total RNA was purified from adipose tissue and red and white portions of skeletal muscle obtained from 3-month old rats (open bars), 12-month old rats (black bars) and 12-month old rats subjected to high-fat feeding (striped bars). 25 μg of total RNA from red and white muscle was applied on gels for Northern blots to detect GLUT-4 mRNA. Representative autoradiograms are shown (panel A). Results are means ± SE of 5-11 observations per group. Data are expressed as a percentage of the 3-month group. * indicates significant differences compared with the 12-month old group, at $P < 0.05$.

fat feeding caused a marked reduction in the concentration of GLUT4 mRNA (79% decrease) in white adipose tissue (Figure 3).

DISCUSSION

In this study we have found that middle-aging, which in the Sprague-Dawley rat is associated with obesity, led to higher circulating levels of glucose and insulin and a reduced insulin-induced stimulation of glucose transport in skeletal muscle and in adipose cells. This pattern indicates insulin resistance affecting muscle and adipose tissue and is in keeping with previous observations (1-3). In parallel to the changes observed in insulin-stimulated glucose transport, middle-aging caused a reduction in GLUT4 glucose transporter expression affecting both white skeletal muscle and adipose tissue but not red skeletal muscle. Furthermore, the mechanisms that lead to a diminished GLUT4 transporter expression seem to be different in white skeletal muscles and in adipose tissue. Thus, whereas middle-aging was associated with decreased levels of GLUT4 mRNA in adipose tissue, no modification of GLUT4 mRNA was detected in white skeletal muscle. These results suggest that whereas GLUT4 expression in adipose cells is regulated at a pretranslational step, regulation in white skeletal muscle is due to alterations lying at translational or post-translational steps.

Previous studies have investigated the expression of GLUT4 in skeletal muscle during middle-aging. Ezaki et al. (6) showed a decreased GLUT4 protein content in gastrocnemius and white quadriceps muscles but not in soleus or red quadriceps muscles from 12-month old Sprague-Dawley rats. In contrast, Kern et al. (21) reported no change in GLUT4 expression in skeletal muscle from 12-month old Fischer rats, a strain that does not develop obesity with age. Taken together, these observations and our data suggest that an important factor leading to GLUT4 reduction in white muscle from 12 month-old rats is not aging per se but obesity associated to aging.

High-fat feeding causes insulin resistance in peripheral tissues (8-11). Here we have studied whether long-term treatment with a high-fat diet causes insulin resistance in aged, obese rats that are already insulin resistant. Our results clearly indicate that high-fat feeding for 6 months caused a marked insulin resistance that affected both skeletal muscle and adipocytes in 12-month old rats. This situation of insulin resistance induced by high-fat feeding was unrelated to alterations in plasma levels of glucose or insulin. An important corollary of these studies is that long-term high-fat feeding causes an insulin resistant state in adipose cells and in skeletal muscle that is additive to the reduced insulin response of aged, obese rats. These results further strenghen the importance of diet as a regulatory factor of insulin sensitivity both under normal and obese states.

Regarding the mechanisms that cause insulin resistance after high-fat feeding, a reduction of GLUT4 expression has been previously reported in skeletal muscle and adipose tissue of rats fed a high-fat diet for periods of time ranging from 7 to 10 weeks (12, 13). Furthermore, our study indicates that long-term high-fat feeding causes additional repression of GLUT4 expression in skeletal muscle and adipose tissue from aged rats. As to the factors involved in GLUT4 repression, in adipose tissue, GLUT4 protein and mRNA levels decreased in parallel, substantiating pretranslational repression triggered by high-fat feeding. In contrast, in skeletal muscle, the reduction of GLUT4 protein content was detected in the presence of normal levels of GLUT4 mRNA suggesting alterations lying at post-transcriptional step/s. This emphasizes the fact that GLUT4 expression is frequently regulated in skeletal muscle by mechanisms that are independent of alterations in mRNA levels (22).

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