Diet-induced insulin resistance precedes other aspects of the metabolic syndrome

R. JAMES BARNARD, CHRISTIAN K. ROBERTS,
SHIRA M. VARON, AND JOSHUA J. BERGER
Department of Physiological Science, University of California, Los Angeles, California 90095-1527

Barnard, R. James, Christian K. Roberts, Shira M. Varon, and Joshua J. Berger. Diet-induced insulin resistance precedes other aspects of the metabolic syndrome. *J. Appl. Physiol.* 84(4): 1311–1315, 1998.—This study was designed to examine the effects of a high-fat refined-sugar (HFS) or a low-fat complex-carbohydrate (LFCC) diet on insulin-stimulated skeletal muscle glucose transport, plasma insulin, blood pressure, plasma triglycerides, plasma glycerol, body weight, and body fat in female Fischer rats. Insulin-stimulated glucose transport was significantly reduced in the HFS group at 2 wk, 2 mo, and 2 yr, whereas serum insulin was significantly elevated at all time points. Blood pressure was not significantly elevated in the HFS group until 12 mo, and all HFS animals were hypertensive by 18 mo. Glycerol, triglycerides, and abdominal fat cell size were not significantly different at 2 wk but were significantly elevated in the HFS rats at 2 and 6 mo. Body weight was similar in both groups until 20 wk on the diet, when the HFS rats started to gain more weight. These results demonstrate that insulin resistance and hyperinsulinemia occur before the other manifestations of the metabolic syndrome and that diet, not obesity, is the underlying cause.

blood pressure; body fat; glycerol; hyperinsulinemia; triglycerides

IT WAS TERMED "syndrome X" in 1988 by Reaven (39), and in 1989 Kaplan (28) called it "the deadly quartet." Both were referring to a cluster of atherosclerotic risk factors, including hyperinsulinemia, hypertriglyceridemia, hypertension, and obesity. This syndrome has been expanded to include abdominal obesity, depressed high-density-lipoprotein cholesterol, and enhanced clotting factor activity (28). The clustering of all these risk factors in the same individual results in a highly atherogenic risk profile. The exact incidence of this risk profile in our society is not known but is suspected to be quite high.

Haffner et al. (26) renamed the syndrome the "insulin resistance syndrome" to stress the fact that insulin resistance is thought to be the underlying defect. DeFronzo and Ferrannini (16) also suggested that insulin resistance is the underlying factor in this syndrome, and once hyperinsulinemia develops, it, coupled with the genetic predispositions, produces the other manifestations of the syndrome. Not all factors are present in all individuals, which leads one to believe that there must be a genetic predisposition for acquiring aspects of the syndrome and that environmental factors play a pivotal role in their subsequent development (25).

Obesity, especially upper body obesity, has traditionally been thought to be the underlying cause of the syndrome (8, 12, 20, 27–31, 35–37, 42, 48). Studies in humans, especially women, have shown that the risk profile or at least some of the risk factors are commonly found with upper body obesity but are rarely found when the obesity is confined to the lower body. Others (2, 3, 16, 19, 24, 39) have suggested that insulin resistance and the resultant hyperinsulinemia are key factors in this risk profile. We recently reported that when female Fischer rats were raised for 2 yr on a high-fat refined-sugar (HFS) diet, similar to the typical US diet, the animals developed insulin resistance and were hyperinsulinemic, hypertriglyceridemic, hypertensive, exhibited enhanced clotting factor activity, and were obese, with an excessive amount of abdominal obesity (2, 6).

Because insulin resistance/hyperinsulinemia can be controlled before obesity is corrected, it would be important to know the true underlying cause of the syndrome (3). The present study was designed to track the development of what is now known as the metabolic syndrome in the female Fischer rat.

METHODS

Animals and diets. Inbred, female Fischer 344 rats were obtained from Harlan Sprague Dawley at 2 mo of age. The animals were housed four per cage with a 12:12-h light-dark cycle starting at 0700 at 76°F. Diets were fed ad libitum, with large bowls placed in the cages to ensure that all animals had access to the food, especially during eating periods. After acclimatization of the animals for 1 wk on standard rat chow, the rats were assigned to the low-fat complex-carbohydrate (LFCC) or the HFS diet group. The diets were prepared in powder form by Purina Mills and contained a standard vitamin and mineral mix with all essential nutrients, as described previously (2). Specifically, the percent distribution of calories and caloric density for the two diets were as follows: 23.0% protein, 9.0% fat, 68.0% starch, 0.0% sucrose, and 13.8 kJ/g for the LFCC diet and 21.0% protein, 39.0% fat, 0.0% starch, 40.0% sucrose, and 19.7 kJ/g for the HFS diet. This experiment was approved by the University of California, Los Angeles, Animal Research Committee.

Insulin resistance. Insulin resistance was assessed by injecting the rats with regular insulin (15 U ip) and 30 min later killing the rats by cervical dislocation. The hindlimb muscles (gastrocnemius, plantaris, and quadriceps groups) were rapidly removed and immediately placed in ice-cold saline for subsequent isolation of skeletal muscle sarcolemmal vesicles to study glucose transport, as described in detail previously (22). In this study we eliminated the incubation with deoxyribonuclease, inasmuch as it has been determined to be unnecessary. Briefly, vesicles from hindlimb muscle were formed by Polytron homogenation after trimming and mincing of the tissue. Sarcolemma were then purified by differential and sucrose-gradient centrifugation. After purification the sarcolemmal vesicles were harvested from fraction 2 (27%) of the sucrose gradient at a density of ~1.1 g/ml. The vesicle suspension was then stored in liquid N_2 until protein content,

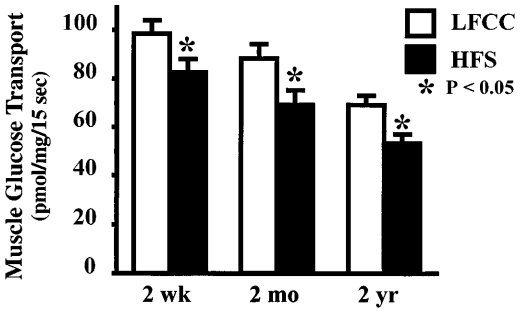

Fig. 1. Effect of diet on muscle glucose transport. Insulin-stimulated transport values were significantly reduced at all time points in group fed high-fat refined-sugar (HFS) diet compared with group fed low-fat complex-carbohydrate (LFCC) diet ($P < 0.05$). Values are means ± SE.

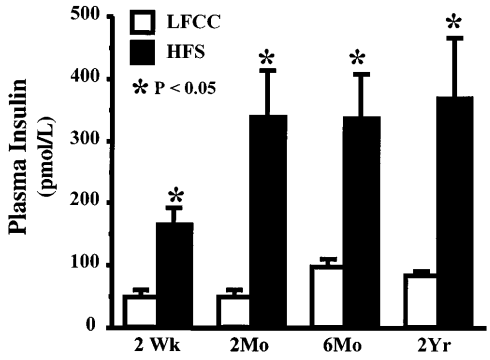

Fig. 2. Effect of diet on plasma insulin. Fasting plasma insulin levels were significantly elevated at all time points in HFS group compared with LFCC group ($P < 0.05$). HFS value was significantly lower at 2 wk than at other times. Values are means ± SE.

K^+-stimulated *p*-nitrophenylphosphatase (KpNPPase), and glucose transport were determined. Protein was measured using the Bradford method (13), and KpNPPase activity was used to determine the purity of the sarcolemmal vesicle preparation (22). Glucose transport into the sarcolemmal vesicles was determined under equilibrium-exchange conditions, as adapted from Ludvigsen and Jarrett (33) as initially reported by Grimditch et al. (22). The tracers, D-[^{3}H]glucose and L-[^{14}C]glucose, with specific activities of 33.1 and 47.0 mCi/mmol, respectively, were purchased from Dupont-New England Nuclear. Transport was measured for 15 s at 37°C at 180 μM D- and L-glucose. Radioactivity was measured using a dual-isotope setting in a Beckman LS-7500 liquid scintillation counter.

Blood chemistry. After an overnight fast, the rats were anesthetized with 10% chloral hydrate (250 mg/kg ip; University of California, Los Angeles, Pharmacy) and blood was obtained via cardiac puncture. Blood samples were centrifuged, and the plasma was frozen at −70°C. Insulin was quantified in the plasma using a double-antibody RIA with materials obtained from Ventrex Laboratories. Plasma glycerol and triglycerides were determined using a kit purchased from Sigma Chemical.

Blood pressure. Blood pressure was measured by the tail cuff method using an IITC Sensor blood pressure system, which has been shown to give readings similar to direct arterial measurement. Initially, the rats were placed in a constant-temperature (29°C) chamber for at least 15 min on 2–3 separate days to acclimate them to the chamber environment. Subsequently, several recordings were made while the animals were quietly resting. During the initial experiments, blood pressure was measured on 2 days, and, if recordings were not similar, then measurements were taken on a 3rd day. The daily means from five to six viable measurements were calculated to obtain a value for each rat.

Body weight and body fat. The rats were weighed initially and at weekly intervals throughout the study. Total body fat was determined by hydrostatic weighing of the carcass according to Rathbun and Pace (38) and as described previously (2, 23). Omental fat samples were taken, rinsed in 0.85% NaCl, and placed in 10% phosphate-buffered Formalin. The samples were then subjected to dehydration, filtration, and embedding in paraffin, as described by Sheehan and Hrapchak (43). Sections were sliced at a thickness of 4 μm at three different depths, at least 200 μm apart, within the tissue sample. The slides were then observed under the microscope, and video prints were taken using a Codonics VP-3500 video printer attached to a Perspective Systems image-analysis system on an Olympus BH2 microscope. By use of the video images, the number of adipocytes in a prescribed area was counted, and the number was used to calculate mean cell number and mean cell volume, as described by Lemonnier (32) and Ashwell et al. (1).

Statistical analysis. Data were analyzed using a Student's *t*-test or an ANOVA. When significant *F* values were noted, post hoc analyses were performed using a repeated-measures *t*-test. Differences were considered statistically significant at $P < 0.05$. Values are means ± SE. There were 8–10 rats per group for each study except body weight, where each group contained 16 rats.

RESULTS

Glucose transport. Insulin-stimulated glucose transport was measured at 2 wk, 2 mo, and 2 yr. The sarcolemmal vesicle insulin-stimulated D-glucose transport for the HFS rats was significantly decreased compared with the LFCC group at 2 wk (82 ± 5 vs. 98 ± 6 pmol·mg^{-1}·15 s^{-1}), 2 mo (69 ± 4 vs. 88 ± 6 pmol·mg^{-1}·15 s^{-1}), and 2 yr (53 ± 4 vs. 69 ± 4 pmol·mg^{-1}·15 s^{-1}) on the diet (Fig. 1). The sarcolemmal protein yields per gram of muscle were 0.38 ± 0.03, 0.42 ± 0.04, and 0.30 ± 0.03 for the LFCC groups and 0.48 ± 0.05, 0.39 ± 0.04, and 0.31 ± 0.04 for the HFS groups at 2 wk, 2 mo, and 2 yr, respectively. The KpNPPase activities (μmol·mg^{-1}·h^{-1}) were 3.45 ± 0.19, 3.86 ± 0.18, and 4.10 ± 0.21 for the LFCC groups and 3.87 ± 0.22, 3.10 ± 0.16, and 3.67 ± 0.23 for the HFS groups at 2 wk, 2 mo, and 2 yr, respectively. There were no significant differences in protein yield or KpNPPase activities

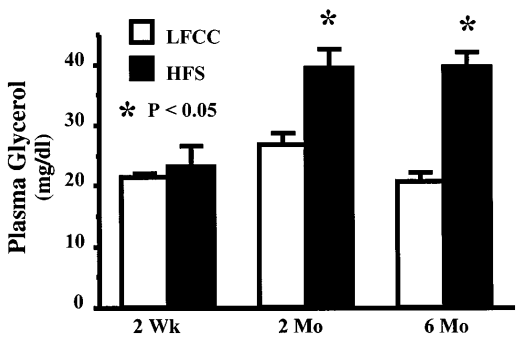

Fig. 3. Effect of diet on plasma glycerol. Fasting plasma glycerol levels were significantly elevated in HFS group at 2 and 6 mo compared with LFCC group ($P < 0.05$). Values are means ± SE.

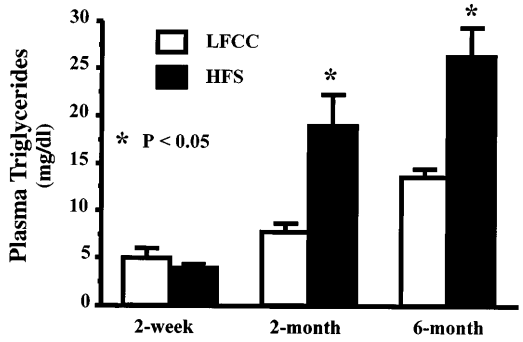

Fig. 4. Effect of diet on plasma triglycerides. Fasting plasma triglycerides were significantly elevated in HFS group at 2 and 6 mo compared with LFCC group ($P < 0.05$). Values are means ± SE.

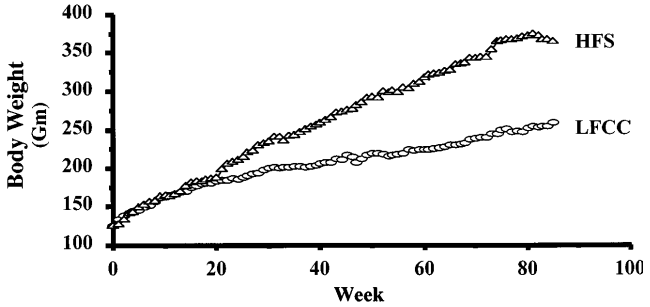

Fig. 6. Effect of diet on body weight over 85 wk. Weights were the same during rapid growth phase, but after 21 wk HFS group gained significantly more weight. Values are means from 16 rats in each group.

between or within the groups, which demonstrates that differences in glucose transport values between the groups cannot be attributed to differences in the protein yield or purity of the sarcolemmal preparations.

Fasting plasma insulin, glycerol, and triglycerides. The plasma insulin levels of both groups of rats were measured at 2 wk, 2 mo, 6 mo, and 2 yr on the diet. Consistent with the decrease in insulin-stimulated glucose transport in the HFS rats, the plasma insulin levels were significantly elevated in the HFS compared with the LFCC group at all time points (Fig. 2). In addition, the HFS insulin levels were significantly higher at 2 mo, 6 mo, and 2 yr than at 2 wk.

Plasma glycerol in the HFS group was not significantly different at 2 wk but was significantly elevated at 2 and 6 mo (Fig. 3). Plasma triglycerides in the HFS group were not significantly elevated at 2 wk but were significantly higher at 2 and 6 mo (Fig. 4).

Blood pressure. Blood pressure was measured in each group at 2, 6, 12, and 18 mo. The development of hypertension was tracked as shown in Fig. 5. Blood pressure was not significantly different at 2 or 6 mo. Blood pressure was significantly elevated in the HFS group at 12 and 18 mo, and at 18 mo all animals fed the HFS diet were hypertensive (>140 mmHg).

Body weight and body fat. Figure 6 shows the body weight changes over 85 wk. There was no significant difference in body weight during the rapid growth phase, but after 21 wk the HFS group gained more weight than the LFCC group. Whole body fat, as assessed by hydrostatic weighing, in the LFCC rats was not different from that in the HFS rats at 2 mo (18.0 ± 0.8 vs. 19.2 ± 0.8%) but was significantly elevated at 6 mo in the HFS rats (17.6 ± 0.6 vs. 22.4 ± 1.5%). Histological studies of the abdominal fat cells showed no differences at 2 wk, but the fat cells of the HFS rats were larger at 2 mo and further increased in size by 6 mo compared with the LFCC rats (Fig. 7).

DISCUSSION

The results of this study clearly indicate that insulin resistance/hyperinsulinemia precedes the other characteristics of the metabolic syndrome. Furthermore, the data demonstrate that obesity is not the cause of insulin resistance/hyperinsulinemia, rather an HFS diet is the true underlying factor. Recent studies by Barnard et al. (2–4) have documented that diet appears to be a major factor in the metabolic syndrome and have shown that the syndrome can be induced in rats by feeding an HFS diet similar to the typical US diet and can be controlled in humans by feeding an LFCC diet combined with aerobic exercise.

Within 2 wk of being placed on the HFS diet, the animals were hyperinsulinemic and demonstrated skeletal muscle insulin resistance, as indicated by a reduction in maximum insulin-stimulated glucose transport. Many others have reported that diets high in fat and/or refined sugar cause insulin resistance, as reviewed earlier (5). Many studies (15, 21, 23, 45, 49) have reported insulin resistance using a high-fat and/or -sucrose diet for as little as 3 wk to 2 mo. Exactly how the diet induces insulin resistance is not known, but defects

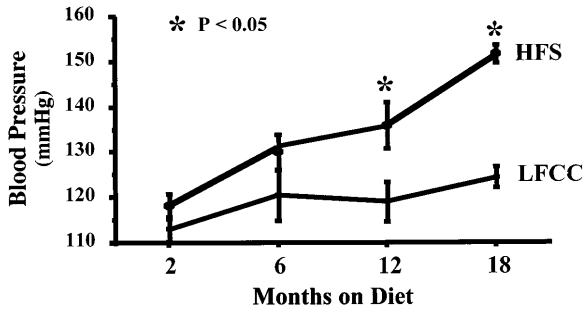

Fig. 5. Effect of diet on systolic blood pressure. Blood pressure values were significantly elevated at 12 and 18 mo in HFS group compared with LFCC group ($P < 0.05$). Values are means ± SE.

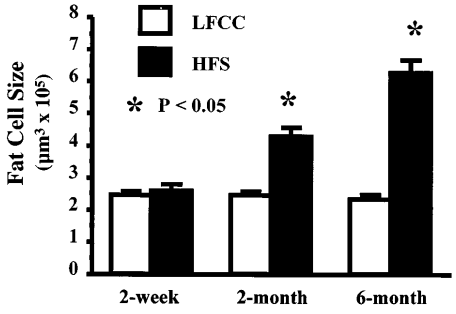

Fig. 7. Effect of diet on fat cell size. Fat cell volume was significantly elevated in HFS group at 2 and 6 mo compared with LFCC group ($P < 0.05$). Values are means ± SE.

in the insulin-signaling pathway have been reported (50). We previously reported that, of the two aspects of the HFS diet, refined sugar caused a greater problem than the high fat content during glucose tolerance tests (21). However, the combination of high fat and high refined sugar resulted in the worse response.

Although insulin resistance/hyperinsulinemia had developed in 2 wk, there was no change in plasma triglycerides, plasma glycerol, or blood pressure, and there was no indication of obesity or adipocyte hypertrophy. Many reports have suggested that obesity is the underlying cause of insulin resistance and the metabolic syndrome and is a result of free fatty acid release into the portal circulation (10, 11). However, this was apparently not the case in our rats, inasmuch as the abdominal fat cells were not enlarged, and plasma glycerol, an indicator of free fatty acid release, was not elevated at 2 wk. The fact that glycerol was not elevated at 2 wk is not surprising, since hyperinsulinemia has been reported to suppress adipocyte hormone-sensitive lipase and the release of free fatty acids (46, 47). In addition, studies in humans have also indicated that insulin resistance precedes the other aspects of the metabolic syndrome (26, 34).

Abdominal obesity, however, may still be a factor in the development of the metabolic syndrome. The fact that hyperinsulinemia preceded obesity suggests that it may be involved in the development of obesity, as we proposed earlier (4). Hyperinsulinemia has been shown to downregulate skeletal muscle lipoprotein lipase and upregulate adipose tissue lipoprotein lipase (4). This would decrease the ability of muscle to take up fat from chylomicrons and would force it into fat cells, leading to obesity. In our previous 2-yr study, the HFS rats developed massive obesity, 38% of their body weight as fat compared with 15% for the LFCC diet group (2). By visual inspection it was determined that a large part of the fat in the HFS rats was located in the abdominal cavity, as was the case in the present study. There was no difference in fat cell size at 2 wk when insulin was elevated in the HFS group. At 2 mo, 6 mo, and 2 yr, plasma insulin was further increased above that seen at 2 wk, and abdominal fat cells were also enlarged. In addition, at 2 and 6 mo, plasma glycerol was elevated, indicating fatty acid release from adipocytes. Thus the abdominal fat cell enlargement at 2 and 6 mo may have contributed to the more severe hyperinsulinemia.

Fasting triglycerides, although very low in this animal model, were significantly elevated at 2 and 6 mo in the HFS rats. We previously reported a significant elevation at 2 yr also (2). The fact that triglycerides were elevated in the HFS rats is not surprising. The high fat content of the diet (40% of energy) combined with the hyperinsulinemia resulting from the high sugar content (40% of energy) would increase the production of apoprotein B_{100} and lead to the hypertriglyceridemia (14, 18).

Many studies have reported a correlation between insulin resistance/hyperinsulinemia and hypertension, as reviewed earlier (9). Although several mechanisms have been proposed to explain the relationship, the precise mechanism(s) remains unknown. Nitric oxide, a vasodilator, is produced by vascular endothelial cells and diffuses to the smooth muscle cells to cause relaxation and vasodilation. Nitric oxide also plays a role in renal hemodynamics and sodium handling (17, 41). Insulin is a well-recognized vasodilator, and its mechanism of action appears to be via the production of nitric oxide (7). Furthermore, it recently has been demonstrated that insulin-resistant individuals demonstrate endothelial dysfunction (44). In our study, blood pressure was the slowest responder to the diet. A significant increase in pressure was not observed until 12 mo on the diet, and hypertension (>140 mmHg) was not present in all HFS animals until 18 mo. If insulin resistance is associated with a decrease in nitric oxide production, as suggested by Baron (7), our studies indicate that an HFS diet is also the underlying cause of the hypertension seen in this syndrome. We recently showed that the HFS diet decreases nitric oxide metabolite excretion (C. K. Roberts and R. J. Barnard, unpublished observations), which may explain the hypertension seen in this syndrome. The prolonged time required for the development of hypertension in our female rats may be due to the protective effect of estrogen, which is known to stimulate the production of nitric oxide (40).

In summary, the results demonstrate that an HFS diet is the underlying factor responsible for the metabolic syndrome. Within 2 wk on the HFS diet, skeletal muscle insulin resistance and plasma hyperinsulinemia develop, and subsequently the rats became hypertriglyceridemic, hypertensive, and obese.

This study was supported by National Institute on Aging Grant AG-07592.

Address for reprint requests: R. J. Barnard, Dept. of Physiological Science, UCLA, PO 951527, Los Angeles, CA 90095-1527.

Received 17 September 1997; accepted in final form 26 November 1997.

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