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

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Exercise early in life in rats born small does not normalize reductions in skeletal muscle PGC-1α in adulthood (13). The skeletal muscle deficits underlying 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
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 (Murchison, 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 Ilum 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 anesthesia/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 Restricted 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 Ilum 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 homogenized 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 Na4P2O7, 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 spectrometry, 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, Tfm, 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-Tfm (1:1,000; Genway, San Diego, CA), mouse monoclonal anti-cytochrome c (1:1,000; BD Biosciences Pharmigen, San Diego, CA), or mouse monoclonal antitubulin (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 (μmol·min⁻¹·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/TRA Mass spectrometer with a turbo-ion spray source. Lipid chromatographic separation was performed on a Zorbax C18, 1.8 μm, 50 × 2.1 mm column at 300°C, 50 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. 1B). 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 dorsal fat weight

<table>
<thead>
<tr>
<th>Age, wk</th>
<th>Group</th>
<th>Exercise Treatment</th>
<th>Body Wt, g</th>
<th>Gastrocnemius Wt, g</th>
<th>Gastrocnemius Wt, %body wt</th>
<th>Dorsal Fat Wt, g</th>
<th>Dorsal Fat Wt, %body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Con</td>
<td>Sedentary</td>
<td>197.16 ± 3.03</td>
<td>0.76 ± 0.04 AB</td>
<td>0.39 ± 0.02 A</td>
<td>1.92 ± 0.19 B</td>
<td>0.99 ± 0.10 B</td>
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<tr>
<td></td>
<td></td>
<td>Early exercise</td>
<td>199.09 ± 4.79</td>
<td>0.72 ± 0.06 A</td>
<td>0.36 ± 0.03 A</td>
<td>1.82 ± 0.13 A</td>
<td>0.92 ± 0.07 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>177.59 ± 5.83</td>
<td>0.67 ± 0.03 A</td>
<td>0.38 ± 0.01 A</td>
<td>1.36 ± 0.14 A</td>
<td>0.77 ± 0.06 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early exercise</td>
<td>171.68 ± 7.64</td>
<td>0.65 ± 0.04 A</td>
<td>0.38 ± 0.01 A</td>
<td>1.11 ± 0.17 A</td>
<td>0.63 ± 0.07 A</td>
</tr>
<tr>
<td>24</td>
<td>Con</td>
<td>Sedentary</td>
<td>187.93 ± 4.18</td>
<td>0.81 ± 0.02 B</td>
<td>0.43 ± 0.01 B</td>
<td>1.51 ± 0.14 A</td>
<td>0.79 ± 0.06 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early exercise</td>
<td>190.25 ± 5.03</td>
<td>0.81 ± 0.04 B</td>
<td>0.43 ± 0.01 B</td>
<td>1.37 ± 0.16 A</td>
<td>0.71 ± 0.07 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>381.59 ± 11.50</td>
<td>1.33 ± 0.06 B</td>
<td>0.35 ± 0.02 B</td>
<td>6.93 ± 0.86 A</td>
<td>1.79 ± 0.18 A</td>
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<tr>
<td></td>
<td></td>
<td>Early exercise</td>
<td>382.68 ± 7.66</td>
<td>1.44 ± 0.07 A</td>
<td>0.37 ± 0.01 B</td>
<td>6.19 ± 0.57 A</td>
<td>1.63 ± 0.16 A</td>
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<tr>
<td></td>
<td></td>
<td>Later exercise</td>
<td>360.72 ± 3.98</td>
<td>1.37 ± 0.07 A</td>
<td>0.38 ± 0.02 B</td>
<td>4.57 ± 0.22 A</td>
<td>1.27 ± 0.07*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>365.29 ± 10.93</td>
<td>1.41 ± 0.06 A</td>
<td>0.39 ± 0.01 B</td>
<td>6.04 ± 0.48 A</td>
<td>1.64 ± 0.10</td>
</tr>
<tr>
<td></td>
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<td>Early exercise</td>
<td>347.54 ± 6.65</td>
<td>1.29 ± 0.10 A</td>
<td>0.37 ± 0.03 B</td>
<td>5.36 ± 0.43 A</td>
<td>1.55 ± 0.11</td>
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<td></td>
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<td>Later exercise</td>
<td>362.88 ± 10.83</td>
<td>1.39 ± 0.09 A</td>
<td>0.38 ± 0.02 B</td>
<td>4.69 ± 0.65* A</td>
<td>1.27 ± 0.14*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restricted</td>
<td>390.59 ± 6.60</td>
<td>1.66 ± 0.03 B</td>
<td>0.37 ± 0.05 B</td>
<td>5.95 ± 0.66 A</td>
<td>1.33 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early exercise</td>
<td>409.52 ± 5.82</td>
<td>1.68 ± 0.05 A</td>
<td>0.41 ± 0.01 B</td>
<td>7.48 ± 0.46 A</td>
<td>1.83 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Later exercise</td>
<td>382.36 ± 7.73</td>
<td>1.61 ± 0.10 A</td>
<td>0.42 ± 0.03 B</td>
<td>5.01 ± 0.24* A</td>
<td>1.31 ± 0.06*</td>
</tr>
</tbody>
</table>

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
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. 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.
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 protein 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. 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.
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, 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 overfeeding 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.

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DISCLOSURES

The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS


REFERENCES


