Prenatally Induced Changes in Muscle Structure and Metabolic Function Facilitate Exercise-Induced Obesity Prevention

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Effective regulation of energy metabolism is vital for the maintenance of optimal health, and an inability to make these dynamic adjustments is a recognized cause of obesity and metabolic disorders. Epidemiological and experimental studies have highlighted the role of prenatal factors in the disease process, and it is now generally accepted that maternal nutrition during pregnancy significantly influences intrauterine development, shaping postnatal health. Consequences of impaired nutrition during fetal development include intrauterine growth restriction (IUGR) and subsequent obesity development in adult life. We have previously shown that prenatal undernutrition has a lasting effect on behavior, with IUGR offspring expressing a higher preference for voluntary exercise, and moderate daily exercise preventing obesity development. The present study investigated skeletal muscle structure in IUGR offspring and how moderate daily exercise drives changes in metabolic pathways that promote obesity prevention. Pregnant Wistar rats were either fed chow ad libitum or undernourished, generating control or IUGR offspring respectively. Although red muscle structure indicated higher oxidative capacity in IUGR offspring, obesity prevention was not due to increased fatty acid oxidation, indicated by decreased peroxisomal proliferator-activated receptor-γ coactivator 1 and carnitine-palmitoyltransferase 1 expression. In contrast, increased protein kinase Cε expression and glycogen content in white muscle of exercised IUGR offspring suggests an enhanced capacity for anaerobic utilization of glucose. Furthermore, exercise-induced lactate accumulation was effectively prevented by stimulation of a lactate shuttle, driven by the increases in monocarboxylate transporters-4 and -1 in white muscle. This enhanced metabolic flexibility in IUGR offspring may facilitate muscle contractile performance and therefore support moderate daily exercise for effective obesity prevention. (Endocrinology 150: 4135–4144, 2009)

Skeletal muscle has a striking ability to modify its functional characteristics to adapt to metabolic need. This high degree of plasticity in structure and metabolic capacity makes skeletal muscle a key determinant of metabolic flexibility and consequently of overall health. Failure by the muscle and intermediary metabolism to adapt to changes in nutrient supply and energy expenditure (termed metabolic inflexibility) leads to the development of obesity and a number of associated pathophysologies, including type 2 diabetes mellitus and cardiovascular disease (1). Because the global societal and economic impact of obesity and related metabolic disorders continues to increase at an alarming rate, their...
treatment and prevention are major challenges for current public health and medicine. Yet the biological basis of an individual’s susceptibility to obesogenic environmental influences is poorly understood.

It is now recognized that environmental changes during fetal development (e.g. maternal undernutrition) play an important role in determining susceptibility to obesity and metabolic disease in adult life (2–4). However, despite the need for urgent measures to combat the rapidly growing obesity epidemic especially in children and young people, our knowledge of the mechanisms underlying the relationship between early-life nutrition and adult health outcomes remains limited. Moreover, very little information exists as to the influence of the prenatal environment on skeletal muscle.

Previous research in the rat has shown that maternal undernutrition during pregnancy leads to intrauterine growth restriction (IUGR) of offspring and subsequent obesity in adult life. Importantly, the obesity that develops in IUGR offspring is metabolically distinct from diet-induced obesity, with the latter characterized by insulin resistance and an increase in fat deposition in nonphysiological, ectopic locations such as liver and muscle (5). In contrast, prenatally induced obesity is characterized by maintained insulin sensitivity, as evaluated by hyperinsulineemic-euglycemic clamp, and enhanced insulin action, as evaluated by hepatic protein kinase C (PKC)-ζ expression (5).

Recently we have shown that prenatally undernourished rats express a lasting change in choice behavior that may influence obesity development (6). When presented with a choice between wheel running and eating, IUGR offspring show a consistently higher preference for exercise vs. food compared with the offspring of ad libitum-fed dams (AD). Furthermore, when IUGR rats were exposed to a moderate amount of daily exercise (56 m in a running wheel) throughout their adult life, prenatally induced obesity was effectively prevented (7). Whereas plasma triglyceride (TG) levels in these exercised IUGR offspring were normalized, this moderate amount of daily exercise had no effect on fat homeostasis in the AD offspring. Therefore, we proposed that exercised IUGR offspring may have an increased facilitated usage of lipid or lipid precursors, e.g. glucose. Such usage would prevent obesity through reduced fat deposition and increased hepatic glycogen availability for exercise (7). This profound change in interactions between choice behavior and intermediary metabolism may represent an effective adaptation that provides a biological advantage in accordance with the thrifty phenotype hypothesis of Hales and Barker (3).

The aims of the present study were to investigate skeletal muscle structure in IUGR offspring and how moderate daily exercise drives changes in metabolic pathways that may explain obesity prevention. We hypothesized that moderate daily exercise prevents obesity development in IUGR offspring through the activation of specific metabolic pathways in muscle tissue that enhance metabolic flexibility. To test this hypothesis, we conducted a study in the rat using maternal undernutrition throughout gestation to induce IUGR in offspring, a model described in detail by Thompson et al. (5). We then used a standardized exercise protocol throughout adult life to investigate the effects of voluntary daily wheel running (56 m/d) on muscle structure and metabolic regulation. The response to moderate daily exercise was explored using a range of histological, molecular, and biochemical markers of metabolic flexibility in both the red, slow, and oxidative *M. tibialis anterior* (SOL) and the white, fast, and glycolytic *M. gastrocnemius superficialis* (GSUP).

### Materials and Methods

#### Experimental design

Tissue and blood plasma samples that were used for the present report came from the same animal experiment, which we have published previously (7), studying hepatic metabolism in response to moderate, daily exercise in prenatally induced obesity (7). Briefly, at 46 d of age, male offspring of AD mothers and mothers fed 30% of *ad libitum* intake during pregnancy (IUGR) were divided into four groups with eight animals per group: AD nonexercised (ANE), AD exercised (AEX), IUGR nonexercised (INE), IUGR exercised (IEX) and were put on a scheduled feeding regimen of 2 h *ad libitum* access per day. From 60 d of age, the exercised groups were individually placed into an operant chamber (Med Associates Inc., St. Albans, VT; model ENV-007) with a running wheel for 1 h/d during the light phase. The running wheel was controlled by a brake system to ensure each animal ran (on average) the same amount each session (56 m/d). All procedures involving animals were carried out with the prior approval of the Animal Ethics Committee of the University of Auckland.

#### Plasma and muscle collection

At 250 d of age, rats were fasted overnight and killed by decapitation under halothane anesthesia. The last exercise bout occurred approximately 18 h before cull. Blood was collected into heparinized tubes and stored on ice (4 C) until centrifugation. Plasma was stored at −20 C until analysis. SOL and GSUP from one leg were snap frozen in liquid nitrogen and stored at −80 C for analysis. The respective muscles from the other leg were isolated, fixed onto cork plates, and frozen in liquid N₂-cooled isopentane, and stored at −80 C to ensure tissue integrity for immunohistochemical studies.

#### Chemicals and antibodies

Analytical grade biochemicals were obtained from BDH Laboratory Supplies (Poole, UK) or Sigma-Aldrich Inc. (St. Louis, MO) unless otherwise specified; reagents and apparatus for SDS-PAGE and immunoblotting were from Bio-Rad (Hercules, CA).
Antibodies for type I fibers and type IIA fibers were obtained from Novocastra Laboratories (Newcastle upon Tyne, UK) and Alexis Biochemicals (San Diego, CA), respectively. Antimouse Cy3-labeled secondary antibody was from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). Primary antibodies for Western blotting were obtained from Sigma [fatty acid synthase (FAS)]; Alpha Diagnostics International [San Antonio, TX; carnitine-palmitoyltransferase (CPT)-1]; Santa Cruz Biotechnology [Santa Cruz, CA; peroxisome proliferator-activated receptor-γ1 coactivator (PGC)-1 and PGCζ]; and Chemicon International [Temecula, CA; glucose transporter (GLUT)-4, monocarboxylate transporter (MCT)-1, and -4].

**Immunohistochemical and morphometrical studies**

To examine muscle fiber type composition, 6-μm slices were cut from the midbelly region of frozen muscles across the fiber direction. Slow and fast myosin heavy chain (MHC) antibodies were used to detect type I fibers (MHC slow positive) and type IIA fibers (MHC fast positive). Type IIB fibers of GSUP were detected as unstained fibers on slices with type I/IIa costaining. The immunodetection procedure was as follows: after thawing, slices were washed in PBS and blocked (4% goat serum, 0.5% Triton X-100, 0.1% NaN₃ in 1× PBS; sterile filtrated) for 1 h at room temperature. After washing slices with PBS, primary antibody (1:100) was incubated in blocking solution overnight in a humidified chamber at 4 C. After washing, slices were incubated for 2 h with secondary antibody [antimouse Cy3 labeled (1:500)] at room temperature, washed again, and covered with covering medium [80% glycerol/1× PBS with 0.1% NaN₃ (pH 7.0)]. Fluorescence signals were detected by a fluorescence microscope (Olympus, Tokyo, Japan) and five pictures per slice per staining were taken with ×20 magnification using a digital camera. For quantification of muscle fiber-type composition, 700–850 fibers/animal were counted, and type-classified fibers were expressed as percent of total counted fibers. The sizes of 50 stained and unstained, randomly selected, fibers per animal were measured.

**Western blotting of muscle proteins involved in lipid and carbohydrate metabolism**

Muscle tissue, ground under liquid N₂, was homogenized on ice in prechilled homogenization buffer [in millimoles per liter: 50 HEPES (pH 7.4), 0.1% Triton X-100, 4 EGTA, 10 EDTA, 100 β-glycerophosphate, 15 tetrasodium pyrophosphate, 5 sodium orthovanadate, 25 sodium fluoride, protease inhibitors (Roche, Mannheim, Germany); 30–50 mg tissue powder per milliliter buffer]. Homogenates were shaken for 1 h at 4 C and subsequently passed 10 times through a syringe with a 22-gauge needle. Protein concentrations of the homogenates were measured according to Bradford (Bio-Rad protein quantification kit). Samples in loading buffer [50 mmol/liter Tris HCl (pH 6.8), 10% glycerol, 2% sodium dodecyl sulfate, 0.1% bromphenol blue, 2% mercaptoethanol] were denatured before loading 40 μg per lane on a 5% stacking/8.1% separation gel. Electrophoresis was performed according to Laemmli (8). Detection of specific proteins was performed after blocking the membranes in 10% fat-free milk/PBS in 0.1% Tween 20 at room temperature. Membranes were incubated overnight at 4 C with rat-specific primary antibodies at the given concentrations: FAS, 1:500; CPT1, 1:260; PGC1, 1:500; PGCζ, 1:300; GLUT4, 1:1500; MCT1, 1:300, and MCT4, 1:200. Detection of the primary antibodies was performed using secondary antibodies: antirabbit-horseradish peroxidase (PGC1, PGCζ, GLUT4, MCT1, MCT4) for 1 h at room temperature or antirabbit-biotin for 1 h at room temperature followed by streptavidin-horseradish peroxidase for 20 min at room temperature (CPT1, FAS). After washing, membranes were incubated with substrate (Pierce, Rockford, IL). After band detection, extent of expression was quantified by densitometry with Quantity One software (Bio-Rad).

**Muscle assays**

Muscle TG (5, 9), and glycogen (5, 10) contents were measured as previously described. Activity of citrate synthase (CS) and lactate dehydrogenase (LDH) were also assayed. Briefly, muscle tissue was ground under liquid N₂ and homogenized in buffer [50% glycerol, 40 mM KCl, 2 mM EDTA, 25 mM Tris-HCl (pH 7.8), 0.2% Triton X-100]. Enzyme activity was then quantified by spectrophotometric assays according to Newsholme and Crabtree (11) (CS assay) and using a commercially available kit (Tos-7 toxicology assay kit, LDH assay; Sigma, St. Louis, MO).

**Plasma metabolite and hormone assays**

Plasma lactate was measured by enzyme colorimetric assay using an automated bioanalyzer (Roche/Hitachi 902; Roche Diagnostics, Penzberg, Germany). Total plasma T₃ was measured by a commercially available coated tube RIA (Spectra T₃; RIA; Orion Diagnostica, Espoo, Finland; sensitivity: 0.1 nmol/liter, intraassay variation: 4.2%) and total plasma T₄ by an enzyme immunoassay (Cedia T₄; Microgenics Corp., Fremont, CA; sensitivity: 6.5 nmol/liter (0.5 μg/dl), intraassay variation: 3.4%) using the automated bioanalyzer. Plasma TSH levels were determined by a commercially available IRMA (Coat-a-Count IKT5. Siemens, Los Angeles, CA; sensitivity: 0.03 μIU/ml, intraassay variation 3.9%).

**Statistical analyses**

All measures were analyzed by two-way ANOVA using StatView for Windows, version 5.0 (SAS Institute Inc., Cary, NC). Differences between groups were compared by Fisher’s projected least significant difference (PLSD) post hoc analysis. If the magnitude of the interaction determined by ANOVA was one third or more of the main effect, this analysis was deemed to be inappropriate, and a method of synergy detection was used (12). Values in figures and tables are given as mean ± SEM (n = 6–8 animals/group). P < 0.05 was considered significant.

**Results**

**Muscle structure and biochemical properties**

Fiber size in both muscles, SOL and GSUP, was significantly reduced by prenatal undernutrition (Table 1). Muscle fiber types were detected by immunohistochemical staining of MHC type. Physiologically, unlike the GSUP, the rat SOL muscle expresses only MHC types I and IIA. The proportion of type I fibers in SOL was signifi-
significantly increased in IUGR offspring, whereas the proportion of type IIA fibers was decreased (Table 1; representative sections are shown in Fig. 1). Fiber type composition of the GSUP muscle was unaffected by prenatal nutrition. Similarly, exercise had no effect on fiber type composition in either SOL or GSUP (Table 1).

Because fiber type composition influences the biochemistry of muscle metabolism, the activity of enzymatic markers of oxidative (CS) and glycolytic (LDH) pathways were measured in muscle homogenates. Neither enzyme in SOL muscle was affected by prenatal nutrition (Table 2). However, enzyme activity in the GSUP did differ, with LDH activity lower in IUGR offspring (Table 2). The activity of CS in GSUP was differentially affected by exercise, increasing with exercise in AD offspring but decreasing with exercise in IUGR (Table 2).

To assess energy storage capacity, intramuscular TG and glycogen concentrations were measured. No significant differences in SOL and GSUP TG content were observed (Table 2). Glycogen content was significantly increased in GSUP of IUGR offspring (Table 2) but unchanged by prenatal nutrition or postnatal exercise in SOL muscle.

Markers of muscular lipid metabolism

CPT1, a marker of fatty acid oxidation, and FAS, a marker of lipid synthesis, were measured semiquantitatively at the protein level in SOL muscle. Prenatal undernutrition significantly decreased CPT1 expression (Fig. 2A). FAS expression was markedly increased by prenatal undernutrition but was differentially influenced by exercise. Whereas FAS expression was increased in AEX offspring, it was reduced in IEX offspring in comparison with their nonexercised counterparts (Fig. 2B). The expression of PGC1, a major regulatory factor of lipid metabolism, was significantly decreased by prenatal undernutrition but not influenced by postnatal exercise (Fig. 2C).

Markers of muscle carbohydrate metabolism

The capacity for glucose uptake is an important feature of muscle energy metabolism, in particular for the glycolytic pathways of white muscle. Therefore, the total expression of glucose trans-

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**TABLE 1.** The effect of maternal undernutrition during pregnancy and postnatal exercise on structure of SOL and GSUP in 250-d-old offspring

<table>
<thead>
<tr>
<th>Group</th>
<th>Fiber size ($\mu m^2$)</th>
<th>Type I (%)</th>
<th>Type II (%)</th>
<th>Type IIA (%)</th>
<th>Type IIB (%)</th>
</tr>
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<tbody>
<tr>
<td>ANE</td>
<td>2263 ± 104$^a$</td>
<td>92.7 ± 1.6$^a$</td>
<td>7.3 ± 1.6$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AEX</td>
<td>2448 ± 169$^a$</td>
<td>90.6 ± 1.9$^a$</td>
<td>9.4 ± 1.9$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INE</td>
<td>1936 ± 86$^b$</td>
<td>97.3 ± 1.0$^b$</td>
<td>2.6 ± 1.0$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IEX</td>
<td>1972 ± 133$^b$</td>
<td>98.0 ± 0.8$^b$</td>
<td>2.0 ± 0.8$^b$</td>
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<td></td>
</tr>
</tbody>
</table>

Two-way ANOVA

<table>
<thead>
<tr>
<th>Undernutrition</th>
<th>Exercise</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are mean ± SEM and calculated using two-way ANOVA and post hoc Fisher’s PLSD test ($n = 6–8/group$). Different superscripts ($a, b$) denote group differences detected by statistical analyses within each column. n.s., Not significant.

$^a$ Fiber size refers to all fibers irrespective of fiber type.

$^b$ Percent refers to percentage of type I or II fibers per totally counted fibers.
TABLE 2. The effect of maternal undernutrition during pregnancy and postnatal exercise on TG and glycogen (Glyc) contents and activities of CS and LDH in SOL and GSUP in 250-d-old offspring

<table>
<thead>
<tr>
<th>Group</th>
<th>SOLTG (mg/g)</th>
<th>SOLGlyc (mg/g)</th>
<th>SOLCS (mU/mg)</th>
<th>SOLLDH (U/mg)</th>
<th>GSUPTG (mg/g)</th>
<th>GSUPGlyc (mg/g)</th>
<th>GSUPCS (mU/mg)</th>
<th>GSUPLDH (U/mg)</th>
</tr>
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<tbody>
<tr>
<td>ANE</td>
<td>0.23 ± 0.06</td>
<td>1.07 ± 0.32</td>
<td>65.5 ± 2.3</td>
<td>15.6 ± 0.4</td>
<td>0.13 ± 0.06</td>
<td>0.51 ± 0.04</td>
<td>23.8 ± 1.8</td>
<td>10.3 ± 2.7</td>
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<tr>
<td>AEX</td>
<td>0.14 ± 0.03</td>
<td>1.24 ± 0.28</td>
<td>71.4 ± 3.1</td>
<td>15.0 ± 0.5</td>
<td>0.04 ± 0.01</td>
<td>0.60 ± 0.05</td>
<td>32.7 ± 2.7</td>
<td>13.1 ± 1.6</td>
</tr>
<tr>
<td>INE</td>
<td>0.26 ± 0.07</td>
<td>0.95 ± 0.15</td>
<td>74.5 ± 2.7</td>
<td>14.5 ± 0.4</td>
<td>0.04 ± 0.01</td>
<td>0.71 ± 0.04</td>
<td>33.6 ± 5.1</td>
<td>7.9 ± 0.9</td>
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<tr>
<td>IEX</td>
<td>0.26 ± 0.07</td>
<td>1.53 ± 0.39</td>
<td>72.2 ± 2.4</td>
<td>15.1 ± 0.5</td>
<td>0.20 ± 0.15</td>
<td>0.81 ± 0.08</td>
<td>28.3 ± 2.9</td>
<td>7.5 ± 0.6</td>
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</table>

Two-way ANOVA:
- Undernutrition: n.s., n.s., n.s., n.s., n.s., 0.001, n.s., 0.05
- Exercise: n.s., n.s., n.s., n.s., n.s., n.s., n.s., n.s.
- Undernutrition: n.s. n.s. n.s. n.s. n.s. 0.001 n.s. 0.05

Values are mean ± SEM and calculated using two-way ANOVA and post hoc Fisher’s PLSD test (n = 6–8/group). Different superscripts (a, b) denote group differences detected by statistical analyses within each column. n.s., Not significant.

* Enzyme activities are given in units per milligram total protein of homogenates.

porter GLUT4 and its regulatory enzyme PKCζ were determined at the protein level in the GSUP muscle. GLUT4 and PKCζ expressions were both decreased by prenatal undernutrition (Fig. 3, A and B). Conversely, PKCζ expression was enhanced by postnatal exercise.

The use of glucose via glycolytic pathways leads to accumulation of lactate within the muscle fiber, which can be efficiently exported from white fibers (type IIB) by MCT4. Expression of this lactate exporter was significantly decreased in IUGR offspring compared with AD rats but increased approximately 3-fold with exercise in IUGR offspring (Fig. 3C). In contrast, exercise had no effect on MCT4 expression in AD offspring. Lactate import into red, type I fibers is mediated by the lactate importer, MCT1. Expression of MCT1 in GSUP was significantly stimulated by exercise in IUGR offspring (Fig. 3D). The effectiveness of this export-import system can be assessed by the measurement of plasma lactate concentrations, which were increased by postnatal exercise in both AD and IUGR offspring (Table 3).

Plasma thyroid hormone concentrations

The concentrations of T3, T4, and TSH, known regulators of whole-body metabolic homeostasis, were measured in plasma. Plasma TSH concentrations were highest in the ANE group (Table 3). Total T3 concentrations were lower in INE offspring compared with ANE animals (Table 3). Interestingly, circulating T3 levels were differentially influenced by exercise, decreasing in response to exercise in AD offspring and increasing with exercise in IUGR offspring (Table 3). Plasma total T4 levels were not influenced by prenatal nutrition or postnatal exercise (Table 3).

Discussion

This study investigated whether the prevention of prenatal obesity by moderate daily exercise is linked to structural and metabolic changes in the muscle of IUGR offspring that facilitate enhanced metabolic flexibility.
Enhanced metabolic flexibility, as demonstrated by an increased use of lipid or lipid precursors (e.g., glucose), and the capability to easily switch between lipid and carbohydrate pathways during exercise, may enhance muscle contractile performance. In the present study, moderate daily exercise was provided to AD and IUGR offspring to increase metabolic demand in skeletal muscle and generate a physiological need for metabolic adaptation. However, it is important to note that the last bout of exercise in this study occurred approximately 18 h before tissue collection. Therefore, the influence of exercise on metabolic and molecular parameters was examined during the recovery period in resting muscle. Furthermore, because exercise was performed from 60 d of age until 250 d of age, observed metabolic adaptations are the consequence of long-term moderate daily exercise.

**Prevention of prenatally induced obesity**

As previously reported, the moderate level of daily exercise used in this study (56 m/d) was sufficient to decrease adiposity in IUGR offspring to prevent obesity but had no effect on fat deposition in AD offspring (7). Results of investigations of hepatic metabolism in this experimental cohort suggested that the offspring of undernourished mothers are able to use glucose and hepatic glycogen efficiently as an energy substrate for exercise. The ability of these offspring to switch efficiently between pathways of energy storage and usage when exercise was available was proposed to decrease the availability of lipid and lipid precursors and thus prevent obesity development (7). However, as a primary site of energy metabolism during exercise, it was important to examine pathways of lipid and glucose use in the skeletal muscle to determine whether alterations in hepatic metabolism are supported by structural or functional changes in lean tissue.

**Lipid metabolism in muscle**

The lipid metabolism of SOL was examined to determine whether fat was preferentially used by IUGR offspring. This muscle has a high capacity to oxidize fatty acids, the preferential energy substrate in red muscle during endurance exercise and recovery. These fatty acids can be derived from either intramuscular TG or the blood plasma after their release from adipose tissue or by liver TG synthesis. SOL muscle is an almost exclusively oxidative muscle, expressing more than 90% type I fibers. In the present study, IUGR offspring had a significantly higher percentage of type I fibers (97%) than AD offspring. This difference has also been reported in sheep (13) and pigs (14), indicating a common adaptive principle of muscle tissue development during prenatal malnutrition. Fast-twitch type IIa fibers were almost nonexistent in the SOL muscle of IUGR rats. Such fast-twitch fibers are predominately derived from secondary myotubes during ontogenesis in the late fetal and early postnatal period of the rat development (15). Therefore, we conclude that prenatal nutrition has long-lasting consequences on muscle structure. Indeed, secondary myotubes are known to be particularly sensitive to nutritional influences during intrauterine development (16). The increase in type I fibers, and concomitant decrease in type II fibers, within the SOL muscle...
Prenatal undernutrition also led to decreased fiber size in both the SOL and GSUP muscles, in agreement with other studies of a prenatal dietary challenge in the rat (17, 18). Smaller muscle fibers may be more metabolically active due to an increase in surface area, providing a better supply of nutrients and oxygen and ensuring an increased clearance of metabolic waste products by red and white muscle tissue, alike. This notion is supported by a recent study in IUGR piglets suggesting that a higher percentage of type I fibers is correlated with a higher capillary density (19).

In addition to morphological changes associated with oxidative capacity, the key enzymes (CPT1 and FAS) and one of the major regulatory factors of oxidative lipid metabolism (PGC1) were also assessed. PGC1 protein expression in the SOL was reduced in IUGR offspring, irrespective of postnatal exercise. This long-term metabolic regulator is known to stimulate β-oxidation of fatty acids in muscle to increase mitochondrial biogenesis and thereby to prevent obesity (20, 21). The gene of the key enzyme of β-oxidation, CPT1, possesses a peroxisome proliferator-activated receptor-responsive element, corroborating the connection between peroxisome proliferator-activated receptor, its coactivator PGC1, and their downstream target, CPT1 (22). Reduced expression of this transcription factor coactivator should result in decreased CPT1 expression (as observed in this study) and thereby a reduction in fatty acid oxidation. Paradoxically and in apparent contradiction with the structural findings, this decrease was observed in both nonexercised and exercised IUGR offspring. However, the capacity for fat synthesis (as measured by FAS) was increased by prenatal undernutrition but decreased in IUGR offspring with postnatal exercise. This is in contrast to AD offspring, which demonstrated the physiologically expected increase in FAS expression in response to exercise to replenish intramuscular TG stores. Importantly, when combined, these differences in lipid metabolism between AD and IUGR offspring indicate that fat is not an important fuel source for exercise in IUGR offspring and further highlights the biological significance of the elevated glucose use observed in the livers of these animals (7).

Thyroid hormones play a permissive role in determining metabolic features of muscles in a diversity of ways. In rats, SOL is especially sensitive to T₃ due to a high expression of thyroid hormone receptors. In turn, enhanced T₃ signaling results in increased oxygen consumption, enhanced activity of citrate synthase and cytochrome oxidase and stimulated mRNA expression of mitochondrial genes including PGC1 and cytochrome oxidase (23). Data from the present study suggest that the responsiveness of oxidative muscle metabolism to T₃ may be altered by prenatal undernutrition. In nonexercised IUGR offspring, lower plasma T₃ and TSH levels may be responsible for the reduced lipid oxidative capacity of SOL muscle, indicating that the storage nature of metabolism in these animals may be based on a decrease in basal metabolic rate. Whereas this notion requires further research, it is tempting to speculate that, when less energy is expended in the absence of an opportunity to exercise, a decreased basal metabolic rate would allow the channeling of excess energy to storage as fat and glycogen. Exercise is known to decrease T₃ levels to spare energy for muscle fueling (24), as observed in the AEX offspring of the present study. Importantly, moderate daily exercise increased T₃ levels in IUGR offspring, although this increase did not affect muscle of adult IUGR offspring in the present study indicates an increased capacity for oxidative metabolism. Whereas a trend toward higher values (P < 0.09) of citrate synthase activity in the SOL muscle of INE offspring supports this notion, further work is warranted to investigate markers of oxidative capacity when exercise regimens are used together with high-energy density diets.

<table>
<thead>
<tr>
<th>Group</th>
<th>Lactate (mmol/liter)</th>
<th>T₃ (nmol/liter)</th>
<th>T₄ (nmol/liter)</th>
<th>TSH (mU/liter)</th>
</tr>
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<tr>
<td>ANE</td>
<td>1.83 ± 0.09</td>
<td>2.46 ± 0.15</td>
<td>46.1 ± 2.2</td>
<td>1.08 ± 0.28</td>
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<tr>
<td>AEX</td>
<td>2.54 ± 0.32</td>
<td>2.10 ± 0.09</td>
<td>46.2 ± 2.4</td>
<td>0.65 ± 0.13</td>
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<tr>
<td>INE</td>
<td>2.09 ± 0.12</td>
<td>2.16 ± 0.11</td>
<td>44.0 ± 2.8</td>
<td>0.41 ± 0.08</td>
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<tr>
<td>IEX</td>
<td>2.91 ± 0.41</td>
<td>2.42 ± 0.19</td>
<td>47.2 ± 3.7</td>
<td>0.76 ± 0.23</td>
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<tr>
<td>Two-way ANOVA</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
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</tbody>
</table>

| Undernutrition | n.s. | n.s. | n.s. | n.s. |
| Exercise       | 0.01 | n.s. | n.s. | n.s. |
| Interaction    | 0.05 | n.s. | n.s. | n.s. |

Values are mean ± SEM and calculated using two-way ANOVA and post hoc Fisher’s PLSD test (n = 6–8/group). Different superscripts (a, b) denote group differences detected by statistical analyses within each column. n.s., Not significant. *<i>P</i><0.05 within the column. #<i>P</i><0.05 within the interaction.
PGC1 and CPT1 expression. Future studies would need to investigate whether the 12% lower plasma T₃ (assessed as total T₃) and its reversal by exercise in the IUGR offspring are reflected in free plasma T₃ and also whether decreased circulating plasma T₃ may be explained, at least in part, by decreased conversion of T₄ to T₃ in oxidative muscles.

These findings confirm that lipid metabolism in muscle of INE offspring reflects the storage character of prenatally undernourished offspring, in agreement with observations made in the liver of these animals (5, 7). This physiological setting exists with no apparent disadvantage on muscle metabolic function. However, exercise did not result in an increase in fat use in IUGR offspring as it was observed in the AD offspring, despite a higher structure-based potential for oxidative capacity. Nevertheless, exercise decreased fat synthesis in IUGR, possibly due to the exercise-induced reduction in lipid precursor availability. It is important to note that rats in the present study were fed a balanced chow diet ad libitum. Further studies are needed to explore whether the potential for enhanced oxidative capacity of IUGR offspring is activated when exercise regimens are used together with high-energy density diets.

### Carbohydrate metabolism in muscle

To study the extent to which glucose was used for muscle fueling in exercised IUGR offspring, key components of the carbohydrate metabolism in the GSUP were examined. The GSUP muscle consists of approximately 45% white type IIB fibers, 30% intermediary type IIA fibers, and 25% red type I fibers in AD and IUGR rats alike. In this muscle, energy is largely produced anaerobically through glycolysis and the subsequent conversion of pyruvate to lactate. The required glucose can be derived from glycogen stores in muscle and liver and plasma glucose. In the present study, GSUP glycogen stores were markedly increased in IUGR offspring, providing a large amount of rapidly available energy within the muscle tissue itself. Therefore, we studied key markers of pathways of muscle glucose uptake (GLUT4 and PKCζ), and anaerobic glucose use (LDH activity, MCT1, MCT4, and plasma lactate) to assess glycolytic capacity. PKCζ is a downstream target of phosphatidylinositol 3-kinase within the insulin signaling cascade and stimulates translocation of GLUT4 into the sarcolemma to increase glucose transport (25, 26). In INE offspring, reduced PKCζ and low GLUT4 expression may contribute to a decrease in overall energy expenditure when exercise is not available. However, exercise had a strong stimulating effect on PKCζ, nearly doubling the protein expression in the IEX group whereas only slightly increasing expression in AEX offspring. Total GLUT4 expression did not reflect this enhancement of PKCζ expression. PKCζ is primarily responsible for the translocation of transporters into the sarcolemma. Therefore, we speculate that the proportion of GLUT4 inserted into the sarcolemma may be increased in IEX muscle in comparison with INE, and glucose transport from plasma into the muscle cell may be enhanced. This notion is supported by the elevated glycogen stores in GSUP of IUGR offspring measured in this study, although future studies are required to quantify plasma membrane GLUT4 concentration.

The overall anaerobic glucose use in GSUP muscle of the IEX group did not appear to be increased in this study because LDH activity was maintained at a lower level in both nonexercised and exercised IUGR compared with AD. Intriguingly, the plasma lactate concentrations showed a significant increase with exercise in both the AD and IUGR offspring. A metabolically meaningful interpretation of the plasma lactate response has to be seen in context with the significant changes of CS activity in GSUP. Exercise caused a significant decrease in CS activity in IUGR offspring, which indicates enhanced glycolytic processes in GSUP of exercised IUGR animals. An increase in glycolytic processes in turn will increase lactate production, which is in agreement with our observation of significantly increased levels of MCT transporters in this tissue. Data from the present study suggest that an increase of MCT4, an effective myocellular lactate exporter (27), may facilitate lactate export from white fibers, and the concomitant increase of MCT1 transporters may enhance the import of lactate into red type I fibers, which can effectively use lactate for oxidative metabolism. Consequently, an intramuscular increase in lactate use in IUGR offspring will prevent excessive lactate accumulation in plasma. This enhanced metabolic flux through glycolytic pathways due to rapid conversion of pyruvate to lactate is an example of the increased metabolic flexibility described in a thrifty phenotype.

Lowering the intracellular levels of lactate by increased MCT4 activity would also diminish the detrimental effects of lactate on muscular function by decreasing acidosis. Avoiding a lowering of intracellular pH is essential for muscle tissue to maintain force and metabolic performance (28). This effective lactate shuttle system in IUGR offspring may ameliorate cellular processes, which would otherwise lead to muscle pain and fatigue (29). When lactate production exceeds the export capacity of MCT4, increases in intracellular lactate levels may influence CPT1 function, which, in turn, leads to decreases in fatty acid oxidation. This further stabilizes the shift to glucose use (29).
highly effective energy production from glucose, increased T₃ may enhance Ca²⁺ uptake into the sarcoplasmic reticulum and the expression of MHC in muscle, resulting in a stimulation of contraction velocity (24). Consequently, we suggest that muscle contractile activity was facilitated by these cellular mechanisms in IUGR offspring, thus making moderate daily exercise easier. This may provide an important link between peripheral metabolism and the increased preference for voluntary exercise observed in IUGR offspring (6). However, this notion requires in-depth analysis in future studies.

Summary and conclusions

In the present study, we investigated skeletal muscle structure in IUGR offspring and show that moderate daily exercise drives changes in metabolic pathways that may explain, at least in part, obesity prevention. Enhanced metabolic flexibility is activated by exercise in IUGR offspring, resulting in an increase in the anaerobic use of glucose, especially in white, GSUP muscle tissue, and is associated with increased intramuscular glycogen stores during recovery from exercise. In addition, data from the present study suggest that SOL of IUGR offspring may use glucose more avidly because it has been observed that SOL tissue treated in vitro with T₃ shows a strong shift from fatty acid oxidation toward glucose use (23). It is tempting to speculate that the use of glucose as a glycogen precursor and fuel for muscle work by IUGR offspring, combined with an increase in overall energy expenditure with exercise, may have reduced energy availability for fat storage. The novel findings from this report warrant future studies that investigate gas exchange in vivo and substrate flux in isolated muscle and fat tissue of IUGR rats.

Despite a predisposition to develop obesity under sedentary conditions, the activation of enhanced metabolic flexibility in muscle through moderate, daily exercise effectively prevented obesity development in IUGR offspring. These findings further highlight the importance of tailoring obesity prevention strategies that improve long-term health.

Acknowledgments

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