High Sucrose Diet and Exercise: Effects on Insulin-Receptor Function of 12- and 24-mo-old Sprague-Dawley Rats¹

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ABSTRACT The purpose of this study was to evaluate the effect of aging (12 vs. 24 mo) on skeletal muscle insulin receptor function of male Sprague-Dawley rats fed either a 33% sucrose (wt/wt) or sucrose-free diet. The effect of exercise in combination with the sucrose diet was also evaluated by exercising half of the sucrose-fed group on a motorized treadmill. Insulin-receptor function was assessed *in vitro* by measuring the binding capacity of [¹²⁵I]-insulin to partially purified receptors of the biceps femoris and vastus lateralis. Tyrosine kinase activity was measured as an index of postreceptor function. Insulin-receptor number was significantly decreased in 24-mo-old sucrose-fed rats compared to 12-mo-old rats fed the sucrose or sucrose-free diets. The affinity of insulin for the receptor did not significantly differ among groups. Maximal tyrosine kinase activity in vastus lateralis was significantly decreased in 12-mo-old sucrose-fed rats compared with sedentary 24-mo-old rats fed the sucrose-free diet or 24-mo-old rats fed the sucrose diet in combination with exercise. Exercise prevented the decrease in receptor function in both 12- and 24-mo-old sucrose-fed rats as measured by insulin binding and tyrosine kinase activity. These data suggest that diet and/or exercise rather than aging *per se* has a greater influence on insulin-receptor function. *J. Nutr.* 121: 1081-1089, 1991.

INDEXING KEY WORDS:

- aging • insulin binding • insulin resistance
- skeletal muscle • rats

Approximately 25 to 30% of those over the age of 65 in the United States exhibit noninsulin-dependent diabetes mellitus and/or impaired glucose tolerance (1). Despite the magnitude of this disorder, the mechanisms leading to the apparent senescence-related glucose intolerance have yet to be elucidated. It is not clear if this phenomenon reflects aging *per se* or lifestyle (i.e., diet composition, physical activity, obesity, decreased lean body mass). The identification of specific mechanisms leading to the senescence-

related glucose intolerance observed in humans has been hindered by the difficulty in controlling environmental factors during the life span. Conversely, small, short-lived rodents, such as mice and rats, provide the researcher with greater control of the environment, allow life-span studies and have been successfully used as models for human aging. Thus, we used mature (12 mo) and aged (24 mo) Sprague-Dawley rats, fed either a high-sucrose or sucrose-free diet, to evaluate the effects of aging and diet on skeletal muscle insulin-receptor function. Furthermore, the impact of exercise in combination with the high sucrose diet was examined to determine if exercise would attenuate any alterations that might be demonstrated with the high sucrose diet.

The association between age and attenuated insulin-receptor function has been suggested by Sinha and Jenquin (2), who reported a decline in the number of insulin receptors and, subsequently, insulin-stimulated receptor function in hepatocytes isolated from adult compared with fetal and neonatal rat tissue. Trishitta and Reaven (3) examined insulin-receptor function in isolated adipocytes of Sprague-Dawley rats aged 2 and 12 mo and concluded that the older rats have a defect in the ability of insulin to stimulate glucose uptake that may be reflected in alterations in insulin-receptor function. The relevance of the data of Trishitta and Reaven (3) to the study of senescence and whole body insulin sensitivity is unclear. That is, 12 mo represents less than half the median life-span of the Sprague-Dawley rat, and results may reflect maturation- rather than senescence-related alterations. Furthermore, the adipocyte only accounts for a

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small percentage of glucose uptake in rats. Because skeletal muscle is responsible for the majority of the storage of excess blood glucose, we believe that evaluation of this tissue's insulin-receptor function may provide a physiologically more relevant marker for insulin sensitivity than adipose tissue. Therefore, to more fully elucidate the effect of senescence on glucose tolerance, we examined skeletal muscle insulin-receptor function of adult (12 mo) and aged (24 mo) rats as a marker of insulin sensitivity.

MATERIALS AND METHODS

Animals and animal care. Male Sprague-Dawley rats, ages 8 and 20 mo, were obtained from Harland Sprague Dawley Laboratory (Indianapolis, IN). Rats were housed individually in stainless steel, wire-bottom hanging cages (20 × 25 × 18 cm), maintained in a temperature-controlled room (25–26°C) on a 12 h light:12 h dark cycle (lights on at 0600 h, off at 1800 h) and given acidified deionized water (pH 3.5). Rats were inspected daily for external signs of disease (i.e., skin lesions or bleeding). Only animals that appeared healthy were used in these experiments. Fifteen 24-mo-old animals (31%) died by the end of the experimental period and were excluded from the study. Necropsy and microbiologic examinations were not done routinely. Serologic examinations (Microbiological Associates, Bethesda, MD) were performed on seven animals for the seven common rat viruses (Kilham rat virus, *toolans H-1*, cilia-associated respiratory bacillus, *Sendai*, pneumonia virus, *mycoplasma pulmonus*, rat coronavirus/*sialodacryoadenitis*). All tested positive for rat coronavirus/*sialodacryoadenitis* and *sendai* viruses and negative for the remaining five viruses.

After a 2-wk adaptation period to our colony conditions (light cycle, temperature, etc.), younger and older rats were assigned to one of three groups: sucrose diet (12 mo, S12;³ 24 mo, S24), sucrose diet and exercised (12 mo, S-Ex12; 24 mo, S-Ex24), and sucrose-free diet (12 mo, SF12; 24 mo, SF24). Rats were assigned to diet/exercise groups so initially there were no significant differences in body weight between animals of the same age. The sucrose-free diet contained cornstarch as the carbohydrate base. In the sucrose diet, half of the cornstarch was replaced with sucrose (Table 1). All groups were given ad libitum access to the diet for 17–20 weeks. At the end of the experimental period, rats were 12 and 24 mo old. The exercised rats ran on a motorized treadmill (SAT 2000, Stanhope Scientific, Davis, CA) 16 m/min, 0% grade, 60 min per day, 5 d per week according to the methods of McDonald et al. (5).

Food intake was measured for 5 consecutive days during wk 1, 3, 6, 10 and 13 of the experimental period. Body weights were recorded weekly. Tail

TABLE 1

Composition of sucrose and sucrose-free diets

Component	g/100 g	
	Sucrose	Sucrose-free
Hydrogenated vegetable oil ¹	6.4	6.4
Sucrose	33.2	0.0
Cornstarch	33.2	66.4
Cascia	17.5	17.5
Fiber (alpha cellulose)	1.8	1.8
DL-Methionine	0.04	0.04
Vitamin mix ²	1.5	1.5
Salt mix ²	6.0	6.0

¹Crisco, Procter and Gamble, Cincinnati, OH.

²Contents of the vitamin and salt mix have been previously reported (4).

blood was collected between 0800 and 1000 h at wk 6 and 10 for nonfasted plasma glucose determinations.

At the end of the 17–20 wk feeding and exercise protocol, rats were killed by decapitation after a 4-h fast. Exercise-trained animals were killed 24–26 h after their last exercise session. Trunk blood was collected and centrifuged at 1520 × g (Beckman TJ-6, Beckman Instruments, Fullerton, CA) in heparinized tubes for plasma glucose and insulin determinations. The vastus lateralis (VL) and biceps femoris (BF) were quickly excised and frozen between aluminum tongs precooled in liquid N₂. The gastrocnemius (GN) was excised and placed on dry ice. All samples were stored frozen at –70°C until analyses of insulin receptor function (VL, BF) and citrate synthase activity (GN) were performed.

The experimental protocol was approved by the University of California Davis Animal Care and Use Administrative Advisory Committee and was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Partial purification of insulin receptors. A modification of the method of Hedo et al. (6) was used for solubilization and partial purification of skeletal muscle insulin receptors from BF and VL. Briefly, frozen muscle samples were ground to a fine powder with a liquid nitrogen dioxide-cooled mortar and pestle. The samples then were homogenized with 2 mL of homogenization/solubilizing buffer (50 mmol/L HEPES, pH 7.6, 150 mmol/L sodium chloride, 1

³Abbreviations used: BF, biceps femoris; GN, gastrocnemius; PEG, polyethylene glycol; S12, sucrose diet 12-mo-old group; S24, sucrose diet 24-mo-old group; S-Ex12, sucrose diet/exercise 12-mo-old group; S-Ex24, sucrose diet/exercise 24-mo-old group; SF12, sucrose-free diet 12-mo-old group; SF24, sucrose-free diet 24-mo-old group; VL, vastus lateralis.

mmol/L bacitracin, 1.1 trypsin inhibitor units/mL aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride and 1% Triton X-100) and incubated for 1 h at 4°C. Homogenates were centrifuged at $100,000 \times g$ for 60 min at 4°C, and the supernatants were applied to columns (8 mm $\times$ 180 mm) of agarose-bound wheat germ agglutinin (Vector Laboratories, Burlingame, CA) to partially purify the receptors. Column eluates were cycled through the column five times each. Columns then were washed extensively with column buffer (50 mmol/L HEPES, pH 7.6, 150 mmol/L sodium chloride and 0.1% Triton X-100) to remove unbound proteins. Bound insulin receptors were eluted from the column with 2.8 mL of column buffer containing 300 mmol/L *N*-acetyl-D-glucosamine. Insulin-binding and insulin-stimulated tyrosine kinase activity then were measured using this eluate containing the receptor. Protein concentration of the eluate containing the receptors was determined by the method of Bradford (7) using bovine serum albumin as the standard.

Insulin binding to solubilized insulin receptors. Insulin-binding activity was determined by a modification of the methods of Pang et al. (8). Briefly, 50- μ L aliquots ($1.47 \pm 0.06 \mu\text{g}$) of the eluates containing the partially purified insulin receptors were incubated with 3- ^{125}I -iodotyrosyl AT4-insulin (74 TBq/mmol; Amersham, Arlington Heights, IL) and unlabeled porcine insulin (0.04–16.0 nmol/L total insulin, Sigma, St. Louis, MO) for 18–24 h at 4°C in a final volume of 200 μ L made up with 50 mmol/L HEPES, pH 7.6, 150 mmol/L sodium chloride and 1 mg/mL bovine serum albumin. Receptor-bound [^{125}I]-insulin was separated from free insulin by polyethylene glycol (PEG) precipitation (9). Bovine gamma globulin (100 μ L) and 20% PEG in 10 mmol/L HEPES, pH 7.6 (300 μ L), were added to each tube—with successive vortexing—and incubated for 15 min at 4°C. After incubation, samples were centrifuged at $1520 \times g$ at 4°C (Beckman TJ-6, Beckman Instruments, Fullerton, CA), and the supernatant was removed. The pellet was washed with 10% PEG in 10 mmol/L HEPES, pH 7.6, centrifuged as before and supernatants were removed. The radioactivity of the precipitated receptor-hormone complex was counted in a Beckman gamma counter model 8500 (Beckman Instruments, Palo Alto, CA). Nonspecific [^{125}I]-insulin binding was determined at 0.7 μ mol/L insulin and subtracted from each point. Receptor binding and affinity were determined according to the methods of Scatchard (10). Scatchard plots were rejected if $r^2 < 0.75$. All [^{125}I]-insulin-binding values are expressed as picomole bound per milligram protein.

Insulin-stimulated tyrosine kinase activity. Poly (Glu, Tyr) 4:1 (Sigma, St. Louis, MO) was used as a substrate to determine tyrosine kinase activity (EC 2.7.1.112) of the partially purified receptors (11, 12). Partially purified insulin receptors were incubated

with or without increasing concentrations of insulin (0.33–100 nmol/L, final concentrations) at 22°C for 60 min. Two mg/mL poly (Glu, Tyr) 4:1, 50 μ mol/L [^{32}P]-ATP (0.37–1.85 TBq/mmol; ICN, Costa Mesa, CA), and 50 mmol/L magnesium chloride (final concentrations) were added to initiate phosphorylation of the artificial substrate. The reaction was terminated after 30 min by application of 50 μ L of the reaction mixture onto Whatman (Clifton, NJ) P81 phosphocellulose paper (2 $\times$ 2 cm). The papers were washed in 75 mmol/L phosphoric acid. The amount of ^{32}P incorporated into poly (Glu, Tyr) 4:1 on the washed papers was determined by liquid scintillation counting (Packard Tricarb 2660, Packard Instrument, Downers Grove, IL). Activity obtained in the absence of exogenous substrate was subtracted from each sample. Data were analyzed by Eadie Hofstee plots (13), using linear regression to estimate tyrosine kinase activity.

Plasma glucose and insulin. Glucose was analyzed by the glucose oxidase method (Beckman Glucose Analyzer 2, Beckman Instruments, Fullerton, CA). Immunoreactive insulin was determined, as previously reported (14), by a single antibody method using [^{125}I] insulin tracer (Amersham, Arlington Heights, IL), a rat insulin standard (20.7 U/mg; Novo Research Institute, Bagvaard, Denmark) and antibody (Radioassay Systems Lab, Carson, CA); PEG was used to separate free and antibody-bound insulin.

Citrate synthase. Citrate synthase (EC 4.1.3.7) activity in GN was determined by a modification of the methods of Bass et al. (15) and Srere (16) as previously described by McDonald et al. (17). Briefly, frozen muscle samples were thawed and homogenized by a Polytron (Brinkmann Instruments, Westbury, NY) in 20 mL of buffer containing 2 mmol/L EDTA and 100 mmol/L sodium phosphate, pH 7.3. The homogenate was centrifuged at $700 \times g$ for 10 min. The supernatant was saved; the pellet was resuspended in 5 mL of phosphate buffer and centrifuged as before. The supernatants were combined and used for the citrate synthase assay. The reaction medium contained 100 mmol/L Tris, 2.5 mmol/L EDTA, 0.1 mmol/L 5,5-dithiobis-(2-nitrobenzoic acid), 0.2 mmol/L acetyl-CoA and 0.5 mmol/L oxaloacetate with a total volume of 0.75 mL. Enzyme activity was measured spectrophotometrically (Shimadzu UV-160, Shimadzu, Kyoto, Japan) as the change in absorbance per minute at 412 nm.

Carcass composition and adipose tissue cellularity. Carcass composition was analyzed according to the methods of Bell and Stern (18). Briefly, eviscerated carcasses were lyophilized and fat was extracted in Soxhlet extractor with circulating distilled ether then acetone. Carcasses were then ashed in a muffle oven. Total body water was calculated as the difference between the weight before and after lyophilization, fat as the difference before and after fat extraction, lean body mass as the difference between

the lyophilized carcass and fat mass and protein as the difference before and after a 7-d ashing.

Adipose tissue cellularity of the epididymal, retroperitoneal, mesenteric and inguinal depots was determined using electronic counting of osmium-fixed cells (19, 20). The data presented for total cell number represent the sum of the number of fat cells in the epididymal, retroperitoneal, mesenteric and inguinal depots. Although some differences in cell number and cell size of individual depots were noted among the groups, no consistent pattern of main effects was established. Therefore, the data for individual depots are not presented here but are available on request.

Statistics. Our original research questions were 1) to determine if 12- and 24-mo-old rats respond to a high sucrose and sucrose-free diet in the same manner, and 2) to determine if those 12- and 24-mo-old rats fed a high sucrose diet respond to exercise in the same manner. Therefore, we analyzed the data by two 2×2 ANOVAs, with age and diet as main effects for one ANOVA and age and exercise as main effects for the other ANOVA (21). (An ANOVA with age, diet, and exercise as main effects was inappropriate as there was no sucrose-free exercise group.) When significant main effects were found, Fischer's least significant difference post hoc test was used to evaluate the differences between the groups (22). Differences were considered significant at $P < 0.05$.

RESULTS

Body weight, carcass composition and adipose cellularity. *Body weight.* Initial body weight did not differ significantly among the diet, exercise, or age groups (Fig. 1). By the 5th wk of the experimental period, body weight of S-Ex24 was significantly lower than the other 24-mo-old groups. Body weight of S-Ex24 remained significantly lower than SF24 and S24 until wk 10 and wk 14 of the experimental period, respectively. No significant differences in body weight were demonstrated between any groups thereafter.

Carcass composition. There was a significant main effect of age [$F(1,45) = 13.52$, $P < 0.006$, ANOVA with age and diet as main effects] on percentage of body fat (Table 2). This reflects the significantly greater percentage of body fat in S24 and SF24 compared with the 12-mo-old animals. Exercise prevented this increase in percentage of body fat in the 24-mo-old animals. The percentage of protein and the percentage of lean body mass in S24 were significantly lower than in S12, SF12 and S-Ex24. No significant main effects were seen in the percentage of ash or the percentage of water (data not presented).

Adipose tissue cellularity. Total fat cell number was significantly increased in the sedentary 24-mo-

FIGURE 1 Body weight means of 12-mo-old (A) and 24-mo-old (B) Sprague-Dawley rats during the 4 mo diet/exercise protocol. For clarity, standard error bars have not been included. SEM was generally 2% of the mean value.

old animals compared with the 12-mo-old animals [significant main effect of age [$F(1,39) = 14.55$, $P < 0.0005$, ANOVA with age and diet as main effects]]. There was no significant difference in total fat cell number in the exercised 24-mo-old animals compared with any of the 12-mo-old groups [means $\pm$ SEM ($\times 10^6$) = SF12, 90.5 ± 3.5 ; S12, 92.5 ± 2.6 ; S-Ex12, 92.5 ± 3.2 ; SF24, 104.2 ± 3.2 ; S24, 105.5 ± 4.0 ; S-Ex24, 96.7 ± 5.8].

Food intake. The sucrose-fed groups ate significantly more food (Table 3) during the 1st wk of the experimental protocol [main effect of diet [$F(1,46) = 6.79$, $P < 0.01$, ANOVA with age and diet as main effects]]. A decrease in food intake in the exercised groups was observed by the 3rd wk of the protocol compared with the sedentary groups [$F(1,43) = 6.6$, $P < 0.01$, ANOVA with age and exercise as main effects]. No significant effects of diet, exercise or age on food intake were seen after wk 3.

Plasma glucose and insulin. Plasma glucose did not significantly differ with age, diet or exercise by the 6th wk or by the end of the experimental period (Table 4). A significant main effect of week of sampling was observed in the glucose measurements

TABLE 2

Carcass composition of younger and older Sprague-Dawley rats after 4 mo of the diet/exercise protocol¹

Parameter	12 mo			24 mo		
	Sucrose-free	Sucrose	Sucrose exercise	Sucrose-free	Sucrose	Sucrose exercise
Fat, g	60.7 ± 4.6 ^a (14)	66.2 ± 7.8 ^{ab} (14)	63.3 ± 5.0 ^{ab} (11)	82.2 ± 8.7 ^b (11)	81.3 ± 9.6 ^b (10)	64.7 ± 6.5 ^{ab} (11)
Fat, %	14.2 ± 0.8 ^a (14)	15.0 ± 1.7 ^a (14)	14.3 ± 0.9 ^a (11)	19.2 ± 1.7 ^{bc} (11)	21.3 ± 1.8 ^b (10)	15.5 ± 1.3 ^{ac} (11)
Lean body mass, g	359.1 ± 6.3 ^{ac} (14)	370.3 ± 10.0 ^a (14)	375.0 ± 7.6 ^a (11)	339.4 ± 9.0 ^{abc} (11)	303.8 ± 29.8 ^b (10)	327.7 ± 6.3 ^{bc} (11)
Lean body mass, %	85.7 ± 0.8 ^a (14)	85.0 ± 1.7 ^a (14)	85.6 ± 0.9 ^a (11)	80.8 ± 1.7 ^{bc} (11)	78.7 ± 1.8 ^b (10)	84.5 ± 1.3 ^{ac} (11)
Protein, g	92.5 ± 1.9 ^{ac} (14)	96.0 ± 2.5 ^a (13)	95.0 ± 2.5 ^{ac} (9)	90.1 ± 3.1 ^{ac} (8)	77.3 ± 8.3 ^b (9)	84.1 ± 2.4 ^{ac} (9)
Protein, %	22.1 ± 0.2 ^a (14)	22.0 ± 0.3 ^a (13)	21.5 ± 0.6 ^{ab} (9)	20.9 ± 0.5 ^{ab} (8)	20.4 ± 0.5 ^b (9)	22.0 ± 0.5 ^a (9)

¹Values are means ± SEM. Numbers in parentheses are numbers of rats. Within a row, values not sharing common superscripts are significantly different ($P < 0.05$) determined by Fischer's least significant difference post hoc test.

[$F(2,197) = 157.1$, $P < 0.0001$], resulting from significantly greater glucose readings at wk 17 compared to wk 6 and 10. No significant differences were observed for plasma insulin due to age, diet or exercise (Table 4).

Insulin-receptor function. There was a significant main effect of age on insulin-receptor number in both BF and VL [$F(1,26) = 4.95$; $P < 0.04$; $F(1,28) = 6.61$; $P < 0.02$ in BF and VL, respectively; ANOVA with age and diet as main effects] due to the generally lower values observed in the older rats (Table 5). Receptor number was significantly reduced in S24 compared with S12 and SF12 in both BF and VL. No significant diet or exercise effect was seen in the 12-mo-old rats. No significant difference in binding affinity of the receptor for insulin was demonstrated for either muscle.

Maximal tyrosine kinase activity was not significantly altered by diet, age or exercise in BF. However,

in VL a significant main effect of diet [$F(1,28) = 4.71$; $P < 0.04$, ANOVA with age and diet as main effects] and a significant main effect of age [$F(1,25) = 5.09$; $P < 0.03$, ANOVA with age and diet as main effects] on maximal tyrosine kinase activity were observed. Maximal tyrosine kinase activity was significantly reduced in S12 vs. S-Ex24 and SF24 and may account for the main effects observed. No significant main effects of diet, age or exercise were observed in the insulin concentration required for half maximal tyrosine kinase activity or endogenous (noninsulin stimulated) tyrosine kinase activity in VL or BF.

Citrate synthase activity. Citrate synthase activity was significantly greater in the S-Ex12 (14.37 ± 2.17 $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100$ mg protein⁻¹) compared with the 12-mo-old sedentary groups (S12, 10.99 ± 1.98 , SF12, 10.35 ± 2.28 $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100$ mg protein⁻¹). No significant differences were observed among the 24-mo-old groups (S-Ex24, 13.99 ± 3.96 ; S24, 13.93 ± 2.30 , SF24, 12.32 ± 3.97 $\mu\text{mol}\cdot\text{min}^{-1}\cdot 100$ mg protein⁻¹).

TABLE 3

Food intake measured for 5 consecutive days during wk 1, 3, 6, 10 and 13 of younger and older Sprague-Dawley rats during 4 mo diet/exercise protocol¹

Week	12 mo			24 mo		
	Sucrose-free (n = 15)	Sucrose (n = 14)	Sucrose exercise (n = 11)	Sucrose-free (n = 11)	Sucrose (n = 10)	Sucrose exercise (n = 12)
	g/d					
1	17.7 ± 0.7 ^{abc}	19.9 ± 0.8 ^a	19.8 ± 0.6 ^{ac}	17.0 ± 1.3 ^{bc}	19.7 ± 1.0 ^{ac}	17.0 ± 1.0 ^b
3	16.2 ± 0.6 ^a	16.7 ± 0.8 ^a	16.3 ± 0.3 ^a	15.9 ± 0.3 ^a	17.1 ± 1.1 ^a	13.7 ± 0.6 ^b
6	14.8 ± 0.9 ^a	15.5 ± 0.4 ^{ac}	15.3 ± 0.4 ^{ac}	16.0 ± 0.6 ^{ac}	15.6 ± 0.7 ^{ac}	14.1 ± 0.4 ^b
10	17.5 ± 0.5 ^{ac}	16.6 ± 0.6 ^a	16.0 ± 0.5 ^{ac}	15.8 ± 0.6 ^b	15.2 ± 0.6 ^{ac}	14.9 ± 0.4 ^a
13	15.0 ± 0.6	14.9 ± 0.5	14.7 ± 0.6	14.5 ± 0.4	15.5 ± 0.5	15.0 ± 0.5

¹Values are means ± SEM; n = number of rats. Within a row, values not sharing common superscripts are significantly different ($P < 0.05$) determined by Fischer's least significant difference post hoc test.

TABLE 4

Plasma glucose measured during wk 6, 10 and 17 and plasma insulin measured during wk 17 of younger and older Sprague-Dawley rats during 4 mo diet/exercise protocol¹

	12 mo			24 mo		
	Sucrose-free	Sucrose	Sucrose exercise	Sucrose-free	Sucrose	Sucrose Exercise
Glucose, mmol/L						
Wk 6	5.8 ± 0.1 (14)	5.5 ± 0.1 (11)	5.8 ± 0.1 (13)	5.5 ± 0.2 (11)	5.5 ± 0.2 (10)	5.2 ± 0.2 (12)
Wk 10	6.0 ± 0.1 ^{ac} (14)	5.9 ± 0.1 ^c (10)	5.3 ± 0.1 ^b (13)	5.4 ± 0.1 ^{bc} (11)	5.5 ± 0.2 ^{bc} (10)	5.9 ± 0.3 ^a (9)
Wk 17	6.2 ± 0.1 (14)	6.6 ± 0.2 (14)	6.6 ± 0.2 (13)	6.1 ± 0.1 (10)	6.1 ± 0.4 (8)	6.2 ± 0.2 (12)
Insulin, pmol/L						
Wk 17	336 ± 31 (13)	252 ± 28 (9)	365 ± 30 (6)	306 ± 38 (9)	349 ± 22 (8)	297 ± 21 (9)

¹Values are means ± SEM. Numbers in parentheses are number of rats. Within a row, values with different superscripts are significantly different ($P < 0.05$) determined by Fischer's least significant difference post hoc test.

DISCUSSION

Alterations in insulin-receptor number, an index of insulin sensitivity, probably cannot be attributed solely to the inherent process of aging. Insulin-receptor number was significantly decreased in the sedentary 24-mo-old rats fed a high sucrose diet compared with 12-mo-old animals. This decline, however, was not evident in 24-mo-old rats exercised on a motor-driven treadmill or fed the sucrose-free diet (compared with the 12-mo-old rats). Thus, these data suggest that aging alone does not account for decreases in insulin sensitivity as measured by insulin-receptor function.

Dietary influences seem to have a role in skeletal muscle insulin-receptor function. Feeding the high sucrose diet resulted in alterations in both the sedentary sucrose-fed 12- and 24-mo-old animals through a decrease in maximal insulin-stimulated tyrosine kinase activity and a decrease in insulin-receptor number, respectively (Table 5). Severe decreases in insulin-receptor number and insulin-stimulated tyrosine kinase activity have been observed in states associated with decreased insulin sensitivity; decreases in skeletal muscle insulin-receptor number have been observed in skeletal muscle of obese compared with lean Sprague-Dawley rats (23); decreases in maximal insulin-stimulated tyrosine kinase activity with no alterations in binding sites have been observed in skeletal muscle of humans with Type II diabetes mellitus (24).

The functional significance of the alterations we observed in skeletal muscle insulin-receptor number and maximal tyrosine kinase activity seems to be minor because these receptor alterations were not accompanied by consistent increases in plasma glucose or insulin. A possible explanation for this

apparent dissociation between tissue and serum markers for insulin resistance is that muscle contains spare receptors (25). That is, maximal insulin effect occurs at an insulin concentration that does not result in the occupation of all the insulin-receptor binding sites. Furthermore, the decrease in maximal tyrosine kinase activity is not likely to be functionally significant because the alteration occurred at insulin concentrations above physiological concentrations, and no significant change in the insulin concentration required for half maximal activity was observed.

Previous studies have demonstrated hyperglycemia and decreased insulin-stimulated glucose uptake in rats fed a high sucrose diet (26). As stated previously, no significant main effect of diet was demonstrated at any of the sampling periods. The inverse relationship between insulin-receptor function and serum glucose and insulin concentrations observed in these rats may reflect the length of time the rats were fed the high sucrose diet. We chose a 4-mo feeding protocol to determine long-term changes in glucose metabolism rather than transient alterations. Gutman and colleagues (27) demonstrated that metabolic alterations in glucose tolerance and triglyceride synthesis in response to a high sucrose diet are multiphasic and result in the greatest decline in glucose tolerance during the first 22 d of the diet, with an attenuated effect on glucose tolerance thereafter. Therefore, the alterations in skeletal muscle insulin receptors may represent long-term adaptations to the high sucrose diet that persist beyond the initial alterations in glucose tolerance. These data are consistent with those of McDonald (28), who reported that intravenous glucose tolerance tests did not differ between 12- and 26-mo-old male Sprague-Dawley rats fed the same sucrose and sucrose-free diets as those in

TABLE 5
Insulin-receptor function of younger and older Sprague-Dawley rats following 4 mo diet/exercise protocol¹

	12 Mo			24 Mo		
	Sucrose-free	Sucrose	Sucrose exercise	Sucrose-free	Sucrose	Sucrose exercise
Biceps femoris						
Insulin binding						
K_d , nmol/L ²	$n = 10$ 0.107 ± 0.026	$n = 10$ 0.122 ± 0.023	$n = 5$ 0.105 ± 0.018	$n = 5$ 0.078 ± 0.007	$n = 5$ 0.076 ± 0.014	$n = 6$ 0.081 ± 0.009
Receptor number, pmol/mg protein	8.83 ± 1.4 ^a	8.18 ± 0.76 ^a	8.29 ± 0.89 ^{ab}	6.83 ± 1.2 ^{ab}	4.61 ± 0.44 ^b	7.70 ± 0.91 ^{ab}
Tyrosine kinase						
K_m , nmol/L ³	$n = 5$ 1.16 ± 0.15	$n = 8$ 1.35 ± 0.17	$n = 6$ 1.02 ± 0.15	$n = 10$ 0.87 ± 0.07	$n = 5$ 1.09 ± 0.14	$n = 8$ 1.37 ± 0.26
V_{max} ⁴	514.0 ± 57.0	552.0 ± 28.0	564.0 ± 79.0	450.0 ± 54.0	454.0 ± 68.0	543.0 ± 97.0
Endogenous activity, ⁵ fmol·min ⁻¹ ·mg protein ⁻¹	39.0 ± 12.0	47.0 ± 12.0	66.0 ± 9.0	37.0 ± 9.0	31.0 ± 10.0	44.0 ± 12.0
Vastus lateralis						
Insulin binding						
K_d , nmol/L ²	$n = 9$ 0.071 ± 0.010	$n = 7$ 0.066 ± 0.011	$n = 9$ 0.059 ± 0.009	$n = 8$ 0.065 ± 0.010	$n = 8$ 0.045 ± 0.008	$n = 6$ 0.066 ± 0.012
Receptor number, pmol/mg protein	11.80 ± 1.3 ^a	12.0 ± 2.1 ^a	9.9 ± 1.0 ^{ab}	8.9 ± 0.8 ^{ab}	8.2 ± 0.7 ^b	9.6 ± 1.5 ^{ab}
Tyrosine kinase						
K_m , nmol/L ³	$n = 9$ 1.10 ± 0.19	$n = 7$ 0.96 ± 0.14	$n = 8$ 0.84 ± 0.13	$n = 7$ 1.37 ± 0.38	$n = 7$ 1.37 ± 0.38	$n = 7$ 1.16 ± 0.23
V_{max} ⁴	635.0 ± 93.0 ^{ab}	414.0 ± 73.0 ^a	607.0 ± 92.0 ^{ab}	779.0 ± 110.0 ^b	601.0 ± 105.0 ^{ab}	792.0 ± 0.23 ^b
Endogenous activity, ⁵ fmol·min ⁻¹ ·mg protein ⁻¹	56.0 ± 18.0	84.0 ± 17.0	61.0 ± 16.0	72.0 ± 23.0	50.0 ± 10.0	47.0 ± 11.0

¹Values are means ± SEM, n = number of animals. Within a row, values with different superscripts are significantly different ($P < 0.05$) as determined by Fischer's least significant post hoc test.

²Binding affinity of insulin receptor.

³Concentration of insulin at half maximal tyrosine kinase activity.

⁴Maximal tyrosine kinase activity in presence of insulin (100 nmol/L) measured by fmol ³²P incorporated into poly (glu, tyr) 4:1·min⁻¹·mg protein⁻¹.

⁵Activity of tyrosine kinase in the absence of insulin measured by fmol ³²P incorporated into poly (glu, tyr) 4:1·min⁻¹·mg protein⁻¹.

the present study.

Exercise seemed to prevent the decline in skeletal muscle insulin-receptor function in both the 12- and 24-mo-old animals. It is possible that the exercise prevented the increase in adiposity seen in the 24-mo-old compared with the 12-mo-old animals which may, in turn, prevent the decrease in insulin-receptor number. The older rats also initially decreased food intake in response to exercise, which may also account for the changes observed. When rats are not allowed to become obese, the decline in insulin sensitivity previously observed is prevented (29). The role of exercise in the younger animals seemed to be one of increasing responsiveness to insulin due to the training effect observed in these animals. It seems, therefore, that younger and older Sprague-Dawley rats may respond to treadmill exercise and high sucrose diet differently, but both groups demonstrate some ability to prevent the attenuated effects of the sucrose diet by exercise.

In conclusion, environmental influences seem to be more important in the onset of insulin insensitivity than age per se. In both the 12- and 24-mo-old animals, minor decreases in insulin-receptor function were demonstrated in the sucrose-fed sedentary groups. The decreases observed were prevented when exercise accompanied the sucrose diet. This indicates that exercise may be involved in the prevention of insulin insensitivity in both young adult and aged adult animals. These alterations, however, were not accompanied by changes in plasma glucose or insulin, indicating that the alterations in skeletal muscle insulin receptor may have marginal functional significance. The lack of significant differences in insulin-receptor function between young and old rats suggest that the age-related declines previously reported for insulin sensitivity may need reassessment.

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