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Am J Physiol Endocrinol Metab 301:E931-E940, 2011. First published 2 August 2011; doi:10.1152/ajpendo.00114.2011

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Short-term exercise training early in life restores deficits in pancreatic β-cell mass associated with growth restriction in adult male rats

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Submitted 7 March 2011; accepted in final form 29 July 2011

Laker RC, Gallo LA, Wlodek ME, Siebel AL, Wadley GD, McConell GK. Short-term exercise training early in life restores deficits in pancreatic β-cell mass associated with growth restriction in adult male rats. *Am J Physiol Endocrinol Metab* 301: E931–E940, 2011. First published August 2, 2011; doi:10.1152/ajpendo.00114.2011.—Fetal growth restriction is associated with reduced pancreatic β-cell mass, contributing to impaired glucose tolerance and diabetes. Exercise training increases β-cell mass in animals with diabetes and has long-lasting metabolic benefits in rodents and humans. We studied the effect of exercise training on islet and β-cell morphology and plasma insulin and glucose, following an intraperitoneal glucose tolerance test (IPGTT) in juvenile and adult male Wistar-Kyoto rats born small. Bilateral uterine vessel ligation performed on *day 18* of pregnancy resulted in Restricted offspring born small compared with sham-operated Controls and also sham-operated Reduced litter offspring that had their litter size reduced to five pups at birth. Restricted, Control, and Reduced litter offspring remained sedentary or underwent treadmill running from 5 to 9 or 20 to 24 wk of age. Early life exercise increased relative islet surface area and β-cell mass across all groups at 9 wk, partially restoring the 60–68% deficit ($P < 0.05$) in Restricted offspring. Remarkably, despite no further exercise training after 9 wk, β-cell mass was restored in Restricted at 24 wk, while sedentary littermates retained a 45% deficit ($P = 0.05$) in relative β-cell mass. Later exercise training also restored Restricted β-cell mass to Control levels. In conclusion, early life exercise training in rats born small restored β-cell mass in adulthood and may have beneficial consequences for later metabolic health and disease.

β-cell; fetal size; insulin secretion

LOW BIRTH WEIGHT IS ASSOCIATED with deficits in pancreatic morphology that contribute to impaired glucose tolerance and diabetes in adulthood (8, 16, 18, 39, 41, 43). In rat models of late-gestation uteroplacental insufficiency, pancreatic β-cell mass is reduced by up to 40% at birth (43) and 70% in adults (39, 41) and although often confounded by obesity, this is associated with a progressive decline in glucose tolerance (8, 24, 42). Studies in sheep have reported clear sex dimorphisms, showing that uteroplacentally restricted males, but not females, are incapable of increasing β-cell mass and insulin secretion in response to an age-related decline in insulin sensitivity (16, 32). Indeed, we have found that following cross-fostering, small-birth weight male, but not female, adult rats have impaired glucose tolerance and compromised β-cell mass and insulin secretion but normal insulin sensitivity in adulthood (39, 40).

Endurance exercise training in diabetic patients and rodent models of diabetes improves whole body insulin sensitivity and glucose tolerance, as well as yielding pancreatic-specific benefits (9, 14, 34, 35). Lowering of blood glucose, as occurs with exercise training, has been suggested to reduce glucotoxicity on pancreatic β-cells, allowing for improved function and regeneration. Indeed, treadmill exercise training in partially pancreatectomized young male rats is associated with increased insulin secretion along with increased β-cell mass (34, 35). As there is significant plasticity of the pancreas during early life (25, 38, 44, 46), this may represent a critical period that responds optimally to intervention. Indeed, only 3 wk of exercise training immediately after weaning in male rats prevented diet-induced obesity for up to 10 wk after exercise cessation (37). This sustained beneficial response was not evident when exercise training was initiated in adult rats (30), illustrating the beneficial effects of early life exercise and implicating the juvenile period as a critical window for interventions that could prevent later disease. Despite this, moderate daily exercise training has been shown to prevent obesity and normalize metabolic parameters in adult growth-restricted male rats in the short-term (29), and regular physical activity in elderly people born small prevents the development of impaired glucose tolerance (12). Together, these data suggest that later life exercise training can confer metabolic benefits, but given the diminished pancreatic plasticity with age (25, 38, 44, 46), the associated benefits are unlikely to be sustained upon cessation of training.

We hypothesized that exercise training during a period of rapid growth and pancreatic plasticity would improve β-cell mass in early life, which would be sustained into adulthood and provide functional metabolic benefits to offspring born small to offset the development of adult disease. A further objective was to establish whether exercise training during adulthood could confer acute pancreatic benefits, thus indicative of the potential benefit of later-life interventions. As an important control, we compared litters exposed to fetal growth restriction (termed Restricted) with sham-operated controls of normal litter size (termed Controls). We have previously shown that reducing litter size from sham-operated dams in an attempt to Control for the smaller litter size associated with bilateral uterine vessel ligation, impairs maternal mammary morphology, lactation, and subsequent postnatal growth of the pups (31). This altered postnatal growth contributes to nephron deficits, elevated blood pressure, and impaired skeletal muscle

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mitochondrial biogenesis, and it is thus not an appropriate Control group, but rather an additional experimental group (termed Reduced litter) (48, 51).

MATERIALS AND METHODS

Ethical approval. All experimental procedures were approved by The University of Melbourne Animal Experimentation Ethics Committee and were conducted in accordance with accepted standards of humane animal care.

Animal procedures. Wistar-Kyoto rats (9–13 wk; $n = 90$ total) were obtained from the Australian Resource Centre (Murdoch, Australia) and were provided with a 12:12 h light-dark cycle with access to standard rat chow and water ad libitum. On *day 18* of gestation, pregnant rats underwent bilateral uterine vessel ligation surgery under anesthesia with an intraperitoneal injection of ilium xylazil-20 (10 mg/kg) and ketamine (50 mg/kg) (Restricted group; $n = 30$). In humans, uteroplacental insufficiency develops throughout gestation; however, to maintain litter survival in the rat, bilateral uterine vessel ligation could not be induced until *day 18* of gestation and, therefore, mimics late-gestation uteroplacental insufficiency. It is important to note that before *day 18* of gestation, fetal and organ growth is normal, and only after bilateral uterine vessel ligation surgery late in gestation is fetal and organ growth impaired. Therefore, organs developing during late gestation and early postnatal growth are likely to be affected and may result in subtle differences in adult disease outcomes compared with humans exposed to uteroplacental insufficiency (31, 50, 52). Sham surgery was performed under identical conditions ($n = 60$), except the uterine vessels were not ligated to obtain Control offspring (31, 50, 52). At birth (*day 22*), half of the sham-operated mothers (litter size 10–14) had their litter size randomly reduced to five pups (Reduced litter) to match that of the Restricted litters (litter size = ~5) (31, 48, 51). Previous studies from our laboratory (39, 40) and others (16, 32) have shown that males are more adversely affected by fetal growth restriction than females, both in terms of metabolic and pancreatic outcomes. Therefore, only male offspring were investigated in this study. At weaning (35 days), male offspring from each of the three experimental groups (Control, Restricted, and Reduced litter) were allocated to remain sedentary with intraperitoneal glucose tolerance test (IPGTT) and post mortem (PM) at *week 9* or *24*, early exercise training (from 5 to 9 wk of age) with IPGTT and PM at *week 9* or *24*, or later exercise training (from 20 to 24 wk of age) with IPGTT and PM at *week 24* ($n = 8$–10 males/group across 15 groups; Fig. 1). Males ($n = 150$ total) from the same litter were allocated to different treatment groups, and up to three males were used from each litter. Body weights at *day 1* were taken as the litter average. Individual body weights were measured on *days 6* and *14* and *weeks 5*, *9*, *20*, and *24*.

Exercise training. Exercise training involved running on a motorized treadmill 5 days/wk for 4 wk (17) ($n = 8$–10 per group). Running duration progressively increased from 20 up to 60 min, with the treadmill speed set at 15 m/min for the first week and 20 m/min thereafter (17).

IPGTT. An IPGTT was performed after an overnight fast in all groups, and 48 h after the final exercise bout in those that trained. Blood samples (200 μl) were collected via tail slice 5 min before, and 5, 10, 20, 30, 45, 60, and 90 min after a 50% glucose injection (25 g in 50 ml ip; Pharmalab, Lane Cove, Australia; 1.0 g/kg body wt), as previously described (40). Plasma was stored at −80°C until further analysis.

Tissue collection. One day after the IPGTT, nonfasted rats were killed with an intraperitoneal injection of Ilium Xylazil-20 (30 mg/kg) and ketamine (225 mg/kg) (52). Whole pancreas was rapidly excised and weighed, and a representative portion (~1 cm^3) from the hepatic end was fixed in 10% neutral buffered formalin (NBF) for immunohistochemical analyses (16, 39).

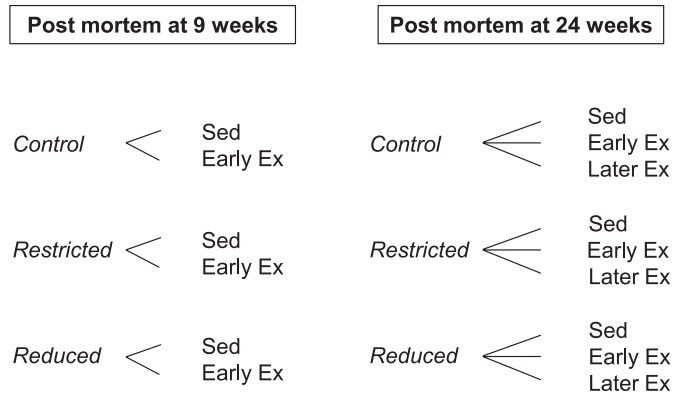

Fig. 1. Experimental design and timeline. Male offspring from each experimental group (Control, Restricted, and Reduced) were allocated to one of the following exercise treatments: *1*) Remained sedentary with postmortem at 9 wk, *2*) early exercise training from 5–9 wk of age with post mortem at 9 wk; *3*) remained sedentary with post mortem at 24 wk; *4*) early exercise training from 5–9 wk of age with post mortem at 24 wk; and *5*) later exercise training from 20–24 wk of age with post mortem at 24 wk. Ex, exercise; Sed, sedentary.

Plasma analysis. Plasma was deproteinized, neutralized, and stored at −80°C, as described previously (36, 40). Glucose concentration was measured using a scaled-down version of the enzymatic fluorometric analysis (36), with volumes reduced to be performed in a 96-well plate. Plasma insulin was measured in duplicate using a high-sensitivity RIA kit (Linco, Sydney Markets, Australia). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as an indication of basal insulin action, which is mostly influenced by hepatic insulin sensitivity. HOMA-IR was calculated by fasting plasma insulin (μU/ml) × fasting plasma glucose (mmol/l) ÷ 22.5 (26, 49). First-phase insulin secretion is indicative of the immediate pancreatic insulin secretory response to the glucose injection (20, 22) and was calculated as the incremental area under the insulin curve from 0 to 5 min (2, 40). Second-phase insulin secretion comprised the remainder of the insulin response and was calculated as the incremental area under the insulin curve from 5 to 90 min (40). Glucose area under the curve (AUC) was calculated as the total AUC from 0 to 90 min. The insulin-to-glucose ratio was calculated by dividing the total insulin AUC by the total glucose AUC.

Pancreatic islet, β-cell morphology, and immunohistochemistry. As previously described (16, 39), one section (10 μm) per pancreas was immunostained to identify and localize insulin-positive β-cells ($n = 5$–8 per group) using polyclonal guinea-pig anti-rat insulin as the primary antibody (1:200 dilution; A0564; DakoCytomation, Ely, UK) and peroxidase-conjugated anti-guinea-pig antibody (P0141; DakoCytomation) as the secondary (1:40 dilution) (K3466; DakoCytomation) with DAB solution, revealing insulin-positive β-cells. The same dilution of nonimmune serum from the same host animal, IgG fraction (X0903; DakoCytomation) was used to incubate negative controls, and a liver sample was immunostained to confirm specific staining. Sections were counterstained with hematoxylin that allowed for clear distinction between islet and exocrine tissue, and slides were code-blinded to remove potential bias.

Pancreatic islet number was expressed relative to total sectional area (per squared millimeter) with islet size arbitrarily classified as small (<5,000 μm^2), medium (5,000–10,000 μm^2), and large (>10,000 μm^2) (8, 39). Random-systematic sampling was then used to select 50 fields per section, and relative islet and β-cell volume density (V_d) was quantified by point-counting morphometry (700 points per field, V_d = number of intercepts on islet or insulin-positive cells as a proportion of intercepts on pancreas) (16, 39). Relative islet surface area was expressed as a percentage of pancreas surface area, and β-cell mass was determined by multiplying V_d and pancreas

weight (6, 39). Percent of islet occupied by β-cells was also calculated.

Terminal transferase-mediated dUTP-digoxigenin nick end-labeling staining was performed on sequential pancreas sections that had been immunostained for insulin at 24 wk. ApopTag kit (Millipore Australia, North Ryde, NSW) was used, which recommended that sections be treated with proteinase K (Qiagen Australia, Doncaster, VIC, Australia) prior to the addition of terminal transferase. Sections were deparaffinized and hydrated prior to blocking of endogenous peroxidase with hydrogen peroxide. Nonspecific binding was removed by washing in PBS before incubating with terminal deoxynucleotidyl transferase-enzyme complex and antidigoxigenin conjugate. Sections were reacted with 3,3′-diaminobenzidine (Sigma Chemical, St. Louis, MO) and counterstained with 0.5% methyl green before analysis by bright-field microscopy (Olympus BX series microscope). Postweaning (4 days) rat mammary tissue was used as a positive control (Millipore, Australia).

Statistical analyses. Two-way ANOVA was performed to determine main effects of experimental group and exercise treatment. Two-way ANOVA with repeated measures was performed for IPGTT analysis of plasma glucose and insulin over time with experimental group and exercise treatment defined as the independent variables. When a significant interaction was observed, one-way ANOVA with Student-Newman-Keuls post hoc or simple effects analyses were performed where appropriate. Data are presented as means ± SE, and $P < 0.05$ was considered statistically significant.

RESULTS

Body and pancreas weights. Bilateral uterine vessel ligation Reduced ($P < 0.05$) litter size (5.6 ± 0.5 vs. 8.2 ± 0.7 pups/litter) and body weight at postnatal *day 1* compared with sham surgery Controls (Table 1). At 35 days of age, the time of commencement of early exercise training, Restricted offspring remained lighter ($P < 0.05$) than Controls and Reduced litter offspring, and there were no differences within experimental groups between those allocated to remain sedentary and those allocated to perform early exercise training. Restricted offspring remained lighter ($P < 0.05$) than Controls until 20 wk (Table 1). Reduced litter offspring showed accelerated growth, such that they were heavier (+5%, $P < 0.05$) than Control and Restricted offspring from 20 wk (Table 1). At the commencement of later exercise training, at 20 wk of age, there were no differences in body weight within experimental groups between those allocated to perform later exercise, remain sedentary, and those that performed early exercise. Relative pancreas weight was not affected by uteroplacental insufficiency, reducing litter size (Table 1) or exercise treatment (data not shown). Body weight at 9 or 24 wk was not affected by early exercise training but was reduced ($P < 0.05$) by ~3.5% at 24 wk of age following later exercise, compared with sedentary offspring (data not shown).

Endocrine pancreas morphology. Relative islet surface area of pancreas and β-cell percent per islet in Controls were an average of 1.3% and 67%, respectively (Fig. 2, *A–D*), consistent with published data (1, 10, 39). Relative islet surface area in Restricted offspring was ~60% less ($P < 0.05$) than Controls at 9 wk of age (Fig. 2*A*), but was not different at 24 wk (Fig. 2*B*). Early life exercise increased ($P < 0.05$) relative islet surface area across all groups at 9 wk, being up to twofold higher in Restricted and Reduced litter offspring (Fig. 2*A*), but was not evident at 24 wk of age (Fig. 2*B*). The proportion of β-cells per islet was not different at 9 wk (Fig. 2*C*), but it was 11% higher ($P < 0.05$) in Reduced litter offspring at 24 wk (Fig. 2*D*). Total islet number was not different at either age (data not shown).

In parallel with the above-mentioned islet deficits at 9 wk of age relative (to body weight) pancreatic β-cell mass was up to 68% lower ($P < 0.05$) in Restricted compared with Control and Reduced litter offspring (Fig. 3*A*). At 9 wk, early exercise training was associated with increased ($P < 0.05$) relative β-cell mass in all groups compared with sedentary, with greater than twofold increases in the Restricted offspring (Fig. 3*A*). At 24 wk of age, relative β-cell mass tended to remain Reduced (−45%, $P = 0.05$) in sedentary Restricted compared with Controls (Fig. 3*B*). Very few apoptotic nuclei (<1 nuclei per islet) were detected at 24 wk, with no changes across groups (Control sedentary, 0.119 ± 0.044; Control early exercise, 0.186 ± 0.033; Control later exercise, 0.129 ± 0.041; Restricted sedentary, 0.119 ± 0.027; Restricted early exercise, 0.188 ± 0.029; Restricted later exercise, 0.114 ± 0.034). Although not statistically significant, exercise training in early life, 15 wk prior, normalized relative β-cell mass in Restricted offspring at 24 wk, reaching values comparable to sedentary Controls (Fig. 3*B*). Later life exercise training also restored β-cell mass in Restricted offspring to values comparable to sedentary Controls (Fig. 3*B*).

Plasma insulin and glucose, insulin sensitivity, and glucose tolerance assessment. Fasting plasma insulin was not different between experimental groups at 9 wk and was unaffected by exercise training (Table 2). At 24 wk, fasting insulin was higher ($P < 0.05$) in Restricted and Reduced litter offspring, with no effect of early or later exercise (Table 2). Fasting plasma glucose at 9 wk was lower ($P < 0.05$) in Restricted and Reduced litter offspring, yet higher ($P < 0.05$) in Restricted compared with Control offspring by 24 wk with no effect of exercise at either age (Table 2). HOMA-IR was not different

Table 1. *Effect of bilateral uterine vessel ligation on body mass and pancreas weight*

		Body Weight, g							Pancreas Weight, %body wt
		Day 1 Total	*Day 6* Males	*Day 14* Males	*Week 5* Males	*Week 9* Males	*Week 20* Males	*Week 24* Males	Post Mortem Males
9 wk	Con	4.11 ± 0.04^{B}	9.18 ± 0.19^{C}	22.05 ± 0.50^{B}	80.38 ± 1.22^{C}	197.47 ± 2.79^{B}			0.219 ± 0.012
	Rest	3.51 ± 0.06^{A}	6.48 ± 0.22^{A}	17.03 ± 0.63^{A}	66.78 ± 1.30^{A}	171.85 ± 4.24^{A}			0.228 ± 0.009
	Red	4.18 ± 0.04^{B}	8.53 ± 0.22^{B}	22.45 ± 0.40^{B}	75.94 ± 1.25^{B}	192.00 ± 2.52^{B}			0.214 ± 0.009
24 wk	Con	4.25 ± 0.04^{B}	8.82 ± 0.16^{B}	21.53 ± 0.34^{B}	79.49 ± 1.12^{B}	202.84 ± 2.29^{B}	360.35 ± 4.92^{A}	370.11 ± 4.01^{A}	0.148 ± 0.004
	Rest	3.52 ± 0.05^{A}	7.39 ± 0.26^{A}	19.34 ± 0.66^{A}	70.85 ± 2.11^{A}	184.62 ± 5.07^{A}	343.37 ± 5.48^{A}	358.77 ± 5.73^{A}	0.166 ± 0.012
	Red	4.33 ± 0.04^{B}	8.78 ± 0.15^{B}	23.26 ± 0.37^{C}	80.15 ± 1.19^{B}	202.27 ± 3.28^{B}	373.29 ± 3.48^{B}	386.78 ± 3.62^{B}	0.143 ± 0.003

Data presented as mean ± SE ($n = 10-12$/group). ^{A,B,C}Significant differences ($P < 0.05$) between groups, within an age, are denoted by letters that differ, such that A is different from B but not different from AB. Con, Control; Rest, Restricted; Red, Reduced litter.

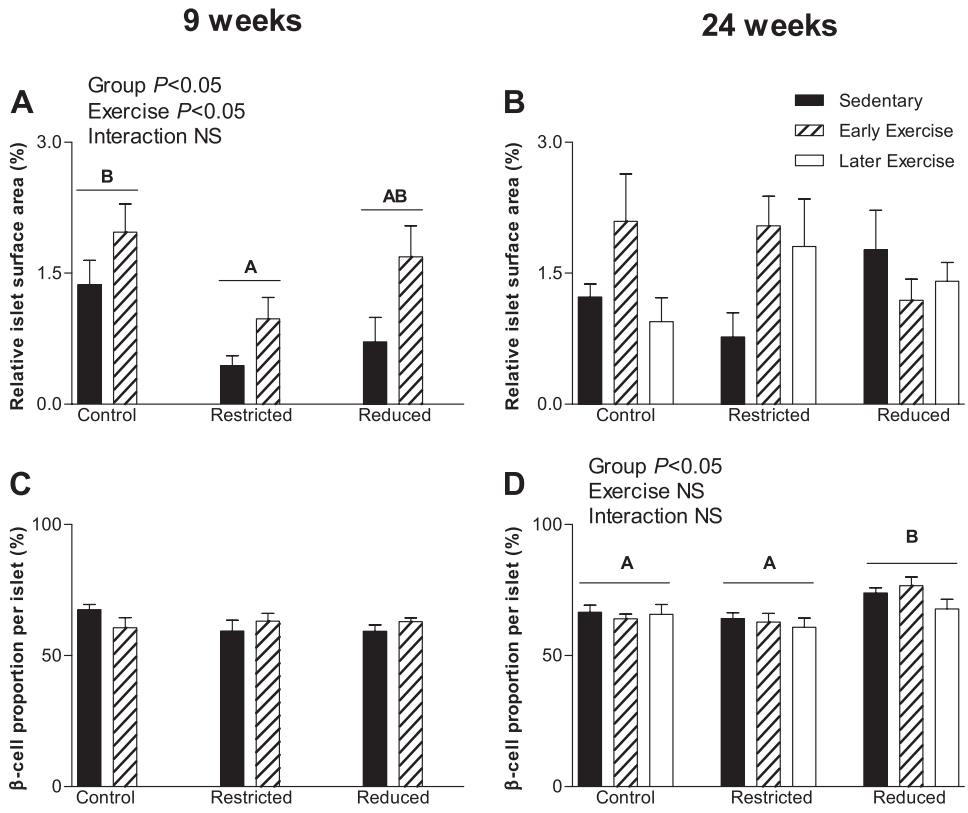

Fig. 2. Relative islet surface area at 9 (*A*) and 24 (*B*) wk and β-cell proportion per islet at 9 (*C*) and 24 (*D*) wk. Filled, hatched, and open bars indicate sedentary, early exercise, and later exercise, respectively. Data are presented as means ± SE (n = 5–8 per group). Different letters denote main effect $P < 0.05$ between groups (A is different from B but not different from AB).

between sedentary groups at 9 wk but was reduced ($P < 0.05$) across all groups following early exercise training (Table 2). At 24 wk, HOMA-IR was higher ($P < 0.05$) in Restricted and Reduced litter offspring, with no effect of exercise training (Table 2). IPGTT plasma glucose AUC was not different between groups at either age, regardless of exercise training (Table 2). The ratio of IPGTT insulin:glucose AUC at 9 wk was higher ($P < 0.05$) in sedentary Reduced litter offspring compared with Controls and remained higher at 24 wk ($P < 0.05$) compared with both Control and Restricted groups with no effect of exercise (Table 2).

First-phase insulin secretion, represented as AUC from basal to 5 min after the glucose bolus, is an indication of β-cell response to glucose during IPGTT. This was not different between experimental or exercise groups at 9 wk (Fig. 4*A*). Second-phase insulin secretion (AUC 5–90 min after glucose bolus), an indirect measure of peripheral insulin sensitivity, was higher ($P < 0.05$) in sedentary Reduced litter offspring compared with sedentary Controls at 9 wk and early exercise training increased ($P < 0.05$) second-phase insulin secretion in Controls (Fig. 4*C*). Although not significant, at 24 wk of age, we observed ~75% reduction in first-phase insulin secretion in sedentary Restricted offspring compared with sedentary Controls (Fig. 4*B*).

At 9 wk, there were no differences in the IPGTT plasma glucose or insulin profile within an exercise treatment (Fig. 5) or within an experimental group (Fig. 5). At 24 wk, plasma glucose was elevated ($P < 0.05$) in sedentary Restricted offspring compared with sedentary Controls and Reduced litter (Fig. 6*A*) but was not different within early or later exercised groups (Fig. 6, *C* and *E*, respectively). However, there were no differences in glucose tolerance between sedentary, early exercised, and later exercised Restricted offspring (Fig. 6). Furthermore, exercise training had no effect on plasma glucose levels during IPGTT in Controls or Reduced litter (Fig. 6). Plasma insulin levels were higher ($P < 0.05$) in Reduced litter compared with Control and Restricted rats that remained sedentary (Fig. 6*B*) but not different in early or later exercise groups (Fig. 6, *D* and *F*). Insulin sensitivity improvements with later exercise training appeared to have been less in Restricted and Reduced litter compared with Controls over the last hour of the IPGTT (Fig. 6*F*). This was indicated by lower plasma insulin at 45 ($P = 0.05$) and 60 ($P < 0.05$) min in later exercised Control rats compared with sedentary and early exercise Controls (Fig. 6, *B*, *D*, and *F*). In addition, exercise training had no overall effect on plasma insulin in Restricted or Reduced litter offspring at 24 wk.

DISCUSSION

This study has shown that a short period of exercise training early in life (from 5 to 9 wk of age) in Restricted male rats can prevent compromised pancreatic β-cell mass later in life (24 wk). This is remarkable considering that the rats remained sedentary between 9 and 24 wk of age. Increased pancreatic β-cell mass with exercise training was also evident in Controls, suggesting that Restricted offspring have intact adaptive responses to exercise training. Despite the substantial morphological improvement, there were no detectable changes in glucose-stimulated first-phase insulin secretion. Interestingly, exercise training from 20 to 24 wk of age in Restricted offspring also allowed β-cell mass to reach levels comparable to Controls. This highlights sustained plasticity of the pancreas in later life, particularly in the face of a compromised β-cell mass.

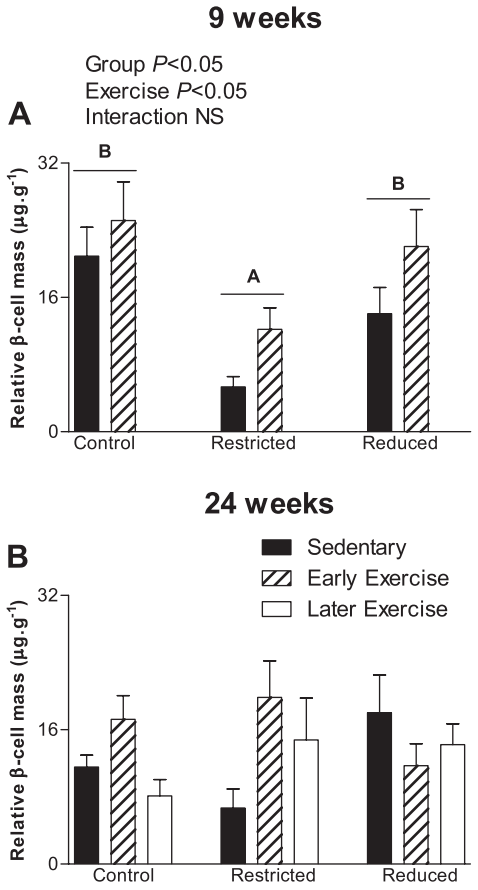

Fig. 3. Relative β-cell mass at 9 (*A*) and 24 (*B*) wk. Filled, hatched and open bars indicate sedentary, early exercise, and later exercise, respectively. Data are presented as means ± SE (n = 5–8 per group). [A,B]Letters that differ denote main effect $P < 0.05$ between groups (A is different from B but not different from AB).

Effect of small birth weight (Restricted) or altered postnatal growth (Reduced litter). Restricted offspring had severely reduced islet surface area and β-cell mass at 9 wk of age and although β-cell mass is generally positively correlated with insulin secretion (1, 23, 27), in the current study, these morphological deficits were not associated with altered first-phase insulin response. This may reflect a functional β-cell reserve that can adequately cater for, what might be, only a modest glucose demand. However, any intrinsic β-cell compensation at 9 wk of age appears to have been exhausted by 24 wk, given the trend toward reduced first-phase insulin secretion by up to 75% in sedentary Restricted offspring when compared with Controls. It is worth noting that islet, and by inference, β-cell densities are not uniformly distributed throughout the pancreas, and analyses of one section per animal may pose a limitation to the study design. Importantly however, the hepatic end of the pancreas was chosen for analyses in all animals, such that differences observed are unlikely to be confounded by variations in islet or β-cell distribution.

Reduced β-cell mass in Restricted offspring at 9 wk tended to persist at 24 wk, with a comparable pattern seen in relative islet surface area. Since β-cell proportion per islet was normal, this suggests that total endocrine mass, and not solely the β-cell, is vulnerable to suboptimal conditions in utero. Thus, despite pancreatic β-cells comprising up to 80% of total islet mass, whole islet mechanisms may be involved in the pathogenesis of diabetes associated with growth restriction. In particular, any effect of glucagon-producing α-cells that work synergistically with β-cells to Control blood glucose should be considered in future studies to confirm this assertion. These β-cell mass reductions appear to be independent of changes in the relative rates of apoptosis at 24 wk; however, we cannot exclude a proliferative and/or perinatal mechanism that is no longer detected in later life.

The morphological deficits observed in Restricted offspring at 9 and 24 wk of age may be associated with altered expression of key genes and proteins important for maintenance of β-cell mass. Reduced *Pdx1* mRNA expression for example, has consistently been reported in adult rats born small and associated with epigenetic modifications established around birth (8, 33). Furthermore, altered expression of GLUT2, glucokinase, VEGF-A, and those involved in insulin/IGF-1

Table 2. *Effect of bilateral uterine vessel ligation and exercise training on fasting plasma insulin, fasting plasma glucose, fasting HOMA-IR, and plasma glucose AUC during IPGTT and plasma insulin:glucose AUC during IPGTT*

Age	Group	Exercise	Fasting Insulin, ng/ml	Fasting Glucose, mmol/l	HOMA-IR	Glucose AUC During IPGTT	Ins:Glu AUC During IPGTT
9 wk	Con	Sed	0.46 ± 0.11	7.25 ± 0.33[B]	3.7 ± 0.96	660.66 ± 70.55	0.08 ± 0.03[a]
		EarlyEx	0.20 ± 0.05	7.17 ± 0.35	1.57 ± 0.42*	706.30 ± 82.06	0.10 ± 0.02
	Rest	Sed	0.29 ± 0.07	6.57 ± 0.31[A]	2.18 ± 0.56	670.07 ± 91.75	0.10 ± 0.02[ab]
		EarlyEx	0.22 ± 0.05	6.32 ± 0.27	1.60 ± 0.39*	704.65 ± 79.66	0.09 ± 0.02
	Red	Sed	0.21 ± 0.04	6.03 ± 0.35[A]	1.44 ± 0.36	713.37 ± 30.08	0.12 ± 0.02[b]
		EarlyEx	0.24 ± 0.05	5.72 ± 0.33	1.46 ± 0.35*	662.90 ± 61.60	0.11 ± 0.03
24 wk	Con	Sed	0.58 ± 0.10[A]	7.73 ± 0.18	4.84 ± 0.92	967.02 ± 84.54	0.05 ± 0.01
		EarlyEx	0.58 ± 0.10	7.46 ± 0.47[A]	4.15 ± 0.90[A]	1108.44 ± 121.18	0.05 ± 0.01[A]
		LaterEx	0.63 ± 0.16	8.45 ± 0.37	5.79 ± 1.69	910.30 ± 148.69	0.05 ± 0.02
	Rest	Sed	0.83 ± 0.11[B]	8.27 ± 0.27[B]	7.42 ± 1.11[B]	1144.31 ± 84.87	0.04 ± 0.01[A]
		EarlyEx	1.11 ± 0.24	9.40 ± 0.68	12.40 ± 3.28	1003.56 ± 54.76	0.04 ± 0.01
		LaterEx	0.95 ± 0.20	8.55 ± 0.42	9.20 ± 2.12	894.74 ± 118.49	0.07 ± 0.02
	Red	Sed	1.43 ± 0.28[B]	8.62 ± 0.54[AB]	12.75 ± 2.87[B]	822.09 ± 70.16	0.12 ± 0.04[B]
		EarlyEx	0.67 ± 0.13	7.83 ± 0.23	5.67 ± 1.20	876.86 ± 95.03	0.06 ± 0.01
		LaterEx	0.92 ± 0.20	8.05 ± 0.35	8.21 ± 2.00	875.95 ± 75.07	0.04 ± 0.01

Data are means ± SE; n = 8–10/group. [A,B]Letters that differ denote main effect, $P < 0.05$, between groups (A is different from B but not from AB). *Significant difference of main effect, $P < 0.05$, between exercise treatments. [a,b]Letters that differ denote significant differences, $P < 0.05$, between groups within exercise treatment (a is different from b but not from ab) following observation of significant ($P < 0.05$) interaction. HOMA-IR, homeostasis model of assessment of insulin resistance; AUC, area under the curve; IPGTT, intraperitoneal glucose tolerance test; Sed, sedentary; ex, exercise.

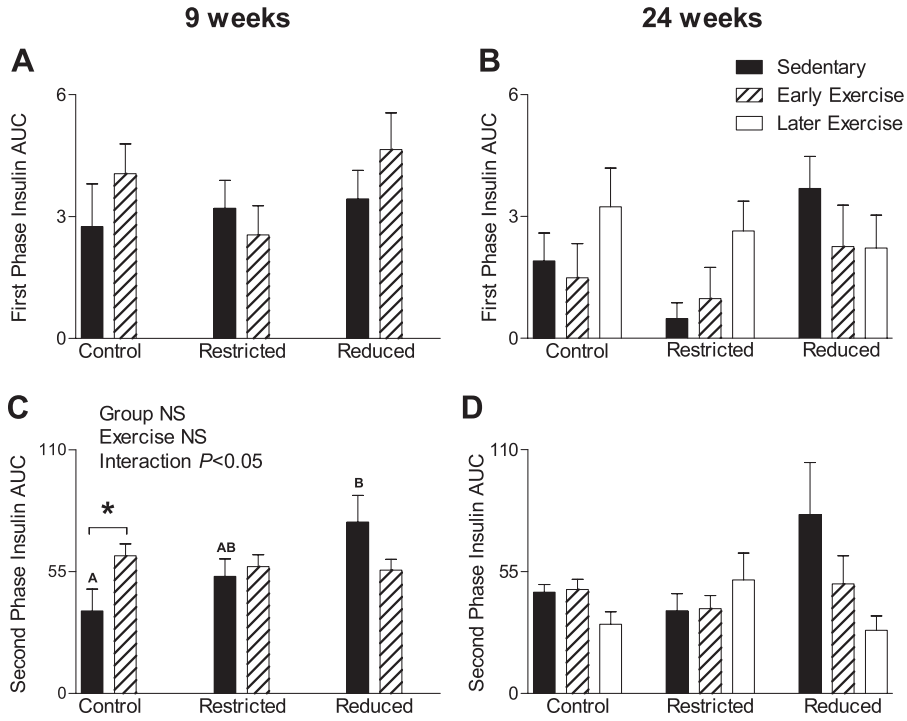

Fig. 4. First- and second-phase area under the insulin curve (AUC) during intraperitoneal glucose tolerance test at 9 (*A* and *C*, respectively) and 24 wk (*B* and *D*, respectively). Filled, hatched, and open bars indicate sedentary, early exercise, and later exercise, respectively. Data are presented as means ± SE (n = 8–10 per group). ^{A,B}Different letters above individual bars denote differences $P < 0.05$ between groups within exercise treatments (A is different from B but not different from AB) following observation of significant $P < 0.05$ interaction. *Significant difference, $P < 0.05$; Control early exercise vs. sedentary following observation of significant $P < 0.05$ interaction.

signaling have been reported in a number of low-birth weight models (16, 19), and are likely to contribute to the reduction in β-cell mass presently observed. Such mechanistic analyses should be a key focus of future work in this field, where pancreatic islets have indeed been isolated.

Restricted offspring had higher fasting insulin at 24 wk compared with Controls, contributing to a significantly higher HOMA-IR, usually suggestive of hepatic insulin resistance (45). Indeed, others have shown that rats exposed to bilateral uterine vessel ligation have a higher rate of basal hepatic

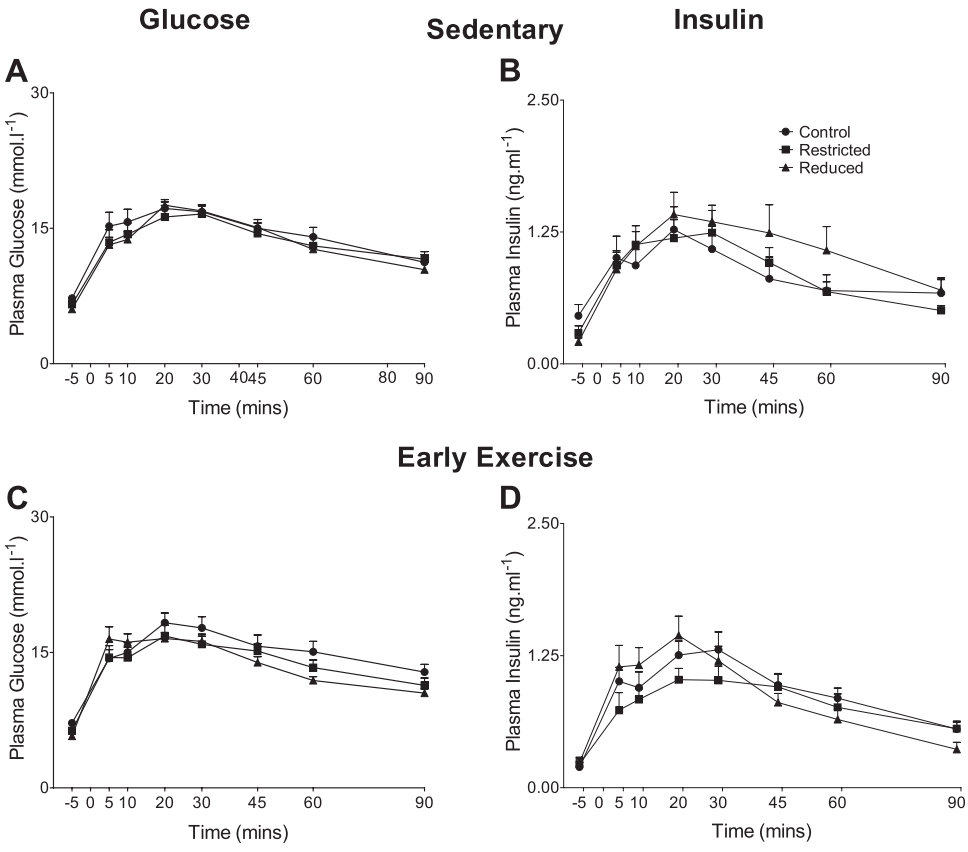

Fig. 5. Plasma glucose (*left*) and insulin (*right*) in response to a glucose load at 9 wk in groups that remained sedentary (*A* and *B*) or performed early exercise training (*C* and *D*). Circles, squares, and triangles represent Control, Restricted, and Reduced litter, respectively. Data are presented as means ± SE (n = 8–10 per group).

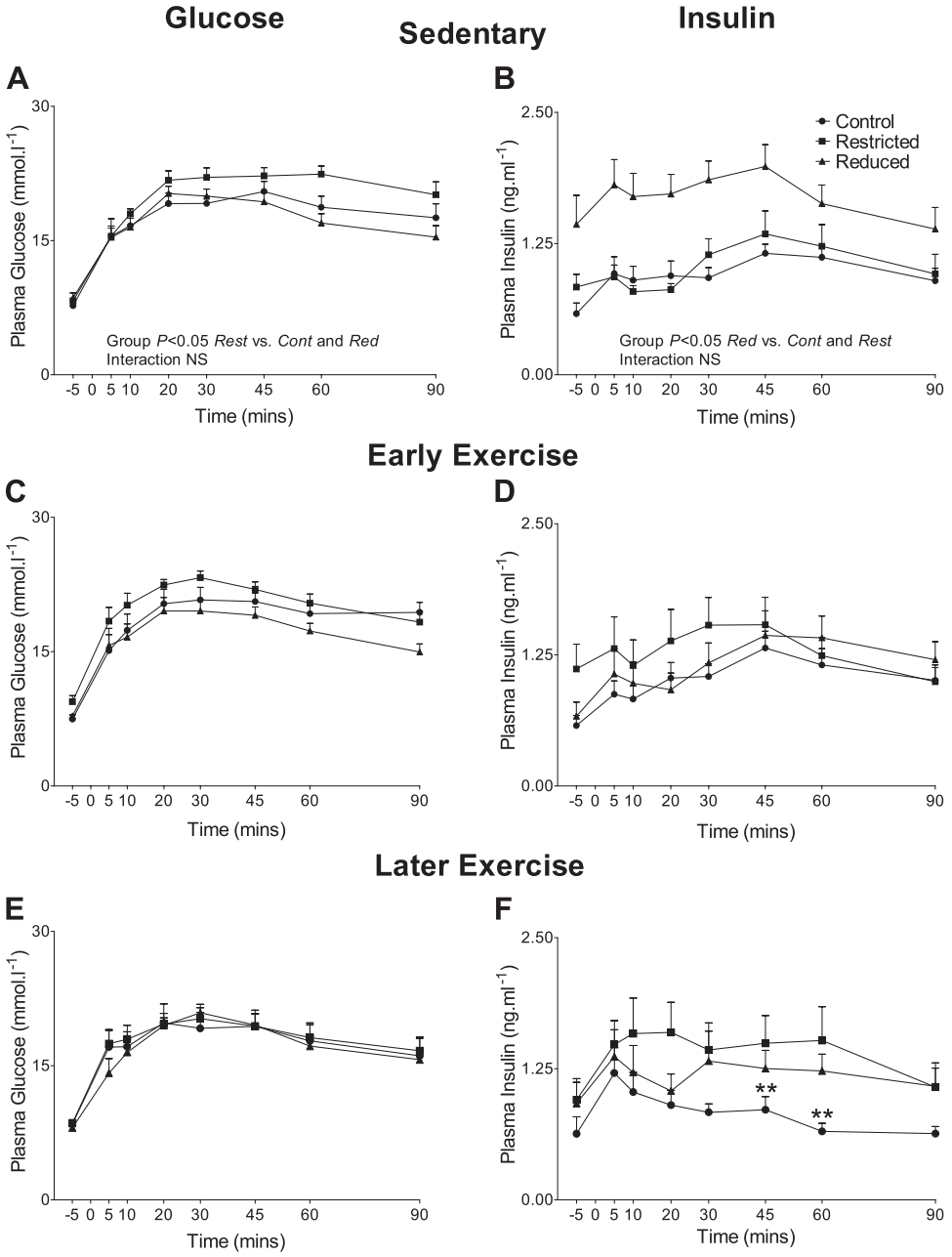

Fig. 6. Plasma glucose (*left*) and insulin (*right*) in response to a glucose load at 24 wk in groups that remained sedentary (*A* and *B*), performed early exercise training (*C* and *D*) or later exercise training (*E* and *F*). Circles, squares, and triangles represent Control, Restricted, and Reduced litter, respectively. Data are presented as means ± SE (n = 8–10 per group). $^{**}P < 0.05$ for Control later exercise (*F*) vs. sedentary (*B*), and early exercise (*D*) following observation of significant $P < 0.05$ interaction.

glucose production and a blunted hepatic response to insulin (47). Although the sedentary Restricted offspring also displayed elevated plasma glucose during IPGTT compared with sedentary Control and Reduced litter offspring, this metabolic phenotype is considerably less severe than that reported in growth-Restricted offspring by Simmons et al. (41). In this previous study, male offspring exposed to uteroplacental insufficiency had overt fasting hyperglycemia and glucose intolerance as early as 7 wk of age. These differences likely arise because of the different rat strain used by Simmons et al. and the metabolic effects of obesity in their model. Simmons *et al.* (41) used Sprague-Dawley rats, which are more prone to developing obesity and associated insulin resistance at a relatively young age (4 mo) on a normal-chow diet (4, 5).

The current study also identified an impaired metabolic phenotype in Reduced litter offspring. Indeed, second-phase insulin secretion was elevated ($P < 0.05$) at 9 wk of age, along with higher fasting insulin ($P = 0.05$), HOMA-IR ($P < 0.05$) and insulin:glucose AUC ($P < 0.05$) at 24 wk of age. Therefore, it appeared that Reduced litter offspring had more severe insulin resistance than Restricted offspring. These metabolic deficits were observed in association with late accelerated growth, which, in humans, has been shown to independently predict later metabolic disease in adulthood (11). Perhaps because of the timing and degree of the postnatal growth restriction, the Reduced litter offspring did not present with pancreatic deficits at 9 wk of age. However, the greater body weight at 24 wk in this group is likely to have contributed to the increased β-cell proportion per islet at this later age.

Effect of short-term exercise training early in life. Early-life exercise training enhanced pancreatic morphology across all groups at 9 wk of age without altering insulin secretion.

Although the literature describing how the pancreas responds to exercise is limited, our findings indicate that Restricted offspring are able to elicit a "normal" response to exercise training with similar fold increases in β-cell mass as Controls at 9 wk of age. Remarkably, in Restricted offspring that exercised early, relative islet surface area and β-cell mass reached values comparable to Controls at 24 wk, despite the rats remaining sedentary from 9 wk of age. Similar to the effects of early exercise at 9 wk, the improved β-cell mass in Restricted offspring at 24 wk was not accompanied by functional benefits as measured by first-phase insulin secretion. However, the elevated plasma glucose during IPGTT in sedentary Restricted offspring compared with Control and Reduced litter, was not evident following early or later exercise and may represent improved glucose handling in these animals. Importantly, this was a subtle effect that was not evident when compared between sedentary, early, and later exercised Restricted offspring. It is possible, however, that morphological benefits associated with early exercise training may translate into greater functional benefits when the demand for insulin is increased. This is likely because insulin secretion is generally sufficient, even following the loss of ~50% of β-cell mass, unless there is insulin resistance present [e.g., due to aging (15, 44) and/or a high-fat diet (28)], which places additional strain on the pancreas to augment insulin secretion. In Wistar rats, impaired insulin-stimulated glucose utilization becomes apparent by 8 mo of age (13). Therefore, future studies may identify functional benefits, following early life exercise training when the demand for insulin is greater; that is in old age and/or following exposure to high-fat diets. This is particularly relevant given the aging population and high levels of fat intake evident in Western populations.

In previous studies using high-fat fed, 90% pancreatectomized rat models, exercise training decreased pancreatic apoptosis (34, 35). In the current study of a more modest pancreatic insult, we found no such changes in the number of apoptotic nuclei within islets, but given that Restricted offspring did not present with increased apoptosis in the sedentary state, it is not surprising that exercise training had no effect. Furthermore, previous studies have reported augmented insulin/IGF-1, IRS-2, Akt, and Pdx1 signaling immediately after exercise (34, 35). Whether these mechanisms mediate our findings of chronic and long-term morphological benefits of exercise training on β-cell mass has yet to be determined. It may also be plausible to investigate whether certain factors released from the skeletal muscle during exercise can target pancreatic tissue and mediate increases in β-cell mass. One possible candidate is IL-6, which is released from contracting skeletal muscle and can result in ~30-fold increases in plasma IL-6 concentration during exercise (7). Indeed, human IL-6 stimulates insulin secretion in isolated Lewis rat islets, while any role for morphological benefits remains to be determined (7).

Importantly, our observations of improved islet morphology with early exercise were independent of changes in absolute and relative pancreas weight, as well as body mass, suggesting a "reprogramming" effect, rather than a secondary effect of weight changes. Previous studies have reported other metabolic benefits of early life exercise, including the prevention of diet-induced obesity in small birth weight rats 10 wk after exercise training cessation (37). Furthermore, in humans, children in the highest quartile of physical activity at age 5 had lower fat mass at 8 and 11 years of age than those in the lowest quartile of activity, even if they were sedentary from 5 to 8–11 years of age (21).

Effect of short-term exercise training later in life. Restricted offspring that performed later-life exercise training demonstrated a restoration of β-cell mass to values comparable to Controls. This was accompanied by a more than five-fold increase in first-phase insulin secretion. Indeed, studies have reported enhanced metabolic flexibility following exercise in those born small compared with normal-birth weight Controls (29), suggesting that an increased demand for insulin allows for a larger than normal response to interventions. Furthermore, regular moderate-intensity exercise training in elderly people who were born small has been shown to protect against impaired glucose tolerance (12), regardless of early life physical activity. Exercise training in later life may, in fact, delay an age-dependent decline in pancreatic plasticity (25, 38, 44, 46). Later exercise training provided functional benefits for Control offspring, with a tendency for lower second-phase insulin secretion, as well as lower plasma insulin at 45 and 60 min of IPGTT, suggesting improved insulin sensitivity. It is also worth noting that later life exercise training was associated with an approximate 3.5% reduction in body weight across all groups. In addition, relative exercise intensity was likely different between the adult and juvenile rats because of body weight and limb length differences, which would impact on weight bearing and stride length. It is possible that adult rats have the capacity to perform a higher intensity of exercise, and therefore, this study may have underestimated the potential effects of exercise training at this age.

On the basis of the plasma insulin levels during the IPGTT in groups that performed later exercise, it is apparent that Restricted and Reduced litter offspring retained some level of peripheral insulin resistance. It is clear that plasma insulin profiles in Restricted and Reduced litter offspring did not return to Control levels 90 min after the IPGTT, despite this being observed in trained pancreatectomized rats (14). These observations may reflect a defect in skeletal muscle insulin signaling in response to exercise training, which will be examined in subsequent studies. Nevertheless, we report some metabolic benefit of later exercise training in all experimental groups and suggest that short-term exercise training, perhaps no matter the age, can improve the adult metabolic phenotype in growth-Restricted offspring. Whether later exercise training is associated with lasting benefits, as was observed with early exercise training, remains to be determined.

Conclusion. Four weeks of exercise training early in life was associated with significant increases in β-cell mass in all groups at 9 wk and, remarkably, this early exercise restored β-cell mass to Control levels in Restricted offspring at 24 wk. This was despite the cessation of exercise training at 9 wk and was in the absence of overt functional benefits. Later life exercise training also restored the tendency for reduced pancreatic β-cell mass in Restricted offspring and was associated with a greater than five-fold increase in first-phase insulin secretion. Because early exercise normalized the reduced β-cell mass at 24 wk, but had little functional consequences on glucose and insulin metabolism, future studies involving older growth-restricted rats exposed to high-fat feeding, with relevance to an aging Western population, may reveal a more severely compromised pancreatic and metabolic phenotype

that may be ameliorated by early exercise training. While the definitive mechanisms remain to be elucidated, exercise training may reprogram the endocrine pancreas in those born small to help prevent the later development of metabolic disease.

ACKNOWLEDGMENTS

We thank Gwyneth Ng and Sarah Heywood for assistance with exercise training, as well as Andrew Jefferies and Kerryn Westcott for assistance with animal surgery and handling.

GRANTS

This research was supported by a grant from the National Health and Medical Research Council of Australia (NHMRC; Grant 454570). R. C. Laker was supported by Sheppard M. Lowe Scholarship (Faculty of Medicine, Dentistry and Health Science, The University of Melbourne; 2007) and cofunded NHMRC/NHF Biomedical Postgraduate Scholarship (2008–2010). A. L. Siebel was supported by a NHMRC Peter Doherty Biomedical Research Fellowship.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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