

## Voluntary running exercise prevents $\beta$ -cell failure in susceptible islets of the Zucker diabetic fatty rat

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<sup>1</sup>Molecular Nutrition Unit and The Montreal Diabetes Research Center, Research Center of the University of Montreal Hospital Center, Montreal, Quebec; <sup>3</sup>Departments of <sup>3</sup>Kinesiology and <sup>4</sup>Nutrition and Biochemistry, University of Montreal, Montreal, Quebec, Canada; and <sup>2</sup>Diabetes and Endocrinology Research Unit, Australian National University Medical School at the Canberra Hospital, Garran, Australian Capital Territory, Australia

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**Delghingaro-Augusto V, Décary S, Peyot M, Latour MG, Lamontagne J, Paradis-Isler N, Lacharité-Lemieux M, Akakpo H, Birot O, Nolan CJ, Prentki M, Bergeron R.** Voluntary running exercise prevents  $\beta$ -cell failure in susceptible islets of the Zucker diabetic fatty rat. *Am J Physiol Endocrinol Metab* 302: E254–E264, 2012. First published November 1, 2011; doi:10.1152/ajpendo.00360.2011.—Physical activity improves glycemic control in type 2 diabetes (T2D), but its contribution to preserving  $\beta$ -cell function is uncertain. We evaluated the role of physical activity on  $\beta$ -cell secretory function and glycerolipid/fatty acid (GL/FA) cycling in male Zucker diabetic fatty (ZDF) rats. Six-week-old ZDF rats engaged in voluntary running for 6 wk (ZDF-A). Inactive Zucker lean and ZDF (ZDF-I) rats served as controls. ZDF-I rats displayed progressive hyperglycemia with  $\beta$ -cell failure evidenced by falling insulinemia and reduced insulin secretion to oral glucose. Isolated ZDF-I rat islets showed reduced glucose-stimulated insulin secretion expressed per islet and per islet protein. They were also characterized by loss of the glucose regulation of fatty acid oxidation and GL/FA cycling, reduced mRNA expression of key  $\beta$ -cell genes, and severe reduction of insulin stores. Physical activity prevented diabetes in ZDF rats through sustaining  $\beta$ -cell compensation to insulin resistance shown in vivo and in vitro. Surprisingly, ZDF-A islets had persistent defects in fatty acid oxidation, GL/FA cycling, and  $\beta$ -cell gene expression. ZDF-A islets, however, had preserved islet insulin mRNA and insulin stores compared with ZDF-I rats. Physical activity did not prevent hyperphagia, dyslipidemia, or obesity in ZDF rats. In conclusion, islets of ZDF rats have a susceptibility to failure that is possibly due to altered  $\beta$ -cell fatty acid metabolism. Depletion of pancreatic islet insulin stores is a major contributor to islet failure in this T2D model, preventable by physical activity.

physical activity; glycerolipid fatty acid cycle; insulin secretion; adrenocorticotrophic hormone; corticosterone

INSULIN RESISTANCE (IR) is a predisposing factor for the development of type 2 diabetes (T2D). However, it must be combined with failure of  $\beta$ -cell compensation for IR for T2D to occur (18, 42). Diet and exercise programs can reduce T2D development in humans with impaired glucose tolerance (35, 54). Thus, it is likely that the exercise interventions prevent islet  $\beta$ -cell failure (18, 42). Despite the abundant literature supporting the beneficial effects of exercise on insulin action (30), much less is known about the effects of exercise on  $\beta$ -cell function. In young individuals, exercise training decreased glucose-stimulated insulin secretion (GSIS) (8, 20, 28). Trained over-

weight to obese subjects also demonstrated a lower acute insulin response during an intravenous glucose tolerance test (GTT) (48). These results most probably reflect lower insulin requirement due to improved insulin sensitivity. Furthermore, Dela and Stallknecht (9) demonstrated that 12 wk of endurance exercise training did not increase GSIS in first-degree offspring of T2D patients. On the other hand, these same authors showed that diabetic subjects with good residual insulin secretory capacity had increased GSIS following endurance training, whereas those with low residual secretory capacity did not improve upon training (10). Of note, Slentz et al. (48), using the disposition index as a reflection of  $\beta$ -cell function, suggested that the latter is improved by moderate-intensity exercise training. All together, the evidence suggests that the health status of subjects and methodologies employed to study  $\beta$ -cell function are key parameters that influenced the outcome of those studies.

The Zucker diabetic fatty (ZDF) rat is a severely insulin-resistant genetic model of obesity-related T2D. Unlike the Zucker fatty (ZF) rat that is able to sustain  $\beta$ -cell compensation for IR, the ZDF rat is prone to  $\beta$ -cell failure. Exercise prevents diabetes in this animal model (6, 21, 22, 41, 49). Pold et al. (41) showed that treadmill-trained ZDF rats did not develop hyperglycemia. In that study, exercise improved peripheral and hepatic insulin action and islet morphology (41). Colombo et al. (6), using a similar exercise protocol, reported that training did not markedly influence gene expression in ZDF rat islets, whereas important gene alterations were found in liver and muscles (6). Kiraly et al. (21, 22) reported that swimming also prevented hyperglycemia in ZDF rats, which was associated with enhanced insulin secretion during intraperitoneal GTT and improved islet morphology with increased  $\beta$ -cell proliferation and  $\beta$ -cell mass. However, the nature of islet susceptibility to failure in the ZDF rat is not known as well as the basis of diabetes prevention by exercise.

Studies conducted in islets of the obese, but diabetes resistant, ZF rat, a model for  $\beta$ -cell compensation, have shown increased  $\beta$ -cell mass and functional islet changes, including enhanced glucose and lipid metabolism, processes that favor production of nutrient-secretion coupling signals (25, 32). Isolated ZF islets have shown enhanced palmitate augmentation of GSIS (32), a finding associated with enhanced glucose-responsive glycerolipid/fatty acid (GL/FA) cycling (32). Both nonesterified fatty acid (NEFA) esterification into glycerolipids such as triglycerides (TG) and diacylglycerols (DAG) and the lipolysis of GLs were increased in ZF islets, particularly at

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increased glucose concentrations. Furthermore, lipolysis inhibition by the lipase inhibitor orlistat markedly reduced GSIS in these islets (32). Thus, GL/FA cycling may provide a mechanism by which the  $\beta$ -cell in ZF rat islets escapes glucolipotoxicity and compensates for IR without the development of diabetes (33).

The aim of the present study was to investigate the biochemical basis of the effects of physical activity on islet function in ZDF rats in which diabetes is prevented by voluntary running in wheel cages (23, 44). Particular focus was placed on whether preserved  $\beta$ -cell performance in exercised ZDF rats could be linked to the compensatory pathway of enhanced GL/FA cycling similar to what was observed in compensating islets of ZF rats. We found that voluntary running markedly improved GSIS in ZDF rat islets, but not quite to the level of function of Zucker lean (ZL) control islets if GSIS was expressed per islet protein content. GL/FA cycling was abnormal in ZDF rats, which was not normalized by physical activity. Voluntary running had a dramatic effect on preventing depletion of islet insulin stores in this diabetes-prone obese rat model.

## MATERIALS AND METHODS

**Animals.** All protocols were approved by the Institutional Animal Care and Use Committee of the University of Montreal. Male obese ZDF rats (ZDF *Lepr<sup>fa/fa</sup>*) and their control ZL (*fa/+* or *+/+*) were purchased from Charles River Laboratories (Saint-Constant, QC, Canada) at 5 wk of age. Rats were housed in a temperature- and humidity-controlled facility on a 12:12-h light-dark cycle with free access to rodent chow (Purina 5008) and tap water.

Animals were assigned to the following three experimental groups: ZL inactive (ZL-I;  $n = 19$ ), ZDF inactive (ZDF-I;  $n = 22$ ), and physically active ZDF (ZDF-A;  $n = 19$ ). All animals were housed in a wheel cage consisting of a stainless steel live-in wheel (circumference: 1.445 m, width: 16 cm) mounted on a shaft containing a low-friction ball-bearing collar with a running surface of 4-mm-wide stainless steel mesh as described earlier (46). Each wheel was equipped with a sensor and wired to a computer where wheel revolutions were recorded continuously for the assessment of the rat's activity. The distance run per day was calculated as the number of wheel revolutions over a 24-h period and was reported two times a week. For the following 6 wk, ZDF-A rats engaged in voluntary running. ZL-I and ZDF-I groups were prevented from running by wheel blocks. Running wheels were blocked 24 h before GTTs and death. An early subset of data collected from  $n = 8$ –9 rats/group has been reported elsewhere (44).

**Body weight, food intake, running distance, and periodically measured plasma parameters.** Running distance, body weight, and food intake were measured two times weekly. Heparinized tail blood was collected weekly at 8:00–9:00 A.M. from awake fed rats for plasma glucose, insulin, TG, NEFA, and corticosterone determinations as indicated in corresponding Figs. 1–6. Plasma glucose, TG, and NEFA were measured by colorimetric enzyme kit assays (respectively, Wako Chemical, Osaka, Japan; GPO Trinder, Sigma-Aldrich, St. Louis, MO; NEFA C kit, Wako Chemical, Neuss, Germany) and plasma insulin and corticosterone by radioimmunoassay kits (respectively, Linco Research, St. Louis, MO, and MP Biomedicals, Orangeburg, NY).

**Glucose tolerance test.** After 37–38 days of the intervention period and at midday after 4 h of fasting, animals were subjected to an oral glucose tolerance test (oGTT) (2 g glucose/kg body wt). Tail blood was collected for glucose measurements (One Touch Ultra; LifeScan) and plasma insulin determinations at the time points as indicated in Figs. 1–6. The insulinogenic index derived from the oGTT was calculated according to the following formula:  $[30 \text{ min insulin } (\mu\text{U/}$

$\text{ml}) - \text{fasting insulin}]/[30 \text{ min glucose (mmol/l)} - \text{fasting glucose}]$ , as previously described (26, 57).

**Death and blood and tissue harvesting.** After 44–46 days of intervention (12-wk-old rats), fed rats were anesthetized between 8:00 and 9:00 A.M. with pentobarbital sodium (60 mg/kg, Somnotol; MTC Pharmaceuticals, Hamilton, ON, Canada) and killed by exsanguination. Blood was collected from the inferior vena cava for the determination of plasma adrenocorticotrophic hormone (ACTH) and active glucagon like peptide-1 (GLP-1), as previously described (27). For ACTH and GLP-1, blood was transferred to, respectively, EDTA-Trasyol and EDTA-dipeptidyl-peptidase IV inhibitor-treated prechilled microtubes. Pancreatic islet isolation was performed by collagenase injection in the bile duct, pancreas resection, digestion at 37°C, histopaque gradient separation, and hand-picking under a stereomicroscope, as previously described (11, 39). Epididymal white adipose tissue (EWAT), muscles (gastrocnemius, plantaris, and soleus), liver, and heart were dissected and immediately frozen under liquid nitrogen, weighed, and stored at  $-80^\circ\text{C}$ .

**Plasma GLP-1, ACTH, and nonislet tissue assays.** Kit assays were used for plasma GLP-1 (active) (Linco Research) and ACTH (MP Biomedicals) measurements. Liver TG content was estimated from glycerol released after ethanolic KOH hydrolysis, employing a commercial kit as described above. Liver and muscle glycogen content and muscle cytochrome oxidase activity were determined as previously described (1, 37).

**Islet insulin secretion and insulin content measurements.** Before experiments, islets were cultured for 1 h in RPMI-1640 medium (11). Isolated islets were distributed into 12-well plates (6 islets/well in triplicates) and incubated for 2 h in 1 ml RPMI complete medium containing 3 mmol/l glucose. They were then washed and preincubated for 40 min at 37°C in 1 ml Krebs-Ringer bicarbonate buffer containing 10 mmol/l HEPES (KRBH; pH 7.4), 0.5% defatted BSA, and 3 mmol/l glucose. The islets were then incubated for 45 min in 0.5 ml of KRBH containing 3, 8, and 16 mmol/l glucose plus 0.5% defatted BSA, in the presence or absence of 0.3 mmol/l palmitate or 3 mmol/l glucose plus KCl (35 mmol/l). At the end of the incubation, media were collected for insulin determination. Total islet insulin content was measured after acid-ethanol (0.2 mmol/l HCl in 75% ethanol) extraction. Batches of 20 freshly isolated islets, representative of those taken for the secretion studies, were used to determine islet protein content, as described previously (11, 39).

**Fatty acid oxidation and esterification.** Total fatty acid oxidation (from exogenous and endogenous NEFA sources) and esterification were measured in isolated islets, as previously described (11).

**Lipolysis.** Islet glycerol released into the incubation medium during the final 2 h of the fatty acid oxidation assessments was used to indirectly determine islet lipolysis, as previously described (39).

**Quantitative real-time PCR.** Total RNA was extracted immediately after islet isolation using a kit (RNeasy Mini; Qiagen, Mississauga, Ontario, Canada) with on-column DNase digestion (Qiagen) according to the manufacturer's instructions. cDNA was synthesized from 2  $\mu\text{g}$  of RNA. PCR amplification of the genes of interest was performed using primers previously described (11). PCR products were normalized by the housekeeping gene cyclophilin A, and results are expressed as arbitrary units.

**Statistical analysis.** All results are expressed as means  $\pm$  SE. Statistical differences of group means were calculated using one- or two-way ANOVA followed by Bonferroni post hoc tests. A  $P$  value of  $<0.05$  was considered significant.

## RESULTS

**Body weight, food intake, and organ weights.** ZDF-A rats ran an average of 4.2 km/day over the 6 wk (Fig. 1A). Differences in food intake or body weight were not observed between ZDF-A and ZDF-I rats (Fig. 1, B and C). Both ZDF groups remained markedly obese and hyperphagic compared

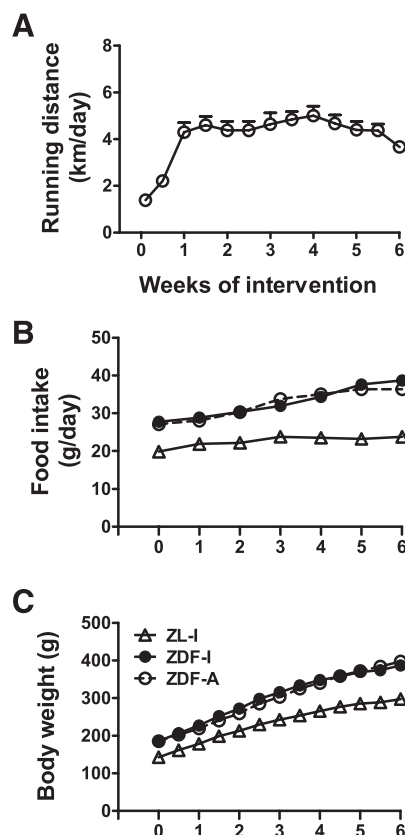


Fig. 1. Running distance (A), food intake (B), and body weight (C) during the 6 wk of inactivity [open triangle, inactive Zucker lean (ZL-I) rats and closed circle, inactive Zucker diabetic fatty (ZDF-I) rats] and voluntary running (ZDF-A rats: open circle). Values are means  $\pm$  SE of 19–21 animals/group.

with the ZL-I rats. There were changes, however, in body composition between the ZDF rat groups at the end of the study (Table 1). Plantaris and soleus muscles were heavier in ZDF-A compared with ZDF-I rats, while the heart followed this tendency ( $P = 0.052$ ) and liver weight was reduced in ZDF-A rats. EWAT tended to weigh less in ZDF-A compared with ZDF-I rats ( $P = 0.064$ ) but was  $\sim 3.5$ -fold heavier than in ZL-I rats.

*Physical activity prevents the development of diabetes but not severe dyslipidemia in ZDF rats.* At 6 wk of age, ZDF-I rats were normoglycemic and hypersulinemic. They became diabetic by 9–10 wk of age while hyperinsulinemia progressively decreased past that age (Fig. 2, A and B). Voluntary exercise prevented diabetes (Fig. 2A). The ZDF-A rats were markedly hyperinsulinemic compared with the ZL-I rats, but the levels in the first 2 wk of intervention were less than half

that required by the ZDF-I rats (Fig. 2B). While physical activity prevented diabetes, it had no effect on the severe hypertriglyceridemia of ZDF rats (Table 2). Fed-state plasma active GLP-1 concentrations were higher in ZDF-A ( $7.8 \pm 1.2$  pM) compared with ZL-I ( $4.6 \pm 0.6$  pM;  $P = 0.02$ ) and tended to be higher than ZDF-I ( $5.1 \pm 0.4$  pM;  $P = 0.06$ ).

*Voluntary running reduced hyperactivation of stress hormones.* Corticosterone levels were not increased in the ZDF-A compared with ZDF-I rats; rather, there was a trend for them to fall in the voluntary exercising group in the last few weeks of the intervention period (significantly reduced at week 4 of the intervention) (Fig. 2C). Plasma ACTH concentrations from samples collected upon death were markedly lower in the ZDF-A compared with ZDF-I rats (Fig. 2D).

*Voluntary exercise improves glucose tolerance through maintenance of the glucose-stimulated insulin response.* The ZDF-I rats had markedly impaired glucose intolerance (Fig. 3A). Voluntary running in the ZDF rats improved glucose tolerance to levels similar to those seen in ZL-I rats (Fig. 3, A and C). Furthermore, ZDF-A rats showed an increase in the plasma insulin response compared with ZDF-I rats (Fig. 3B). Improved pancreatic islet  $\beta$ -cell function in ZDF-A vs. ZDF-I rats was also evident from the insulinogenic index (Fig. 3E). The insulin response of ZDF-A rats was markedly higher than that of the ZL-I rats (Fig. 3, B and D).

*Elevated nonislet tissue glycogen and TG contents are not lowered by exercise in ZDF rats, but skeletal muscle mitochondrial oxidative capacity is increased.* Red and white gastrocnemius muscle glycogen levels were not affected by 6 wk of voluntary running, being similar across all three rat groups (data not shown). Liver glycogen content was lower in ZL-I rats ( $130 \pm 9$   $\mu$ mol/g;  $P < 0.01$ ) compared with both ZDF rats (ZDF-A:  $273 \pm 13$  and ZDF-I:  $261 \pm 13$   $\mu$ mol/g). Liver TG content was also lower in ZL-I rats compared with ZDF rats (ZL-I:  $8.0 \pm 0.4$  mg/g vs. ZDF-A:  $22.5 \pm 1.5$  and ZDF-I:  $21.1 \pm 1.0$  mg/g;  $P < 0.05$ ). Higher liver glycogen and TG contents observed in the ZDF rats were not affected by physical activity. However, muscle cytochrome oxidase activity, an index of mitochondrial oxidative capacity, was increased in the gastrocnemius in ZDF-A compared with ZDF-I rats (respectively,  $0.318 \pm 0.051$  and  $0.168 \pm 0.011$  U/ $\mu$ g in red gastrocnemius;  $P < 0.05$ ;  $0.086 \pm 0.021$  and  $0.036 \pm 0.003$  U/ $\mu$ g in white gastrocnemius;  $P = 0.059$ ).

*Severe depletion of islet insulin stores in ZDF-i rats is prevented by physical activity.* Islets from ZDF-I rats were four times larger than those of ZL-I rats based on protein content with no difference in size evident between ZDF-I and ZDF-A rats (Fig. 4A). Importantly, when insulin content was normalized to protein content, islets from ZDF-I rats showed an 86%

Table 1. Tissue weight after 6 wk of intervention

	ZL-I	n	ZDF-I	n	ZDF-A	n
Muscles						
Plantaris, g	$0.27 \pm 0.01^{\dagger\dagger}$	18	$0.22 \pm 0.01^*$	18	$0.25 \pm 0.01$	18
Soleus, g	$0.14 \pm 0.01^{\dagger\dagger}$	12	$0.12 \pm 0.01^{**}$	19	$0.16 \pm 0.01$	19
Liver, g	$10.60 \pm 0.18^{\dagger\dagger}$	17	$20.14 \pm 0.51^*$	19	$17.32 \pm 0.40$	19
Heart, g	$0.94 \pm 0.03^{\dagger\dagger}$	9	$1.06 \pm 0.04$	7	$1.17 \pm 0.03$	12
EWAT, g	$0.87 \pm 0.06^{\dagger\dagger}$	17	$3.34 \pm 0.11$	19	$3.08 \pm 0.08$	19

Values are expressed as means  $\pm$  SE; n, no. of measurements. ZL-I, inactive Zucker lean; ZDF-I, inactive Zucker diabetic fatty; ZDF-A, active ZDF; EWAT, epididymal white adipose tissue.  $^*P < 0.05$  and  $^{**}P < 0.01$  vs. ZDF-A.  $^{\dagger\dagger}P < 0.01$  vs. other two groups.



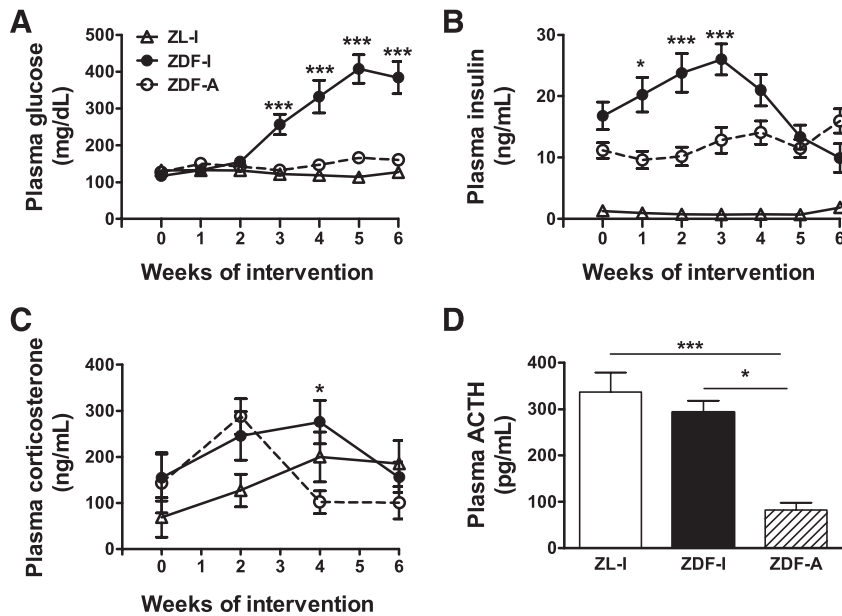


Fig. 2. Plasma glucose (A), insulin (B), corticosterone (C), and adrenocorticotrophic hormone (ACTH, D) during the intervention period in nonfasted ZL-I, ZDF-I, and active ZDF (ZDF-A) rats. Values are means  $\pm$  SE of 15 rats/group, with the exception of corticosterone ( $n = 5-10$ ) and ACTH ( $n = 4-9$ ). ACTH was measured at the end of the intervention period only. \* $P < 0.05$  and \*\*\* $P < 0.001$  vs. ZDF-A.

reduction compared with ZL-I islets. In contrast, partial but significant preservation of the insulin stores was observed in ZDF-A compared with ZDF-I islets (Fig. 4B).

*Physical activity prevents loss of compensatory insulin hypersecretion to glucose and palmitate in isolated islets from ZDF rats.* Insulin secretion in control ZL-I islets, expressed per islet (Fig. 4C), was increased at intermediate (8 mmol/l) and further increased at high (16 mmol/l) glucose concentrations with potentiation by palmitate. Insulin secretion in ZDF-I islets was similar to ZL-I islets at 8 mmol/l glucose with or without palmitate, but further increases in secretion at 16 mmol/l glucose were not seen either in the presence or absence of palmitate (Fig. 4C). In contrast, islets from physically active ZDF-A rats showed an enhanced glucose and fatty acid-potentiated insulin secretion compared with ZL-I rats at both 8 and 16 mmol/l glucose (Fig. 4C). No significant effect of KCl on insulin secretion was observed between the three groups (Fig. 4C). Interestingly, if insulin secretion was normalized per islet protein content (Fig. 4D), the ZL-I islets were found to have significantly better insulin secretion in response to 16 mmol/l glucose in the presence or absence of palmitate than both ZDF-I and ZDF-A islets. Expressed this way, ZDF-A islets still had improved GSIS compared with ZDF-I islets, although the difference did not reach statistical significance (Fig. 4D). When GSIS was expressed as a percentage of corrected total islet insulin content (sum of insulin content measured at the end of the incubation period and the secreted insulin within that period), the impairment of GSIS in ZDF-I

islets was no longer evident and was actually increased compared with ZL-I islets in response to KCl at 3 mmol/l glucose (Fig. 4E).

*Glycerolipid cycling and fatty acid oxidation in islets from ZDF rats is unresponsive to glucose and is not influenced by physical activity.* All islet fatty acid metabolism data have been corrected for islet protein content. The inhibitory effect of high glucose concentrations on fatty acid oxidation was observed in ZL-I rat islets (Fig. 5A). However, in both ZDF-I and ZDF-A, fatty acid oxidation was reduced at both 3 and 8 mmol/l compared with ZL-I islets. In addition, no inhibitory effect of high glucose on islet fatty acid oxidation was observed in the ZDF groups (Fig. 5A). After 16 h incubation (equilibrium phase), net exogenous palmitate accumulation into intracellular NEFAs was increased by  $\sim 60-70\%$  in islets of the ZDF-I and ZDF-A rats compared with ZL-I islets ( $P < 0.05$ ; 2-way ANOVA: group effect) (Fig. 5B). Net exogenous palmitate esterification into total glycerolipids (monoacylglycerol + DAG + TG) was increased by  $\sim 65-70\%$  in islets of both ZDF rat groups compared with ZL-I islets ( $P < 0.05$ ; 2-way ANOVA, group effect) (Fig. 5C). Lipolysis of islet glycerolipids, as assessed by measurement of glycerol release, was increased by 85% at 16 compared with 3 mmol/l glucose in islets from ZL-I rats (Fig. 5D). Basal lipolysis was not different between islets of the three rat groups, but, in contrast to ZL-I islets, islets of both ZDF groups were unresponsive to the stimulatory effect of high glucose (Fig. 5D).

Table 2. Plasma NEFA and TG

	ZL-I	<i>n</i>	ZDF-I	ZDF-A	<i>n</i>
NEFA, mM					
Start (week 0)	0.32 $\pm$ 0.03	9	0.47 $\pm$ 0.09	0.42 $\pm$ 0.03	7
End (week 6)	0.39 $\pm$ 0.03#	9	0.61 $\pm$ 0.06	0.52 $\pm$ 0.06	6
TG, mM					
Start (week 0)	0.52 $\pm$ 0.09##	17	1.32 $\pm$ 0.19	1.25 $\pm$ 0.25	14
End (week 6)	0.34 $\pm$ 0.05##	14	2.17 $\pm$ 0.25	1.72 $\pm$ 0.22	11

Values are expressed as means  $\pm$  SE; *n*, no. of measurements. NEFA, nonesterified fatty acids; TG, triglycerides. # $P < 0.05$  and ## $P < 0.01$  vs. ZDF-I.

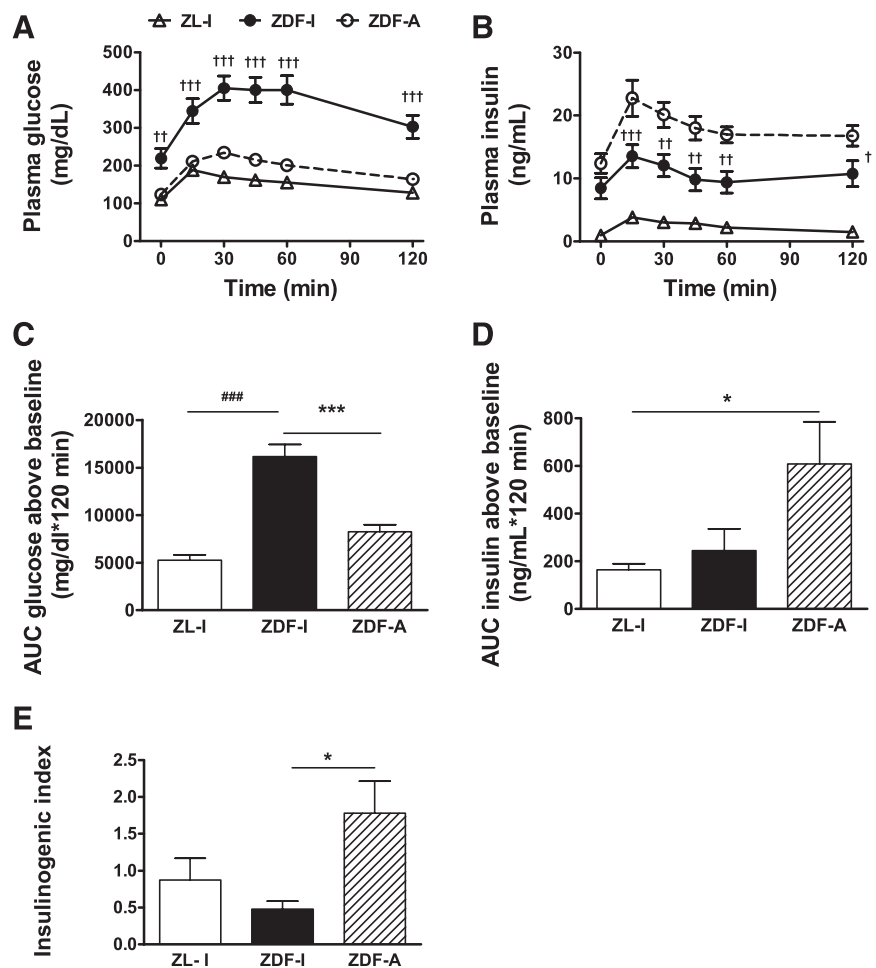


Fig. 3. Improvement in oral glucose tolerance and the insulin response to oral glucose measured after 37 days of voluntary running in ZDF rats. Plasma glucose (A) and insulin (B) concentrations and respective areas under the curves (AUC) above baseline (C and D) in ZL-I, ZDF-I, and ZDF-A rats during a 2 g/kg oral glucose tolerance test (oGTT). E: insulinogenic index calculated from plasma glucose and insulin concentrations obtained during the oGTT (refer to MATERIALS AND METHODS for calculation formulas);  $n = 14-15$  animals/group. \* $P < 0.05$  and \*\*\* $P < 0.001$  vs. ZDF-A. ### $P < 0.001$  vs. ZDF-I. † $P < 0.05$ , †† $P < 0.01$ , and ††† $P < 0.001$  vs. the other two groups.

Islets of ZDF-i rats show alterations in expression of key  $\beta$ -cell genes that are minimally prevented by physical activity. Reduction in islet insulin mRNA was observed in the ZDF-I, with partial prevention of this being evident in the ZDF-A group. The mRNA expressions of glucagon-like peptide-1 receptor (GLP1r), glucose-dependent insulinotropic polypeptide receptor (GIPr), G protein-coupled receptor 40 (GPR40), MafA, pancreatic and duodenal homeobox factor-1 (PDX-1), and pyruvate carboxylase (PC) were markedly downregulated in ZDF-I islets, and physical activity did not prevent these changes (Fig. 6). Among the tested genes, the ones that were upregulated in ZDF-I compared with ZL-I rats were the ones that were normalized by exercise. These included stearoyl-CoA desaturase (SCD-1), carnitine palmitoyltransferase 1 (CPT-1), and peroxisomal proliferator-activated receptor- $\gamma$  co-activator-1 $\alpha$  (PGC-1 $\alpha$ ) (Fig. 6).

## DISCUSSION

As in other studies using forced exercise in the ZDF rat (21, 22, 41), voluntary running prevented the development of diabetes despite continuing hyperphagia, obesity, and hyperlipidemia. Compensatory insulin secretion was preserved by exercise, and, this was best characterized by enhanced insulin secretion per islet and prevention of severe depletion of islet insulin stores. Interestingly, islets of both ZDF-I and ZDF-A rats had significant abnormalities in key  $\beta$ -cell gene expression

as well as loss of glucose responsiveness of fatty acid oxidation and the lipolysis arm of GL/FA cycling.

Voluntary exercise does not reverse hyperphagia and obesity but prevents hyperglycemia in ZDF rats. Food intake and body weight were not altered by exercise despite the greater exercise-related energy expenditure in the ZDF-A rats, which ran an average 4.2 km/day. However, this finding is not surprising because ZDF-A maintained glycemia below 180 mg/dl throughout the study. Therefore, less calories from food intake would be wasted into urine, making ZDF-A rats more metabolically efficient than ZDF-I rats, which had mean glycemia  $>250$  mg/dl for most of the study. Such improvement in metabolic efficiency via reduced glucose excretion in urine has been reported previously (6, 41). On the other hand, there were changes in body composition with increased skeletal muscle weights, reduced liver mass, and a trend for EWAT depots to be smaller (8% reduced,  $P = 0.064$ ) in ZDF-A compared with ZDF-I rats. Such favorable changes in body composition have been observed in T2D patients who are physically active (43). At 2 wk into the intervention, ZDF-I rats were still normoglycemic but had two times the insulinemia of ZDF-A rats (Fig. 2A), consistent with some lessening of IR in the physically active rats. Other studies using homeostasis model assessment-IR and the hyperinsulinemic-euglycemic clamp technique have reported increased insulin sensitivity in exercise-trained ZDF rats (23, 41). Although not the focus of the present

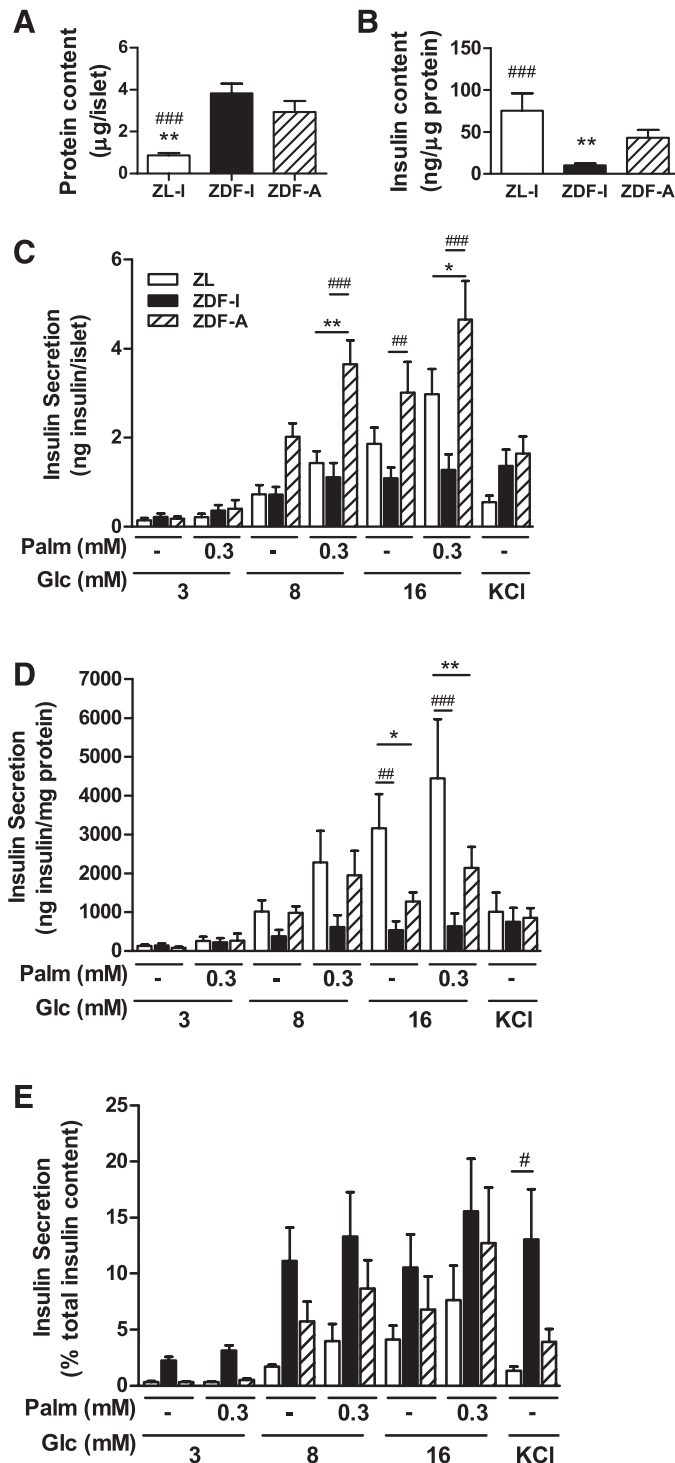


Fig. 4. Voluntary exercise prevents depletion of islet insulin stores and the loss of compensatory enhanced insulin secretion in response to glucose (Glc) and palmitate (Palm) in isolated islets from ZDF rats. Insulin secretion (IS) was measured in groups of 6 islets/well in triplicates in the presence and absence of 0.3 mM palmitate at 3, 8, and 16 mmol/l glucose and 3 mmol glucose with 35 mmol/l KCl. *A*: islet protein,  $n = 13-18$ . *B*: islet insulin content normalized by protein,  $n = 5-6$ . *C*: IS in 45 min expressed as ng insulin/islet,  $n = 5-7$ . *D*: IS in 45 min normalized by mg of islet protein,  $n = 5-7$ . *E*: IS expressed as percentage of total islet insulin content secreted in 45 min,  $n = 5-7$ . Data are means  $\pm$  SE of 4 independent experiments. \* $P < 0.05$  and \*\* $P < 0.01$  vs. ZDF-*A*. ## $P < 0.05$ , ### $P < 0.01$ , and #### $P < 0.001$  vs. ZDF-I.

experiment, we expect that insulin sensitivity was improved in physically active ZDF rats.

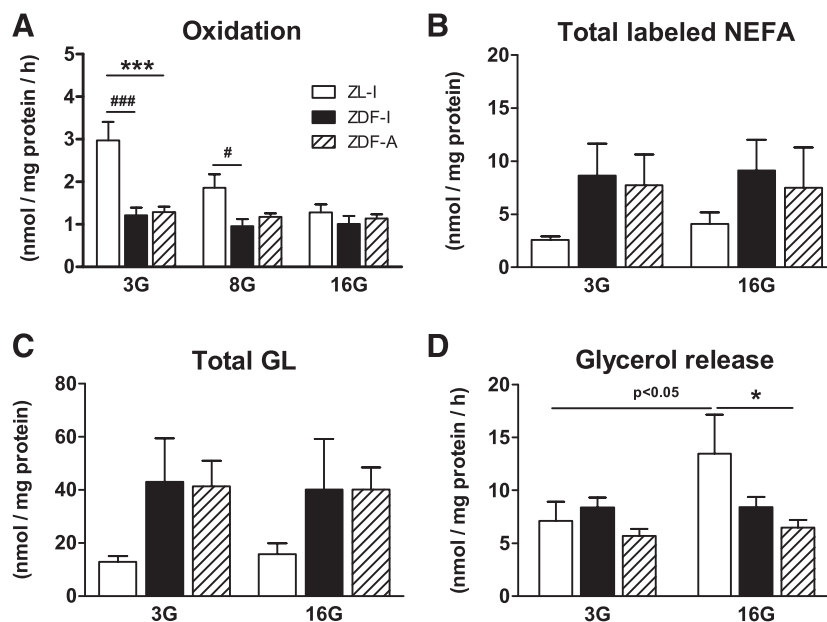
*Voluntary exercise is not causing elevation of stress hormones.* All but two exercise studies (23, 44) in male ZDF rats have used forced treadmill running or swimming. During forced exercise, often conducted during the lights-on cycle, animals experience severe psychological stress and disruption of the normal circadian rhythm of the hypothalamic-pituitary-adrenal (HPA) axis (12). In addition to beneficial adaptations, such as increased oxidative capacity and improved insulin sensitivity, forced treadmill running is associated with unwanted physiological adaptations indicative of chronic stress (29). Importantly, glucose homeostasis itself can be affected by nonexercise forms of stress (19). Hence, ZDF rats bathing in 5 cm of water and serving as a sham control group to a swimming group of rats had increased plasma corticosterone concentrations, which was associated with delayed onset of postprandial hyperglycemia (22). Such results stemming from forced swimming exercise make the interpretation of the data ambiguous.

In the present study, we measured plasma corticosterone concentrations in the morning at times of rest for the rats (basal corticosterone) rather than during times of exercise, and these were not altered by voluntary running in ZDF rats. Moreover, plasma ACTH was decreased in physically active ZDF rats in the face of similar plasma corticosterone concentrations compared with inactive ZDF rats. This may be due to an enhanced sensitivity of the adrenal glands to ACTH, as suggested by Fediuc et al. (12) based on data obtained from Sprague-Dawley rats that had been trained by voluntary running for 5 wk. Alternatively, increased conversion of 11-dehydrocorticosterone to corticosterone through the action of 11 $\beta$ -hydroxysteroid dehydrogenase 1 (11 $\beta$ -HSD1) dehydrogenase activity may have contributed to the maintenance of plasma corticosterone. However, Campbell et al. (3) assayed 11 $\beta$ -HSD1 dehydrogenase protein content in liver, muscle, and adipose tissues of ZDF rats and found that voluntary exercise did not affect it. Voluntary running is intuitively a more physiological exercise model, since the animals are not forcibly exercised through stressful means and are permitted to exercise at the intensity, duration, and frequency patterns that they choose during their normal awake time at night. Nonetheless, a careful study directly comparing the response of the HPA axis to voluntary wheel running and forced treadmill exercise training would be helpful.

Voluntary exercise preserves capacity of ZDF islets to compensate for IR. Insulin secretion to oral glucose was markedly enhanced in ZDF-A compared with both ZDF-I and ZL-I rats. This was confirmed in the isolated islet studies. GSIS in the presence or absence of fatty acids and expressed per islet was higher in ZDF-A islets compared with the ZL-I and ZDF-I islets. Although GSIS was similar at 8 mmol/l in ZDF-I and ZL-I islets, compensatory enhanced secretion as seen in ZDF-A islets was not evident in ZDF-I islets, and there was a complete failure of further enhancement of secretion at 16 mmol/l glucose in the absence or presence of fatty acids.

Although the results can only be considered preliminary, the effect of exercise to increase active GLP-1 levels in the ZDF-A compared with ZDF-I rats in this study could potentially be important in the mechanisms by which exercise preserves insulin secretion in ZDF rats.

Fig. 5. Glycerolipid (GL) cycling in ZDF rats is unresponsive to glucose, and it is not reversed by physical activity. **A**: fatty acid (FA) oxidation ( $n = 6$ ). **B** and **C**: accumulation of [ $^{14}\text{C}$ ]palmitate during FA esterification experiments into nonesterified fatty acid (NEFA) and total GLs (monoacylglycerol + diacylglycerol + triglyceride) ( $n = 7-10$ ). **D**: glycerol release, an indirect indicator of lipolysis ( $n = 5-6$ ). All results were normalized by protein. Two-way ANOVA, group effect  $P < 0.05$  (**B** and **C**). \* $P < 0.05$  and \*\*\* $P < 0.001$  vs. ZDF-A. # $P < 0.05$  and ### $P < 0.001$  vs. ZDF-I.



**ZDF islet failure or islet  $\beta$ -cell failure?** GSIS was expressed in three different ways, per islet, per islet protein content, and per islet total insulin content, as shown in Fig. 4, C-E. Almost all previous studies of GSIS in isolated islets of ZDF rats have expressed their results as insulin release per islet only (13, 14, 16, 24, 34, 47, 51, 52, 55). GSIS expressed per islet reflects both the effects of islet size and function on insulin secretion, and this was clearly enhanced in islets of the ZDF-A rats compared with the islets of both the ZL-I and ZDF-I rat groups. Secretion per islet, however, is not a good measure of individual islet  $\beta$ -cell function, particularly if islet size is very different between groups. Islet size was approximately fourfold greater in ZDF-I and ZDF-A islets compared with ZL-I islets. Correcting GSIS for islet protein content and for islet total insulin content should give closer approximations to individual  $\beta$ -cell function.

Correcting for islet protein content showed improved function in ZDF-A compared with ZDF-I islets, but not to the same level of GSIS of ZL-I islets. The islet protein content measurement, however, is a reflection of not only  $\beta$ -cells but all cells in the islet. Insulin is present only in  $\beta$ -cells and, for this reason, correcting for islet insulin content may be the best measure of individual  $\beta$ -cell function depending on whether insulin content per individual  $\beta$ -cell is equivalent across groups. Interestingly, correcting GSIS for islet total insulin content showed no deficiencies in either the ZDF-I or ZDF-A islets, and the ZDF-A islets were not better than the ZDF-I islets. Although not significant, there appeared to be a left shift in glucose dependency for insulin secretion in ZDF-I islets. Also, insulin secretion in response to KCl was greatest in the ZDF-I islets when insulin secretion was expressed in this way. Only one previous study reported insulin secretion based on the fraction of the islet insulin content released in ZDF rats (36). They observed enhanced basal secretion and reduced GSIS.

Altogether these results suggest that increased islet size plays a major role in islet compensation for IR in the ZDF-A rat, since GSIS per islet protein was still mildly impaired in ZDF-A islets. Unclear from this data, however, is whether

individual islet  $\beta$ -cell function is impaired in ZDF-I islets and whether this is altered by exercise. To determine this, it would be necessary to have accurate assessment of the proportion of  $\beta$ -cells per islet.

Morphological studies of sedentary ZDF rat islets have previously shown them to be enlarged with increased nonendocrine cells and fibrosis with reduced immunostaining for insulin (21, 22, 41). The latter appears to be a consequence of both reduced  $\beta$ -cells per islet area and reduced intensity of insulin staining. Furthermore, these features are reversed by exercise (21, 22, 41). The 86% reduction in islet insulin content in the current study is consistent with these findings and those of Zhou and colleagues (59) who showed that insulin content expressed per islet DNA in ZDF rats was only 30% of that in ZL rat islets. Thus, the poor GSIS of ZDF-I islets is likely to be contributed to by both reduced  $\beta$ -cells per islet and reduced individual  $\beta$ -cell insulin stores, and both are at least partly improved by exercise. This view of the ZDF rat model is of significance for diabetes research since it is widely used.

The finding that insulin secretion corrected for total insulin content is not impaired suggests that the main defect in ZDF rat islets is not its secretory machinery or glucose signaling per se. However, the failure of further enhancement of insulin secretion at the highest glucose concentration in the presence or absence of fatty acids suggests some defects in nutrient-secretion coupling mechanisms.

Unclear in the current data is whether the loss of insulin stores is an early pathogenic factor in the  $\beta$ -cell failure of ZDF-I rats or a consequence of progressive  $\beta$ -cell damage due to glucolipotoxic effects after early  $\beta$ -cell failure from other causes. In the ZF-Px rat model, we also showed marked loss of pancreatic insulin content when the rats were moderately hyperglycemic (11), such that the depletion of insulin stores is not an exclusive feature of the ZDF rat. It is generally accepted that  $\beta$ -cell degranulation is a common late pathogenic factor in T2D (56). In favor of depletion of insulin stores as being a more primary factor is a known insulin-promoter mutation



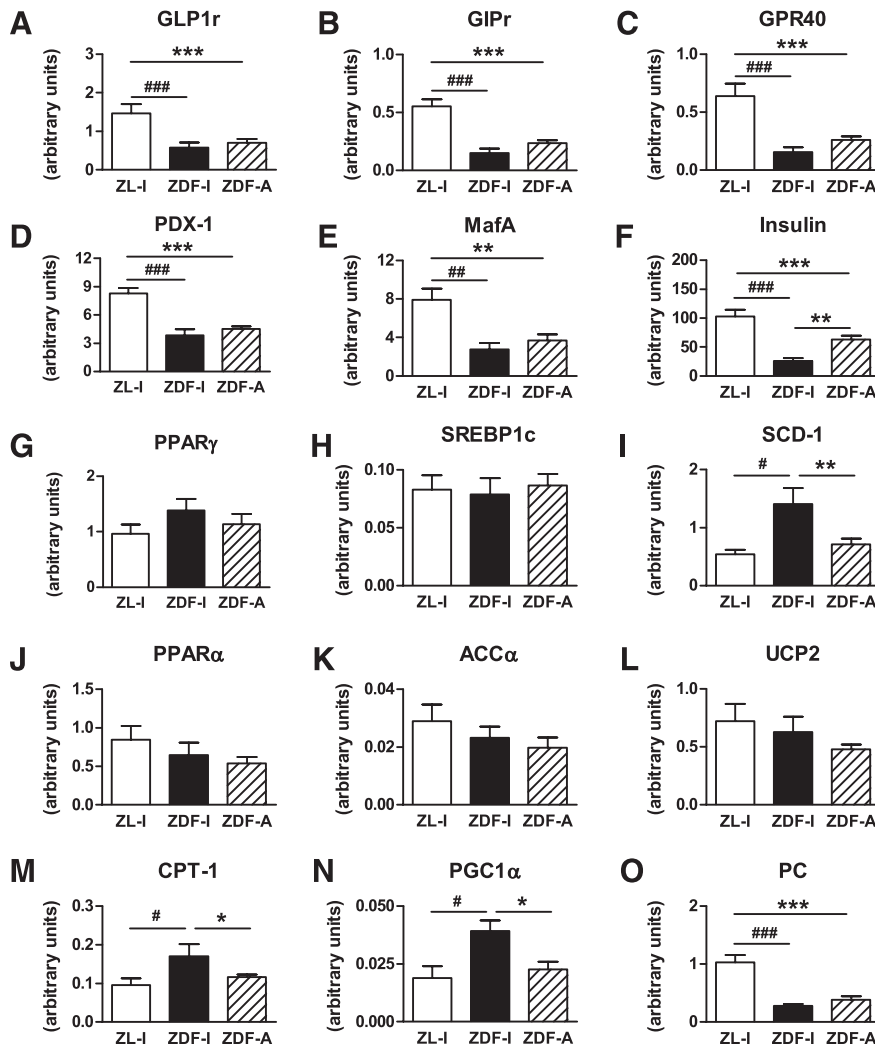


Fig. 6. The expression of key islet  $\beta$ -cell genes at the mRNA level was analyzed in freshly isolated islets by real-time RT-PCR. Results were normalized for the expression level of the housekeeping gene cyclophilin and expressed in arbitrary units;  $n = 5$ –11 rats/group. GLP1r, glucagon like peptide-1 receptor; GIPr, glucose-dependent insulinotropic polypeptide receptor; GPR40, G protein-coupled receptor 40; PDX-1, pancreatic and duodenal homeobox factor-1; MafA, musculoaponeurotic fibrosarcoma oncogene homolog A; PPAR $\gamma$ , peroxisome proliferator-activated receptor- $\gamma$ ; SREBP1c, sterol regulatory element binding protein 1c; SCD-1, stearoyl-CoA desaturase; PPAR $\alpha$ , peroxisome proliferator-activated receptor- $\alpha$ ; ACC $\alpha$ , acetyl-CoA carboxylase- $\alpha$ ; UCP2, uncoupling protein 2; CPT-1, carnitine palmitoyltransferase 1; PGC1 $\alpha$ , peroxisomal proliferator-activated receptor- $\gamma$  coactivator-1 $\alpha$ ; PC, pyruvate carboxylase. \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  vs. ZDF-A. # $P < 0.05$ , ## $P < 0.01$ , and ### $P < 0.001$  vs. ZDF-I.

specific to the ZDF rat (15), whereas both the ZDF and ZF animals show a mutated leptin receptor gene.

**Role of altered lipid partitioning and GL/FA cycling in ZDF rats.** Fatty acid oxidation at low glucose and the lipolysis arm of GL/FA cycling at high glucose, both proposed as mechanisms for cellular lipid detoxification (33), were reduced in ZDF-I compared with ZL-I rats. Furthermore, glucose responsiveness of both processes was lost. Importantly, these defects were also present in the ZDF-A rats, such that exercise did not prevent them. These defects in islet fatty acid partitioning, therefore, are not induced by hyperglycemia but appear as early defects in ZDF islets such that they could underlie islet susceptibility in this model. Consistent with these findings, we observed a lack of glucose regulation of islet fatty acid oxidation and lipolysis in ZDF rat islets at the diabetes transition phase (9 wk of age) (53). We also found defective glucose responsiveness of GL/FA cycling in islets of hyperglycemic diet-induced obese mice (C57Bl6) but enhanced GL/FA cycling and fat oxidation at elevated glucose in compensating ZF rat islets (32). Thus, glucose responsiveness of islet lipid partitioning pathways appears to be robust in the ZF rat (diabetes resistant) but poor in the ZDF rat strain and high-fat-fed C57Bl6 mice (both diabetes prone).

The reduced fatty acid oxidation at low and intermediate glucose concentrations and the reduced lipolysis at high glucose are consistent with the increased accumulation of intracellular NEFA and neutral esterified lipids in ZDF-I islets. A consequence is a greater pool of lipid signaling molecules, such as DAG, to cope with the increased demand of insulin secretion, but this dysregulation could also result in lipotoxicity. In particular, the lack of glucose-stimulated lipolysis in ZDF islets may contribute to an imbalance between fat esterification and lipolysis. The consequent glucolipotoxicity in this model could cause both a reduction in the expression of the insulin gene (40) as well as other key  $\beta$ -cell genes and lead to  $\beta$ -cell apoptosis.

**Physical activity and  $\beta$ -cell gene expression.** Reduced mRNA expression of MafA, PDX-1, insulin, GPR40, GLP1r, GIPr, and PC in islets from ZDF-I compared with islets from ZL-I rats suggests a state of moderate “dedifferentiation” (reduced expression of key genes that characterize a normal  $\beta$ -cell phenotype) of  $\beta$ -cells in diabetic rats. Surprisingly, except for the insulin gene, physical activity did not reverse these modifications, but this is not inconsistent with some mild impairment of  $\beta$ -cell function in ZDF-A islets compared with ZL-I islets when the analysis is corrected for protein. In



contrast, pancreatectomized female Sprague-Dawley rats fed a high-fat diet showed increased islet PDX-1 mRNA expression and improved  $\beta$ -cell function following 8 wk of exercise training (4). However, the ZDF rat does not seem to fit with the theory proposing that  $\beta$ -cell dedifferentiation plays a central causal role in  $\beta$ -cell dysfunction and failure (7, 17, 56). We speculate that the reduced expression of these genes is a marker of islet stress and to some degree has a protective role to prevent excessive insulin biosynthesis/secretion and associated ER stress (45) causing cell death. The enhanced expression of the SCD-1, CPT-1, and PGC-1 $\alpha$  genes in diabetic ZDF-I rat islets could be attempts by the  $\beta$ -cell to protect themselves in the face of hyperglycemia favoring  $\beta$ -cell lipodetoxification by simultaneously enhancing both fat oxidation (CPT-1 and PGC-1 $\alpha$ ) and diverting fatty acids to safe TG deposition (SCD-1) rather than to the accumulation of complex lipids such as ceramides that are more toxic to the cell (5, 31, 38). Fatty acid oxidation, however, was not increased in the hyperglycaemic ZDF-I rats, suggesting upregulation of these genes was not an effective compensation.

**Role of exercising muscle in  $\beta$ -cell protection.** A key question arises as to how physical activity preserves insulin stores and  $\beta$ -cell function. A possible explanation is that the over-nourished but physically active male ZDF-A rats avoided hyperglycemia by improving insulin sensitivity, as suggested by the diminutions in weekly plasma glucose and insulin concentrations. Direct demonstration of improved whole body insulin sensitivity in trained ZDF rats was shown by Pold et al. (41) using the hyperinsulinemic-euglycemic clamp technique. Alternatively, exercise can enhance glucose partitioning to exercising muscle by activating AMP-activated protein kinase (AMPK) in muscle, which is known to promote glucose disposal independently of insulin (2, 30). Because voluntary running ZDF rats spent  $\sim$ 300 min/night exercising, this would reduce the insulin required to compensate for IR, as well as favor lower glycemia. The finding of an increased marker of muscle oxidative capacity in ZDF-A rats is in keeping with a role for enhanced muscle glucose usage being important. With respect to the potential role of AMPK activation, it is interesting to note that forced treadmill running in ZDF rats led to upregulation of AMPK $\alpha_1$  subunit in red gastrocnemius (41) and that AICAR (58) and thiazolidinediones (50), both known to affect AMPK activity, also prevent diabetes in ZDF rats.

In conclusion, the ZDF rat, unlike the ZF rat, has islets that are susceptible to failure in the face of nutrient overload. The underlying  $\beta$ -cell susceptibility factor(s) in ZDF rats is unknown, but the results of this study provide evidence that dysregulation of  $\beta$ -cell lipid metabolism is an attractive possibility, since exercise could not reverse this and also because the defect is already apparent at the diabetes transition phase at 9 wk of age (53) and thus is independent of hyperglycemia. Another possibility is the mutation in the insulin promoter in ZDF rats (15) that combines with glucolipotoxicity-induced reduction in insulin gene expression (40). Severe depletion of islet insulin stores due to either loss of islet  $\beta$ -cells or reduced insulin content per  $\beta$ -cell, but most likely both, is a major contributor to insulin secretion failure in this rodent model of obesity-associated T2D, preventable by voluntary running.

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## DISCLOSURES

No conflicts of interest are declared by the authors.

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