Accelerated contractile function and improved fatigue resistance of calf muscles in newborn piglets with IUGR

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Wank, Veit, Reinhard Bauer, Bernd Walter, Harald Kluge, Martin S. Fischer, Reinhard Blickhan, and Ulrich Zwiener. Accelerated contractile function and improved fatigue resistance of calf muscles in newborn piglets with IUGR. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R304–R310, 2000.—Asymmetrical intrauterine growth restriction is denoted by disproportional reduction of muscle mass compared with body weight reduction. However, effects on contractile function or tissue development of skeletal muscles were not studied until now. Therefore, isometric force output of serial-stimulated hindlimb plantar flexors was measured in thiopental-anesthetized normal weight (NW) and intrauterine growth-restricted (IUGR) 1-day-old piglets under conditions of normal, reduced (aortic cross clamping), and reestablished (clamp release) blood supply (measured by colored microspheres technique). Furthermore, muscle fiber type distribution was determined after histochemical staining, specific muscle force of the plantar flexors (quotient from absolute force divided by muscle mass (N/g)) was calculated, and glycogen content and morphometric data of the investigated muscles were estimated. Regional blood flow of hindlimb muscles was similar in NW (6 ± 2 ml·min⁻¹·100 g⁻¹) and IUGR piglets (8 ± 1 ml·min⁻¹·100 g⁻¹). Isometric muscle contractions induced a marked increase in regional blood flow of 4.1-fold in NW and 5-fold in stimulated hindlimb muscles of IUGR piglets (baseline blood flow). Specific force of NW piglet muscles (5.2 ± 0.2 N/g) was significantly lower than IUGR piglet muscles (6.1 ± 0.6 N/g; P < 0.05). Isometric muscle contractions (NW: 32.7 ± 4.7 N; IUGR: 21.7 ± 4.0 N) resulted in a higher rate of force decrease in the calf muscles of NW animals compared with IUGR piglets (8 ± 2 vs. 3 ± 1%; P < 0.01). Functional restoration of contractile performance after hindlimb recirculation was nearly complete in IUGR piglets (98 ± 1%), whereas in NW piglets a deficit of 9 ± 3% was found (P < 0.01). Muscle fiber type estimation revealed an increased proportion of type I fibers in flexor digitorum superficialis and gastrocnemius medialis in IUGR piglets (P < 0.05). These data clearly indicate that contractile function is accelerated in newborn IUGR piglets.

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IUGR obviously exhibits a distinct impact on fetal muscle development and differentiation. Weight reduction of skeletal muscles is disproportionately caused by IUGR compared with body weight restriction (4). Furthermore, it was recently shown that the mitochondrial function of rat skeletal muscles was postnatally altered by IUGR (23). In addition, the fractional rate of protein synthesis in skeletal muscle of IUGR piglets was not altered, but skeletal protein synthesis rate and the estimated absolute synthesis rates of longissimus dorsi muscle protein did not accelerate compared with normal weight (NW) piglets (9). However, until now, the effect of IUGR on muscle differentiation and contractile properties has not been elucidated.

In this study, contractile performance and fiber type composition of hindlimb skeletal muscles in NW and IUGR newborn piglets were investigated. For that purpose, the isometric force output of supramaximal-stimulated plantar flexors was measured at different states of muscle fatigue induced by changed blood supply. Additionally, glycogen content and morphometric data of the investigated muscles were compared between NW and IUGR piglets. We used a morphometrically well-characterized state of asymmetrical IUGR in newborn piglets (4, 16, 30) and included animals with optimal vital conditions early after birth (10).

**MATERIALS AND METHODS**

Subjects and surgical instrumentation. The committee of the Thuringian State government for animal research approved the protocol of the study. The animals were managed in accordance with the guidelines of the American Physiological Society.

In this study, 14 NW (body wt 1,465 ± 125 g) and 13 IUGR (body wt 807 ± 44 g) 1-day-old Piétrain × German Landrace cross-breed piglets were investigated. Animals were obtained from a breeding farm. Delivery was observed, and viability of neonatal swine was checked so that animals with a score of clinical evaluation of viability ≥ 7 (10) were included in the study. Immediately before the onset of the experiments, animals were carried to the laboratory in a climatized transport incubator at environmental temperature of 33–34°C. The time for transportation needed 30–60 min.

Seven NW and seven IUGR piglets were randomly selected and used for measurements of hindlimb contractile function. The animals were initially anesthetized with 1.5% isoflurane in 70% nitrous oxide and 30% oxygen. Moreover, all skin incisions were preceded by local anesthesia with hypodermic lidocaine injection (Xylocitin 2%, Jenapharm). A central venous catheter was introduced through the left external jugular vein and was used for administration of drugs and for volume substitution (Ringer-lactate solution: 5 ml·kg body wt⁻¹·h⁻¹). An appropriate endotracheal tube (12–16 French Charrier) was inserted through a tracheotomy. Animals breathed spontaneously throughout the experiment. End-expiratory CO₂ content was continuously recorded (CO₂ analyzer 930, Servo Ventilator, Siemens-Elema, Solna, Sweden). A polyurethane catheter (PU-3.5 Charier, Sherwood, UK) was introduced into the left brachial artery to measure blood gases and pH, record blood pressure, and to withdraw reference samples from the arterial line for regional muscle blood flow measurements using the colored microsphere method. The left ventricle was cannulated retrogradely via the right common carotid artery with a polyurethane catheter (PU-3.5 Charier). Correct positions of the catheter tip were checked by continuous pressure trace recordings and by autopsy at the end of the experiment.

Through a diagonal incision in the flank of the right site located in the middle between the twelfth rib and the pelvic rim, the abdominal aorta just proximal to the hypogastric branches was exposed by a retroperitoneal approach and entangled by a thread to induce temporal ischemia of the lower limbs by temporal aortic cross clamping usingatrauma cardiovascular forceps according to the experimental protocol.

Rectal temperature was maintained throughout the experiment at 38°C using a heating pad and a feedback-controlled heating lamp. Arterial and left ventricular catheters were connected with pressure transducers (P23Db, Statham Instruments). Electrocardiogram recordings were made from standard limb leads using stainless steel needle electrodes. Physiological parameters were recorded on a multichannel polygraph (MT95K2, Astro-Med).

For measurements of isometric muscle force of the piglet plantar flexors, the piglets were placed in supine position. The tibia bone was carefully fixed on the proximal and distal ends with steel mandrils, and the hip was fixed with a horizontal iron rod so that the femur and, consequently, the origins of the stimulated muscles (soleus, flexor digitorum superficialis, medial and lateral gastrocnemius) were kept unchanged (Fig. 1). The muscle force was transferred to a force transducer via a calcaneus clamp and a steel wire. The lower leg of the animal and the transducer were aligned in such a way that the longitudinal axis of the calf muscles and the wire to the transducer ran horizontally (Fig. 1). The force signal of a strain gauge transducer (type 11E-100N0, Sensotec; ± 100 N, 0.5 µm/N compliance) was amplified by a direct-current amplifier (ME-10, Hottinger-Baldwin). After analog-to-digital conversion (12 bit), the data were sampled with 1,000 Hz and stored on a personal computer.

For calf muscle stimulation, the sciatic nerve of the left hindlimb was used. For this purpose, a skin incision was made in the lateral upper leg. The sciatic nerve was carefully prepared and attached to a platinum-electrode pair immersed in Ringer solution to avoid desiccating the nerve. Bipolar stimulation of the sciatic nerve was used for supramaximal muscle contraction (voltage-constant rectangular pulses, 150 Hz repetition frequency, 100-µs impulse width; Physiostimulator, Hugo Sachs Elektronik).

A series consisted of 21 nerve stimulations of 2 s each interrupted by pauses of 13 s each. During the experiments, stimulation nerve and electrodes were superfused with warmed physiological saline (37°C). To avoid an influence of...
force output measured on the calcaneus by ankle torque due to a simultaneous contraction of the muscle antagonists, the distal tendons of tibialis anterior and extensor digitorum longus were sectioned. After the surgical preparation was completed, general anesthesia was changed by exchange of isoflurane inhalation with intravenous thiobarbital infusion (12.5 mg·kg body wt\(^{-1}\)·h\(^{-1}\)). Furthermore, an epidural blockade was done after lumbar puncture below the fourth lumbar vertebra by instillation of 0.5–1.0 ml of the local anesthetic bupivacaine hydrochloride (Curotozine). Exact setting of the transmission blockade was verified by an immediate tonus loss of the hindlimb muscles. Then the piglets were allowed to rest for \(\sim 30\) min.

Experimental protocol. First, the optimal muscle length of the calf muscles had to be estimated. For that purpose, a series of isometric force measurements at different muscle lengths (1-mm step width) was carried out. On the basis of these data, the muscle length was adjusted to the length with highest isometric force recording.

This procedure was followed by estimation of physiological parameters and muscle blood flow under baseline conditions. Two minutes later, a series of 21 isometric muscle contractions under normal blood flow conditions was performed, which was immediately followed by a second muscle blood flow measurement.

A hindlimb ischemia was then induced by abdominal aortic cross clamping, as described before. Ten minutes later, a further data set of physiological parameters and muscle blood flows was obtained. At the 15th minute of ischemia, a second series of 21 isometric muscle contractions was performed, which was immediately followed by a further muscle blood flow measurement. At the 25th minute, aortic cross clamping was released to enable a hindlimb reperfusion. Ten minutes later, the last measurements of physiological parameters and muscle blood flow were performed, followed by a series of isometric muscle contractions.

Physiological measurements. Regional muscle blood flow was measured by means of the reference sample color-labeled microsphere technique (22). Application in piglets and methodological considerations have been presented and discussed in detail elsewhere (34). Briefly, in random sequence, a known amount (\(-1.5 \times 10^7\) per injection) of colored polystyrene microspheres (diameter 15.5 ± 0.33 \(\mu\)m) in 0.01% Tween 80, surface coated with one of five dyes (white, yellow, red, violet, blue; Dye-Trak, Triton Technology, San Diego, CA), was thoroughly vortexed and sonicated and immediately injected within 20 s into the left ventricle. The injection line was then flushed with 2 ml saline. A blood sample was withdrawn from the brachial aorta as the reference sample (27) beginning 15 s before the microsphere injection and continuing for 2 min with a rate of 1.5 ml/min (syringe pump SP210iW, World Precision Instruments, Sarasota, FL). At the end of each experiment, the piglet was killed with KCl. The calf muscles from both lower legs were obtained, adjacent connective tissue was carefully removed, and samples were weighed. Reference blood samples and tissue samples were covered with digestive solution (4 N KOH with 4% Tween 80 in deionized water). To retain the microspheres, each digested sample was then filtered under vacuum suction through an 8-\(\mu\)m pore polyester-membrane filter. Colored microspheres were quantified by their dye content. The dye was recovered from the microspheres by adding 150 \(\mu\)l of dimethylformamide. The photometric absorption of each dye solution was measured by a diode-array, ultraviolet-visible spectrophotometer (model 7500, Beckman Instruments). Calculations were performed using the MISS software (Triton Technology, San Diego, CA). The number of microspheres was calculated using the specific absorbance value of the different dyes. All reference and tissue samples contained \(> 400\) microspheres.

Heart rate, arterial blood pressure, arterial pH, \(P_{O_2}\), and \(P_{CO_2}\), oxygen saturation, and hemoglobin values were measured at each study period. Blood \(P_{O_2}\), \(P_{CO_2}\), and \(P_{CO_2}\) were measured with a blood gas analyzer (model ABL50, Radiometer, Copenhagen, Denmark), and blood hemoglobin and oxygen saturation were measured using a hemoxymeter (model OSM2, Radiometer). Absolute flows to tissues measured by colored microspheres were calculated by the formula:

\[
\text{flow}_{\text{tissue}} = \frac{\text{number of microspheres}_{\text{tissue}}}{\text{flow}_{\text{reference}}} \times \frac{\text{flow}_{\text{reference}}}{\text{number of microspheres}_{\text{reference}}}
\]

Flows are expressed in milliliters per minute per 100 g tissue by normalizing for muscle tissue weight.

Parameters of isometric muscle contractions. The maximum force (\(F_{\text{max}}\)) was used for determination of isometric force amount under conditions of the predetermined optimal muscle length of calf muscles. The specific muscle force of the plantar flexors was calculated as \(F_{\text{max}}\) divided by the sum of muscle masses of the whole plantar flexor group. \(F_{\text{max}}\) was determined from the force-time curve of the first contraction cycle under normal blood flow conditions.

Histochemistry and fiber type determination. Plantar flexors of the contralateral hindlimb were removed immediately after killing the animals. Muscles were prepared, powdered with talcum, and fixed at the origin and the insertion of the muscles using two artery forceps so that in vivo resting length was roughly reestablished. Then muscles were covered with aluminum foil, frozen in liquid nitrogen, and stored at \(-80\)°C. Transverse sections 12-\(\mu\)m thick were cut from the midsection of every four muscles at \(-22\)°C using a cryostat microscope. Complete cross sections of every muscle were obtained. Muscle fiber type determination was based on the alkaline combination staining method of Ref. 37. This method combines the myosin ATPase reaction after alkaline preincubation (pH 10.4) and the NADH tetrazolium salt reductase reaction on the same section. The calcium-activated myosin ATPase reaction described by Mejer and Elias (27a) produces a brown staining (lead sulfide). NADH tetrazolium reductase generates a blue final reaction product (formazan) following the protocol of Ref. 32. With the use of these procedures in the combination the fibers from NW and IUGR newborns, fiber type I and II could be distinguished by different staining (type I: blue; type II: brown). The muscle fiber type distribution was determined by counting the fiber number of each type within the cross-sectional area. In each slice, all myofibers within a fasciculus were counted. About 600 fibers were counted per muscle sample, and the mean percentages of type I and type II fiber number were calculated. Microscopic examination included superficial and deep aspects with similar frequency.

In four additional NW and three IUGR piglets, architecture of the four plantar flexors was determined by measurement of their fiber angles. For that purpose, longitudinal sections of all four muscles were made and photographed while the knee joint and ankle joint were fixed in a natural position (angle between femur and tibia: \(-120\)°; angle between tibia and foot: \(-140\)°). To determine a representative value of muscle fiber angles in every muscle studied, 10 equidistant angle measurements along the proximal-distal extension of longitudinal sections were performed and the mean value was calculated.

Measurement of glycogen content in calf muscles. In additional experiments, glycogen content in calf muscles was determined. Therefore, three NW piglets and three IUGR piglets were initially anesthetized with 1.5% isoflurane in 70% nitrous oxide and 30% oxygen, a central venous catheter
was introduced through the left external jugular vein, and general anesthesia was changed by exchange of isoflurane inhalation with intravenous thiobarbital infusion (12.5 mg·kg body wt \(^{-1}\)·h \(^{-1}\)). Calf muscles were prepared carefully and adjacent connective tissue was removed and pieces of \(-0.5\) g were obtained, immediately frozen in liquid nitrogen, and stored at \(-80^\circ\text{C}\) until analysis. Determination of tissue glycogen content was performed enzymatically. Glycogen was initially broken down into glucose using amyloglucosidase reaction (21) and then measured fluorometrically as glucose equivalents using hexokinase and glucose-6-phosphate dehydrogenase reactions (26).

Statistical analysis. If not otherwise indicated, data are reported as means \(\pm\) SD. One-way ANOVA with repeated measures was used to determine effects of isometric hindlimb muscle contraction alone and after hindlimb blood flow reduction and recirculation on hemodynamic and metabolic values. Because the normality test failed for muscle blood flow data, one-way repeated-measures ANOVA on ranks was used. Post hoc comparisons were made with the Student-Newman-Keuls method. Comparisons between groups were made with unpaired \(t\) tests and with the use of the Bonferroni correction for multiple use, if necessary. Differences were considered significant when \(P < 0.05\).

RESULTS

Physiological parameters and regional blood flows. Table 1 summarizes the physiological values of newborn NW and IUGR newborn piglets during baseline conditions before and after a series of isometric muscle contractions, before and after a series of isometric muscle contractions during hindlimb ischemia due to cross clamping of the abdominal aorta, and after 10 min of recirculation. The baseline prestimulation values of NW piglets are within the physiological range and consistent with other data obtained from anesthetized and artificially ventilated newborn piglets (25). There was no significant difference between NW and IUGR piglets for regional blood flow of hindlimbs (Table 1). Under baseline conditions, isometric muscle contractions led to an increase of mean arterial blood pressure in IUGR piglets (\(P < 0.05\)). Isometric muscle contractions induced a marked increase in regional blood flow of 4.1-fold in NW and 5-fold in IUGR piglets. Acute cross clamping of abdominal aorta was accompanied with only mild changes of physiological values, but regional blood flow of hindlimbs muscles was reduced significantly (\(P < 0.05\)). Isometric muscle contraction did not induce a relevant change in these parameters.

Release in aortic cross clamping was followed by a moderate regional hyperperfusion in NW piglets and an increase of the arterial lactate content in NW and IUGR piglets (\(P < 0.05\)).

Isometric muscle contraction and muscle fiber estimation. Muscle mass of plantar flexors is presented in Table 2. Muscles of IUGR piglets showed consistently lower values compared with those from NW piglets (\(P < 0.05\)). However, compared with the reduced body mass (IUGR piglets showed a reduction of 55% com-

Table 1. Physiological values of newborn NW and IUGR newborn piglets

<table>
<thead>
<tr>
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<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tr>
<td></td>
<td>BL-prestimulation</td>
<td>BL-poststimulation</td>
<td>I-prestimulation</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>68 ± 8</td>
<td>69 ± 8*</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>NW</td>
<td>63 ± 11</td>
<td>75 ± 12*</td>
<td>71 ± 21</td>
</tr>
<tr>
<td>IUGR</td>
<td>183 ± 22</td>
<td>192 ± 21</td>
<td>193 ± 14</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>159 ± 23</td>
<td>185 ± 12</td>
<td>188 ± 34</td>
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<tr>
<td></td>
<td>8 ± 1</td>
<td>25 ± 15*</td>
<td>3 ± 2*</td>
</tr>
<tr>
<td>Calf muscle blood flow, ml·min(^{-1})·100 g(^{-1})</td>
<td>105 ± 20</td>
<td>113 ± 28</td>
<td>104 ± 19</td>
</tr>
<tr>
<td>NW</td>
<td>116 ± 21</td>
<td>130 ± 23</td>
<td>126 ± 36</td>
</tr>
<tr>
<td>IUGR</td>
<td>39 ± 2</td>
<td>38 ± 2</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Arterial PO(_2), mmHg</td>
<td>38 ± 2</td>
<td>39 ± 3</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>NW</td>
<td>75 ± 0.02</td>
<td>75 ± 0.02</td>
<td>75 ± 0.02</td>
</tr>
<tr>
<td>IUGR</td>
<td>75.1 ± 0.03</td>
<td>74.9 ± 0.03</td>
<td>74.8 ± 0.02</td>
</tr>
<tr>
<td>Arterial oxygen content, mmol/l</td>
<td>6.3 ± 1.3</td>
<td>6.3 ± 1.1</td>
<td>6.4 ± 1.2</td>
</tr>
<tr>
<td>NW</td>
<td>6.0 ± 1.0</td>
<td>6.0 ± 0.8</td>
<td>5.9 ± 0.7</td>
</tr>
<tr>
<td>IUGR</td>
<td>3.5 ± 0.8</td>
<td>3.7 ± 0.7</td>
<td>3.9 ± 1.0</td>
</tr>
<tr>
<td>Arterial glucose content, mmol/l</td>
<td>3.3 ± 1.2</td>
<td>2.9 ± 0.7</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>NW</td>
<td>1.9 ± 0.6</td>
<td>2.1 ± 0.5</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>IUGR</td>
<td>2.2 ± 0.7</td>
<td>2.6 ± 0.6</td>
<td>2.6 ± 0.8</td>
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</table>

Values are means ± SD. NW, normal weight; IUGR, intrauterine growth restriction. *Indicates comparison between baseline values (BL-prestimulation) and values obtained immediately after isometric hindlimb contraction (BL-poststimulation). 10 min after induced hindlimb ischemia due to abdominal aortic cross clamping (I-prestimulation), after isometric hindlimb contraction during ischemia (I-poststimulation) and 10 min after recirculation (R-prestimulation) (\(P < 0.05\)).
pared with NW piglets), muscle mass was slightly less reduced (on average 58%).

Representative records of isometric force traces for each of the three different experimental conditions are shown in Fig. 2. Isometric force of the plantar flexors was always considerably higher in NW than in IUGR piglets (P < 0.05; Fig. 3). However, specific force of NW muscles (5.2 ± 0.2 N/g) was significantly lower than for IUGR muscles (6.1 ± 0.6 N/g; P < 0.05).

Under baseline conditions, a series of 21 isometric contractions resulted in a higher rate of force decrease in the calf muscles of NW animals of 8 vs. 3% in IUGR piglets (Table 3; P < 0.01). A similar amount of muscular blood flow reduction due to aortal cross clamping led to an initial reduction of isometric force that was markedly stronger in IUGR piglets (37 vs. 17% in NW piglets; P < 0.01). The amount of fatigue during a series of isometric muscle contractions was similar in both animal groups. Therefore, there was a similar difference of isometric force at the end of the contraction series with a reduction of 65% in NW piglets and a reduction of 77% in IUGR piglets. The functional restoration after hindlimb recirculation was nearly complete in IUGR piglets, whereas in NW piglets, a deficit of ~9% was found (P < 0.01). Furthermore, in NW animals, a markedly higher increase of fatigue during the final series of isometric muscle contraction occurred (reduction by 8%) compared with IUGR piglets, which showed a reduction of only 4% (P < 0.01).

Table 2. Muscle mass and mean muscle fiber angle of plantar flexor muscles in NW and IUGR newborn piglets

<table>
<thead>
<tr>
<th>Muscle Mass, g</th>
<th>Muscle Fiber Angle, °</th>
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<tr>
<td>NW (n = 5)</td>
<td>IUGR (n = 5)</td>
</tr>
<tr>
<td>NW (n = 4)</td>
<td>IUGR (n = 3)</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.95 ± 0.15</td>
</tr>
<tr>
<td>Flexor digitalis superficialis</td>
<td>1.09 ± 0.13</td>
</tr>
<tr>
<td>Gastrocnemius lateralis</td>
<td>1.9 ± 0.06</td>
</tr>
<tr>
<td>Gastrocnemius medialis</td>
<td>2.2 ± 0.18</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Indicates comparisons between NW and IUGR piglets (P < 0.05).

Fig. 2. Time course of representative isometric force (F) recordings of first (0) and last (20) isometric contraction of series consisting of 21 nerve stimulations during normal conditions of hindlimb blood flow (BL), after 15 min of compromised hindlimb blood flow due to abdominal aorta cross clamping (I), and after 15 min of reperfusion (R) obtained from a normal weight piglet (NW; A) and an intrauterine growth-restricted piglet (IUGR; B).

Fig. 3. Isometric force of plantar flexors in NW (filled bars) and IUGR (open bars) newborn piglets during repeated isometric contractions (2-s stimulation, 13-s resting period) during normal conditions of hindlimb blood flow (A), after 15 min of compromised hindlimb blood flow due to abdominal aorta cross clamping (B), and after 15 min of reperfusion (C). Data are means ± SD, n = 7 in both groups; significant differences of isometric force between NW and IUGR piglets at every contraction cycle (P < 0.05).

Table 3. Normalized isometric muscle force of plantar flexors at first and last contraction cycle in each series in NW and IUGR newborn piglets

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<tr>
<td>Contraction cycle 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NW</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IUGR</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Contraction cycle 20</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NW</td>
<td></td>
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<td>IUGR</td>
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Values are means ± SD. Force values are related to maximum force at first baseline contraction. *Indicates comparisons between baseline and 15 min after induced hindlimb ischemia due to abdominal aortal cross clamping (ischemia) or 15 min after recirculation (reperfusion), respectively (P < 0.05). Comparisons between NW and IUGR piglets: †P < 0.01, ‡P < 0.05.
The muscle fiber type estimation revealed an increased proportion of type I fibers in flexor digitalis superficialis and gastrocnemius medialis in IUGR piglets compared with NW piglets (Fig. 4; P < 0.05). However, glycogen content of the muscle tissue was similar in NW and IUGR piglets: 139 ± 30 (NW) and 145 ± 15 µmol/g wet wt (IUGR).

**DISCUSSION**

Data presented herein demonstrate for the first time that IUGR is accompanied by an accelerated development of skeletal muscle function combined with precocious muscle fiber differentiation of calf muscles. This view is supported by our results of isometric muscle contraction efficiency under normal hindlimb blood supply, increased fatigue resistance, and precocial type II to type I muscle fiber conversion in newborn IUGR piglets. Indeed, the increased specific muscular force of IUGR piglets of ~18% (P < 0.05) and also the reduced amount of fatigue at the end of serial isometric muscle contraction, which was indicated by markedly less force decrease in IUGR piglets (P < 0.01), reflect accelerated muscular development. An increase in fatigue resistance at serial tetanic calf muscle contraction was also reported at regular developmental progress in ovine fetuses (20). Presumably, the increased muscle fatigue resistance of IUGR piglets due to isometric muscle contraction may be elevated by the slightly but significantly higher proportion of type I muscle fibers of calf muscles involved. The higher proportion of type I fibers in calf muscles was shown to be a sign of developmental progress in both precocious and altricial species, including swine (5, 11, 12, 15, 24, 33).

Altogether, no remarkable difference in muscle architecture and fiber angle was found between IUGR and NW piglets (Table 2). Therefore, the distinct specific muscle force values of IUGR and NW should be caused by different contractile properties of muscle tissue. Indeed, the development of muscle tissue structure seems to be more advanced in IUGR than in NW muscles, although the mean fiber diameter in IUGR muscles was smaller. This is supported by the fact that muscle fibers in IUGR muscles showed a more polygonal form compared with NW muscles, where round fibers were predominating. Because of geometric reasons, a lower portion of connecting tissue between muscle fibers in IUGR piglets is suggested, but the present study did not focus on this point. Furthermore, the increase in type I muscle fiber (Fig. 4) indicates that there is obviously an accelerated developmental differentiation in distinct muscles of IUGR piglets, because muscle maturation is marked by this kind of conversion (5). This process is obviously heterogeneously in time and/or muscle specific, because significant differences in the proportion of type I fibers in NW and IUGR muscles were found only in flexor digitalis superficialis and gastrocnemius medialis. Previous observations reported yet a reduced content of type I fibers in the deep part of semitendinosus of IUGR piglets but no differences in the superficial part of semitendinosus and the lumbar part of longissimus between NW and IUGR piglets younger than 12 h. Differences were abolished in 3-day-old piglets (1).

Moreover, the aerobic metabolism in plantar flexors of IUGR animals seems to be more pronounced. This is supported by higher fatigue resistance of IUGR muscles against repeated isometric contractions under normal blood supply and after reperfusion and the higher rate of fatigue under ischemia compared with muscles of NW newborns.

Ordinarily, for calculation of specific muscle tension, fiber angle and physiological cross-sectional area have to be considered. Because it was not possible to separate the total force maximum for each muscle, specific muscle force of the whole plantar flexor group for IUGR and NW piglets was determined by division of total isometric force maximum by the sum of muscle masses of the plantar flexors.

To study contractile function during compromised blood supply, aortic cross clamping just above the bifurcation of the iliac arteries was performed. Regional blood flow was markedly reduced but not abolished and the muscle work-related pronounced blood flow increase was impeded. Concomitantly, oxygen delivery to calf muscles was dramatically reduced at muscle contraction during hindlimb ischemia, whereas the lower rate was more pronounced in IUGR (9% of control) compared with NW piglets (18% of control) of values during baseline conditions. This stronger restriction of oxygen delivery appeared to be partly responsible for the pronounced decline in isometric force of hindlimb plantar flexors during the contraction cycle in IUGR piglets. A further reason could be a reduced glycolytic capacity in hindlimb muscles of these animals. However, a related study using similar histochemical methods to examine muscle fiber composition as used in this study was ambiguous. Because of rather low activity of glycolytic enzymes (7, 8), a differentiation of type II fibers in fast-twitch oxidative (type IIa) or fast-twitch glycolytic (type IIb) muscles was impossible, which is in line with findings in former studies (5, 33, 35). A distinct substrate deficit may also cause pronounced development of stronger reduced isometric muscle work in IUGR piglets, because glucose availability was significantly lower in those piglets during ischemia even though muscle glycogen content was similar in both animal groups, as shown in this study.

**Perspectives**

Our results indicate that IUGR is accompanied by advanced muscle development soon after birth. This opinion is supported by an increased specific muscle force, increased specific muscular force of IUGR piglets, newborn piglets. Values are means ± SD. §Comparisons between NW and IUGR piglets (P < 0.05).

Fig. 4. Percentage of slow-twitch fiber number in soleus (Sol), flexor digitalis superficialis (FDS), and gastrocnemius medialis (GM) between NW (n = 5; filled bars) and IUGR (n = 5; open bars) newborn piglets. Values are means ± SD. §Comparisons between NW and IUGR piglets (P < 0.05).

**Table 2.** Total isometric force maximum of hindlimb plantar flexors in NW and IUGR piglets.

<table>
<thead>
<tr>
<th>Muscle</th>
<th>NW (n = 5)</th>
<th>IUGR (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexor digitalis superficialis</td>
<td>19.4 ± 1.3</td>
<td>17.2 ± 1.1</td>
</tr>
<tr>
<td>Gastrocnemius medialis</td>
<td>20.5 ± 1.5</td>
<td>18.3 ± 1.2</td>
</tr>
</tbody>
</table>

Data presented herein demonstrate for the first time that IUGR is accompanied by an accelerated development of skeletal muscle function combined with precocious muscle fiber differentiation of calf muscles. This view is supported by our results of isometric muscle contraction efficiency under normal hindlimb blood supply, increased fatigue resistance, and precocial type II to type I muscle fiber conversion in newborn IUGR piglets. Indeed, the increased specific muscular force of IUGR piglets of ~18% (P < 0.05) and also the reduced amount of fatigue at the end of serial isometric muscle contraction, which was indicated by markedly less force decrease in IUGR piglets (P < 0.01), reflect accelerated muscular development. An increase in fatigue resistance at serial tetanic calf muscle contraction was also reported at regular developmental progress in ovine fetuses (20). Presumably, the increased muscle fatigue resistance of IUGR piglets due to isometric muscle contraction may be elevated by the slightly but significantly higher proportion of type I muscle fibers of calf muscles involved. The higher proportion of type I fibers in calf muscles was shown to be a sign of developmental progress in both precocious and altricial species, including swine (5, 11, 12, 15, 24, 33).

Altogether, no remarkable difference in muscle architecture and fiber angle was found between IUGR and NW piglets (Table 2). Therefore, the distinct specific muscle force values of IUGR and NW should be caused by different contractile properties of muscle tissue. Indeed, the development of muscle tissue structure seems to be more advanced in IUGR than in NW muscles, although the mean fiber diameter in IUGR muscles was smaller. This is supported by the fact that muscle fibers in IUGR muscles showed a more polygonal form compared with NW muscles, where round fibers were predominating. Because of geometric reasons, a lower portion of connecting tissue between muscle fibers in IUGR piglets is suggested, but the present study did not focus on this point. Furthermore, the increase in type I muscle fiber (Fig. 4) indicates that there is obviously an accelerated developmental differentiation in distinct muscles of IUGR piglets, because muscle maturation is marked by this kind of conversion (5). This process is obviously heterogeneously in time and/or muscle specific, because significant differences in the proportion of type I fibers in NW and IUGR muscles were found only in flexor digitalis superficialis and gastrocnemius medialis. Previous observations reported yet a reduced content of type I fibers in the deep part of semitendinosus of IUGR piglets but no differences in the superficial part of semitendinosus and the lumbar part of longissimus between NW and IUGR piglets younger than 12 h. Differences were abolished in 3-day-old piglets (1).

Moreover, the aerobic metabolism in plantar flexors of IUGR animals seems to be more pronounced. This is supported by higher fatigue resistance of IUGR muscles against repeated isometric contractions under normal blood supply and after reperfusion and the higher rate of fatigue under ischemia compared with muscles of NW newborns. Ordinarily, for calculation of specific muscle tension, fiber angle and physiological cross-sectional area have to be considered. Because it was not possible to separate the total force maximum for each muscle, specific muscle force of the whole plantar flexor group for IUGR and NW piglets was determined by division of total isometric force maximum by the sum of muscle masses of the plantar flexors.

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**Perspectives**

Our results indicate that IUGR is accompanied by advanced muscle development soon after birth. This opinion is supported by an increased specific muscle.
force of the plantar flexors and an accelerated progress of muscle fiber type differentiation toward type I fibers in plantar flexors of newborn IUGR piglets. The more mature functional state of muscle development obviously includes an increase in muscular oxidative capacity, because IUGR piglets showed an improved tolerance to fatigue throughout a series of isometric contractions. Similar observations of accelerated brain (2) and lung (14) maturation suggest that compromised intrauterine nutrition and related hormonal alterations may induce an improved adaptation to extrauterine life.

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