Exercise early in life in rats born small does not normalize reductions in skeletal muscle PGC-1 α in adulthood

Rhianna C. Laker, Mary E. Wlodek, Glenn D. Wadley, Linda A. Gallo, Peter J. Meikle and Glenn K. McConell

Exercise early in life in rats born small does not normalize reductions in skeletal muscle PGC-1α in adulthood

Rhianna C. Laker,[1] Mary E. Wlodek,[1] Glenn D. Wadley,[1,2] Linda A. Gallo,[1] Peter J. Meikle,[3] and Glenn K. McConell[1,4]

[1]*Department of Physiology, The University of Melbourne, Parkville, Victoria;* [2]*Centre for Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria;* [3]*Baker IDI Heart and Diabetes Institute, Melbourne, Victoria; and* [4]*Institute of Sport, Exercise and Active Living and the School of Biomedical and Health Sciences, Victoria University, Victoria, Australia*

Laker RC, Wlodek ME, Wadley GD, Gallo LA, Meikle PJ, McConell GK. Exercise early in life in rats born small does not normalize reductions in skeletal muscle PGC-1α in adulthood. *Am J Physiol Endocrinol Metab* 302: E1221–E1230, 2012. First published February 21, 2012; doi:10.1152/ajpendo.00583.2011.—We have previously shown that 4 wk of exercise training early in life normalizes the otherwise greatly reduced pancreatic β-cell mass in adult male rats born small. The aim of the current study was to determine whether a similar normalization in adulthood of reduced skeletal muscle mitochondrial biogenesis markers and alterations in skeletal muscle lipids of growth-restricted male rats occurs following early exercise training. Bilateral uterine vessel ligation performed on *day 18* of gestation resulted in Restricted offspring born small ($P < 0.05$) compared with both sham-operated Controls and a sham-operated Reduced litter group. Offspring remained sedentary or underwent treadmill running from 5–9 (early exercise) or 20–24 (later exercise) wk of age. At 24 wk of age, Restricted and Reduced litter offspring had lower ($P < 0.05$) skeletal muscle peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) protein expression compared with Control offspring. Early exercise training had the expected effect of increasing skeletal muscle markers of mitochondrial biogenesis, but, at this early age (9 wk), there was no deficit in Restricted and Reduced litter skeletal muscle mitochondrial biogenesis. Unlike our previous observations in pancreatic β-cell mass, there was no "reprogramming" effect of early exercise on adult skeletal muscle such that PGC-1α was lower in adult Restricted and Reduced litter offspring irrespective of exercise training. Later exercise training increased mitochondrial biogenesis in all groups. In conclusion, although the response to exercise training remains intact, early exercise training in rats born small does not have a reprogramming effect to prevent deficits in skeletal muscle markers of mitochondrial biogenesis in adulthood.

fetal programming; mitochondrial biogenesis

LOW BIRTH WEIGHT IN HUMANS is associated with skeletal muscle insulin resistance in early adulthood (29) as well as an increased propensity for developing type 2 diabetes later in life (3, 16, 20, 22). Skeletal muscle is the major site for insulin-stimulated glucose disposal and as a consequence is also largely responsible for the development of insulin resistance in type 2 diabetes (13). The skeletal muscle deficits underlying this insulin resistance are poorly characterized; however, there is evidence to suggest that increased intramuscular lipids (26, 27, 31, 48, 51, 59) and elevated reactive oxygen species

production (23) may be contributing factors. Lipid accumulation in the skeletal muscle may interfere with the insulin-signaling pathway via serine phosphorylation of insulin receptor substrate 1 (IRS-1), which then inhibits IRS-1 tyrosine phosphorylation and the subsequent downstream insulin-signaling pathway (39). Mitochondria are the primary controllers of cellular energy metabolism and are responsible for the oxidation of fats within the cell. Reduced skeletal muscle gene expression of key regulators of mitochondrial biogenesis, including the master regulator peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α), has been reported in humans with insulin resistance and type 2 diabetes (38, 46).

Selak and colleagues (53) reported that uteroplacental insufficiency and fetal growth restriction in the rat resulted in adult insulin resistance and diabetes. Furthermore, small-birth-weight offspring showed impaired oxidative phosphorylation capacity from isolated skeletal muscle mitochondria. Subsequently, our laboratory has reported marked reductions of both gene and protein expression of mitochondrial biogenesis markers PGC-1α, cytochrome *c* oxidase subunit III and IV (COX IV), and transcription factor A mitochondrial (Tfam) in the skeletal muscle of male, but not female, 6-mo-old rat offspring exposed to uteroplacental insufficiency and consequently born small (62).

We also reported that normal-birth-weight male offspring that had their litter size randomly reduced to five pups at birth (Reduced litter group), to match that of growth-restricted litters (4–6 pups/litter), showed lower skeletal muscle mitochondrial biogenesis markers and elevated intramuscular triglycerides (62). These rats had altered postnatal growth as a result of the litter size manipulation such that body weight was lower than controls at 3 days after birth but caught up to, and exceeded, the controls during the juvenile period (41, 62, 63). Reduced litter offspring also displayed mild insulin resistance (32), which may predispose them to future diabetes risk. Therefore, Reduced litter offspring are not appropriate controls, but rather an additional experimental group that allows us to investigate the impact of altered postnatal growth on later health (62, 63).

It is known that exercise can be preventative against the development of type 2 diabetes. Given that being born small increases the likelihood of developing type 2 diabetes, exercise training may be a logical lifestyle intervention that could normalize the impaired skeletal muscle mitochondrial biogenesis and improve fat oxidation in small-birth-weight offspring. One study reported in offspring exposed to maternal undernutrition that long-term daily moderate exercise improved overall energy expenditure and enhanced skeletal muscle substrate metabolism, without changes to fiber size or distribution. This

Address for reprint requests and other correspondence: G. McConell, Institute of Sport, Exercise and Active Living and The School of Biomedical and Health Sciences, Victoria Univ., Victoria, 8001, Australia (e-mail: glenn. mcconell@vu.edu.au).

improved muscle metabolism likely aided in the prevention of obesity development also reported in that study and provides evidence to show that exercise elicits beneficial adaptations in offspring born small (25).

Because we have previously reported that our model of uteroplacental insufficiency demonstrates significantly attenuated markers of skeletal muscle mitochondrial biogenesis (62), we propose that exercise may be effective to upregulate this signaling pathway and provide metabolic benefits to small-birth-weight offspring in later life. Indeed, it is well documented that acute bouts of exercise upregulate key components of the mitochondrial biogenesis signaling pathway (2, 58, 61) and that endurance exercise training increases skeletal muscle mitochondrial volume (24). Furthermore, exercise in patients with type 2 diabetes increases PGC-1α to the same extent as healthy controls (56). Finally, exercise training in patients with type 2 diabetes increases insulin sensitivity in association with reduced skeletal muscle triglyceride content and increased mitochondrial enzyme activity (10).

We propose that exercise training during a period of rapid growth and development may improve the metabolic phenotype and these improvements could be maintained into adulthood. Indeed, a recent study showed that 3 wk of relatively low-intensity exercise training in rats immediately after weaning prevented the onset of diet-induced obesity for at least 10 wk after the cessation of exercise training (45). Furthermore, in humans, individuals within the highest quartile of moderate-to-vigorous physical activity at 5 yr of age had the lowest fat mass at 8 and 11 yr of age compared with those in the lowest quartile, even if they returned to a sedentary lifestyle (28). It is possible that exercise training in offspring born small could also provide metabolic benefits to prevent later disease development. Indeed, we have shown that exercise training early in life, from 5 to 9 wk of age, fully normalizes the pancreatic β-cell mass deficit at 24 wk of age in male rats born small, despite no exercise after 9 wk of age (32).

Therefore, the main aim of this study was to determine whether short-term exercise training in early life in male growth-restricted rats normalized the reduced markers of mitochondrial biogenesis we have previously observed in adulthood (62). A secondary aim of this study was to determine whether exercise later in life could elicit normal training adaptations in small-birth-weight rats. We hypothesised that exercise training during a period of rapid growth would increase skeletal muscle mitochondrial biogenesis in early life, which would be sustained into adulthood and provide metabolic benefits to offspring born small to offset the development of adult disease.

MATERIALS AND METHODS

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

Animal procedures. The animals used in this study are a subset of a cohort previously published in Laker et al. (32). Briefly, Wistar Kyoto rats (9–13 wk of age; $n = 75$ total) were obtained from the Australian Resource Centre (Murdoch, WA, Australia) and 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 or sham surgery under anesthesia with an intraperitoneal injection of Ilium Xylazil-20 (10 mg/kg) and ketamine (50 mg/kg) (Restricted group; $n = 25$) (32, 41, 62). Sham surgery was used to control for the known effects of anaesthesia/surgery alone on fetal growth. Therefore, we could confirm that any later health outcomes are indeed due to impaired uterine blood flow during late gestation. At birth (*day 22*), half of the sham-operated mothers (litter size 6–10) had their litter size randomly reduced to five pups (termed Reduced litter) to match that of the Restricted litters (litter size ~5) (32, 41, 62). Because we have previously reported skeletal muscle mitochondrial biogenesis deficits in adult male but not female rats that were born small (62), this study investigated the effects of exercise training on male offspring only. Males from the same litter were allocated to different treatment groups within the same age group, and up to three males were used from each litter. At 5 wk of age, male offspring ($n = 120$ total) from each experimental group (Control, Restricted, and Reduced litter) were allocated to one of the following exercise treatments: remained sedentary with postmortem at 9 or 24 wk, early exercise training (from 5 to 9 wk of age) with postmortem at 9 or 24 wk, or later exercise training (from 20 to 24 wk of age) with postmortem at 24 wk ($n = 8$ males/group) (32). Body weight at *day 1* was taken as the average of all males in each litter. Individual body weight was measured on *days 6* and *14* and *weeks 5*, *9*, *12*, *16*, *20*, and *24*.

Exercise training. The exercise training protocol was identical for all rats that were allocated to either early or later exercise training such that all exercise groups performed the same absolute workload. Rats exercised 5 days/wk followed by 2 days of rest, for 4 wk. On the first day of training, rats ran on a motorized treadmill (Columbus Instruments, Columbus, OH) for 20 min at a speed of 15 m/min and 0° incline for familiarization. Each subsequent day an additional 10 min were applied to the running time until on *day 5* of *week 1* the rats were exercised for 60 min. On *day 1* of *week 2* and thereafter, rats ran for 60 min/day at a speed of 20 m/min with an incline of 0° (32). Rats were encouraged to run by blowing compressed air near the base of their tail (60).

Preparation of rat tissue. At 9 or 24 wk of age, rats were killed with an intraperitoneal injection of Ilium Xylazil-20 (30 mg/kg) and ketamine (225 mg/kg). Gastrocnemius muscle was rapidly excised (62), weighed, snap-frozen in liquid nitrogen, and stored at −80°C for later analysis. Dorsal fat was excised and weighed. For immunoblotting and enzyme activity, frozen gastrocnemius muscle was homogenised in ice-cold lysis buffer [10 μl buffer/mg muscle; 50 mM Tris at pH 7.5 containing 1 mM EDTA, 10% vol/vol glycerol, 1% vol/vol Triton X-100, 50 mM NaF, 5 mM $Na_4P_2O_7$, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 5 μl/ml protease inhibitor cocktail (P8340; Sigma, St. Louis, MO)]. The lysates were centrifuged at 16,000 g for 20 min at 4°C, and the supernatant was stored at −80°C for later analysis (62). For mass spectroscopy, frozen gastrocnemius muscle was homogenised in ice-cold PBS solution using a Dounce homogenizer. Protein concentration of the homogenate for each of the above procedures was determined using the bicinchoninic acid protein assay (Pierce, Rockford, IL), and BSA was used as the standard.

Immunoblotting. For determination of PGC-1α, Tfam, and cytochrome *c* (Cyt C) protein abundance, muscle lysates were solubilized in Laemmli solution and separated by SDS-PAGE. Protein was then electrotransferred onto polyvinylidene difluoride membranes and probed with rabbit polyclonal anti-PGC-1α (1:1,000; Chemicon, Temecula, CA), rabbit polyclonal anti-Tfam (1:1,000; Genway, San Diego, CA), mouse monoclonal anti-cytochrome *c* (1:1,000; BD Biosciences Pharmigen, San Diego, CA), or mouse monoclonal anti-tubulin (1:10,000; Sigma-Aldrich, St. Louis, MO). Fluorescent secondary anti-rabbit antibody IRDye 800 nm (Rockford, Gilberstville, PA) or anti-mouse antibody IRDye 700 nm (Molecular Probes, Eugene, OR) was used to detect primary antibody binding. The fluorescent signal was detected using the Odyssey infrared imaging system and computer software (LI-COR Biosciences, Lincoln, NE).

Proteins of interest were normalized to tubulin, and data were expressed as integrated intensity (62).

Enzyme activities. Muscle homogenates that were extracted for immunoblotting were diluted to 1 mg/ml of total protein. Citrate synthase activity was determined by measuring the increase in 5,5-dithiobis-2-nitrobenzoate (DTNB) spectrophotometrically at 412 nm at room temperature (55). β-Hydroxyacyl-CoA dehydrogenase (β-HAD) activity was determined by measuring the disappearance of NADH spectrophotometrically at 340 nm (35). All enzyme activities are expressed in micromoles per minute per gram ($\mu mol \cdot min^{-1} \cdot g^{-1}$) of total protein (61, 62).

Skeletal muscle triglycerides, diglycerides, and ceramides. Lipids were extracted from muscle homogenates (~5 μl containing 50 μg protein) with chloroform-methanol (2:1, 20 volumes) following the addition of internal standards [100 pmol of (ceramide) 17:0 (Matreya, Pleasant Gap, PA), together with 200 pmol of (diglyceride) 15:0/15:0 and 100 pmol of (triglyceride) 17:0/17:0/17:0 (Sigma Aldrich)] as previously described (6, 7, 40). Lipid analysis was performed by electrospray ionization-tandem mass spectrometry using a PE Sciex API 4000 Q/TRAP mass spectrometer with a turbo-ion spray source and Analyst 1.5 data system. *Solvent A* and *B* consisted of tetrahydrofuran-methanol-water in the ratios 30:20:50 and 75:20:5, respectively, both containing 10 mM NH$_4$COOH. Prior liquid chromatographic separation was performed on a Zorbax C18, 1.8 μm, 50 × 2.1 mm column at 300 μl/min using the following gradient conditions: 0% B to 100% B over 8.0 min followed by 2.5 min at 100% B, a return to 0% B over 0.5 min then 3.0 min at 0% B before the next injection. Tri- and diglycerides were separated using the same solvent system with an isocratic flow (100 μl/min) of 85% B. Quantification of individual lipid species was performed using scheduled multiple-reaction monitoring in positive ion mode (6, 7, 40). Each ion pair was monitored for 10–50 ms with a resolution of 0.7 atomic mass units at half-peak height and averaged from continuous scans over the elution period. Lipid concentrations were calculated by relating the peak area of each species to the peak area of the corresponding internal standard and expressed as picomoles per gram of protein (36). Total lipids of each class were calculated by summing the individual lipid species.

Statistical analyses. Two-way ANOVA was performed to determine main effects of experimental group and exercise treatment. If a significant interaction was observed, one-way ANOVA with Student-Newman-Keuls post hoc were performed where appropriate. Data are presented as means ± SE, and $P < 0.05$ was considered statistically significant.

RESULTS

Body, dorsal fat, and muscle weights. Bilateral uterine vessel ligation (Restricted) reduced ($P < 0.05$) litter size compared with sham-operated Controls (5.6 ± 0.5 vs. 8.2 ± 0.7) and reduced birth weight compared with Controls and Reduced litters (3.63 ± 0.06 vs. 4.31 ± 0.03 and 4.43 ± 0.04 g, respectively). There were no differences in offspring gender ratios within litters between Control, Restricted, or Reduced litter groups (data not shown). At weaning (35 days), Restricted offspring remained lighter ($P < 0.05$) than Controls and Reduced litter, and this remained the case at all time points measured (Table 1 and Fig. 1). Reduced litter offspring accelerated their growth such that they were heavier ($P < 0.05$) than Controls from 16 wk of age onward (Fig. 1*B*). Neither early nor later exercise training had any impact on body weight. Restricted offspring were significantly shorter ($P < 0.05$), as indicated by crown-to-rump length, than Control and Reduced litter offspring from 6 days of age (40.56 ± 0.82 vs. 44.58 ± 0.32 and 44.25 ± 0.75 mm, respectively). This remained the case at 9 wk of age (153.47 ± 1.08 vs. 160.90 ± 1.39 and 161.65 ± 0.88 mm, respectively; $P < 0.05$), but, by 24 wk of age, there were no differences between Restricted, Control, and Reduced litter offspring (193.59 ± 1.42, 196.06 ± 0.84, and 197.97 ± 0.86 mm, respectively).

At 9 wk of age, absolute and relative (to body weight) gastrocnemius weight was similar between Restricted and Control offspring but were significantly lower ($P < 0.05$) than Reduced litter (Table 1). All other absolute and relative muscle weights [soleus, tibialis anterior, extensor digitorum longus (EDL), plantaris] were similar between groups at 9 wk of age, and there was no effect of early exercise training (data not shown). At 24 wk of age, relative gastrocnemius weight was not different between groups; however, absolute gastrocnemius, tibialis anterior, and EDL weights were greater ($P < 0.05$) in Reduced litter offspring (Table 1; data not shown). Exercise training had no effect on muscle weight (Table 1). At 9 wk of age, absolute and relative dorsal fat mass was lower ($P < 0.05$) in both Restricted and Reduced litter offspring compared with Controls, and there was no effect of early

Table 1. *Body, gastrocnemius, and dosal fat weight*

Age, wk	Group	Exercise Treatment	Body Wt, g		Gastrocnemius Wt, g		Gastrocnemius Wt, %body wt		Dorsal Fat Wt, g		Dorsal Fat Wt, %body wt	
9	Con	Sedentary	193.16 ± 3.03	B	0.76 ± 0.04	AB	0.39 ± 0.02	A	1.92 ± 0.19	B	0.99 ± 0.10	B
		Early exercise	199.09 ± 4.79		0.72 ± 0.06		0.36 ± 0.03		1.82 ± 0.13		0.92 ± 0.07	
	Restr	Sedentary	177.89 ± 5.83	A	0.67 ± 0.03	A	0.38 ± 0.01	A	1.38 ± 0.14	A	0.77 ± 0.06	A
		Early exercise	171.68 ± 7.64		0.65 ± 0.04		0.38 ± 0.01		1.11 ± 0.17		0.63 ± 0.07	
	Red	Sedentary	187.93 ± 4.18	B	0.81 ± 0.02	B	0.43 ± 0.01	B	1.51 ± 0.14	A	0.79 ± 0.06	A
		Early exercise	190.25 ± 5.03		0.81 ± 0.04		0.43 ± 0.01		1.37 ± 0.16		0.71 ± 0.07	
24	Con	Sedentary	381.59 ± 11.50	B	1.33 ± 0.08	A	0.35 ± 0.02		6.93 ± 0.86		1.79 ± 0.18	
		Early exercise	382.68 ± 7.66		1.44 ± 0.07		0.37 ± 0.01		6.19 ± 0.57		1.63 ± 0.16	
		Later exercise	360.72 ± 3.98		1.37 ± 0.07		0.38 ± 0.02		4.57 ± 0.22*		1.27 ± 0.07*	
	Restr	Sedentary	365.29 ± 10.93	A	1.41 ± 0.06	A	0.39 ± 0.01		6.04 ± 0.48		1.64 ± 0.10	
		Early exercise	347.54 ± 6.65		1.29 ± 0.10		0.37 ± 0.03		5.36 ± 0.43		1.55 ± 0.11	
		Later exercise	362.88 ± 10.83		1.39 ± 0.09		0.38 ± 0.02		4.69 ± 0.65*		1.27 ± 0.14*	
	Red	Sedentary	390.59 ± 6.60	C	1.66 ± 0.03	B	0.37 ± 0.05		5.95 ± 0.66		1.33 ± 0.23	
		Early exercise	409.52 ± 5.82		1.68 ± 0.05		0.41 ± 0.01		7.48 ± 0.46		1.83 ± 0.11	
		Later exercise	382.36 ± 7.73		1.61 ± 0.10		0.42 ± 0.03		5.01 ± 0.24*		1.31 ± 0.06*	

Data presented as means ± SE; n = 8 mice/group. Con, Control litter; Restr, Restricted litter; Red, Reduced litter. Different capital letters denote main effect ($P < 0.05$) between groups ("A" is different from "B" but not different from "AB"). *Main effect $P < 0.05$ between exercise treatments.
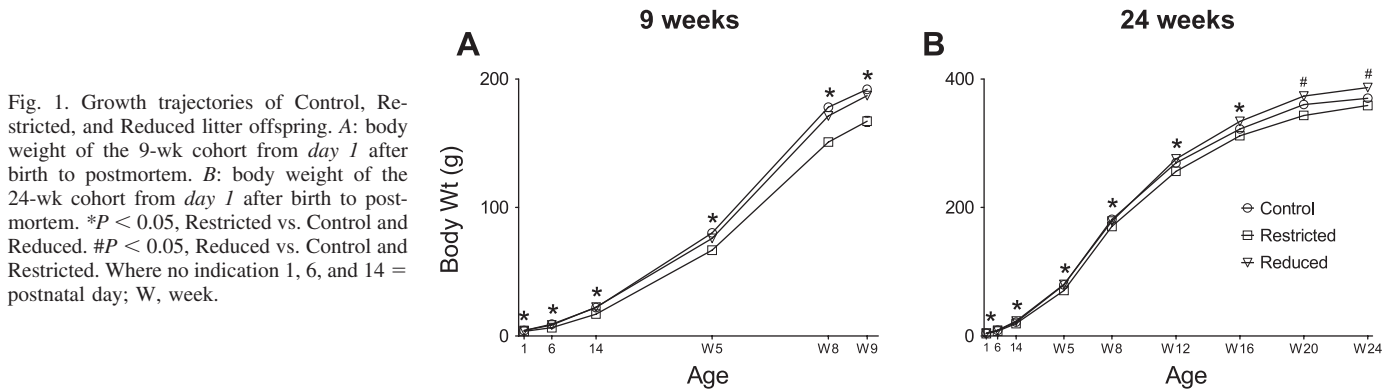
Fig. 1. Growth trajectories of Control, Restricted, and Reduced litter offspring. *A*: body weight of the 9-wk cohort from *day 1* after birth to postmortem. *B*: body weight of the 24-wk cohort from *day 1* after birth to postmortem. *P < 0.05, Restricted vs. Control and Reduced. #P < 0.05, Reduced vs. Control and Restricted. Where no indication 1, 6, and 14 = postnatal day; W, week.

exercise (Table 1). At 24 wk of age, absolute and relative dorsal fat weight was similar between Control, Restricted, and Reduced litter groups and was decreased (P < 0.05) by ~25% following later exercise training in all groups (Table 1). Absolute and relative liver (data not shown) and pancreas (32) weights were similar between groups at 9 wk. At 24 wk of age, absolute liver weight was lower (P < 0.05) in Reduced offspring (data not shown), whereas pancreas weight was similar between Control, Restricted, and Reduced groups (32).

Skeletal muscle markers of mitochondrial biogenesis protein expression. At 9 wk of age, PGC-1α protein was similar between Control, Restricted, and Reduced litter groups. Consistent with an endurance-training effect, PGC-1α was increased (P < 0.05) in all groups following early exercise training at 9 wk of age (Fig. 2A). At 24 wk of age, the Restricted and Reduced litter groups had lower (P < 0.05) PGC-1α protein expression compared with the Control group (Fig. 2B). Those that performed early exercise, but then remained sedentary until postmortem at 24 wk, showed similar PGC-1α protein as their sedentary counterparts (Fig. 2B). Later exercise, from 20 to 24 wk, increased (P < 0.05) PGC-1α protein expression in all groups (Fig. 2B). At 9 wk of age, skeletal muscle protein expression of both Tfam and Cyt C was similar between Control, Restricted, and Reduced litter groups and was not affected by early exercise training (Fig. 2, C and E). At 24 wk of age, Tfam and Cyt C protein was also similar between the sedentary Control, Restricted, and Reduced litter groups, and there was no effect of early exercise training at the later age (Fig. 2, D and F). However, consistent with the endurance-training effect observed with PGC-1α, both Tfam and Cyt C protein expression were increased (P < 0.05) following later exercise training at 24 wk of age in all groups (Fig. 2, D and F).

Skeletal muscle mitochondrial enzyme activity. At 9 and 24 wk of age, citrate synthase activity in the sedentary state was similar between Control, Restricted, and Reduced litter groups (Fig. 3A). Following early exercise training, citrate synthase activity was increased (P < 0.05) only in the Restricted and Reduced litter groups at 9 wk of age (Fig. 3A) and at 24 wk had returned to sedentary levels. Following later exercise training, citrate synthase activity increased (P < 0.05) ~13% in all groups (Fig. 3B). β-HAD activity was similar in all experimental groups at 9 wk of age and increased (P < 0.05) with early exercise training in all groups (Fig. 3C). At 24 wk of age, β-HAD activity was similar between experimental groups and

did not change significantly in groups that performed later exercise training (Fig. 3D).

Skeletal muscle lipid content and composition. At 9 wk of age, total measured skeletal muscle triglycerides were similar among experimental groups and were increased (P < 0.05) by approximately twofold following early exercise training in all groups (Fig. 4A). At 24 wk of age, total measured skeletal muscle triglycerides were approximately fourfold higher (P < 0.05) in the Reduced litter and, although not statistically significant (P = 0.10), were more than twofold greater in Restricted offspring compared with Controls (Fig. 4B). Interestingly, at 24 wk of age, 26 of the 44 triglyceride species were higher (P < 0.05) in early exercise groups compared with later exercise, with sedentary rats showing intermediate levels (Supplementary Table S1; supplementary tables are found linked to the online version of the paper). This observation tended (P = 0.05) to persist in the total sum of the measured triglycerides also (Fig. 4B).

Total skeletal muscle diglycerides were similar between sedentary groups at 9 wk of age (Fig. 4C) and were ~50% higher (P < 0.05) following early exercise in all groups (Fig. 4C). At 24 wk of age, total measured diglycerides were up to 80% higher (P < 0.05) in Reduced litter and tended (P = 0.06) to be elevated by ~30% in Restricted offspring compared with Controls (Fig. 4D). At 24 wk of age, 11 diglyceride species were elevated (P < 0.05) in early exercise groups compared with later exercise, with sedentary rats showing intermediate levels (Supplementary Table S2). The higher levels of individual diglycerides tended (P = 0.07) to persist in the total sum of the measured diglycerides (Fig. 4D).

Total measured skeletal muscle ceramides were similar between groups at 9 wk of age and not affected by early exercise (Fig. 4E). However, four of the seven individual ceramides were elevated (P < 0.05) by up to 35% following early exercise at 9 wk (Supplementary Table 3). At 24 wk, there were no differences in skeletal muscle ceramides between experimental or exercise groups (Fig. 4F).

DISCUSSION

This study has shown that small-birth-weight (Restricted) offspring have normal skeletal muscle adaptations to early life exercise training with increased PGC-1α and mitochondrial enzyme activities at 9 wk of age. However, unlike our previous observations in the pancreas of these same rats (32), early exercise did not "reprogram" the skeletal muscle to offset the deficits observed later in life. A possible explanation for this
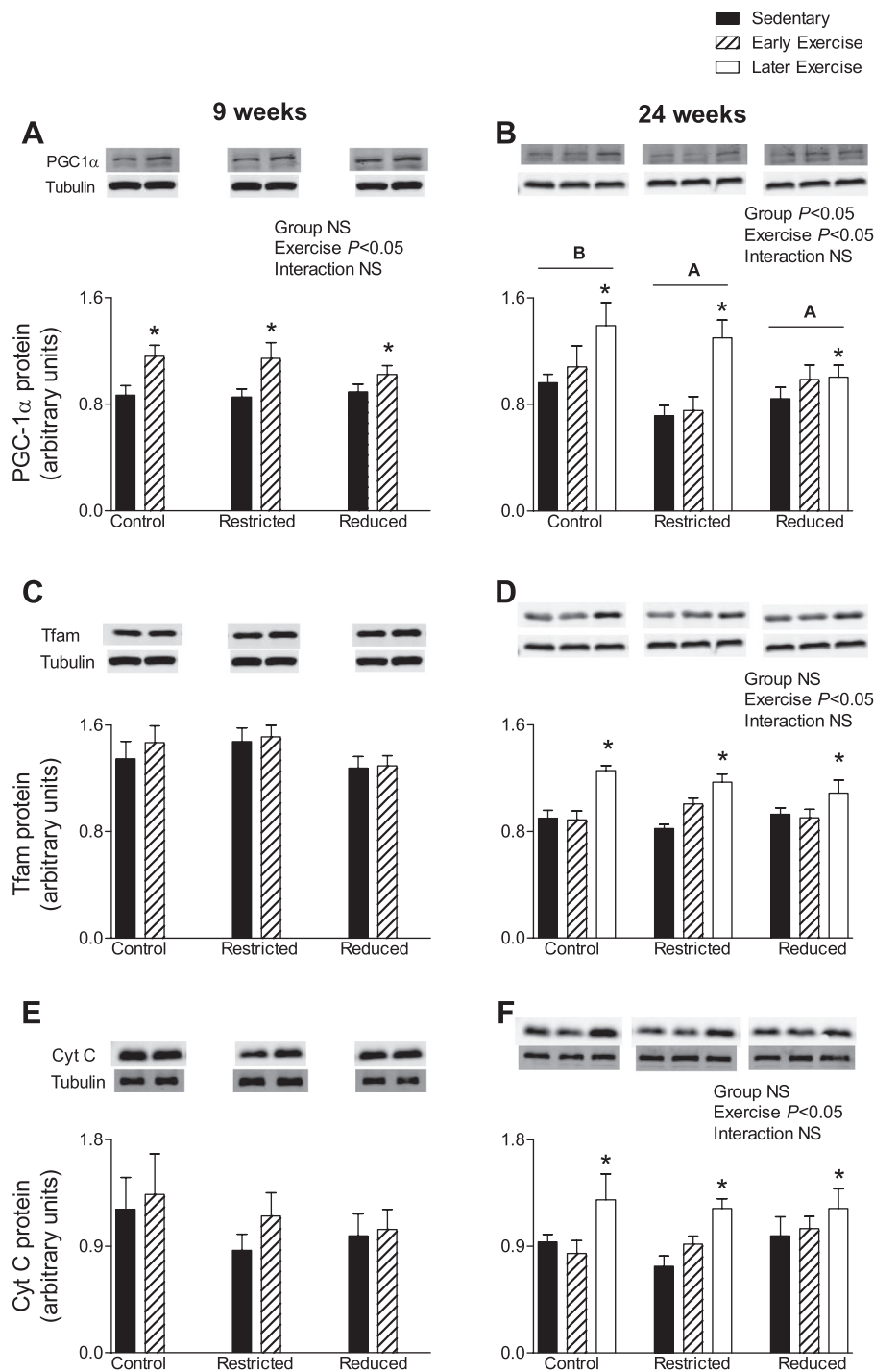
Fig. 2. Skeletal muscle protein expression of markers of mitochondrial biogenesis. *A* and *B*: peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α) protein expression. *C* and *D*: transcription factor A mitochondrial (Tfam) protein expression. *E* and *F*: cytochrome C (Cyt C) protein expression at 9 and 24 wk of age, respectively. Representative blots for the protein of interest and loading control (tubulin) are presented above their respective graphs. Note that PGC-1α and Tfam were immunoblotted on the same membrane and therefore have the same loading control. Dark, hatched, and open bars indicate sedentary, early exercise, and later exercise, respectively. NS, not significant. Data are presented as means ± SE ($n = 8$ mice/group). Different letters denote main effect ($P < 0.05$) between experimental group ("A" is different from "B"). *$P < 0.05$, exercise treatment vs. sedentary.

apparent absence of reprogramming effects of early exercise on skeletal muscle mitochondrial biogenesis could be because, unlike the pancreas (32), there were no deficits in skeletal muscle mitochondrial biogenesis at the time of early exercise, so exercise-induced "reprogramming" was not necessary in early life. Importantly, in adulthood, when deficits in skeletal muscle PGC-1α were observed in Restricted offspring, later exercise training was associated with skeletal muscle mitochondrial adaptations comparable to levels observed in exercised Controls, and therefore Restricted offspring have normal exercise-training adaptations.

Effect of small birth weight (Restricted) and/or altered postnatal growth (Reduced litter). At 9 wk of age, there were no overt impairments in the skeletal muscle phenotype of Restricted offspring. Although Lane and colleagues (33) have previously reported increased PGC-1α mRNA in the EDL muscle and decreased PGC-1α in the soleus at 3 wk of age in offspring born small, that study was performed using a reduced litter group as the control. Furthermore, their study used Sprague Dawley rats, which have an increased propensity for obesity and insulin resistance (4, 5) and therefore limits interpretation between strains. Although we found no changes in
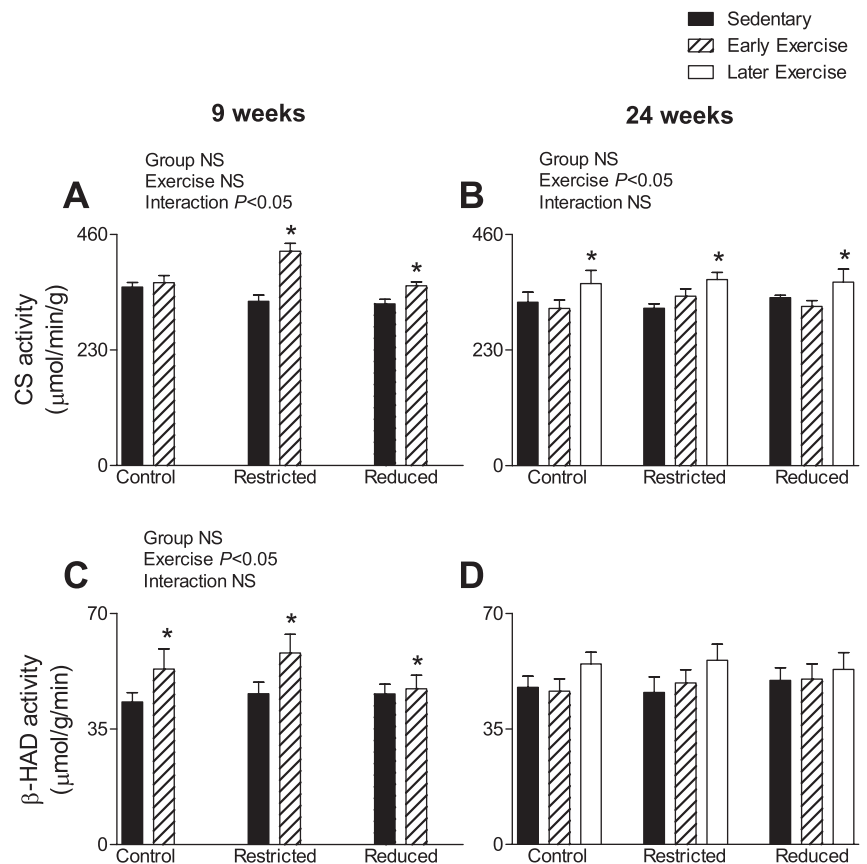
Fig. 3. Skeletal muscle mitochondrial enzyme activity. *A* and *B*: citrate synthase (CS) activity. *C* and *D*: β-hydroxyacyl-CoA dehydrogenase (β-HAD) activity at 9 and 24 wk of age, respectively. Dark, hatched, and open bars indicate sedentary, early exercise, and later exercise, respectively. Data are presented as means ± SE (*n* = 8/group). *$P < 0.05$, exercise treatment vs. sedentary.

PGC-1α at 9 wk in the mixed-fiber gastrocnemius muscle, we also investigated the predominately slow-twitch soleus muscle and found no differences at any age (data not shown). Furthermore, no studies have investigated the skeletal muscle phenotype in a rat model of uteroplacental insufficiency at the juvenile age of 9 wk. In early adulthood, at 24 wk of age, we detected a 20% reduction in the mitochondrial biogenesis master regulator, PGC-1α, in Restricted offspring, and, although not as great, this deficit is consistent with our previous findings (~50% deficit) (62). The relevance of a 20% decrease in PGC-1α is supported by studies in humans that report increases in PGC-1 protein by 23% following acute endurance exercise, which plateaus at ~30–40% following endurance training (47). Thus we believe that a reduction of 20% in PGC-1α protein is of considerable functional importance.

Consistent with previous findings (62), the maximal citrate synthase and β-HAD enzyme activities were not altered in Restricted and Reduced offspring. The maximal in vitro activity of mitochondrial enzymes reflects their abundance and suggests that components of the mitochondrial respiratory chain (tricarboxylic acid cycle, β-oxidation) remain intact but other components such as COX IV in our previous study were not (62). Unlike previous findings by Wadley and colleagues (62), in the current study, there were no impairments detected in Tfam and COX IV protein expression. For unknown reasons, there was no deficit detected in other markers of skeletal muscle mitochondrial biogenesis in the current cohort of offspring. However, we also report altered skeletal muscle lipid levels that are consistent with the early indications of metabolic dysfunction reported previously in these same animals (32).

Together these data represent a programming effect of fetal growth restriction or reducing litter size. Other studies have reported both reductions (25) and no change (44) in skeletal muscle markers of mitochondrial biogenesis in adult Wistar rats exposed to fetal undernutrition, and no impairments were reported in young adult male humans born of low weight (9). A more recent study, however, showed that a deficit in skeletal muscle PGC-1α was observed following 5 days of high-fat feeding in young adult men born of low birth weight (8), which suggests that an added metabolic stress may be required to unmask skeletal muscle deficits programmed in utero in humans. In the current study, Restricted offspring also showed tendencies for higher muscle triglyceride content by up to twofold, which may contribute to impaired glucose utilization and insulin resistance (27, 31, 48, 51, 59), particularly in the face of lower oxidative capacity.

Although these observations are subtle, they represent a programming effect of small birth weight on skeletal muscle. Future studies should consider investigating skeletal muscle programming effects at a later age or following an additional metabolic stress such as high-fat feeding that may exacerbate the deficit in markers of mitochondrial biogenesis. It is possible that, with a secondary insult such as aging (18, 19) or high-fat feeding (37, 42, 54, 57), the capacity to respond to the elevated metabolic stress would be limited, and an exacerbated disease phenotype would likely become apparent.

Reduced litter offspring showed a similar skeletal muscle phenotype to Restricted offspring such that markers of mitochondrial biogenesis were intact in early life, but, at 24 wk of age, PGC-1α protein was lower compared with Control animals.
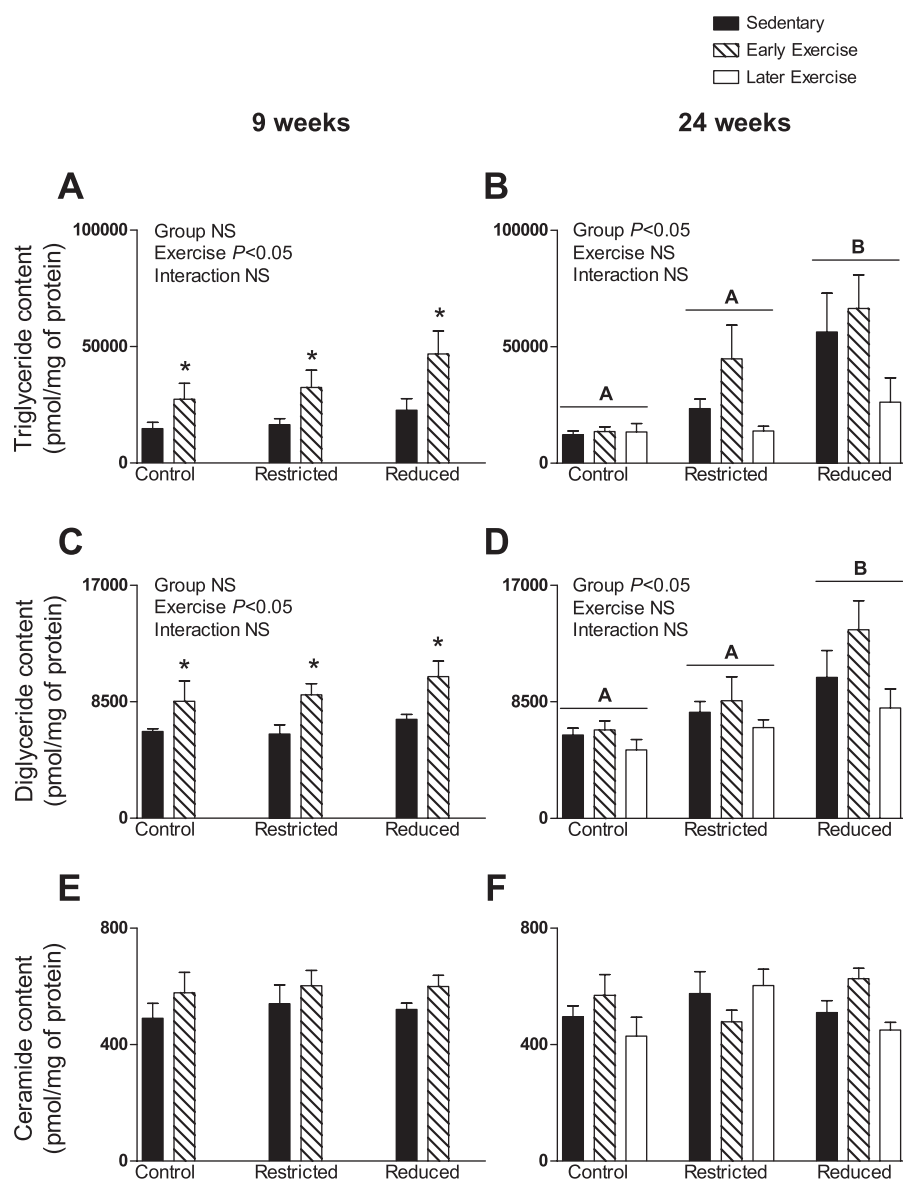
Fig. 4. Skeletal muscle lipid content. *A* and *B*: total measured triglyceride concentration. *C* and *D*: total measured diglyceride concentration. *E* and *F*: total measured ceramide concentration at 9 and 24 wk of age, respectively. Dark, hatched, and open bars indicate sedentary, early exercise, and later exercise, respectively. Data are presented as means ± SE (*n* = 8/group). Different letters denote main effect (*P* < 0.05) between groups (A is different from B). *P < 0.05, exercise treatment vs. sedentary.

Reduced litter offspring actually had significantly higher tri- and diglycerides at 24 wk compared with Control and Restricted offspring. Similar changes to total intramuscular lipids were also observed in the individual levels of all lipid species and showed trends in those that were not statistically different (data not shown). Therefore, we cannot attribute the overall changes in total lipids to specific changes in de novo synthesis, as would be indicated, for example, by changes in relative abundance of specific lipid species such as the fatty acid palmitoleate 16:1 (14, 21). It is likely that the increased skeletal muscle tri- and diglycerides observed in the Reduced litter offspring may contribute to impaired insulin sensitivity (27, 31, 48, 51, 59). Indeed, we have previously published data from our extended cohort showing higher homeostasis model assessment-insulin resistance and tendencies for elevated plasma insulin during an intraperitoneal glucose tolerance test in Reduced litter offspring (32), indicative of early insulin resistance.

It is likely that the early postnatal environment has an independent impact on the later skeletal muscle lipid profile, since the greatest effect in this study was observed in the Reduced litter offspring. Indeed, previous studies from our laboratory have reported that reducing litter size after birth impairs maternal mammary morphology and lactation, resulting in slowed early postnatal growth of the pups that is followed by accelerated growth in the juvenile period (41, 62, 63). Although one might expect this reduction of litter size to result in offspring overfeeding, this is a mild reduction of litter size, which is implemented 1 day after birth. This reduction of litter size has less severe consequences for suckling stimulus and lactational response compared with other interventions such as those induced by Plagemann and colleagues (12, 50) where litter size is reduced to only three pups per litter, which are compared with control litters of 12 pups in Sprague Dawley rats. Furthermore, the reduction of litter size induced by Plagemann and colleagues (12, 50) occurs on *postnatal day 3* after the mother has adapted levels of lactation to accommodate a larger litter (12, 50). Therefore, when litter size is reduced in the Plagemann model, lactation

far exceeds the needs of the remaining offspring, and over-feeding occurs as a consequence.

Effect of short-term exercise training early in life. Early exercise training elicited normal skeletal muscle adaptations with increased protein expression of PGC-1α and elevated β-HAD and citrate synthase enzyme activity in all groups at 9 wk of age and confirms that the exercise protocol was of sufficient intensity to induce adaptations. The normal increase in PGC-1α, β-HAD, and citrate synthase enzyme activity indicates that the Restricted offspring's response to exercise training was intact at a young age. All groups that performed early exercise training exhibited higher total skeletal muscle tri- and diglycerides. Increases in skeletal muscle triglycerides with endurance exercise training are well documented (30, 49, 52, 59), since they are used as a source of fuel and in an exercise training setting are accompanied by increased fat oxidation. Fewer studies have investigated the effects of exercise on skeletal muscle diglyceride levels. A recent study in humans reported higher levels of diglycerides in athletes compared with normal-weight sedentary and obese sedentary subjects (1). Other studies in obese (11) and overweight older (15) humans report lower skeletal muscle diglyceride levels following exercise training, whereas studies in Sprague Dawley rats have shown no change following 4 wk of treadmill running (34). In the current study, skeletal muscle diglycerides were elevated following early exercise, perhaps because of higher basal triglyceride hydrolysis, since muscle was collected 3 days after the final exercise bout. Despite the higher diglyceride levels, the concomitant potential for increased oxidative capacity of the muscle suggests that this will not be detrimental to the whole body metabolic phenotype.

There were no sustained effects of early exercise training in any group, such that PGC-1α, β-HAD, and citrate synthase were no longer elevated by 24 wk of age. Indeed, PGC-1α protein expression was similarly reduced at 24 wk in Restricted offspring that either remained sedentary or performed early exercise. Therefore, early exercise did not elicit a reprogramming effect on skeletal muscle mitochondrial biogenesis since the increase in markers observed at 9 wk of age were reversed following cessation of training. This may be because of the high degree of plasticity of skeletal muscle and the fact that markers of mitochondrial biogenesis in skeletal muscle were not impaired at the time of early exercise training, and so did not require exercise-induced mitochondrial reprogramming. In the pancreas, however, we have previously observed a remarkable reprogramming effect of early exercise training such that, at 24 wk of age, pancreatic islet surface area and β-cell mass were doubled and restored to Control levels (32). Indeed, at the time of early exercise training, pancreatic islet surface area and β-cell mass were reduced by up to 68% in sedentary offspring (32) and perhaps were more receptive to reprogramming. On the other hand, and as would be predicted from previous studies showing the reversibility of exercise training effects on skeletal muscle, there was no evidence of a reprogramming effect in terms of skeletal muscle mitochondrial biogenesis. It is surprising that the effects of exercise training, an activity that is directly reliant on skeletal muscle, has greater reprogramming effects on the pancreas than skeletal muscle. Further studies are required to understand the mechanisms involved.

Effect of short-term exercise training later in life. As was the case with early exercise training, later exercise training increased skeletal muscle markers of mitochondrial biogenesis and mitochondrial enzyme activities in all groups. Importantly, although Restricted offspring had significantly lower PGC-1α protein at 24 wk of age, their response to later exercise training was intact, such that PGC-1α, Tfam, and Cyt C protein and citrate synthase activity increased to levels observed in exercised Controls. Whether PGC-1α protein would have returned to the lower levels observed in sedentary Restricted offspring following exercise cessation remains unknown although this seems likely based on the reversibility of the early exercise effect. Later exercise training was also associated with an ~25% reduction in dorsal fat weight in all groups and, along with the skeletal muscle mitochondrial biogenesis adaptations, would be expected to contribute to improved peripheral insulin sensitivity. However, as previously reported (32) in our larger cohort of rats, improved insulin sensitivity was observed in Control and Reduced litter offspring that performed later exercise but not in Restricted offspring. Considering the normal skeletal muscle adaptations to exercise training in Restricted offspring, the absence of improved insulin sensitivity may represent a divergence between skeletal muscle mitochondrial biogenesis and insulin sensitivity. Indeed, although increased mitochondrial biogenesis markers were observed with later exercise training, skeletal muscle lipid profile was not improved. Therefore, the tendencies for elevated tri- and diglycerides in Restricted offspring may contribute to the absence of a more insulin-sensitive phenotype, as would normally be expected with exercise training. Importantly, elderly people born of low birth weight who performed regular moderate exercise were protected from glucose intolerance (17, 43). Therefore, our results in rats, along with previous epidemiological studies, suggest that exercise training may be used as an effective therapeutic approach to offset metabolic dysfunction in offspring that have experienced altered pre- and/or postnatal growth.

In conclusion, unlike our previously reported findings of early exercise in rats born small restoring pancreatic β-cell mass in adulthood, there was no benefit of early exercise training on skeletal muscle PGC-1α later in life, such that skeletal muscle PGC-1α was lower in Restricted and Reduced offspring in adulthood regardless of early exercise training. This may have been attributed to the fact that skeletal muscle PGC-1α levels were normal at the time of early exercise training. Importantly, in later life, when PGC-1α deficits are observed in sedentary Restricted and Reduced litter offspring, later exercise elicited normal skeletal muscle adaptations as observed in exercised Control rats. Future studies are required to determine how short-term exercise early in life in our rat model of small born weight "reprograms" the pancreas but not skeletal muscle.

DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS

Author contributions: R.C.L., M.E.W., G.D.W., and G.K.M. conception and design of research; R.C.L. and L.A.G. performed experiments; R.C.L. analyzed data; R.C.L., M.E.W., G.D.W., P.J.M., and G.K.M. interpreted results of experiments; R.C.L. prepared figures; R.C.L. drafted manuscript; R.C.L., M.E.W., G.D.W., L.A.G., P.J.M., and G.K.M. edited and revised manuscript; R.C.L., M.E.W., G.D.W., L.A.G., P.J.M., and G.K.M. approved final version of manuscript.

REFERENCES

1. **Amati F, Dubé JJ, Alvarez-Carnero E, Edreira MM, Chomczynski P, Coen PM, Switzer GE, Bickel PE, Stefanovic-Racic M, Toledo FG, Goodpaster BH.** Skeletal muscle triglycerides, diacylglycerols, and ceramides in insulin resistance: another paradox in endurance-trained athletes? *Diabetes* 60: 2588–2597, 2011.

2. **Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, Holloszy JO.** Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 16: 1879–1886, 2002.

3. **Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM.** Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* 36: 62–67, 1993.

4. **Barzilai N, Banerjee S, Hawkins M, Chang CJ, Chen W, Rossetti L.** The effect of age-dependent increase in fat mass on peripheral insulin action is saturable. *J Gerontol* 53: B141–B146, 1998.

5. **Barzilai N, Rossetti L.** Relationship between changes in body composition and insulin responsiveness in models of the aging rat. *Am J Physiol Endocrinol Metab* 269: E591–E597, 1995.

6. **Borg ML, Andrews ZB, Duh EJ, Zechner R, Meikle PJ, Watt MJ.** Pigment epithelium-derived factor regulates lipid metabolism via adipose triglyceride lipase. *Diabetes* 60: 1458–1466, 2011.

7. **Boslem E, MacIntosh G, Preston AM, Bartley C, Busch AK, Fuller M, Laybutt DR, Meikle PJ, Biden TJ.** A lipidomic screen of palmitate-treated min6 beta-cells links sphingolipid metabolites with endoplasmic reticulum (er) stress and impaired protein trafficking. *Biochem J* 435: 267–276, 2011.

8. **Brons C, Jacobsen S, Nilsson E, Ronn T, Jensen CB, Storgaard H, Poulsen P, Groop L, Ling C, Astrup A, Vaag A.** Deoxyribonucleic Acid Methylation and Gene Expression of PPARGC1A in Human Muscle Is Influenced by High-Fat Overfeeding in a Birth-Weight-Dependent Manner. *J Clin Endocrinol Metab* 95: 3048–3056, 2010.

9. **Brons C, Jensen CB, Storgaard H, Alibegovic A, Jacobsen S, Nilsson E, Astrup A, Quistorff B, Vaag A.** Mitochondrial function in skeletal muscle is normal and unrelated to insulin action in young men born with low birth weight. *J Clin Endocrinol Metab* 93: 3885–3892, 2008.

10. **Bruce CR, Kriketos AD, Cooney GJ, Hawley JA.** Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type-2 diabetes. *Diabetologia* 47: 23–30, 2004.

11. **Bruce CR, Thrush AB, Mertz VA, Bezaire V, Chabowski A, Heigenhauser GJF, Dyck DJ.** Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. *Am J Physiol Endocrinol Metab* 291: E99–E170, 2006.

12. **Davidowa H, Li Y, Plagemann A.** Altered response to orexigenic (AGRP, MCH) and anorexigenic (α-MSH, CART) neuropeptides of paraventricular hypothalamic neurons in early postnatally overfed rats. *Eur J Neurosci* 18: 613–621, 2003.

13. **DeFronzo RA, Ferrannini E, Sato Y, Felig P.** Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest* 68: 1468–1474, 1981.

14. **Dimopoulos N, Watson M, Sakamoto K, Hundal HS.** Differential effects of palmitate and palmitoleate on insulin action and glucose utilisation in rat L6 skeletal muscle cells. *Biochem J* 399: 473–481, 2006.

15. **Dubé JJ, Amati F, Stefanovic-Racic M, Toledo FGS, Sauers SE, Goodpaster BH.** Exercise-induced alterations in intramyocellular lipids and insulin resistance: the athlete's paradox revisted. *Am J Physiol Endocrinol Metab* 294: E882–E888, 2008.

16. **Eriksson JG, Osmond C, Kajantie E, Forsén TJ, Barker DJP.** Patterns of growth among children who later develop type 2 diabetes or its risk factors. *Diabetologia* 49: 2853–2858, 2006.

17. **Eriksson JG, Yliharsila H, Forsen T, Osmond C, Barker DJ.** Exercise protects against glucose intolerance in individuals with a small body size at birth. *Prev Med* 39: 164–167, 2004.

18. **Escriva F, Agote M, Rubio E, Molero JC, Pascual-Leone AM, Andres A, Satrustegui J, Carrascosa JM.** In vivo insulin-dependent glucose uptake of specific tissues is decreased during aging of mature Wistar rats. *Endocrinology* 138: 49–54, 1997.

19. **Escriva F, Gavete ML, Fermin Y, Perez C, Gallardo N, Alvarez C, Andres A, Ros M, Carrascosa JM.** Effect of age and moderate food restriction on insulin sensitivity in Wistar rats: role of adiposity. *J Endocrinol* 194: 131–141, 2007.

20. **Forsen T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D.** The fetal and childhood growth of persons who develop type 2 diabetes. *Ann Inter Med* 133: 176–182, 2000.

21. **Green CD, Ozguden-Akkoc CG, Wang Y, Jump DB, Olson LK.** Role of fatty acid elongase in determination of de novo synthesized monounsaturated fatty acid species. *J Lipid Res* 51: 1871–1877, 2010.

22. **Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD.** Fetal and infant growth and impaired glucose tolerance at age 64. *Br Med J* 303: 1019–1022, 1991.

23. **Hoehn KL, Salmon AB, Hohnen-Behrens C, Turner N, Hoy AJ, Maghzal GJ, Stocker R, Van Remmen H, Kraegen EW, Cooney GJ, Richardson AR, James DE.** Insulin resistance is a cellular antioxidant defense mechanism. *Proc Natl Acad Sci USA* 106: 17787–17792, 2009.

24. **Holloszy JO.** Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 242: 2278–2282, 1967.

25. **Huber K, Miles JL, Norman AM, Thompson NM, Davison M, Breier BH.** Prenatally induced changes in muscle structure and metabolic function facilitate exercise-induced obesity prevention. *Endocrinology* 150: 4135–4144, 2009.

26. **Itani SI, Ruderman NB, Schieder F, Boden G.** Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IκB-α. *Diabetes* 51: 2005–2011, 2002.

27. **Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU.** Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. *Diabetes* 48: 1113–1119, 1999.

28. **Janz KF, Kwon S, Letuchy EM, Eichenberger Gilmore JM, Burns TL, Torner JC, Willing MC, Levy SM.** Sustained effect of early physical activity on body fat mass in older children. *Am J Prev Med* 37: 35–40, 2009.

29. **Jensen CB, Storgaard H, Madsbad S, Richter EA, Vaag AA.** Altered skeletal muscle fiber composition and size precede whole-body insulin resistance in young men with low birth weight. *J Clin Endocrinol Metab* 92: 1530–1534, 2007.

30. **Kiens B, Essen-Gustavsson B, Christensen NJ, Saltin B.** Skeletal muscle substrate itilization during submaximal exercise in man:effect of endurance training. *J Physiol* 469: 459–478, 1993.

31. **Krssak M, Falk Patersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI.** Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia* 42: 113–116, 1999.

32. **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.

33. **Lane RH, Maclennan NK, Daood MJ, Hsu JL, Janke SM, Pham TD, Puri AR, Watchko JF.** IUGR alters postnatal rat skeletal muscle peroxisome proliferator-activated receptor-γ coactivator-1 gene expression in a fiber specific manner. *Pediatr Res* 53: 994–1000, 2003.

34. **Lessard SJ, Rivas DA, Chen ZP, Bonen A, Febbraio MA, Reeder DW, Kemp BE, Yaspelkis BBI, Hawley JA.** Tissue-specific effects of rosiglitazone and exercise in the treatment of lipid-induced insulin resistance. *Diabetes* 56: 1856–1864, 2007.

35. **Lowry OH, Passonneau JV.** *A Flexible System of Enzymatic Analysis.* New York, NY: Academic, 1972.

36. **Meikle PJ, Duplock S, Blacklock D, Whitfield PD, MacIntoch G, Hopwood JJ, Fuller M.** Effect of lysosomal storage on bis(monoacylglycero)phosphate. *Biochem J* 411: 71–78, 2008.

37. **Milburn JLJ, Hirose H, Lee YH, Nagasawa Y, Oqawa A, Ohneda M, BeltrandelRio H, Newgard CB, Johnson JH, Unger RH.** Pancreatic Beta-cells in obesity. *J Biol Chem* 270: 1295–1299, 1995.

38. **Mootha VK, Hosker JP, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstrale M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC.** PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* 34: 267–273, 2003.

39. **Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, Neschen S, White MF, Bilz S, Sono S, Pypaert M, Shulman GI.** Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J Clin Invest* 115: 3587–3593, 2005.

40. **Murphy RC, James PF, McAnoy AM, Krank J, Duchoslav E, Barkley RM.** Detection of the abundance of diacylglycerol and triacylglycerol molecular species in cells using neutral loss mass spectrometry. *Anal Biochem* 366: 59–70, 2007.

41. **O'Dowd R, Kent JC, Moseley JM, Wlodek ME.** Effects of uteroplacental insufficiency and reducing litter size on maternal mammary function and postnatal offspring growth. *Am J Physiol Regul Integr Comp Physiol* 294: R539–R548, 2008.

42. **Oakes ND, Bell KS, Furler SM, Camilleri S, Saha AK, Ruderman NB, Chisholm DJ, Kraegen EW.** Diet-induced muscle insulin resistance in rats is ameliorated by acute dietary lipid withdrawal or a single bout of exercise: parallel relationship between insulin stimulation of glucose uptake and suppression of long-chain fatty acyl-CoA. *Diabetes* 46: 2022–2028, 1997.

43. **Ortega FB, Ruiz JR, Hurtig-Wennlöf A, Meirhaeghe A, González-Gross M, Moreno LA, Molnar D, Kafatos A, Gottrand F, Widhalm K, Labayen I, Sjöström M.** Physical activity attenuates the effect of low birth weight on insulin resistance in adolescents: findings from two observational studies. *Diabetes* 60: 2295–2299, 2011.

44. **Ozanne SE, Jensen CB, Tingey KJ, Storgaard H, Madsbad S, Vaag AA.** Low birthweight is associated with specific changes in muscle insulin-signalling protein expression. *Diabetologia* 48: 547–552, 2005.

45. **Patterson CM, Dunn-Meynell AA, Levin BE.** Three weeks of early-onset exercise prolongs obesity resistance in DIO rats after exercise cessation. *Am J Physiol Regul Integr Comp Physiol* 294: R290–R301, 2008.

46. **Patti ME.** Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA* 100: 8466–8471, 2003.

47. **Perry CGR, Lally J, Holloway GP, Heigenhauser GJF, Bonen A, Spriet LL.** Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle. *J Physiol* 588: 4795–4810, 2010.

48. **Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L.** Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48: 1600–1606, 1999.

49. **Phillips SM, Green HJ, Tarnopolsky MA, Heigenhauser GJF, Grant SM.** Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *Am J Physiol Endocrinol Metab* 270: E265–E272, 1996.

50. **Plagemann A, Harder T, Brunn M, Harder A, Roepke K, Wittrock-Staar M, Ziska T, Schellong K, Rodekamp E, Melchior K, Dudenhausen JW.** Hypothalamic proopiomelanocortin promoter methylation becomes altered by early overfeeding: an epigenetic model of obesity and the metabolic syndrome. *J Physiol* 587: 4963–4976, 2009.

51. **Roden M, Price TB, Perseghin G, Petersen KF, Rothman DL, Cline GW, Shulman GI.** Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 97: 2859–2865, 1996.

52. **Schrauwen-Hinderling VB, Schrauwen P, Hesselink MKC, van Engelshoven JMA, Nicolay K, Saris WHM, Kessels AGH, Kooi ME.** The increase in intramyocellular lipid content is a very early response to training. *J Clin Endocrinol Metab* 88: 1610–1616, 2003.

53. **Selak MA, Storey BT, Peterside I, Simmons RA.** Impaired oxidative phosphorylation in skeletal muscle of intrauterine growth-retarded rats. *Am J Physiol Endocrinol Metab* 285: E130–E137, 2003.

54. **Sone H, Kagawa Y.** Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia* 48: 58–67, 2005.

55. **Srere P.** *Citrate Synthase.* New York, NY: Academic, 1969.

56. **Sriwijitkamol A, Coletta DK, Walcberg E, Balbontin GB, Reyna SM, Barrientes J, Eagan PA, Jenkinson CP, Cersosimo E, DeFronzo RA, Sakamoto K, Musi N.** Effect of acute exercise on AMPK signalling in skeletal muscle of subjects with type 2 diabetes: a time-course and dose-response study. *Diabetes* 56: 836–848, 2007.

57. **Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW.** Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol Endocrinol Metab* 251: E576–E583, 1986.

58. **Terada S, Tabata I.** Effects of acute bouts of running and swimming exercise on PGC-1α protein expression in rat epitrochlearis and soleus muscle. *Am J Physiol Endocrinol Metab* 286: E208–E216, 2004.

59. **van Loon LJC, Goodpaster BH.** Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Eur J Physiol* 451: 606–616, 2006.

60. **Wadley GD, Bruce CR, Konstantopoulos N, Macaulay SL, Howlett KF, Hawley JA, Cameron-Smith D.** The effect of insulin and exercise on c-Cbl protein abundance and phosphorylation in insulin-resistant skeletal muscle in lean and obese Zucker rats. *Diabetologia* 47: 412–419, 2004.

61. **Wadley GD, Choate J, McConell GK.** NOS isoform-specific regulation of basal but not exercise-induced mitochondrial biogenesis in mouse skeletal muscle. *J Physiol* 585: 253–262, 2007.

62. **Wadley GD, Siebel AL, Cooney GJ, McConell GK, Wlodek ME, Owens JA.** Uteroplacental insufficiency and reducing litter size alters skeletal muscle mitochondrial biogenesis in a sex specific manner in the adult rat. *Am J Physiol Endocrinol Metab* 294: E861–E869, 2008.

63. **Wlodek ME, Westcott K, Siebel AL, Owens JA, Moritz KM.** Growth restriction before or after birth reduces nephron number and increases blood pressure in male rats. *Kidney Int* 74: 187–195, 2008.