The Effects of High-Fat Diet on Exercise-Induced Changes in Metabolic Parameters in Zucker *fa/fa* Rats

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The objectives of this study were to document the effects of moderate aerobic exercise on insulin secretion and other metabolic indices in *fa/fa* rats and to determine if a high-fat (HF) diet altered these effects. Six-week-old *fa/fa* and lean Zucker rats were either sedentary or exercised by daily swimming for 4 weeks. Half of the exercised and sedentary rats were fed a diet with 16% fat and 44% carbohydrate, while the control groups were fed a diet with 4.5% fat and 49% carbohydrate. At the end of 4 weeks, caloric intake, weight gain, plasma hormone and nutrient levels, and oral glucose tolerance were measured. The pancreatic islet β-cell function was assessed by measuring glucose-stimulated insulin secretion, glucose phosphorylating activity, and free fatty acid (FFA) oxidation in cultured islets. In *fa/fa* rats fed the control diet, exercise reduced weight gain, caloric intake, and fasting plasma triglyceride (TG) concentrations without affecting fasting glucose and insulin concentrations. HF diet blocked the effects of exercise on weight gain and food intake and worsened insulin resistance of *fa/fa* rats. In vitro, neither exercise nor HF diet alone affected islet β-cell function. However, in combination, exercise and high dietary fat reduced glucokinase sensitivity to glucose and increased islet cell response to mannoheptulose inhibitory actions. We conclude that beneficial effects of moderate exercise on metabolism are not mediated by effects on pancreatic β cells. Diets elevated in fat decrease the beneficial effects of exercise on metabolic indices in vivo.

OBESITY OF BOTH humans and experimental animals is associated with hyperinsulinemia, insulin resistance, hypertriglyceridemia, and impaired glucose tolerance.[1] The causes of fasting hyperinsulinemia are still not clearly understood, but in obese rodents, an increased pancreatic β-cell response to stimulation plays a significant role.[2] Increased parasympathetic nervous system drive to pancreatic β cells is shown to greatly enhance glucose-stimulated insulin secretion (GSIS) in obese animals and may contribute to the early onset of hyperinsulinemia.[3,4]

GSIS from pancreatic islets isolated from genetically obese Zucker (*fa/fa*) rats is elevated and fails to respond appropriately to inhibitors of glucose metabolism, such as starvation and mannoheptulose.[5,6] In normal rats, insulin secretion is acutely inhibited by intense aerobic exercise,[7,8] perhaps due to the increased sympathetic nervous system in the endocrine pancreas. Chronic aerobic exercise (treadmill running) is reported to reduce GSIS in normal rats by inhibiting glucokinase gene expression and glucokinase activity.[9]

In contrast to exercise, high-fat (HF) diets may reduce sympathetic nervous system activity.[10] However, prolonged exposure of islets to free fatty acids (FFA) in vitro reduces insulin secretion at high glucose concentrations, while maintaining basal hypersecretion,[11] a phenomenon also observed in glucose-intolerant animals.[11,12] Long chain acyl CoA esters inhibit glucokinase activity in the liver,[13] and their involvement in the inhibition of acetyl CoA carboxylase in insulin-secreting cells may be due to their action on glucokinase activity.[14] Chronic feeding of a HF diet to normal rodents reduces proinsulin, glucokinase, and GLUT2 mRNA and suppresses GSIS.[15,16]

The interaction between exercise and HF diet in a model of pre-existing obesity has not been examined, particularly with respect to β-cell function. While exercise reduces insulin secretion during exercise, the long-term effects during the inter-exercise period are less well characterized. We hypothesized that swimming exercise would improve glucose tolerance by having a direct, chronic effect on the islet β cells of *fa/fa* rats and that these effects would be negated by feeding the rats a HF diet.

MATERIALS AND METHODS

Animals

Five-week-old Zucker lean (Ln) and *fa/fa* (fa) rats were purchased from Charles River Laboratories (St Constant, QC, Canada) and fed rat chow for 1 week. All animals were housed at a temperature of 22°C to 25°C with a 12-hour dark:light cycle. At 6 weeks of age, the animals were separated and housed individually in plastic cages. Sixteen of the rats of each phenotype were fed rat chow (3.74 kcal/g, 4.5% fat), while the others were fed a diet with moderately elevated fat content (HF, 3.96 kcal/g, 16% fat) ad libitum for 4 weeks. The animals were further subdivided into exercise (EX or HF-EX) or sedentary (SED or HF-SED) groups for both phenotypes. The HF diet (condensed milk diet) was made according to Triscari et al[17] by mixing ground rat chow, Eagle's brand sweetened condensed milk, and Mazola corn oil. Diet composition is shown in Table 1. Animals had access to tap water ad libitum.

Exercise

At 6 to 10 weeks of age, the exercise groups swam daily (Monday to Friday) from 9:00 to 10:00 AM in a 150-cm diameter pool. A water depth of greater than 30 cm prevented the rats from resting at the bottom of the pool. Water and room temperatures were maintained at 34°C to 35°C to eliminate cold-induced stress.[18,19] The protocol started with the animals swimming 20 minutes on the first day, gradually increasing so that after 5 days they could swim for 60 minutes. After swimming, the animals were towel dried and returned to their cages. To ensure that the animals were adapting to the exercise routine without excessive chronic stress, weekly blood samples were collected from the

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Table 1. Diet Composition of the LF (Purina rat chow) and HF (condensed milk) Diet-fed Zucker rats

Components	LF Diet	HF Diet
Crude protein (%)	23.4	14.7
Carbohydrate (%)	49	44.2
Lipid (%)	4.5	15.8
Fiber (%)	5.8	2.5
Vitamin mix/ash (%)	7.3	1.2
Water (%)	10	19

NOTE. Data from Triscari et al.[17]

tail vein between 9:00 to 10:00 AM at 6 (before) and 7 weeks of age (after starting exercise) for measurement of glucose and corticosterone.

Caloric Intake and Weight Gain

Food intake was measured on 3 consecutive days of each week and then averaged to calculate daily food intake and calories consumed. Animals were weighed weekly.

Oral Glucose Tolerance Test

After 4 weeks of exercise and diets, the rats were fasted overnight but had ad libitum access to water. The animals were weighed the next day and a 0.5-mL blood sample was collected in a heparinized tube from the tail vein of conscious rats using minimal gentle restraint (this was referred to as 0 minute). Glucose (40% wt/vol solution) at a dose of 1 g/kg body weight was administered using a gastric feeding tube. Blood samples were collected after 10, 20, 30, 40, and 60 minutes. Plasma was collected by centrifugation and stored frozen. Plasma glucose concentrations were measured by the glucose oxidase method using a Beckman glucose analyzer II (Beckman Instruments, Fullerton CA). Plasma triglyceride (TG) and FFA concentrations were determined spectrophotometrically with commercially available kits (Sigma Diagnostics, St Louis, MO and Boehringer Mannheim, Laval, QC, Canada, respectively). Corticosterone was determined by radioimmunoassay (RIA) following the kit manufacturer's manual (ICN Biomedical Inc, Costa Mesa, CA).

Islet Isolation and Insulin Release

Immediately after the last blood collection, the animals were anesthetized by intraperitoneal (IP) injection with sodium pentobarbital (65 mg/kg body weight). The pancreas was excised after ductal distension with collagenase type XI. The pancreas was dissected out and chopped into small pieces. The islets were isolated by sequential digestion with collagenase and dextran step-density gradient and cultured overnight as previously described.[6] Insulin release was measured by replacing the culture medium with 1.0 mL of fresh Dulbecco's modified Eagle's medium (DME) containing various glucose concentrations (0 to 25 mmol/L) and 0.1% gelatin. To measure the effect of the glucokinase inhibitor mannoheptulose (MH) on GSIS, concentrations of 1 to 100 mmol/L MH were added to some samples incubated in the presence of 16.5 mmol/L glucose. Samples were statically incubated for 90 minutes at 37°C (95% air, 5% CO_2, saturated with water vapor). At the end of 90 minutes, the supernatant was collected by aspiration following centrifugation and stored at -20°C until secreted insulin and islet insulin content were measured as previously described.[5]

Glucose Phosphorylating Activity and Fatty Acid Oxidation in Islets

Isolated pancreatic islets in batches of 20 to 30 were cultured as described above. Glucokinase and hexokinase activities were determined by measuring phosphorylation of [^{14}C] glucose as described.[20] Data were normalized to islet protein (Lowry method, Sigma). Pancreatic islet fatty acid oxidation was measured as described by Chen et al.[21]

Statistical Analysis

Data are expressed as means ± SEM and analyzed using repeated measures analysis of variance (ANOVA) (SAS statistical package; SAS Institute, Cary, NC). Differences between means were assessed using the Student Newman Keuls test. All results were considered significant at $P \leq .05$.

RESULTS

Effects of Exercise With Control Diet

Caloric intake and weight gain. Caloric intake was influenced by phenotype, age ($P < .001$, ANOVA) and exercise ($P < .05$). Caloric intake in *fa/fa* rats was approximately 1.5-fold to 2-fold higher than in lean rats (Fig 1A). At 8 weeks of age, fa-SED rats consumed 30% more kcal/d than fa-EX rats ($P < .05$). Food intake decreased with age in fa-SED, but not in fa-EX rats ($P < .05$) so by 10 weeks of age, fa-SED and fa-EX caloric intake was similar. Caloric intake of lean rats was not affected by exercise (Fig 1A).

Body weight gain was significantly influenced by phenotype

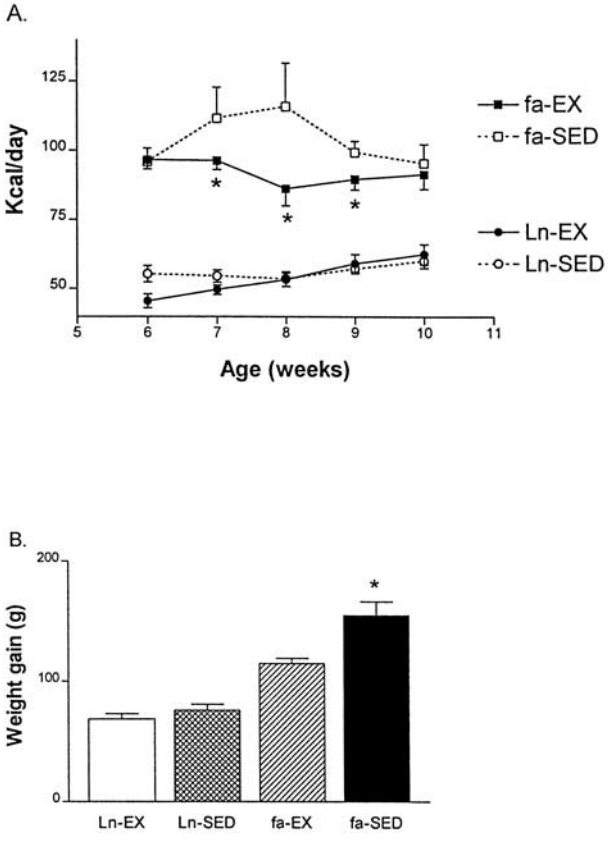


Fig 1. Effects of swimming exercise on (A) food intake in kcal/d and (B) weight gain in lean and fa/fa Zucker rats. *Indicates a significant ($P < .05$) effect of exercise within phenotype. Obese fa/fa rats ate significantly more ($P < .05$ at all time points) and weighed significantly more ($P < .05$) than lean rats, independent of exercise.

Table 2. Plasma Corticosterone and Glucose Concentrations in Zucker Rats Before (6-weeks-old) and During (7-weeks-old) Exercise

	Corticosterone (ng/mL)		Glucose (mmol/L)	
Animals	6 Weeks	7 Weeks	6 Weeks	7 Weeks
Lean rats				
EX	381 ± 82	328 ± 61	7.66 ± 0.23	8.31 ± 0.22
SED	483 ± 72	489 ± 74	7.85 ± 0.27	7.88 ± 0.21
Obese rats				
EX	446 ± 63	252 ± 74	7.17 ± 0.30	7.21 ± 0.33
SED	394 ± 47	479 ± 76	7.41 ± 0.22	7.22 ± 0.37

NOTE. Data are expressed as means ± SEM for 8 animals/group. Rats were fed normal rodent chow.

and age (all $P < .0001$). Exercise reduced weight gain in *fa/fa* rats by 26% compared with fa-SED rats (Fig 1B, $P < .05$), which was similar to the decrease in caloric intake. In lean rats, exercise had no effect on weight gain (Fig 1B).

Effects of exercise on stress indicators. Ambient plasma corticosterone and glucose levels were measured before (age 6 weeks) and after (age 7 weeks) beginning the exercise regimen. Exercise did not affect plasma corticosterone or glucose (Table 2). Fasting plasma corticosterone levels measured at the completion of the study (10 weeks) were uniformly higher than the nonfasting values reported in Table 2, but there were no effects of phenotype or exercise (data not shown).

Effects of exercise on plasma lipid concentrations. Fasting plasma TG concentrations (Table 3) were significantly affected by phenotype ($P < .0001$) and exercise ($P < .05$). Sedentary *fa/fa* rats had approximately 17-fold higher plasma TG concentrations than did lean sedentary rats. In *fa/fa* rats, exercise reduced TG concentrations by 49% ($P < .05$). In lean rats, exercise did not affect plasma TG concentrations. The fasted FFA concentrations of *fa/fa* rats were more than 2-fold higher than those in the lean group independent of exercise ($P < .05$) (Table 3).

Oral glucose tolerance. Fasting glucose concentrations were not different among *fa/fa* or lean rats (Table 3). Plasma glucose concentrations after an oral glucose load were negatively influenced by exercise only in lean rats (Fig 2, $P < .0001$). Fasting plasma insulin concentrations were approximately 5-fold higher in *fa/fa* rats than in lean rats and were not affected by exercise (Table 3). The integrated insulin response to oral glucose was significantly influenced by phenotype ($P < .05$). Exercise did not have any effect on the integrated insulin response in *fa/fa* rats, but in lean rats, exercise lowered the integrated insulin response by 70% (Fig 2, $P < .05$).

Table 3. Effects of Exercise on Fasting Plasma Corticosterone, Glucose, Insulin, TG, and FFA Concentrations in Zucker Rats at Age 10 Weeks

Animals	Insulin (pmol/L)	Glucose (mmol/L)	TG (mg/dL)	FFA (mmol/L)
Lean rats				
EX	86 ± 13*	7.24 ± 0.32	1.45 ± 0.38*	0.51 ± 0.15*
SED	128 ± 38*	7.49 ± 0.42	2.22 ± 0.98*	0.30 ± 0.09*
Obese rats				
EX	513 ± 149	7.77 ± 0.51	20.83 ± 3.11†	1.30 ± 0.23
SED	683 ± 139	8.60 ± 0.37	39.05 ± 5.15	1.28 ± 0.24

NOTE. Data are expressed as means ± SEM; N = 8 animals in each group.

*$P < .05$ lean compared with *fa/fa* rats.

†$P < .05$ exercise compared with sedentary rats.

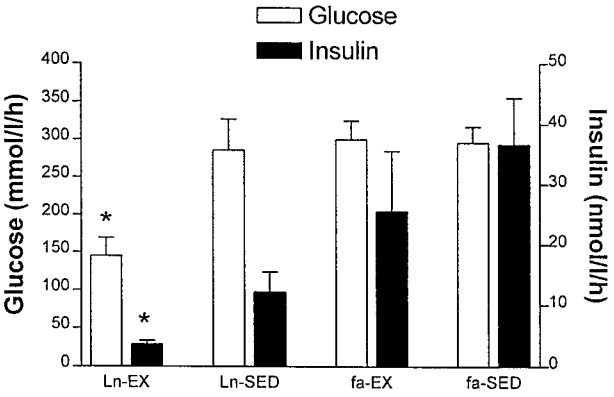


Fig 2. Effects of swimming exercise on oral glucose tolerance. The AUC of both glucose and insulin responses to oral glucose are given. *Indicates a significant ($P < .05$) effect of exercise within phenotype.

Isolated pancreatic islet studies. Overall, islet insulin content in *fa/fa* rats was greater than 2-fold that of lean rats ($P < .005$), but exercise had no effect (Table 4). Exercise did not alter islet glucose sensitivity (EC_{50}) (Table 4), nor were either basal or maximal insulin secretion levels changed. However, the ability of an intermediate concentration of glucose (11 mmol/L) to stimulate insulin secretion was depressed in fa-EX rat islets (Fig 3). The EC_{50} of *fa/fa* rat islets tended to be lower than that of lean rat islets, but this did not achieve statistical significance.

Glucose (16.5 mmol/L)-stimulated insulin secretion from isolated islets of lean and *fa/fa* rats was measured in the absence or presence of MH (30 mmol/L). The MH response was significantly influenced by phenotype and MH concentration (both $P < .0001$). Insulin secretion in response to 16.5 mmol/L glucose in *fa/fa* islets was not inhibited by MH. In all lean rat islet groups, as expected, MH inhibited GSIS ($P < .05$) (Fig 4).

Exercise lowered glucose phosphorylation at low glucose concentrations in *fa/fa* rats ($P < .05$) (Fig 5A). In lean rats, total

Table 4. Effect of Exercise on β-Cell Sensitivity to Glucose (EC_{50}) and Islet Insulin Content

Animals	N	Insulin Content (nmol/islet)	EC_{50} (mmol/L)
Lean rats			
EX	8	10.52 ± 2.75*	8.70 ± 5.19
SED	5	9.54 ± 2.35*	6.28 ± 3.60
Obese rats			
EX	5	23.82 ± 2.53	5.98 ± 2.77
SED	8	25.52 ± 1.95	4.54 ± 0.22

NOTE. Values are means ± SEM. N = number of animals.

*$P < .05$ lean *v fa/fa* rats receiving similar treatment.

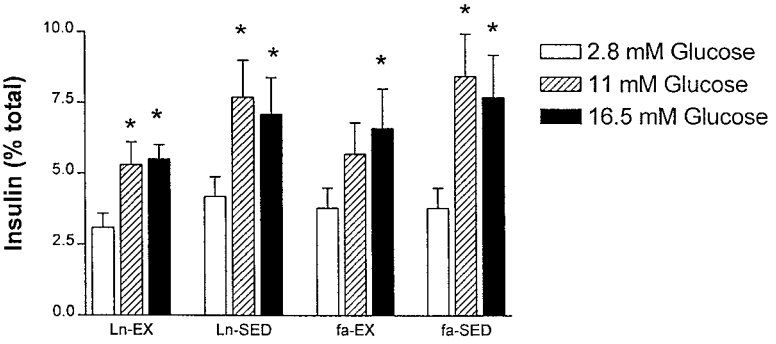


Fig 3. Effects of swimming exercise on glucose-stimulated insulin secretion from isolated islets, expressed as percent of total islet content. *Indicates a significant effect of glucose compared with 2.8 mmol/L, within phenotype. There was no significant effect of exercise in any group.

hexokinase activity was not different among the groups. At physiologic glucose concentrations (6 to 16 mmol/L), glucose phosphorylation was similar in all groups of lean and *fa/fa* rats (Fig 5B). Likewise, in islets from *fa/fa* (4.93 ± 1.26 *v* 3.91 ± 0.74 fmol/islet/h) or lean rats (8.38 ± 3.26 *v* 5.05 ± 1.37 fmol/islet/h), there were no effects of exercise on FFA oxidation.

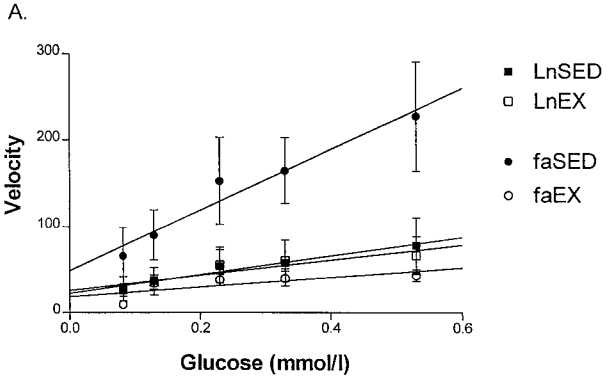
Effects of HF Diet on Total Dietary Fat Intake and Caloric Intake

Fat intake was calculated from the total caloric intake at each time period in the study (Fig 6B). Fat intake could be stratified into 4 distinct groups, corresponding to lean < fa < lean-HF < fa-HF. In lean rat groups, total fat intake was 3.5-fold to 4-fold higher in the HF groups. Since the HF diet had 3.5-fold more calories as fat, the increase in dietary fat intake was achieved without significant change in total intake (Fig 6A). Likewise, in fa-HF-SED and fa-HF-EX rats, there was a 3.5-fold to 5-fold increase in fat intake. In the fa-HF-EX diet, there was a 5-fold increase in fat intake compared with fa-EX on the control diet. This difference was due to a marked reduction in food intake of the latter group ($P < .005$ at weeks 7 to 9 compared with all other fa groups).

Effects of HF Diet on Exercise-Induced Changes In Vivo

Weight gain. HF diet blocked the preventative effects of exercise on weight gain in *fa/fa* rats (Table 5). After 5 weeks, fa-HF-EX rats gained 85% more weight than fa-EX rats. Unexpectedly, Ln-HF-EX rats gained approximately 15% more weight ($P < .05$) than Ln-HF-SED rats.

Plasma lipid concentrations. Feeding HF diet to exercised *fa/fa* rats increased plasma TG concentrations by 66% ($P <$



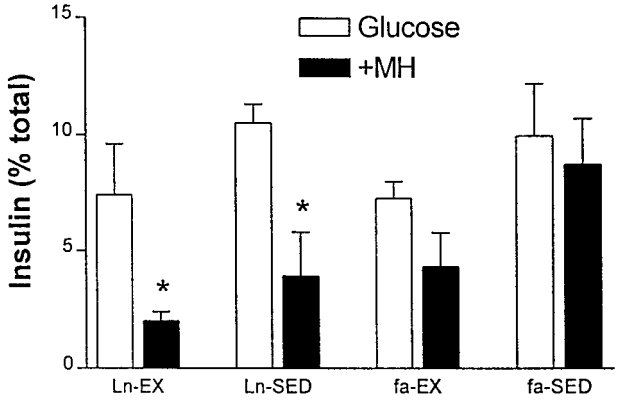


Fig 4. Effects of MH on GSIS from isolated islets in sedentary and exercised rats. *Indicates a significant effect of MH. There was no significant effect of exercise, although insulin secretion was decreased by MH by 40% in the fa-EX group compared with 10% in the fa-SED group.

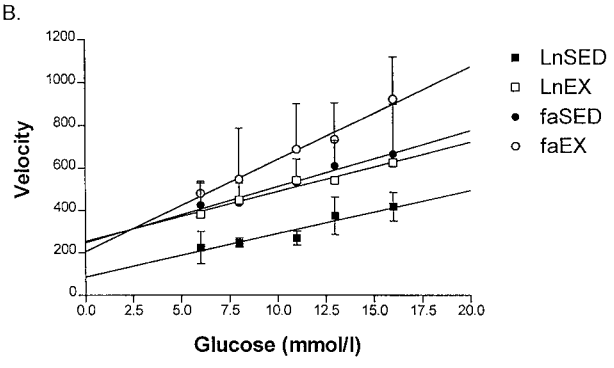


Fig 5. Effects of swimming exercise on (A) hexokinase and (B) glucokinase phosphorylating activity in isolated islets from lean and fa/fa rats. *Indicates a significant effect of exercise ($P < .05$), within phenotype.

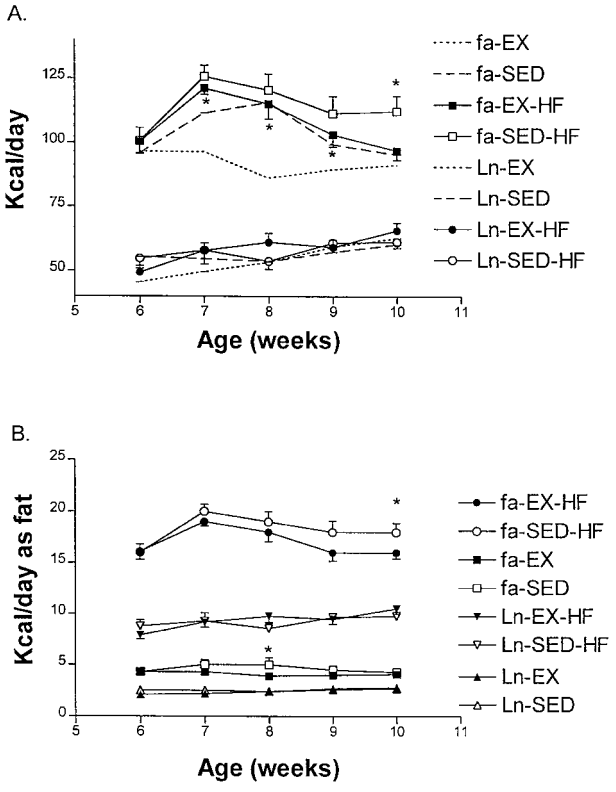


Fig 6. (A) Effects of HF diet on total food intake in kcal/d in lean and fa/fa Zucker rats. For comparison, data presented in Fig 1. are shown in the top panel as dashed (sedentary) or dotted (exercised) lines for each phenotype. *Indicates a significant ($P < .05$) effect of diet compared with the matching phenotype-activity group. (B) Fat intake of all rat groups. *Significant effect of exercise ($P < .05$).

.05) to the same level as sedentary *fa/fa* rats, thereby blocking the beneficial effects of exercise. In lean rats, HF diet did not negate the TG-lowering effects of exercise. Diet had no significant effect on plasma FFA concentrations in either lean or *fa/fa* rats.

Oral glucose tolerance. Fasting plasma glucose concentrations were not significantly changed by diet in *fa/fa* rats. During the oral glucose tolerance test (OGTT), fa-HF-EX rats had significantly higher plasma glucose excursions than fa-EX rats on control diet. Glucose area under the curve (AUC) was elevated by a similar magnitude in the sedentary groups on HF diet (data not shown), indicating that increased dietary fat induced glucose intolerance independent of exercise status. The HF diet also induced glucose intolerance in lean rats and negated the glucose-lowering effects of exercise.

Feeding a HF diet to exercised *fa/fa* rats caused an increase in fasting insulin concentrations by 4-fold ($P < .05$), but had no effect on circulating insulin of lean rats. However, the mean integrated insulin responses of fa-HF-EX and fa-EX rats were not significantly different. In lean rats, HF diet opposed the exercise effect on insulin during an OGTT ($P < .05$).

Isolated pancreatic islet studies. HF diet did not affect insulin content, glucose-stimulated insulin secretion, or islet glucose sensitivity of islets from exercised *fa/fa* or lean rats. Although a combination of HF diet and exercise partially restored the MH inhibitory response in fa-HF-EX rat islets ($P < .05$ *v* SED), this was achieved at a very high concentration of 30 mmol/L (3.37 ± 0.90%, $P < .05$ compared with 16.5 mmol/L glucose, 8.86 ± 1.15%).

In *fa/fa* rat islets, HF diet induced a further 50% reduction in hexokinase V_{max} in islets from HF-EX compared with EX rats (velocity at 0.5 mmol/L glucose = 44.0 ± 6.8 fmol/μg protein/h, $P < .05$ *v* EX, see Fig 5). Glucose phosphorylation was decreased by a HF-associated 60% increase in the glucokinase K_m in exercised *fa/fa* rats (velocity at 16 mmol/L = 365 ± 113 fmol/μg protein/h, $P < .02$ *v* EX, see Fig 5). In lean rat islets, a HF diet did not alter glucokinase activity.

Dietary fat did not affect islet fatty acid oxidation in *fa/fa* rat islets (4.63 ± 1.22 *v* 3.91 ± 0.74 fmol/islet/h), but in lean rat islets, HF diet decreased FFA oxidation by approximately 50% in exercising animals (2.67 ± 0.72 *v* 5.05 ± 1.37 fmol/islet/h, $P < .05$).

DISCUSSION

HF diet and decreased physical activity are considered the most important environmental factors contributing to the increasing prevalence of obesity throughout the world.[22] Moreover, reduction of energy intake through dieting plus increased energy expenditure through exercise is the most commonly prescribed intervention for obesity. It is therefore important to understand the potential interactions between these factors. High-intensity exercise can increase lipolysis and thereby protect an individual from the effects of HF diet under certain circumstances.[23] However, less is known about the benefits of moderate exercise on parameters, such as glucose tolerance and islet function. Impaired glucose tolerance places stress on the pancreatic islets to produce more insulin and may therefore contribute to developing diabetes mellitus. Last, obesity is a condition particularly refractory to intervention. It is important to study the benefits of exercise in models of pre-existing obesity rather than in normal animals to determine how best to prevent further morbidity.

This study examined the effects of exercise on β-cell function in a pre-existing model of obesity and insulin resistance, the *fa/fa* rat. Based on previous findings in normal rats,[9] we expected that exercise would affect islet glucose or fat metabolism to alter insulin secretion. Together with the expected

Table 5. Effects of HF diet (% change *v* control diet) on Exercise-Induced Changes in Metabolic Parameters Measured In Vivo

Parameter	Lean	*fa/fa*
Food intake	+14.4 ± 0.8*	+34.4 ± 5.4*
Weight gain	+15.8 ± 0.8*	+84.8 ± 4.0*
Fasting plasma TG	−43.6 ± 5.6	+66.3 ± 15.7
Fasting plasma FFA	−45.1 ± 13.7	+11.2 ± 2.1
Fasting plasma glucose	−14.0 ± 0.5*	+9.8 ± 0.5
Integrated plasma glucose	+73.1 ± 4.3*	+78.2 ± 8.6*
Fasting plasma insulin	−17.8 ± 2.2	+452.0 ± 115.0*
Integrated plasma insulin	+400 ± 93.3*	No change

NOTE. Data are mean % change ± SEM compared with exercised rats on control diet.

*$P < .05$.

beneficial effects of exercise on glucose sensitivity of peripheral tissues, decreased stress on the β cells was predicted, leading to a lowering of insulin secretion. It was further anticipated that HF diet would negate these beneficial effects. The discussion will first examine the effects of exercise alone, followed by an examination of interactions between diet and exercise. Of note, considerable differences in the responses of lean versus obese animals were obtained.

Effects of Exercise Combined With Control (low-fat) Diet

Although less intensive than many chronic exercise regimens reported in earlier studies of insulin secretion,[9] the swimming regimen was selected because it more closely mimics the low to moderate intensity activity likely to be adopted by obese human patients advised to exercise. Therefore, the outcomes on metabolism are more likely to be representative than those resulting from high-intensity activities. The animals subjected to this routine showed no overt signs of stress, as indicated by normal circulating corticosterone and glucose concentrations.

The moderate level of exercise attained by swimming did improve several metabolic parameters in the *fa/fa* rats. The most striking result was an exercise-associated anorexia leading to significantly lower caloric intake and a modest reduction in weight gain. These data mimic human studies that showed a combination of low-fat diet and exercise induced a caloric deficit that achieved weight maintenance.[24] The anorectic effects of exercise are shown to be due to increased hypothalamic secretion of corticotropin-releasing hormone relative to neuropeptide Y.[25] Neither caloric reduction nor lowered weight gain was observed in lean rats.

Exercise by treadmill running normalized glucose tolerance in an obese diabetic rat model[26] and in *fa/fa* rats.[27] The swimming exercise used in this study did not improve glucose tolerance in the *fa/fa* rats, indicating a dissociation of tissue insulin sensitivity from weight gain under these conditions. In contrast, lean rats did display an increase in glucose sensitivity, as evidenced by lowered excursions of both glucose and insulin during the OGTT. Exercise is reported to increase muscle glucose metabolism by increasing glucose-6-phosphatase levels, GLUT-4 protein, and hexokinase II activity.[28]

Lowering the stress on the β cell by increasing tissue insulin sensitivity is considered desirable in preventing later development of diabetes. To determine how exercise might directly affect β-cell function, isolated islet secretory and metabolic properties were studied. Unlike after intense exercise regimens,[9,29] no marked changes in GSIS were observed in this study in either *fa/fa* or lean rats. This outcome might be expected based on the finding of null difference in either ambient or fasting plasma glucose concentrations between control and exercised groups. Glucose is a major regulator of the expression of glucokinase in islets.[30] Moreover, when fasting plasma glucose concentrations of *fa/fa* rats were lowered by adrenalectomy, a decrease in basal insulin secretion was observed.[31] Insensitivity to competitive inhibition by MH is a feature of obese, hyperinsulinemic rat islets[2] that is also ameliorated by adrenalectomy.[32] However, exercise did not improve the MH response in *fa/fa* islets in these experiments.

Certain biochemical parameters are considered key indicators of β-cell health. Glucokinase is the glucosensor of the β cell[33] and therefore is the ultimate controller of glucose metabolism. In *fa/fa* rats, glucokinase activity can be altered by in vitro glucose exposure[34] or by adrenalectomy.[31,32] However, moderate exercise had no consistent effects on glucokinase kinetics, as expected from the insulin secretion data. On the other hand, hexokinase activity was highest in the fa-SED rat islets and normalized by the exercise regimen. However, the contributions of hexokinase-catalyzed glucose phosphorylation at physiologic glucose levels are minor because of end-product inhibition.[33]

The TG-laden islets of *fa/fa* rats[11,31] may have increased β-oxidation rates under some conditions that lead to elevated basal insulin secretion.[35] However, as shown when glucose was maintained at 3 mmol/L, no differences in palmitic acid oxidation were observed between phenotype or exercise groups. Similar results were found at high (25 mmol/L) glucose (data not shown). Thus, pancreatic islets maintained stable fat metabolism despite a 10-fold increase in plasma TG and 3-fold increase in plasma FFA levels.

Comparing the results of this study to others, we conclude that vigorous exercise is required to substantially affect β-cell metabolism or insulin secretion. Even in lean rats that had improved glucose tolerance after exercise, there was no lowering of GSIS or any changes in glucose phosphorylation or FFA oxidation.

Effects HF Diet on Exercise-Induced Changes in Metabolic Parameters

In sedentary rats, HF diet induced similar effects in lean and *fa/fa* rats. Weight gain was increased without any increase in caloric intake. The rats became glucose intolerant, despite an increase in the in vivo insulin response to glucose. However, the intrinsic insulin-secreting activity of isolated islets was not affected nor were major changes in biochemical parameters detected (data not shown).

Examination of the interactions between exercise and diet revealed that HF diet blocked the ability of exercise to decrease weight gain and energy intake in *fa/fa* rats. It has been hypothesized that animals eat to maintain a stable level of glycogen stores.[36] Exercise utilizes glycogen as energy, which may then lead to increased consumption to regain the balance. Although physical activity has been shown to alter food preference to carbohydrates,[37] thereby easily allowing the glycogen balance to be regained,[22] the rats in this study had no choice but to increase consumption of the HF diet. However, exercised lean rats did not increase food consumption, as would be predicted from the model. Lean rats may have compensated for increased caloric expenditure by decreasing activity during the nonexercise period. Since *fa/fa* rats are already more sedentary than lean rats,[38] this mechanism for energy balance compensation would be less available to them.

Intake of total fat is correlated with markers of insulin resistance in human subjects.[39] The increase in HF diet consumption is the probable cause of the equally impaired glucose tolerance in sedentary versus exercised groups of *fa/fa* rats,

whereas, in lean rats, the beneficial effects of exercise on glucose tolerance were retained despite the HF diet. These data indicate that pre-existing obesity may require a higher intensity of exercise to improve glucose tolerance, particularly if the diet is elevated in fat content. It should be pointed out that the diet chosen has only a modest increase in fat compared with many studies, yet negative effects on overall metabolism were still observed.

The HF diet used in this study appeared to affect insulin sensitivity without marked changes in β-cell function. This is in contrast to higher fat diets, which induce an increased response to basal glucose and loss of response to high glucose.[40] No consistent changes in islet glucose sensitivity, insulin content, or glucokinase activity were observed. Insensitivity to the inhibitory effects of MH is a common feature of obese rodent islets,[2] although the reason for this phenomenon is unknown. Certain interventions, such as adrenalectomy, restore MH effects in *fa/fa* rats.[31,32] Curiously, a combination of HF diet and exercise partially restored MH inhibition of insulin secretion in the *fa/fa* rats. Unlike in adrenalectomy, this could not be attributed to a decrease in plasma corticosterone levels, which were similar in fa-HF-EX (837 ± 76 ng/mL) and faSED rats (798 ± 68 ng/mL).

In conclusion, low-moderate intensity exercise over a 5-week period resulted in decreased food intake, reduced weight gain, and lowered plasma TG in *fa/fa* Zucker rats. However, more widespread improvements on indicators of the metabolic state, such as glucose tolerance, were not observed nor were marked changes documented in insulin secretion patterns. In contrast, the most pronounced effect in lean exercised rats was improved oral glucose tolerance without changes in body weight or insulin secretion. However, increasing daily fat intake by 3-5-fold negated any beneficial metabolic effects of the exercise in both lean and *fa/fa* rats, particularly a decrease in oral glucose tolerance and an increase in weight gain. The most likely explanation for these opposite results of exercise and diet is the opposite effects of each on glucose uptake in muscle,[28] rather than effects on pancreatic islet function. Thus, the benefits of low-moderate intensity exercise are best realized if dietary fat is restricted.

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