The high-fat-fed lean Zucker rat: a spontaneous isocaloric model of fat-induced insulin resistance associated with muscle GSK-3 overactivity

Erik J. Henriksen, Mary K. Teachey, Katherine A. Lindborg, Cody J. Diehl, and Alan N. Beneze

Muscle Metabolism Laboratory, Department of Physiology, University of Arizona College of Medicine, Tucson, Arizona

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Henriksen EJ, Teachey MK, Lindborg KA, Diehl CJ, Beneze AN. The high-fat-fed lean Zucker rat: a spontaneous isocaloric model of fat-induced insulin resistance associated with muscle GSK-3 overactivity. Am J Physiol Regul Integr Comp Physiol 294: R1813–R1821, 2008. First published April 2, 2008; doi:10.1152/ajpregu.00178.2008.—High-fat feeding (HFF) is a well-accepted model for nutritionally-induced insulin resistance. The purpose of this investigation was to assess the metabolic responses of female lean Zucker rats provided regular chow (4% fat) or a high-fat chow (50% fat) for 15 wk. HFF rats spontaneously adjusted food intake so that daily caloric intake matched that of chow-fed (CF) controls. HFF animals consumed more (p < 0.05) calories from fat (31.9 ± 1.2 vs. 24 ± 0.2 kcal/day) and had significantly greater final body weights (280 ± 10 vs. 250 ± 5 g) and total visceral fat (24 ± 3 vs. 10 ± 1 g). Fasting plasma insulin was 2.3-fold elevated in HFF rats. Glucose tolerance (58%) and whole body insulin sensitivity (75%) were markedly impaired in HFF animals. In HFF plantaris muscle, in vivo insulin receptor β-subunit (IR-β) and insulin receptor substrate-1 (IRS-1) tyrosine phosphorylation and phosphorylation of Akt Ser473 and glycogen synthase kinase-3β (GSK-3β) Ser9, relative to circulating insulin levels, were decreased by 40–59%. In vitro insulin-stimulated glucose transport in HFF soleus was decreased by 54%, as were IRS-1 tyrosine phosphorylation (26%) and phosphorylation of Akt Ser473 (38%) and GSK-3β Ser9 (25%), the latter indicative of GSK-3 overactivity. GSK-3 inhibition in HFF soleus using CT98014 increased insulin-stimulated glucose transport (28%), IRS-1 tyrosine phosphorylation (28%) and phosphorylation of Akt Ser473 (38%) and GSK-3β Ser9 (48%). In summary, the female lean Zucker rat fed a high-fat diet represents an isocaloric model of nutritionally-induced insulin resistance associated with moderate visceral fat gain, hyperinsulinemia, and impairments of skeletal muscle insulin-signaling functionality, including GSK-3β overactivity.

DEFECTS IN THE ABILITY OF insulin to effectively engage the elements of the insulin-signaling pathway leading to activation of the glucose transport system in skeletal muscle, termed insulin resistance, is a primary dysfunction leading to the development of type 2 diabetes (reviewed recently in Ref. 18). Insulin resistance is one of several cardiometabolic dysfunctions, including hypertension, type 2 diabetes, dyslipidemia, atherosclerosis, and central obesity, that contributes to the development of a pathophysiological state known variously as the “insulin resistance syndrome” (5), or the “cardiometabolic syndrome” (17), which represents a condition of markedly increased risk of cardiovascular disease (5, 17). It is therefore critical to more thoroughly understand the etiology of skeletal muscle insulin resistance and to develop interventions that are effective in increasing insulin action on the glucose transport system.

Several rodent models of obesity-associated insulin resistance have been utilized to investigate the etiology of skeletal muscle defects in insulin action over the past decades. These include rat models of monogenic defects leading to hyperphagia and ultimately massive obesity and insulin resistance, such as the obese Zucker rat (18, 29), which expresses a defective leptin receptor gene (22, 34, 41). Compared with the lean Zucker rat, the obese Zucker rat displays marked central obesity, dyslipidemia, insulin resistance of skeletal muscle glucose transport and metabolism, and defective insulin signaling (1, 18, 29). Rats undergoing high-fat feeding (HFF) (~50% of calories derived from fat for more than 3 wk) display marked metabolic dysfunctions (14, 16, 25), including large increases in body weight gain. However, only a limited number of investigations have pursued a long-term (>3 wk) HFF regimen in lean Zucker rats (24, 27, 28, 30). These previous HFF studies utilized male lean Zucker rats, and none of these investigations have adequately addressed the potential metabolic consequences of a long-term high-fat diet in the lean Zucker rats, including an assessment of skeletal muscle insulin signaling.

A distal element of the insulin-signaling pathway that has received much attention recently is a potential negative modulator of tissue insulin action and as a site of intervention in insulin-resistant states is the serine/threonine kinase glycogen synthase kinase-3 (GSK-3) (see reviews in refs. 3, 9, 18, 42). GSK-3 exists in tissues as highly homologous α- and β-isoforms (43), and plays a role in the regulation of a diverse array of cellular functions, including glycogen synthesis, protein synthesis, gene transcription, and cell differentiation (3, 18, 38). Importantly, GSK-3 has been shown to directly phosphorylate IRS-1 on serine residues and impair insulin signaling (10, 26), and GSK-3 overactivity is associated with insulin resistance in the HFF mouse (11), in the female obese Zucker rat (8), in the male Zucker diabetic fatty rat (21), and in obese type 2 diabetic humans (33). Moreover, selective inhibition of GSK-3 leads to enhanced insulin action at the whole body level in insulin-resistant rodents (2, 7, 20, 23, 35–37) and in isolated skeletal muscle tissue or cells from insulin-resistant rats (8, 20, 21, 37) or humans (32). However, the potential role of GSK-3 as a contributor to an insulin-resistant state induced by HFF in lean Zucker rats has not yet been investigated.

In the context of the foregoing information, a primary purpose of the present investigation was to investigate the potential negative impact of chronic HFF on whole body and
skeletal muscle glucose disposal in female lean Zucker rats, including an assessment of both proximal and distal elements of the insulin-signaling pathway. An associated purpose of this study was to evaluate, utilizing a selective GSK-3 inhibitor, the specific contribution of GSK-3 to the etiology of insulin resistance of skeletal muscle glucose transport in HFF female lean Zucker rats.

METHODS

Animals and treatments. Female lean Zucker (Fz/–) rats were obtained at 4–5 wk of age, and 1 wk later were assigned to either a regular chow diet (model 7001 mouse/rat diet with 4% of calories derived from fat; Teklad, Madison, WI) or a high-fat diet (modified model AIN-76A with 32% lard and 18% corn oil representing 50% of calories derived from fat; Dyets, Bethlehem, PA). The rats were allowed ad libitum access to Chow and water throughout the period of the study, except as described below. All animals were housed in a temperature-controlled room (20°–22°C) with a 12:12-h light-dark cycle at the Central Animal Facility of the University of Arizona. Food consumed by the animals and body weight were determined every other day and calories consumed were calculated from the amount of food consumed and the caloric content of the respective diets (regular chow, 3.88 kcal/g; high-fat diet, 4.66 kcal/g). All procedures were approved by the University of Arizona Animal Care and Use Committee.

Assessment of glucose tolerance and plasma variables. At the end of the 15-wk treatment period, animals were food restricted overnight (chow was restricted to 4 g at 5 PM and was consumed immediately) and subjected to an oral glucose tolerance test (OGTT) using a 1 g/kg-glucose feeding by gavage. Blood (~0.25 ml) was collected from a cut at the end of the tail immediately before and at 15, 30, 60, and 120 min after glucose administration. Whole blood was then thoroughly vortexed with EDTA (18 mM final concentration) and centrifuged at 13,000 g for 30 s to isolate the plasma. The plasma was then removed and stored at −80°C until analysis. Following the blood collection, animals were administered 2.5 ml of 0.9% saline solution subcutaneously to compensate for plasma loss during the OGTT, and animals were again food restricted overnight (4 g chow at 5 PM). At 8 AM, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip; Nembutal, Abbott Laboratories, North Chicago, IL). Tissues collected initially included soleus muscle, plantaris muscle, a piece of omental fat, and the lateral lobe of the liver. Except for soleus muscle used for in vitro treatments (see below), all tissues were immediately frozen between aluminum blocks cooled to the temperature of liquid nitrogen and stored at −80°C until analysis. Following dissection of these tissues, the total amount of visible fat in the abdominal cavity was measured and used.

Two strips (25–35 mg) from one isolated soleus muscle were prepared for in vitro incubation in the unmounted state without tension. Glucose transport activity, measured as 2-deoxyglucose uptake, was then assessed in the absence or presence of a maximally effective concentration of insulin (5 mU/ml Humulin R; Eli Lilly, Indianapolis, IN) exactly as described previously (19). This method for assessing glucose transport activity in isolated muscle has been validated (15). In some incubation experiments, the selective GSK-3 inhibitor CT98014 (kindly provided by Chiron, Emeryville, CA) was used.

Measurement of signaling factors. In addition, soleus strips were incubated in the absence or presence of insulin as above and then frozen, weighed, and stored at −80°C for the subsequent assessment of insulin-signaling factors. Tyrosine phosphorylation of IR-β and IRS-1, as well as phospho-Akt1/2 Ser473, phospho-GSK-3α/β Ser21/9, phospho-p38 MAPK Thr180/Tyr182, and phospho-c-jun NH2-terminal kinase (JNK1/2) Thr183/Tyr185 were determined in homogenates of muscle and fat exactly as described previously (8, 21). The level of the phosphorylated signaling element was expressed relative to the total amount of that protein from the same sample.

Assessment of tissue triglyceride concentrations. Triglyceride concentrations were determined in the plantaris muscle and liver tissue using the extraction method of Folch et al. (12) and the processing method of Frayn and Maycock (13), as modified by Denton and Randle (6). Pieces of tissue (30–40 mg) were homogenized in extraction buffer (20:10.3 of chloroform/methanol/butylated hydroxytoluene) and incubated at 4°C for 16 h. Separation of phases was obtained after addition of 0.9% saline, with centrifugation at 3,000 g for 60 min. The lower (organic) phase was evaporated to dryness under N2 gas at 60°C for 60 to 90 min. The sample was reconstituted in extraction buffer, and triglyceride concentration was determined spectrophotometrically using an enzymatic colorimetric assay (Sigma, St. Louis, MO).

Statistical analysis. All values are expressed as means ± SE. Unpaired Student t-tests were used to determine statistical differences between the CF and HFF groups. The effect of the GSK-3 inhibitor CT98014 was determined using a paired Student’s t-test. A level of \( P < 0.05 \) was set for statistical significance.

RESULTS

Over the final 4 wk of the 15-wk intervention period, the female lean Zucker rats in the HFF group adjusted their food intake, such that total caloric intake over this period was nearly identical to that of the CF group (Fig. 1). However, the calories derived from fat consumed by the HFF animals over this final 4-wk period were more than 12-fold greater (\( P < 0.05 \)) than those consumed by the CF group. This isocaloric, high-fat dietary intervention had a significant impact on body weight gain over the 15-wk intervention period. Whereas the initial body weights in these groups were not different, the final average body weight of the HFF group was 30 g greater than in the CF group. Importantly, the amount of total intra-abdominal fat was more than twofold greater in the HFF group compared with the CF group, whether expressed in absolute terms (Fig. 1) or relative to body weight (8.8 ± 0.7 vs. 4.0 ± 0.4%, \( P < 0.05 \)).

The HFF intervention did not induce a significant increase in fasting plasma glucose (Fig. 2). In contrast, fasting plasma insulin was more than twofold greater (\( P < 0.05 \)) in the HFF group compared with the CF group. The homeostasis model assessment of insulin resistance, an index of fasting insulin resistance (31), was 153% greater in the HFF group than in the CF group. The HFF intervention induced a moderate 20% increase in fasting plasma FFA levels and did not affect the triglyceride concentrations in either skeletal muscle or liver (Fig. 3).

Whole body glucose tolerance and insulin sensitivity were assessed during an OGTT (Fig. 4). The responses for glucose (Fig. 4A) and insulin (Fig. 4B) to the glucose load were significantly (\( P < 0.05 \)) elevated at all time points during the 2-h test. The total integrated areas under the curve for glucose (glucose AUC; Fig. 4C) was 59% greater in the HFF group relative to the CF group, while the insulin AUC was 32% greater. The
The glucose-insulin index is defined as the product of the glucose AUC and the insulin AUC and is inversely related to whole body insulin sensitivity under a glucose load (4) and is shown in Fig. 4E. Compared with the CF group, the glucose-insulin index was fourfold greater in the HFF group, indicating that this dietary intervention in the lean Zucker rat significantly impaired whole body insulin sensitivity.

The in vivo protein expression and functionality of several critical elements of the insulin-signaling pathway in skeletal muscle and abdominal fat from the CF and HFF groups were determined (Fig. 5). In both plantaris muscle and omental fat, the HFF intervention did not alter the protein expression of the IR-β subunit, IRS-1, Akt1/2, or GSK-3α or GSK-3β relative to the CF groups (data not shown). The absolute level of the phosphorylation state of these insulin-signaling elements in muscle and fat in vivo did not differ significantly between the CF and HFF groups (data not shown). However, when expressed relative to the prevailing plasma insulin concentration, there were significant reductions in tyrosine phosphorylation of IR-β (40%) and IRS-1 (59%) and of Akt Ser473 phosphorylation (47%), and phosphorylation of GSK-3α on Ser21 (48%) and GSK-3β on Ser9 (53%) were all significantly reduced compared with the CF group when expressed relative to the prevailing plasma insulin concentration. Protein expression of GLUT-4 was not altered in muscle of the HFF animals nor was the absolute protein expression or the phosphorylation status of stress-activated kinases p38 MAPK or JNK (data not shown).

The direct effects of insulin to enhance glucose transport activity and alter the functional status of insulin-signaling elements were investigated in isolated soleus muscle preparations from CF and HFF lean Zucker rats (Figs. 6 and 7). Whereas basal glucose transport activity was not different between CF and HFF groups, insulin-stimulated glucose transport activity in the HFF soleus was 35% less \((P < 0.05)\) than in the CF soleus (Fig. 6), and the increase above basal due to insulin (Fig. 6, inset) was 54% less in the HFF soleus compared with the CF soleus. Interestingly, insulin-stimulated IR-β tyrosine phosphorylation in the HFF soleus was reduced 40% compared with the CF soleus. Phosphorylation of GSK-3α on Ser21 was undetectable in skeletal muscle, as we have reported previously (7, 8, 21).

**Fig. 1.** Effect of chronic high-fat feeding (HFF) on total caloric intake, consumed calories derived from fat, body weight, and total abdominal fat in female lean Zucker rats. Caloric intake and consumed calories derived from fat were determined over the final 4 wk of the 15-wk intervention period in chow-fed (CF) and HFF lean Zucker rats. In addition, initial and final body weights were determined, as well as the amount of abdominal fat at the end of the study. Values are means \(\pm\) SE for 8 animals per group. \(*P < 0.05\), HFF group vs. CF group.

**Fig. 2.** Effects of HFF on fasting plasma variables and insulin sensitivity in female lean Zucker rats. Plasma values for glucose, insulin, and free fatty acids (FFA) were determined after an overnight fast. Fasting whole body insulin sensitivity was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) by using the formula: [fasting plasma glucose (mg/dl) \(\times\) fasting plasma insulin (\(\mu\)U/ml)]/405 (31). Values are means \(\pm\) SE for 8 animals per group. \(*P < 0.05\), HFF group vs. CF group.
rosine phosphorylation did not differ between CF and HFF soleus (Fig. 7). In contrast, insulin-stimulated IRS-1 tyrosine phosphorylation (26%), Akt Ser473 phosphorylation (38%), and GSK-3β Ser9 phosphorylation (25%) were all significantly less than the CF group. The reduced insulin-stimulated GSK-3 phosphorylation indicates an enhanced GSK-3 activity in the isolated HFF soleus.

As both the in vivo and in vitro results in skeletal muscle indicated that the HFF induced a state of GSK-3 overactivity, we investigated the utility of selective inhibition of GSK-3 using CT98014 on insulin action in isolated soleus muscle from the HFF animals. As shown in Fig. 8, treatment of control CF muscle with this selective GSK-3 inhibitor, either in the absence or the presence of insulin, had no effect on glucose transport activity, in agreement with our previous findings in insulin-sensitive muscle from lean Zucker rats (8, 20). However, whereas the GSK-3 inhibitor did not alter basal glucose transport activity in HFF soleus, the CT98014 did significantly increase insulin-stimulated glucose transport activity by 28% (P < 0.05). This effect of the GSK-3 inhibitor on insulin-stimulated glucose transport activity in the HFF soleus was associated with similar enhancements of IRS-1 tyrosine phosphorylation (28%) and phosphorylation of Akt Ser473 (38%) and GSK-3β Ser9 (48%). In addition, the phosphorylation of IRS-1 on Ser307 tended to be lower (24%, P = 0.10) in insulin-stimulated HFF soleus incubated with the GSK-3 inhibitor (data not shown).

**DISCUSSION**

In the present investigation, we have for the first time provided an evaluation of both the systemic and local adaptive responses of the female lean Zucker rat to a chronic HFF regimen (15 wk on a 50% fat diet), including an assessment of the functionality of critical insulin-signaling elements in muscle and fat. An interesting initial outcome of this study was our observation that the lean Zucker rat spontaneously reduced the intake of the high-fat diet, so that the absolute caloric consumption (in kcal/day) of the HFF and the CF groups was essentially identical (Fig. 1). This observation is consistent with the findings of a previous study using male lean Zucker rats, in which a less pronounced high-fat diet (20% of calories derived from fat) was provided over 30 wk (30). However, it is clear that the proportion of consumed calories derived from fat was markedly higher in the HFF group (Fig. 1) and the HFF diet elicited significantly greater final body weights and total abdominal fat (Fig. 1) compared with the CF group.

After 15 wk on the high-fat diet, the HFF animals, compared with the CF group, displayed numerous characteristics of the cardiometabolic syndrome (17), including increased visceral...
fat (Fig. 1), marked hyperinsulinemia (Fig. 2), systemic insulin resistance in both the fasting state (Fig. 2) and following a glucose load (Fig. 4), glucose intolerance (Fig. 4), and marginal dyslipidemia, as reflected by a 20% increase in plasma FFA (Fig. 2). Interestingly, the marginal dyslipidemia is consistent with our observation that tissue triglycerides were not elevated in the liver or muscle of the HFF animals (Fig. 3). Overall, the HFF female lean Zucker rat represents a very useful rodent model with which to investigate the etiology of, and relevant interventions for the treatment of, this clustering.

Fig. 5. Effects of HFF by on in vivo insulin signaling in skeletal muscle and abdominal fat in female lean Zucker rats. The functionality of the indicated insulin-signaling factors, expressed relative to the circulating insulin concentration, was assessed in plantaris muscle (A) and abdominal fat (B) obtained after an overnight fast. IR-β, insulin receptor β-subunit; IRS-1, insulin receptor substrate-1; GSK-3, glycogen synthase kinase-3. Values are means ± SE for 8 animals per group. *P < 0.05, HFF group vs. CF group.
of atherogenic risk factors. Whereas the female obese Zucker rat, the dysfunctional counterpart of the lean Zucker rat that expresses a defective leptin receptor (22, 34, 41), has provided numerous important insights into the mechanisms underlying obesity-associated insulin resistance at the systems and cellular levels (18), it is admittedly a model of extreme obesity (~65% body fat) (40) and tissue lipid accumulation (39), and its derangements in insulinemia (8- to 10-fold greater) and dyslipidemia (2- to 3-fold greater plasma FFA) (7, 29) are much larger than in the typical insulin-resistant overweight or obese human subject. The HFF female lean Zucker rat may be a more suitable rodent model for investigations translatable to human obesity and insulin resistance.

We have demonstrated in the present investigation that the HFF lean Zucker rat is characterized by impairments of insulin signaling in both muscle and abdominal fat. In vivo insulin signaling, relative to the prevailing fasting plasma insulin concentration, was decreased in the plantaris muscle, as hallmarked by relative decreases in tyrosine phosphorylation of IR-β and IRS-1 and in serine phosphorylation of Akt and GSK-3 (Fig. 5A). Similar decreases in these signaling elements in vivo were observed in omental fat (Fig. 5B). Likewise, in isolated soleus muscle from the HFF animals, insulin action on IRS-1 tyrosine phosphorylation, Akt Ser473 phosphorylation, and GSK-3β Ser9 phosphorylation was significantly reduced (Fig. 7), and these insulin-signaling defects were associated with a diminution of insulin-stimulated glucose transport activity (Fig. 6). It is probable that the central adiposity that characterizes the HFF animal is mechanistically linked with the development of the impaired whole body glucose tolerance and the reduced insulin-dependent skeletal muscle glucose transport capacity documented in these animals, as has been demonstrated in male rat models of HFF (14, 25).

An important novel finding in the present investigation is the significant contribution of defective GSK-3 regulation to the insulin resistance of glucose transport activity in skeletal mus-
cle of the HFF lean Zucker rat. The reduced insulin-stimulated GSK-3 Ser9 phosphorylation in soleus muscle of the HFF rat (Fig. 7) allows GSK-3 to retain a more active form, and overactivity of GSK-3 in skeletal muscle can reduce the functionality of critical upstream insulin-signaling elements, such as IRS-1 in the obese Zucker rat (8) and IR and IRS-1 in the Zucker diabetic fatty rat (21). We demonstrate in the present study that selective in vitro inhibition of GSK-3 in HFF soleus using CT98014 (see reviews in Refs. 18 and 42) was associated with increased insulin-stimulated glucose transport activity (Fig. 8). IRS-1 tyrosine phosphorylation and phosphorylation of Akt Ser473 and GSK-3 Ser9 (Fig. 9). These data indicate that ~40–50% of the insulin resistance of skeletal muscle glucose transport activity can be attributed to this defective GSK-3 regulation in the HFF animals, consistent with our previous findings related to GSK-3 dysfunction and insulin resistance in the prediabetic obese Zucker rats (7, 8) and the type 2 diabetic Zucker diabetic fatty rat (20, 21, 37).

Previous investigations of chronic HFF in lean Zucker rats have involved only males and have addressed metabolic adaptations to only a limited extent. For example, Matsuda et al. (30) fed male lean Zucker rats a 20% fat diet for 30 wk and reported a greater body weight gain, increased plasma triglycerides, and enhanced plasma thiobarbituric acid-reacting substrates, an index of oxidative stress. Maher et al. (28) fed male lean Zucker rats a 48% fat diet for 6 wk and observed an enhanced body weight gain, but no effects on plasma glucose or insulin. Finally, Loh et al. (27) placed male lean Zucker rats on a 65% fat diet for 8 wk, but did not observe any discernable effects on tissue lipid accumulation. It is clear that results from the present investigation using female lean Zucker rats may differ from these previous investigations because of the gender difference as well as a result of differences in the duration and composition (% fat) of the high-fat diets offered to the animals.

**Perspectives and Significance**

The present investigation has demonstrated that the female lean Zucker rat provided a high-fat diet for 15 wk produces a novel and highly relevant spontaneous isocaloric model of nutritionally induced insulin resistance, characterized by dysregulation of glucose disposal at the systemic and skeletal muscle levels and by marginal dyslipidemia. Moreover, the HFF female lean Zucker rat displays moderate visceral fat gain, hyperinsulinemia, and impairments of skeletal muscle insulin-signaling.
functionality, including GSK-3β overactivity. Importantly, this GSK-3 dysregulation contributes significantly to the defects in insulin action on the glucose transport system documented in skeletal muscle of these animals. Based on these results, the HFF female lean Zucker rat may prove useful as a model of obesity-associated insulin resistance that is reflective of a substantial segment of the human insulin-resistant population with moderate central adiposity.

**GRANT**

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