Gerontology 1995;41:205–211

*R. James Barnard*
*Jack F. Youngren*
*Deborah A. Martin*

Department of Physiological Science, University of California, Los Angeles, Calif., USA

# Diet, Not Aging, Causes Skeletal Muscle Insulin Resistance

**Key Words**
Glucose transport
GLUT-4
Glucose
Obesity

**Abstract**
The purpose of this study was to compare the effects of raising female Fischer rats on a low-fat, high-complex-carbohydrate diet (LFCC) versus a high-fat, sucrose diet (HFS) on serum glucose and insulin as well as skeletal muscle glucose transport. No significant differences were observed between 6- and 24-month-old rats raised on the LFCC diet for serum glucose (3.6 ± 0.1 vs. 3.7 ± 0.2 m*M*) and insulin (88 ± 6 vs. 98 ± 10 p*M*) or for basal (35 ± 3 vs. 39 ± 6 pmol/mg protein/15 s) or insulin-stimulated (74.2 ± 7.6 vs. 69.4 ± 3.8 pmol/mg protein/15 s) glucose transport. These data indicate that aging per se does not lead to insulin resistance. When the 24-month-old animals raised on the HFS diet were compared with those on the LFCC diet, major differences were observed. Fasting serum insulin was significantly higher in the HFS group (437 ± 118 vs. 98 ± 10 p*M*) and insulin-stimulated glucose transport was significantly reduced (52.5 ± 3.7 vs. 69.4 ± 3.8 pmol/mg protein/15 s). Fasting glucose (3.7 ± 0.2 vs. 3.6 ± 0.1 m*M*) and basal glucose transport (38 ± 6 vs. 39 ± 6 pmol/mg protein/15 s) were unchanged. These results indicate that diet and not aging per se caused insulin resistance.

## Introduction

Glucose intolerance and insulin resistance have classically been ascribed to aging. This relationship was first described by Spence [1] in 1920. In 1979, Davidson [2] reviewed the literature and from 62 reports concluded that the aging process was linked to an impaired ability to handle glucose. Using World Health Organization standards, Harris et al. [3] re-

Supported by NIH grant AG 07529.

Received:
July 7, 1994
Accepted:
November 23, 1994

R. James Barnard, PhD
Department of Physiological Science
University of California
1804 Life Sciences Building
Los Angeles, CA 90024-1527 (USA)


0304–324X/95/
0414–0205$8.00/0

ported finding impaired glucose tolerance or diabetes in 41.5% of individuals 65–74 years of age. However, life-style factors including diet, obesity and physical inactivity have also been linked to glucose intolerance and insulin resistance. In a more recent study, Zavaroni et al. [4] performed oral glucose tolerance tests on 732 factory workers aged 22–73 years. They correlated the responses with percent ideal body weight, leisure physical activity and work activity. The partial correlation coefficients left the effect of age on plasma glucose insignificant in women and accounting for less than 10% of the variance in men. In another large study, Shimokata et al. [5] conducted glucose tolerance tests on 743 men and women aged 17–92 years. They also measured skin-fold fat, waist-to-hip ratio, physical activity levels and maximum oxygen uptake. Multiple regression analysis revealed that age accounted for only 3–6% of the variance in the 2-hour glucose values. In a smaller study, Pacini et al. [6] compared a group of physically active, elderly men with healthy young men and found that neither oral nor intravenous glucose tolerance tests were different between the two groups. Similar results were found by Seals et al. [7].

Unfortunately, none of these studies were longitudinal; therefore, they cannot conclude that aging per se does not lead to glucose intolerance. It may well be that when older individuals exercise or lose weight, they reverse preexisting glucose intolerance. Unfortunately, no detailed dietary information was obtained in any of these studies. Three animal studies, one by Goodman et al. [8], one by Gulve et al. [9] and one from our laboratory [10], show that when rats are raised on a low-fat, starch diet some insulin resistance develops with maturation, but after the rapid growth phase, age has little effect on the glucose transport capacity of skeletal muscle. These data, from both human and animal studies, suggest that life-style, as opposed to aging per se, is the cause of the commonly observed glucose intolerance associated with aging. Skeletal muscle is the most important target tissue for insulin action and is the major site responsible for whole-body glucose intolerance. The purpose of the present study was to test the effects of prolonged feeding (2 years) of a high-fat, refined-sugar diet on body weight, fasting glucose and insulin, as well as on skeletal muscle glucose transport using an isolated skeletal muscle sarcolemmal vesicle preparation.

## Materials and Methods

*Materials.* Porcine insulin was donated by Eli Lilly (D-[$^3$H]glucose, *L*-[$^{14}$C]glucose and $^{125}$I protein A were obtained from DuPont-New England Nuclear.) Reagents for sodium dodecylsulfate-polyacrylamide gel electrophoresis were obtained from Bio-Rad (Richmond, Calif., USA), GLUT-4 polyclonal antibody was purchased from East Acres Biologicals (Southbridge Mass., USA). Other reagents were obtained from Sigma (St. Louis, Mo., USA). Autoradiography cassettes and intensifying screens were purchased from Fisher Scientific (Los Angeles, Calif., USA) and X-Omat AR film from Eastman Kodak (Rochester, N.Y., USA).

*Animals.* Female Fischer 344 rats were obtained from the National Institute on Aging colony at Harlan Sprague Dawley. The animals arrived at 4 weeks of age and were housed 5 per cage with water and food ad libitum. During the 1st week, all animals were placed on a diet (LFCC) low in fat (6% calories) and high in complex-carbohydrates (68% calories). After 1 week half the animals were placed on a diet (HFS) that contained 40% of calories from fat, mostly saturated fat and 40% of calories from sucrose. Details concerning the diets have been published previously [11]. Both diets contained standard vitamin and mineral mix as well as methionine and were provided ad libitum. Studies were conducted on animals from the LFCC diet group at 6 and 24 months and from the HFS group at 24 months.

*Blood Glucose and Insulin.* The animals were anesthetized with 7% chloral hydrate (0.5 ml/100 g i.p.) and a blood sample obtained following an overnight fast. Glucose measurements were made immediately using a One Touch blood glucose analyzer (Milpitas

Calif., USA). The remaining sample was centrifuged and the plasma stored at −70°C until analyzed for insulin using a double-antibody radioimmunoassay from Ventrex Laboratories (Portland, Me., USA).

*Sarcolemma Isolation and Glucose Transport.* Skeletal muscle sarcolemmal vesicles were prepared from basal and insulin-stimulated (2 U/kg i.v.) animals. For the insulin-stimulated preparations, the muscles were removed 10 min after the intravenous injection. Gastrocnemius, planaris and quadriceps muscles were combined for each preparation. The sarcolemmal isolation procedure involves differential and sucrose-gradient centrifugation as described in detail previously [12], with the exception that deoxyribonuclease incubation was not used. Protein was determined according to Bradford, and $K^+$-stimulated-*p*-nitrophenylphosphatase (KpNPPase) activity was measured to assess purity [12]. Glucose transport was measured under equilibrium-exchange conditions using both *D*- and *L*-glucose as described previously [12]. Measurement was made for 15 s at 37°C at a glucose concentration of 180 μ*M*.

*Total Cellular Membrane Preparation and Western Blotting.* Isolation of total cellular membranes was done using a slight modification of the method of Charron and Kahn [13]. The hindlimb muscles (gastrocnemius, plantaris, quadriceps) were removed and immediately frozen in liquid nitrogen. The frozen muscles were pulverized in liquid nitrogen using a mortar and pestle. The powder was suspended in sarcolemmal homogenizing medium and subjected to Teflon homogenization. The solution was centrifuged (1,200 *g*, 10 min), the pellet resuspended and centrifuged (1,200 *g*, 10 min) and the two supernatants combined and centrifuged at 9,000 *g* for 10 min. The supernatant was then centrifuged at 227,000 *g* for 75 min and the pellet resuspended in homogenizing medium and used for GLUT-4 determination via Western blotting.

The membrane samples (30 μg protein) were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis using an 8% gel. The proteins were then electrophoretically transferred to a nitrocellulose membrane. Following transfer, the membranes were incubated overnight at 4°C in a blocking buffer consisting of 150 m*M* NaCl, 50 m*M* Tris, 0.2% gelatin, 3% powdered milk, 0.05% Tween 20, and 0.1% sodium azide at pH 7.6. The membranes were then rinsed with buffer A (50 m*M* Tris, 150 m*M* NaCl, 0.04% NP-40, pH 7.4) and overlaid with a polyclonal antibody to GLUT-4, the insulin responsive glucose transporter, diluted 1:500 in buffer A. After 1 h incubation, the membranes were washed for 1 h in buffer A and then overlaid with $^{125}I$ protein A (1 μCi in 5 ml buffer A). Following 1 h of washing in buffer A, the membrane was dried and exposed to XAR-5 film for approximately 48 h. The developed film was then analyzed on a LBK 2202 Ultroscan laser densitometer (Bromma, Sweden). Two different gels were run; one contained 6- and 24-month samples, while the other contained the two different diet groups at 24 months.

*Statistical Analysis.* Data were analyzed by a Student t test or by analysis of variance with post hoc analysis by a Tukey's test. A $p < 0.05$ was considered significant. Values are given as means ± SE.

## Results

At 6 months of age, 10 rats from the LFCC group were taken for serum and for muscle glucose transport studies. The mean weight of all the LFCC rats at 6 months was 187 ± 1.2 g. This was the end of the rapid growth phase, as the remaining animals gained only an additional 25 g in the next 1.5 years, ending at 223 ± 2.7. The HFS rats at 6 months weighed 210 ± 2.2 g but continued to gain weight and by 24 months weighed 287 ± 5.8 g, significantly more than the LFCC group.

As can be seen in table 1, there was no significant difference in fasting blood glucose among the three groups. Fasting serum insulin was significantly elevated in the 24-month-old HFS group compared to the other two groups, which were not significantly different from each other.

Table 2 gives the data pertaining to sarcolemma yield (mg protein/g muscle) and purity as assessed by the marker enzyme KpNPPase activity. No significant differences were observed among the different age or diet groups.

Figure 1 shows both basal and insulin-stimulated glucose transport by isolated skeletal muscle sarcolemmal vesicles. Basal glucose transport was similar in all three groups. Insulin-stimulated transport was similar for both

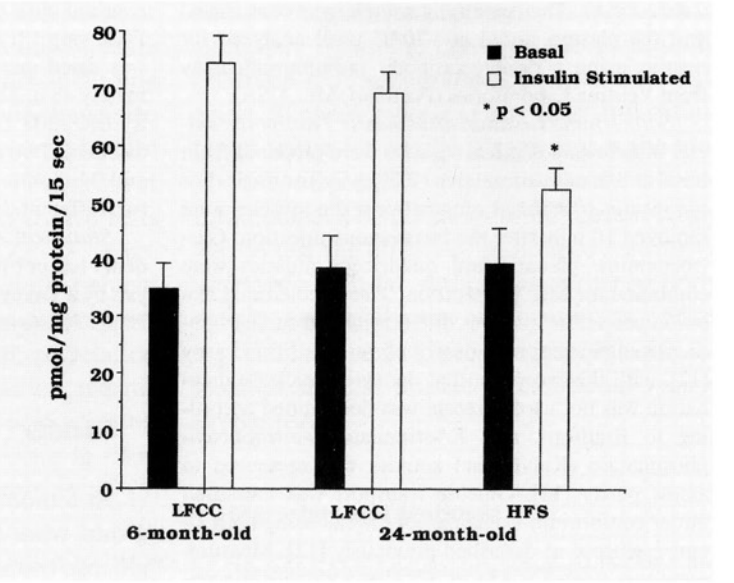


**Fig. 1.** Basal and insulin-stimulated glucose transport in skeletal muscle sarcolemmal vesicles. Insulin-stimulated transport was significantly depressed in the HFS rats compared to the LFCC rats. Each group contained 5 animals.

**Table 1.** Fasting glucose and insulin values

| | LFCC | | HFS |
|---|---|---|---|
| | 6 months | 24 months | 24 months |
| Blood glucose, m*M* | 3.6 ± 0.1 | 3.7 ± 0.2 | 3.5 ± 0.2 |
| Serum insulin, p*M* | 87.5 ± 6.4 | 97.5 ± 10.0 | 437.3 ± 118.3* |

* $p < 0.05$. There were 9 rats in each group.

**Table 2.** Sarcolemmal yield and purity

| | LFCC | | HFS |
|---|---|---|---|
| | 6 months | 24 months | 24 months |
| Yield, mg protein/g muscle | | | |
| Basal | 0.30 ± 0.3 | 0.23 ± 0.02 | 0.32 ± 0.06 |
| Insulin stimulated | 0.28 ± 0.4 | 0.21 ± 0.04 | 0.25 ± 0.05 |
| KpNNPase, µmol/mg/h | | | |
| Basal | 4.82 ± 0.38 | 4.62 ± 0.24 | 5.11 ± 0.43 |
| Insulin stimulated | 4.20 ± 0.51 | 3.82 ± 0.30 | 3.92 ± 0.69 |

There were 5 rats in each group and no significant difference among the groups.

the 6- and 24-month LFCC animals (74 ± 5 vs. 69 ± 4 pmol/mg protein/15 s) but was significantly reduced in the 24-month-old HFS diet group (53 ± 4 pmol/mg protein/15 s).

Quantitation of total cellular membrane GLUT-4 transporters via Western blots revealed no significant difference between the 6- and 24-month-old animals (1.31 ± 0.23 vs. 1.23 ± 0.07 units) nor between the 24-month-old LFCC and HFS diet groups (0.80 ± 0.07 vs. 0.83 ± 0.09 units).

## Discussion

Numerous studies in humans, as reviewed by Davidson [2], have reported glucose intolerance in aged individuals. Recent studies have, however, indicated that life-style, as opposed to aging per se, may be responsible for the glucose intolerance [4–7]. The results of the present study indicate that in the female Fischer rat, aging is not associated with a significant reduction in insulin sensitivity when animals are raised on the LFCC diet. Fasting glucose and insulin levels were not significantly different nor was basal or maximally insulin-stimulated glucose transport between the 6- and 24-month-old animals. However, when the rats were raised on a high-fat, refined-sugar diet, the 24-month-old animals were hyperinsulinemic and insulin-stimulated glucose transport was significantly depressed. These rats were not likely to be diabetic as fasting glucose was very low and not significantly different among the three groups. The fact that aging did not result in a significant reduction in insulin-stimulated glucose transport confirms our earlier study [10] with sarcolemmal membranes and agrees with the data from Goodman et al. [8] using hindlimb perfusion techniques and Gulve et al. [9] using isolated muscle preparations.

Skeletal muscle is responsible for removing up to 75% of the blood glucose during hyperinsulinemic clamp studies [14]. Thus, insulin resistance in skeletal muscle, as demonstrated in the present study, should have a major impact on glucose regulation. In humans, skeletal muscle insulin resistance has been observed in obese individuals as well as in patients with non-insulin-dependent diabetes mellitus [14, 15]. Classically, insulin resistance has been attributed to obesity [16]. In the present study, the rats raised on the HFS diet weighed considerably more than the rats raised on the LFCC diet. However, in an earlier study with young rats, we induced skeletal muscle insulin resistance by using the HFS diet without any change in body weight or percent body fat [17]. These results, combined with the present study, indicate that insulin resistance precedes obesity; thus indicating that the widely held concept that obesity is the cause of insulin resistance is false. When obese individuals are placed on a low-fat, complex-carbohydrate diet combined with daily walking, fasting insulin drops by 30–40% within 3 weeks, indicating a significant improvement in insulin resistance, while the individuals are still obese [18]. The same diet that induces insulin resistance (HFS) also will eventually lead to obesity, and not vice versa. In addition, Rowe et al. [19] and Jackson et al. [20] reported insulin resistance in aged humans who were not obese. This does not mean that obesity has no influence on glucose regulation. In fact, Coon et al. [21] recently reported that insulin sensitivity did not decline with aging unless abdominal obesity, as measured by the waist-to-hip ratio, increased. Unfortunately, they did not collect dietary information. In our rats on the HFS diet for 2 years, obesity, with a large increase in fat in the abdominal cavity, was observed [11].

The exact mechanisms by which the HFS diet induces skeletal muscle insulin resistance

are not known. This is not surprising as the exact mechanisms by which insulin stimulates glucose transport in skeletal muscle are unknown as well [22, 23]. Under basal conditions, glucose is thought to be transported into muscle cells primarily by the GLUT-1 transporter isoform, normally present in the cell membrane. When insulin binds to its receptor, the receptor is phosphorylated and the tyrosine kinase of the beta subunit activated. The tyrosine kinase, by unknown mechanisms, leads to the translocation of GLUT-4 transporters from an intracellular pool out to the sarcolemmal membrane and possibly the T-tubules [22, 23]. There is also some evidence that insulin activates transporters to increase the turnover rate [22]. Studies using skeletal muscle from non-insulin-dependent diabetes mellitus patients have demonstrated a lack of insulin-stimulated glucose uptake [15]. A decrease in the total number of skeletal muscle insulin receptors as well as a decrease in the insulin-stimulated tyrosine kinase of receptors have been reported [22, 24]. The total cellular pool of GLUT-4 transporters in patients with non-insulin-dependent diabetes mellitus and obese Zucker rats has been reported to be unchanged in most studies [22, 25]. Thus, it was not surprising that GLUT-4 was unchanged in the HFS, insulin-resistant muscles. Others [9, 26] also have reported no change in GLUT-4 with aging, as we found. In our previous study with young rats, the HFS-diet-induced insulin resistance was found without any change in the number of insulin receptors; there was, however, a reduction in the affinity of the receptors for insulin binding [17].

In summary, the results of this study show that when rats were raised on a low-fat, high complex-carbohydrate diet, insulin resistance and hyperinsulinemia were not observed. Fasting insulin and glucose remained well within normal limits and obesity did not develop. However, when rats were raised on a high-fat, refined-sugar diet, similar to the typical US diet, insulin resistance, hyperinsulinemia and obesity developed, which indicates that diet and not the aging process per se is responsible for insulin resistance and the related factors.

## References


1 Spence JM: Some observations on sugar tolerance with special reference to variations found at different ages. Am J Med 1920;14:314–326.

2 Davidson MB: The effect of aging on carbohydrate metabolism: A review of the English literature and a practical approach to the diagnosis of diabetes mellitus in the elderly. Metabolism 1979;28:688–705.

3 Harris MI, Hadden WC, Knowler WC, Bennett PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 years. Diabetes 1987;36:523–534.

4 Zavaroni I, Dall-Aglio E, Bruschi F, Bonora E, Alpi O, Pezzarossa A, Butturini U: Effect of age and environmental factors on glucose tolerance and insulin secretion in a worker population. J Am Geriatr Soc 1986;34:271–275.

5 Shimokata H, Muller DC, Fled JL, Sorkin J, Ziemba AJ, Andres R: Age as independent determinant of glucose tolerance. Diabetes 1991;40: 44–51.

6 Pacini G, Valerio A, Beccaro F, Nosadini R, Cobelli C, Crepaldi G: Insulin sensitivity and beta-cell responsivity are not decreased in elderly subjects with normal OGTT. J Am Geriatr Soc 1988;36:317–323.

7 Seals DR, Hageberg JM, Allen WK, Hurley BF, Dalsky GP, Ehsani AA, Holloszy JO: Glucose tolerance in young and older athletes and sedentary men. J Appl Physiol 1984;56: 1521–1525.

8 Goodman MN, Dluz SM, McElaney MA, Belur E, Ruderman NB: Glucose uptake and insulin sensitivity in rat muscle: Changes during 3–96 weeks of age. Am J Physiol 1983; 244:E93–E100.

9 Gulve EA, Henriksen EJ, Rodnick KJ, Youn JH, Holloszy JO: Glucose transporters and glucose transport in skeletal muscles of 1- to 25-month-old rats. Am J Physiol 1993; 264:E319–E327.
10 Barnard RJ, Lawani LO, Martin DA, Youngren JF, Singh R, Scheck SH: Effects of maturation and aging on the skeletal muscle glucose transport system. Am J Physiol 1992;262: E619–E626.
11 Barnard RJ, Faria DJ, Menges JE, Martin DA: Effects of a high-fat, sucrose diet on serum insulin and related atherosclerotic risk factors in rats. Atherosclerosis 1993;100:229–236.
12 Grimditch GK, Barnard RJ, Kaplan SH, Sternlicht E: Insulin binding and glucose transport in rat skeletal muscle sarcolemmal vesicles. Am J Physiol 1985;249:E398–E408.
13 Charron MJ, Kahn BB: Divergent molecular mechanisms for insulin-resistant glucose transport in muscle and adipose cells in vivo. J Biol Chem 1990;265:7994–8000.
14 DeFronzo RA: The triumvirate: β-Cell, muscle, liver. A collusion responsible for NIDDM. Diabetes 1988;37:667–687.
15 Dohm GL, Tapscott EB, Paries WJ, Dobbs DJ, Flickinger EG, Meelheim D, Fushiki T, Atkinson SM, Elton CW, Caro JF: An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport from morbidly obese and diabetic subjects. J Clin Invest 1988;82:486–494.
16 Pedersen O: The impact of obesity on the pathogenesis of non-insulin-dependent diabetes mellitus: A review of current hypotheses. Diabetes Metab Rev 1989;5:495.
17 Grimditch GK, Barnard RJ, Sternlicht E, Whitson RH, Kaplan SA: Effect of diet on insulin binding and glucose transport in rat sarcolemmal vesicles. Am J Physiol 1987;252: E420–E425.
18 Barnard RJ, Ugianskis EJ, Martin DA, Inkeles SB: Role of diet and exercise in the management of hyperinsulinemia and associated risk factors. Am J Cardiol 1992;69:440–444.
19 Rowe JW, Minaker KL, Pallotta JA, Flier JS: Characterization of the insulin resistance of aging. J Clin Invest 1983;71:1581–1587.
20 Jackson RA, Hawa MI, Roshania RD, Sim BM, DiSilvio L, Jaspan JB: Influence of aging on hepatic and peripheral glucose metabolism in humans. Diabetes 1988;37:119–129.
21 Coon PJ, Rogus EM, Drinkwater D, Muller DC, Goldberg AD: Role of body fat distribution in the decline in insulin sensitivity and glucose tolerance with age. J Clin Endocrinol Metab 1992;75:1125–1132.
22 Barnard RJ, Youngren JF: Regulation of glucose transport in skeletal muscle. FASEB J 1992;6:3238–3244.
23 Klip A, Marette A: Acute and chronic signals controlling glucose transport in skeletal muscle. J Cell Biochem 1992;48:51–60.
24 Häring H, Obermaier-Kusser B: Insulin receptor kinase defects in insulin-resistant tissues and their role in the pathogenesis of NIDDM. Diabetes Metab Rev 1989;5:431–444.
25 King PA, Horton ED, Hirshman MF, Horton ES: Insulin resistance in obese Zucker rat (fa/fa) skeletal muscle is associated with a failure of glucose transporter translocation. J Clin Invest 1992;90:1568–1575.
26 Kern M, Dolan PL, Mazzeo RS, Wells JA, Dohm GL: Effect of aging and exercise on GLUT-4 glucose transporters in muscle. Am J Physiol 1992;263:E362–E367.