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ORIGINAL CONTRIBUTION

Enhanced sensitivity of skeletal muscle growth in offspring of mice long-term selected for high body mass in response to a maternal high-protein/low-carbohydrate diet during lactation

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Abstract
Aim To investigate the effects of a high-protein/low-carbohydrate diet fed to mice of different genotypes during pregnancy and/or lactation on offspring skeletal muscle growth and metabolism.
Methods Pregnant mice from strains selected for high body mass (DU6) or endurance running performance (DUhLB) and from an unselected control strain (DUK) were fed iso-energetic diets containing 20 % (C) or 40 % protein and low carbohydrate (HP) from mating to weaning at day 21 of age. At birth, offspring were cross-fostered resulting in different exposure to maternal prenatal-pre-weaning diets (C–C, HP–C, C–HP, HP–HP). *Rectus femoris* muscle of male mice ($n = 291$) was examined at day 23, 44, 181 and 396 of age for cellular growth and metabolism.
Results At day 23 of age, body and muscle growth was retarded by 30–40 % ($P < 0.0001$) in response to the C–HP and HP–HP, but not to the HP–C diet, due to reduced fibre size ($P < 0.0001$) but not fibre number. DNA was highly reduced in DU6, less in DUhLB, but not in DUK muscle (strain × diet; $P < 0.0001$). Despite some compensation, muscle growth was still impaired ($P < 0.001$) in adulthood (day 44; day 181), but at senescence only in DU6 mice (strain × diet; $P < 0.05$). Only at weaning, isocitrate and lactate dehydrogenase activities were increased or decreased ($P < 0.0001$), respectively, without influence on fibre type composition.
Conclusion A high-protein/low-carbohydrate diet fed to dams during lactation, but not during pregnancy, retards skeletal muscle growth in offspring with greater response of a heavy, obese compared with a physically fit and a control genotype and causes a transient shift towards oxidative versus glycolytic muscle metabolism.

Keywords Genetic selection · Protein/carbohydrate intake · Pregnancy · Lactation · Genotype–nutrition interaction · Muscle metabolism

Introduction

Obesity during pregnancy is increasingly recognized as a determinant for the developmental programming of obesity and metabolic disorders in the offspring [1, 2]. A high-protein/low-carbohydrate diet is considered very effective to combat obesity and therefore might be practised also by women in child-bearing age [3–5]. In addition, both nutrient deficiency and nutrient excess during early development could lead to later obesity and related disorders [6]. As a consequence of a high-protein/low-carbohydrate diet fed to mothers during gestation, intrauterine growth retardation (IUGR) has been observed in rodents

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[7, 8] and in pigs [9]. Also epidemiological studies in women showed that high-protein intakes during pregnancy resulted in IUGR [10, 11]. When a high-protein diet was fed to mouse dams during lactation, adverse effects on offspring during early postnatal growth were observed as a consequence of impaired lactation [8, 12], and offspring mortality was more than eightfold increased in the same mouse model [13].

In general, long-term effects of maternal high-protein/low-carbohydrate maternal diets on the offspring are not well described, and studies elucidating effects of these diets on structural and functional properties of skeletal muscle that plays a major role in regulating metabolic homoeostasis [14, 15] are scarce. We have shown recently that a high-protein intake at the level of 250 % of daily requirements together with a very low carbohydrate intake in pregnant primiparous sows caused IUGR, but did not adversely affect foetal myogenesis [9]. Moreover, reduced body mass of piglets at birth was completely compensated until 4 weeks of age, after they have been cross-fostered to sows fed an adequate diet [9]. On the other hand, recent results suggest that an impairment of myogenesis by reducing myofibre number and/or myonuclear accumulation during early life may have long-term negative effects on skeletal muscle growth and body composition in favour of adiposity [9, 16–18].

It is commonly known that there is a great diversity of body mass, body composition (lean to fat ratio) and physical fitness and that these body conditions can be related to health status [e.g., 19, 20]. High-protein/low-carbohydrate diets lead to hypoglycaemia, lipolysis and ketogenesis [21, 22], and higher utilization of fat reserves and improved glucose homoeostasis were observed in obese and diabetic individuals [23–25]. We hypothesized that a maternal high-protein/low-carbohydrate diet may cause different responses in the offspring depending on genetically determined body conditions. For this purpose, we used mice from one of the heaviest and most adipose mouse strains worldwide [26], and mice selected for high endurance fitness in terms of running activity on a treadmill [27, 28] along with unselected control mice.

The objectives of this study were (1) to characterize the growth as well as cellular and metabolic properties of skeletal muscle of mouse strains highly differing in body mass, body composition and physical endurance fitness and (2) to identify in which way high-protein/low-carbohydrate diets fed to mouse dams during pregnancy and/or lactation changed these characteristics in the offspring and whether dietary responses depended on the genotype of mice. For this purpose, we used a cross-fostering design that allowed separating the effects of the diets fed during pregnancy and lactation.

Materials and methods

Animals and diets

The study was conducted with approval of the Animal Care Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, State Mecklenburg-Vorpommern, Germany (LVL-MV/TSD/7221.3-1.1-033/05).

We used mice of an unselected strain (DUK), a strain selected for high endurance running performance at day 70 of age (DUhLB) and a strain selected for high body mass at day 42 of age (DU6), all bred at the Leibniz Institute of Farm Animal Biology (FBN), Dummerstorf, Germany. The initial population of mice was established in 1975 by crossing four outbred (NMRI orig., Han/NMRI, CFW, CF1) and four inbred (CBA/Bln, AB/Bln, C57BL/Bln, XVII/Bln) populations [29, 30]. Mice of the control DUK strain used in this experiment were mated randomly over 125 generations, while DU6 mice were selected for high body mass by sib selection [31]. The strain DUhLB has been generated by selection for high treadmill performance over 93 generations. Endurance fitness was measured in 70-day-old male mice by a computer-controlled treadmill using a submaximal test with a start speed of about 15 m/min and a final speed of 38 m/min [27].

Within the genetic strains, nulliparous mouse dams were mated at 9 weeks of age. Different iso-energetic semi-synthetic experimental diets with control (C; 20 %) and high protein concentration made iso-energetic by low carbohydrate level (HP; 40 %) were fed. The diets similar to those used earlier [8] consisted of casein (Molkereigesellschaft Lauingen mbH, Lauingen, Germany; C: 212 g/kg; HP: 426 g/kg) supplemented with DL-methionine (4 g/kg; LAH GmbH & CO. KG, Cuxhaven, Germany), wheat starch (Ferdinand Kreutzer Sabamühle GmbH, Nürnberg, Germany; C, 443.9; HP 225.9 g/kg), sucrose (Nordzucker GmbH, Hamburg, Germany; 160 g/kg), soy oil (Sedina ADM, Hamburg, Germany; 50 g/kg), microcellulose (50 g/kg), vitamin mixture (20 g/kg; SSNIFF Spezialdiäten GmbH, Soest, Germany), mineral mixture (60 g/kg; SSNIFF Spezialdiäten GmbH) and butylhydroxytoluene (0.1 g/kg; LAH GmbH & CO. KG). Dietary crude protein content was 19.8 and 39.1 %, and protein/carbohydrate ratio was 0.36 and 1.12 in the C and HP diets, respectively.

Female full-sibs were allocated to either the C or HP diet, and immediately afterwards, one female and one male were put together to mate. Males were withdrawn immediately after appearance of a vaginal plug, which was denoted day 1 of pregnancy. After birth, litters were cross-fostered to different dams fed the C or HP diet from mating to weaning (day 21 of age). Thus, we obtained four groups of offspring that were exposed to different prenatal/pre-weaning diet combinations: C–C, HP–C, C–HP and

HP–HP (Online Resource, Fig. S1). Litters were standardized to 10 pups immediately after birth and weaned on a standard rodent diet (21 % protein, 0.4 % L-Met, 55 % starch, 5 % sucrose, 5 % fat; 5 % cellulose, 2 % vitamin and 6 % mineral mixture; Altromin 1314, Altromin Spezialfutter GmbH & Co. KG, Lage, Germany). Pregnant and lactating dams were housed individually in standard rodent cages (Makrolon, Type II, EBECO, Castrop-Rauxel, Germany) in a controlled environment of 22 °C with a 12:12 h light/dark cycle. Water was available ad libitum.

In experiment 1 (*EXP1*), male offspring at day 23 ($n = 149$) and 44 of age ($n = 142$) was examined to address effects of a maternal diet on early postnatal muscle growth and metabolism. For this purpose, mice were anesthetized after 4–6 h of food withdrawal, by s.c. injection of ketamine hydrochloride (87 mg/kg BM, Ursotamin®, Serum-Werk-Bernburg AG, Bernburg, Germany) and xylazine hydrochloride (13 mg/kg BM, Xylazine® 2 %, Riemser Arzneimittel AG, Greifswald, Insel Riems, Germany) and then decapitated. Subsequently, left and right *M. rectus femoris* (RF) was dissected, weighed, rapidly frozen in liquid nitrogen and stored at −80 °C until analyses. The low number of HP–HP mice was due to higher early postnatal losses in this group. The experimental design of *EXP2* was the same as for *EXP1*, with the exception that the effects of the maternal C–C, HP–C and C–HP diets were studied in male offspring at day 181 ($n = 89$) and 396 ($n = 81$) of age.

Biochemical and enzyme analyses (EXP1 and EXP2)

The left-side RF muscles were homogenized on ice in 2 ml potassium phosphate buffer (pH = 6.9) using a Potter–Elvehjem Tissue Grinder (Wheaton, Milleville, NJ, USA). The homogenates were used at different degrees of dilution to quantify DNA (1/20), protein (1/80), creatine kinase (CK; EC 2.7.3.2.; *EXP1* 1/100; EXP2 1/300), isocitrate dehydrogenase (ICDH; E.C. 1.1.1.42; 1/1) and lactate dehydrogenase (LDH; E.C. 1.1.1.27; 1/15) activities. DNA was measured fluorometrically using Hoechst 33258 (Sigma-Aldrich, Taufkirchen, Germany) [32]. Protein concentration was determined according to Peterson [33] and CK activity was measured in supernatants of diluted muscle homogenates at 37 °C using a commercial kit (Pointe Scientific, Canton, MI, USA). ICDH and LDH activities were measured using modified assay protocols according to SIGMA as described previously in detail [34].

Histology/(immono-)histochemistry, microscopy (EXP1)

Because of significant differences between C–C and C–HP in all characteristics of muscle growth and sufficient size of groups within strains, a subset of animals (C–C = 31, C–HP = 26 at day 23; C–C = 31, C–HP = 29 at day 44) in *EXP1* was examined for RF microstructure as described previously [35]. Briefly, serial transverse frozen sections (10 μm) of right-side RF were stained by NADH-tetrazolium reductase for metabolic fibre types (red, intermediate, white) or for cytoplasm by eosin and alkaline phosphatase to visualize capillaries. Muscle cross-sectional area (MCSA) was measured by image analysis (TEMA, Checkvision, Stoevring, Denmark). To determine the total muscle fibre number per cross-section, fibres were counted over an area of 0.754 (day 23) or 3.047 mm^2 (day 44) and extrapolated to total MCSA. Fibre type distribution, fibre cross-sectional area (FCSA) and capillary density were determined separately on 100–150 muscle fibres each in the dark (deep) and bright (superficial) regions of the muscle by image analysis (AMBA, IBSB, Berlin, Germany). The averages of both regions were calculated and given as an overall estimate for the muscle. To detect proliferating nuclei, serial transverse frozen sections (8 μm) from RF of 23-day-old C–C ($n = 29$) and C–HP ($n = 28$) mice of EXP1 were immunostained as described previously in detail [36] using the primary rabbit anti-human monoclonal antibody to Ki-67 (clone SP6, Biozol, Eching, Germany). Another section was stained with haematoxylin and eosin (HE). Nuclei were counted in HE sections on four fields spread over the muscle cross-section (∼16–27 % of the total MCSA), and Ki-67-positive nuclei within (mitogenic active satellite cells) and between (mitogenic active non-myofibre nuclei) myofibres were counted over the whole cross-section by image analysis (TEMA, Checkvision) to obtain the number and percentage of proliferating nuclei.

Statistical analysis

Data of each stage of age within *EXP1* and *EXP2* were subjected to analyses of variance, using the GLM model of SAS (SAS System for Windows Release 9.2; SAS Institute Inc., Cary NC 27513 USA) including mouse strain and maternal dietary regime and their interaction as fixed factors. Data given in tables and figures are least squares mean ± SE. Differences between least squares means were tested post hoc by the Tukey–Kramer test. Differences were considered significant if $P < 0.05$ and as tendencies if $0.05 < P < 0.10$. Pearson correlation coefficients were estimated using the CORR procedure of SAS.

Results

Effects of long-term genetic selection for high body mass

Body weight of the DU6 mice was doubled at day 23 (204 %) and more than doubled (265 %) at day 44 of age

compared with unselected DUK mice (*EXP1*; Table 1). The differences in RF muscle weight were similar to the differences in body weight at day 23 (201 %) but less at day 44 (193 %) resulting in a lower relative muscle weight in DU6. At both stages of age, the relative increase in muscle protein content in DU6 versus DUK was greater than the increase in DNA content, which led to lower DNA/protein ratios. The activities of key enzymes of muscle metabolism were similar in DU6 and DUK mice at day 23 of age, whereas DU6 muscles showed less CK, but greater LDH and ICDH activities at day 44 of age. Further studies on muscular microstructure in a subset of animals (C–C and C–HP groups only) revealed that RF muscle of DU6 mice exhibited greater total fibre numbers and fibre size (FCSA) compared with DUK mice both at day 23 and day 44 of age (*EXP1*; Table 2) with fibres of all metabolic types being larger (data not shown). The number of capillaries per fibre was higher in DU6 than DUK muscle at day 23 of age without change in FCSA supplied by one capillary, whereas both traits of capillary density were not different at day 44 of age. Frequencies of red, intermediate (data not shown) and white fibres were not affected by body mass selection at day 23 of age. At day 44, however, the percentage of white glycolytic fibres was greater in DU6 than DUK muscle by 3.5 % units. Interestingly, the proportions of Ki-67 positive, proliferating nuclei measured at day 23 of age were considerably less in the DU6 strain (−60 %) compared with the DUK strain.

The differences in body weight of DU6 compared with DUK mice were 221 and 211 % at day 181 and day 396 of age, respectively (*EXP2*, Table 3). The RF muscle weighed still more in DU6 than DUK mice at day 181, but even less (by 8 %) at day 396 of age due to a significant loss by 37 % in muscle mass from day 181 to day 396. Finally, the relative muscle mass was only 0.23 % in DU6 compared with 0.54 % in DUK. At day 181, the difference in muscle protein content (+58 %) was greater than the difference in DNA content (+24 %) resulting in a reduced DNA/protein ratio, as observed already at day 23 and 44. At day 396, DNA/protein ratio was even higher in DU6 compared with

Table 1 Body weight and characteristics of muscle growth and metabolism in *Rectus femoris* muscle in 23- and 44-day-old mouse offspring of different genotypes born to dams exposed to a standard diet during pregnancy and lactation (C–C) or a high-protein/low-carbohydrate diet during lactation (C–HP), during pregnancy (HP–C), or during pregnancy plus lactation (HP–HP)–*EXP1*

	Age (day)	Diet				Strain			SE	*P*		
		C–C	C–HP	HP–C	HP–HP	DUK	DUhLB	DU6		Diet	Strain	Strain × diet
N	23	54	41	39	15	49	50	50				
	44	54	35	39	14	47	45	50				
Body weight (g)	23	15.93^{A}	10.97^{B}	15.97^{A}	11.46^{B}	10.33^{C}	9.30^{C}	21.11^{D}	0.46	<0.0001	<0.0001	0.01
	44	40.51^{A}	37.18^{B}	40.53^{A}	36.51^{B}	24.68^{C}	25.80^{C}	65.57^{D}	0.68	<0.0001	<0.0001	0.03
Muscle weight (mg)	23	69.9^{A}	42.8^{B}	68.0^{A}	46.3^{B}	44.3^{C}	36.9^{D}	89.1^{E}	2.0	<0.0001	<0.0001	0.01
	44	199.6^{A}	170.6^{B}	190.7^{A}	165.6^{B}	138.8^{C}	137.9^{C}	268.2^{D}	5.3	<0.0001	<0.0001	0.74
Muscle weight (%)	23	0.44^{A}	0.38^{B}	0.43^{A}	0.40^{AB}	0.42^{C}	0.39^{D}	0.42^{C}	0.01	<0.01	<0.0001	0.33
	44	0.52^{A}	0.49^{B}	0.51^{AB}	0.48^{AB}	0.56^{C}	0.53^{C}	0.41^{D}	0.01	0.03	<0.0001	0.80
Total DNA (μg)	23	125.2^{A}	89.3^{B}	127.8^{A}	90.6^{B}	95.6^{C}	79.8^{D}	149.3^{E}	2.91	<0.0001	<0.0001	<0.0001
	44	427.4^{A}	405.3^{A}	411.0^{A}	344.9^{B}	335.3^{C}	368.0^{C}	488.17^{D}	11.40	<0.0001	<0.001	0.80
Total protein (mg)	23	12.72^{A}	7.64^{B}	12.38^{A}	8.20^{B}	8.02^{C}	6.70^{D}	15.97^{E}	0.40	<0.0001	<0.0001	0.001
	44	40.05^{A}	34.73^{B}	38.04^{A}	32.56^{B}	28.57^{C}	28.68^{C}	51.79^{D}	1.11	<0.0001	<0.0001	0.87
DNA/protein (μg/mg)	23	10.4^{A}	13.3^{B}	10.7^{A}	12.0^{B}	12.6^{C}	12.7^{C}	9.6^{D}	0.27	<0.0001	<0.0001	<0.0001
	44	11.1^{A}	12.4^{B}	11.5^{AB}	11.2^{AB}	12.0^{C}	13.2^{D}	9.5^{E}	0.36	0.04	<0.0001	0.87
CK (IU/mg protein)	23	17.6	18.0	17.8	17.9	18.3^{C}	17.3^{D}	17.9^{CD}	0.24	0.62	0.01	<0.0001
	44	16.4^{A}	16.8^{AB}	17.4^{AB}	18.2^{B}	18.4^{C}	16.4^{D}	16.7^{D}	0.38	0.02	<0.0001	0.28
LDH (IU/g protein)	23	$1{,}071^{A}$	924^{B}	$1{,}059^{A}$	975^{B}	941^{C}	$1{,}151^{D}$	930^{C}	17.2	<0.0001	<0.0001	0.02
	44	1,422	1,457	1,460	1,424	$1{,}327^{C}$	$1{,}442^{D}$	$1{,}553^{E}$	28.4	0.64	<0.0001	0.07
ICDH (IU/g protein)	23	11.9^{A}	14.5^{B}	12.0^{A}	12.9^{AB}	12.6^{C}	13.8^{D}	12.1^{C}	0.36	<0.0001	<0.01	<0.01
	44	8.1	8.4	8.7	8.3	7.4^{C}	8.9^{D}	8.8^{D}	0.29	0.48	<0.001	0.30

DUK unselected control strain, *DUhLB* selected for high endurance running performance at day 70 of age, *DU6* selected for high body mass at day 42 of age. Data are least squares mean ± SE from ANOVA with factors diet, strain, strain × diet. AB: Within a row least squares means without a common superscript differ among diets ($P < 0.05$) CDE: Within a row least squares means without a common superscript differ among mouse strains ($P < 0.05$)

Table 2 Microstructural characteristics and proportions of proliferating nuclei in *Rectus femoris* muscle cross-sections of 23- and 44-day-old mouse offspring of different genotypes born to dams exposed to a standard diet during pregnancy and lactation (C–C) or a high-protein/low-carbohydrate diet during lactation (C–HP)–*EXP1*

	Age (day)	Diet		Strain			SE	*P*		
		C–C	C–HP	DUK	DUhLB	DU6		Diet	Strain	Strain × diet
N	23	31	26	14	23	20				
N	44	31	29	20	20	20				
MCSA (mm^2)	23	5.1^{A}	3.4^{B}	3.8^{C}	3.4^{C}	5.5^{D}	0.12	<0.0001	<0.0001	0.18
	44	9.8^{A}	8.9^{B}	8.6^{C}	7.5^{D}	12.0^{E}	0.30	<0.01	<0.0001	0.43
FCSA (μm^2)	23	886^{A}	615^{B}	687^{C}	617^{C}	947^{D}	41	<0.0001	<0.0001	0.24
	44	$2{,}176^{A}$	$1{,}974^{B}$	$2{,}018^{C}$	$1{,}704^{D}$	$2{,}503^{E}$	51	<0.01	<0.0001	0.90
Total fibre no.	23	$5{,}564^{A}$	$5{,}195^{A}$	$4{,}895^{C}$	$5{,}356^{CD}$	$5{,}887^{D}$	190	0.09	<0.01	0.05
	44	3,995	4,080	$3{,}834^{C}$	$3{,}980^{CD}$	$4{,}299^{D}$	118	0.53	0.02	0.32
White glycolytic fibres (%)	23	44.8	46.3	46.2^{CD}	42.9^{C}	47.6^{D}	1.1	0.25	<0.01	0.07
	44	50.4	50.3	49.8^{C}	48.1^{C}	53.3^{D}	1.0	0.94	0.001	0.82
Capillaries/fibre	23	0.24	0.24	0.23^{C}	0.18^{C}	0.31^{D}	0.02	0.97	<0.001	0.51
	44	0.63	0.67	0.60	0.67	0.68	0.04	0.35	0.23	0.58
FCSA/capillary (μm^2)	23	$4{,}252^{A}$	$2{,}861^{B}$	3,584	3,817	3,269	371	<0.01	0.60	0.15
	44	$3{,}709^{A}$	$3{,}091^{B}$	3616^{C}	$2{,}634^{D}$	$3{,}949^{C}$	226	0.02	<0.001	0.68
Ki-67 + nuclei within fibres (%)	23	1.90^{A}	3.34^{B}	3.69^{C}	2.66^{CD}	1.53^{D}	0.57	0.02	0.02	0.80
Ki-67 + nuclei between fibres (%)	23	0.37^{A}	0.59^{B}	0.64^{C}	0.58^{C}	0.21^{D}	0.11	0.05	<0.01	0.30
Ki-67 + nuclei total (%)	23	2.27^{A}	3.93^{B}	4.33^{C}	3.23^{CD}	1.74^{D}	0.67	0.02	0.02	0.73

DUK unselected control strain, *DUhLB* selected for high endurance running performance at day 70 of age, *DU6* selected for high body mass at day 42 of age, *MCSA* muscle cross-sectional area, *FCSA* fibre cross-sectional area. Data are least squares mean ± SE from ANOVA with factors diet, strain, strain × diet; AB, Within a row least squares means without a common superscript differ among diets ($P < 0.05$); CDE, Within a row least squares means without a common superscript differ among mouse strains ($P < 0.05$)

DUK because of similar protein and greater DNA (+8 %) contents. DU6 exhibited greater LDH and ICDH activities than DUK muscle similar to findings at day 44 of age. In 396-day-old mice, ICDH activity was also greater, but LDH activity was less in DU6 muscle. CK activity was not different between DU6 and DUK mice at both ages.

Altogether long-term growth selection caused a tremendous increase in body and muscle mass, but was associated with negative allometric muscle growth. Also, muscle protein accretion exceeded DNA accumulation in youth and adulthood, and proliferative activity was decreased in response to selection. Increases in muscle mass resulted from increases in fibre size and fibre number with emphasis on fibre size. Selection led to a higher activity of both oxidative and anaerobic glycolytic muscle metabolism together with a slight shift to white glycolytic myofibres in adulthood, whereas effects on capillary supply were not detectable. At senescence, the growth-selected mice showed a tremendous loss in muscle mass, but not in body mass, thereby reaching the level of the unselected control mice in muscle mass and protein, which was associated with a 50 % reduction in relative muscle mass and a shift to oxidative muscle metabolism.

Effects of long-term genetic selection for high endurance running performance

At day 23 of age, DUhLB mice selected for high endurance running performance exhibited similar body weight, but less absolute (−17 %) and relative muscle mass compared with DUK mice, which was associated with less DNA and protein contents at similar degree (*EXP1*; Table 1). In contrast, DUhLB mice did not differ from DUK mice in body and muscle weight as well as muscle total protein and DNA contents at day 44 of age. However, RF muscle showed a higher DNA/protein ratio at day 44. At both stages of age, differences in metabolic traits were apparent in that CK activity was less (−5 %; −11 %), but LDH (+22 %; +9 %) and ICDH (+9 %; +20 %) activities were greater in DUhLB than DUK muscle. Differences in muscle microstructure between DUhLB and DUK mice were only detectable at day 44, but not at day 23 of age (*EXP1*; Table 2). Muscle cross-sectional area (MCSA) was smaller in DUhLB versus DUK mice due to smaller fibres (FCSA) but not less numbers of fibres. FCSA supplied by one capillary was less in DUhLB compared with DUK muscles, whereas no differences occurred in the number of

Table 3 Body weight and characteristics of muscle growth and metabolism in *Rectus femoris* muscle in 181 and 396 days old mouse offspring of different genotypes born to dams exposed to a standard control diet during pregnancy and lactation (C–C) or a high-protein/low-carbohydrate diet during lactation (C–HP) or pregnancy (HP–C)–*EXP2*

	Age (day)	Diet			Strain			SE	*P*		
		C–C	C–HP	HP–C	DUK	DUhLB	DU6		Diet	Strain	Strain × diet
N	181	30	29	30	29	29	31				
	396	25	31	25	24	32	25				
Body weight (g)	181	61.81^{A}	55.74^{B}	62.69^{A}	44.04^{C}	38.86^{D}	97.31^{E}	1.39	0.001	<0.0001	0.06
	396	64.61^{ab}	60.01^{a}	64.69^{b}	46.45^{C}	44.64^{C}	98.21^{D}	1.94	0.06	<0.0001	0.24
Muscle weight (mg)	181	256.3^{a}	235.2^{b}	258.0^{a}	218.9^{C}	167.1^{D}	363.4^{E}	7.2	0.05	<0.0001	0.44
	396	236.6	219.8	232.1	247.5^{C}	212.3^{D}	228.6^{D}	8.0	0.15	0.001	<0.01
Muscle weight (%)	181	0.54	0.44	0.43	0.51	0.43	0.47	0.06	0.34	0.67	0.44
	396	0.42	0.41	0.42	0.54^{C}	0.48^{D}	0.23^{E}	0.02	0.68	<0.0001	0.11
Total DNA (μg)	181	584.2^{a}	557.1^{b}	585.2^{a}	548.0^{C}	500.0^{D}	678.5^{E}	9.31	0.05	<0.0001	0.58
	396	550.9	536.3	536.8	526.2^{C}	528.2^{C}	569.5^{D}	12.56	0.48	0.01	0.02
Total protein (mg)	181	53.05	49.42	52.19	45.24^{C}	36.71^{D}	71.37^{E}	1.40	0.16	<0.0001	0.46
	396	50.08^{a}	46.01^{b}	48.43^{ab}	50.35^{c}	46.21^{d}	47.97^{cd}	1.74	0.11	0.11	0.02
DNA/protein (μg/mg)	181	11.86	11.82	12.20	12.26^{C}	14.16^{D}	9.45^{E}	0.31	0.64	<0.0001	0.06
	396	11.13	12.09	11.20	10.53^{Cc}	11.64^{CDd}	12.24^{Dd}	0.45	0.11	0.01	0.05
CK (IU/mg protein)	181	22.27	21.91	23.59	23.33^{C}	20.89^{D}	23.56^{C}	0.64	0.13	<0.01	0.29
	396	24.10	23.41	25.47	24.48	24.82	23.68	0.91	0.22	0.59	0.18
LDH (IU/g protein)	181	$1{,}321^{a}$	$1{,}233^{b}$	$1{,}316^{ab}$	$1{,}234^{Cc}$	$1{,}147^{Cd}$	$1{,}488^{De}$	29.0	0.06	<0.0001	0.90
	396	1,294	1,309	1,350	$1{,}479^{C}$	$1{,}181^{D}$	$1{,}293^{D}$	48.5	0.65	<0.0001	0.57
ICDH (IU/g protein)	181	11.3	10.1	11.5	9.8^{Cc}	10.6^{CDc}	12.4^{Dd}	0.55	0.17	<0.01	0.88
	396	16.6^{a}	17.3^{ab}	19.2^{b}	16.0^{C}	17.6^{CD}	19.5^{D}	0.96	0.09	0.03	0.71

DUK unselected control strain, *DUhLB* selected for high endurance running performance at day 70 of age, *DU6* selected for high body mass at day 42 of age. Data are least squares mean ± SE from ANOVA with factors diet, strain, strain × diet. AB, Within a row least squares means without a common superscript differ among diets ($P < 0.05$); ab: ($P < 0.10$). CDE, Within a row least squares means without a common superscript differ among mouse strains ($P < 0.05$); cde: ($P < 0.10$)

capillaries per fibre. The remaining microstructure traits were not different between the strains.

At day 181 of age, DUhLB mice weighed less (−18 %) as did RF muscle (−24 %) compared with DUK due to less protein (−19 %) and DNA (−9 %) resulting in a greater DNA/protein ratio (*EXP2*; Table 3). At day 396 of age, body weight of DUhLB mice was not different, whereas RF weighed less in both absolute and relative terms compared with DUK mice due to less protein. At both ages, lower LDH activity, but similar ICDH activity, was observed in DUhLB compared with DUK mice. CK activity was less in DUhLB at day 181 as seen at day 23 and 44, but remained unaffected in old mice at day 396.

Altogether, long-term selection for high running performance resulted in reduced muscle accretion during growth with the exception of early adulthood (day 44). This was associated with less muscle protein accretion and/or DNA accumulation. In youth and adulthood, the muscle metabolic rate related to energy generation was consistently increased by this type of selection, whereas CK activity as mediator of energy storage in muscle was diminished. These effects were no longer apparent at senescence.

Diet effects at day 23 and 44 of age (EXP1)

Body and muscle growth was significantly affected by the experimental diets (Table 1). Considering the results over all three mouse strains at day 23 of age, body and muscle growth was retarded by 30–40 % in response to the C–HP and HP–HP diets, but not in response to the HP–C diet. This means that growth inhibition occurred when the dams received the HP diet during pregnancy plus lactation or during lactation only, but not when administered during pregnancy only. Muscle protein accretion (−36 to 44 %) was more affected than DNA accumulation (−28 to −29 %) resulting in greater DNA/protein ratios. Significant strain × diet interactions revealed that there were mouse strain-specific responses to HP diets at day 23 of age. In the case of body weight, muscle weight and muscular DNA and protein contents, the magnitude but not the direction of the dietary effects differed among the mouse

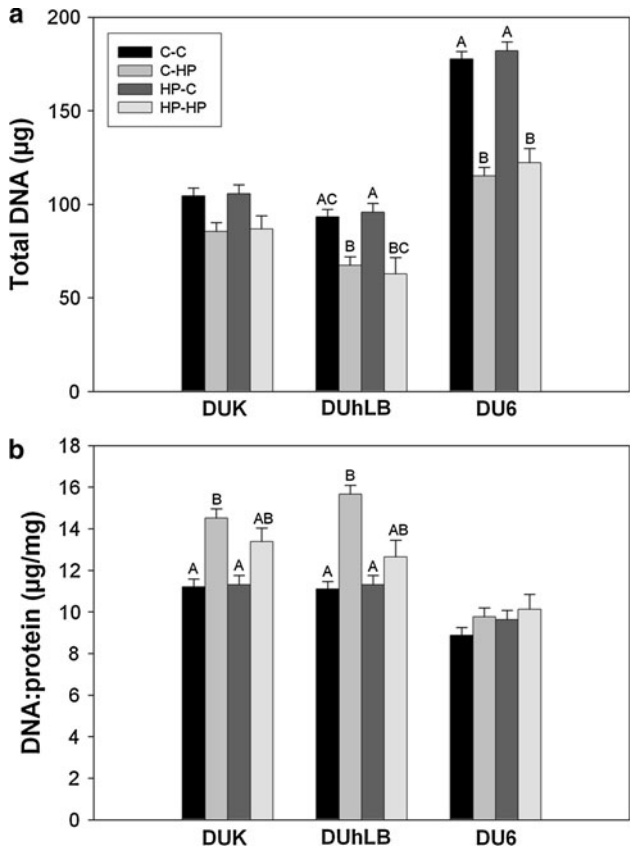

Fig. 1 **a** Total DNA and **b** DNA/protein ratio in *Rectus femoris* muscle of 23-day-old mouse offspring of different genotypes born to dams exposed to a standard control diet (C–C) during pregnancy and lactation or a high-protein/low-carbohydrate diet during lactation (C–HP), during pregnancy (HP–C), or during pregnancy plus lactation (HP–HP) and fed a standard diet after weaning at day 21 of age. *DUK*: unselected control strain; *DUhLB*: selected for high running performance at day 70 of age; *DU6*: selected for high body mass at day 42 of age. Within mouse strains least squares means without common superscripts differ (ABC; $P < 0.05$)

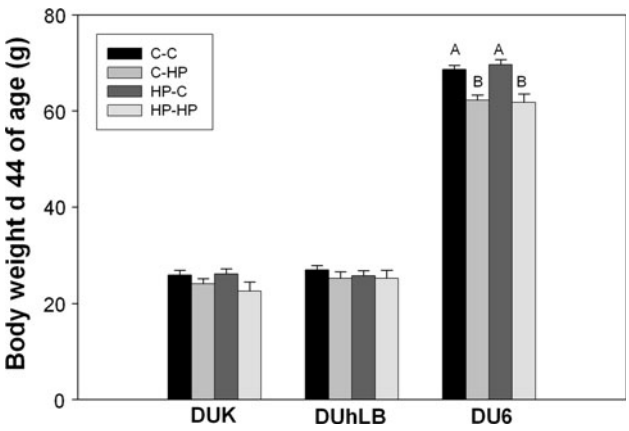

Fig. 2 Body weight of 44-day-old mouse offspring of different genotypes born to dams exposed to a standard control diet (C–C) or a high-protein/low-carbohydrate diet fed during lactation (C–HP), during pregnancy (HP–C), or during pregnancy plus lactation (HP–HP) and fed a standard diet after weaning at day 21 of age. *DUK*: unselected control strain; *DUhLB*: selected for high running performance at day 70 of age; *DU6*: selected for high body mass at day 42 of age. Within mouse strains least squares means without common superscript differ (AB; $P < 0.05$)

strains. More importantly, the reduction in total DNA in response to both C–HP and HP–HP diets was non-significant in DUK mice (17–18 %; $P > 0.05$), highest in DU6 mice (38–39 %) and intermediate in DUhLB mice (28–33 %) (Fig. 1a). Because of similar extent of decreases in DNA and protein contents, no difference in DNA/protein ratio was seen in DU6, but increases were apparent in C–HP offspring of DUK and DUhLB strains (Fig. 1b). At day 44 of age, effects of the experimental diets were still apparent after feeding a standard diet to all experimental groups from weaning at day 21 to day 44 of age (Table 1). The effects were genotype-dependent for body weight only (strain × diet; $P = 0.03$). Body weight was still reduced in the C–HP and HP–HP compared with C–C mice of the DU6 strain by 9 %, whereas reduction in body weight was no longer apparent in DUK and DUhLB mice (Fig. 2). This is indicative of catch-up growth from day 23–44 in all strains with incomplete compensation in DU6 mice. No strain-specific effects of the diets were observed for all muscle characteristics at day 44 of age. In contrast to body weight, muscle weight was still reduced in C–HP and HP–HP mice by 15 and 17 %, respectively, compared with C–C mice. In addition, relative muscle weight was reduced in C–HP mice, while the reduction was not significant for HP–HP mice because of low animal number. Total muscle protein amount was still reduced by 13 and 19 % in the C–HP and HP–HP mice, respectively. A reduction in total DNA, however, was apparent in HP–HP (by 19 %), but not in C–HP mice. Because of disproportional reductions in total muscle DNA and protein, a greater DNA/protein ratio was observed in C–HP compared with C–C mice.

Evaluating enzyme activities as indicators of muscle metabolism at day 23 of age, less LDH activity was observed in C–HP and HP–HP groups, while ICDH activity was enhanced only in C–HP compared with C–C mice. Strain × diet interactions revealed genotype-specific responses to the diets. Increases in ICDH activity and decreases in LDH activity in response to C–HP and HP–HP treatments differed in magnitude, but not in direction among the mouse strains. The largest changes were seen in DUhLB mice, whereas only medium and small differences were observed in DUK and DU6 mice, respectively (data not shown). At day 44 of age, the activities of LDH and ICDH were no longer altered in C–HP and HP–HP groups. The tendency for a strain × diet interaction for LDH activity ($P = 0.06$) revealed no differences between the dietary groups within strains. As average of all mouse strains, CK activity remained unchanged by the dietary

treatments at day 23 and the strain × diet interaction revealed no significant effects of the diets within the different genotypes (data not shown). The slight increase in CK activity seen in HP–HP offspring at day 44 of age is not overestimated, because CK activity per g muscle tissue remained unchanged (HP–HP: 3,507 ± 99 vs. C–C: 3,391 ± 50 IU/g, $P = 0.72$).

Analyses of muscular microstructure in C–C and C–HP groups showed that feeding the dams a HP diet during lactation reduced MCSA and the growth of myofibres in size both at day 23 (by 31 %) and day 44 (by 9 %), but did not reduce myofibre number in the offspring (Table 2). The number of capillaries per fibre was not changed by the C–HP diet resulting in a smaller fibre area supplied by one capillary at both ages. Estimation of Pearson correlation coefficients within mouse strains revealed positive values of $r = 0.59$ to 0.68 ($P < 0.01$) between LDH and FCSA and negative values of $r = -0.63$ to—0.73 ($P < 0.01$) between ICDH and FCSA at day 23, whereas the correlations were only significant for ICDH at day 44 of age (data not shown). The frequencies of oxidative (red + intermediate) and white glycolytic fibres were not affected by the experimental diets. The absolute numbers of Ki-67 positive, proliferating nuclei examined at day 23 of age tended to be increased (data not shown) and their proportions related to the total number of nuclei was more than doubled in C–HP compared with C–C muscles (Table 2). Both nuclei between myofibres and nuclei within myofibres (satellite cells) were equally affected.

Diet effects at day 181 and 396 of age (EXP2)

When mouse offspring (C–C, C–HP and HP–C) were examined at day 181 and 396 of age, some effects of the maternal C–HP diet compared to the control diet (C–C) were still detectable. Body weight of C–HP mice was still reduced by 9 % at day 181 and tended to be reduced by 7 % at day 396 (Table 3). On day 181, the reduction was only pronounced in the DU-6 strain (101.0 vs. 88.2 g; strain × diet $P = 0.06$).

On day 181, RF muscle weight ($P = 0.10$) and total DNA content ($P = 0.09$) tended to be reduced in C–HP mice in a similar degree without any strain-specific responses indicating that no full catch-up growth has occurred since day 44 of age. In old mice at day 396, however, the traits of muscle growth showed a clear strain-specific response to the C–HP diet indicated by significant strain × diet interactions. Muscle weight, protein and DNA contents were only reduced in mice of the DU-6 strain by 26 % (192.4 ± 10.4 vs. 260.5 ± 9.9 mg; $P < 0.001$), 24 % (40.9 ± 2.4 vs. 54.0 ± 2.2 mg; $P = 0.01$) and 10 % (552.7 ± 16.2 vs. 617.4 ± 15.4 µg; $P = 0.11$), respectively, compared with C–C mice (Online Resource, Fig. S2a–c). Regarding the metabolic enzymes, LDH activity tended to be lower in C–HP versus C–C mice ($P = 0.08$) on day 181, while at day 396, HP–C mice tended to exhibit a more oxidative muscle metabolism in terms of ICDH activity compared with C–C mice ($P = 0.07$), which was irrespective of the strain. The remaining traits were not affected by the diets, and there were no strain-specific differences in the dietary responses.

Discussion

This is the first study investigating the effects of a high-protein/low-carbohydrate, iso-energetic diet fed during pregnancy and/or lactation on skeletal muscle growth and metabolism in offspring of mice of different genotypes selected for specific body conditions. Consistently, in mice of three different genotypes that represent high growth and obesity, high physical endurance fitness, and normal growth, body composition and fitness, a high protein together with a low-carbohydrate intake by the dams during pregnancy had no effects on offspring postnatal muscle growth and metabolism. In contrast, a high-protein/low-carbohydrate diet fed to dams during lactation substantially reduced body and muscle growth in all strains of mice. After weaning, the recovery of body mass occurred faster than the recovery of muscle mass in terms of the RF as the largest femoral muscle. Interestingly, offspring of the high-growth mouse strain showed a higher sensitivity to sub-optimal nutrition in terms of reduced myonuclear accumulation, which allowed transient compensatory growth but led to significantly less muscularity in 1-year-old mice.

Genotypes

The selection for high day-42 body mass over 125 generations (DU6) resulted in a doubling of body mass and led to considerable changes in skeletal muscle characteristics compared with the unselected control strain DUK. Differences in muscle mass were less pronounced than differences in body mass, due to an enormous body fat deposition in this strain [26, 37]. The increases in muscle mass resulted from increases in number and girth of the muscle fibres, which confirms results obtained previously in generation 78 of this selection strain [37]. In response to selection, protein accumulation was increased to a higher extent than DNA accumulation as reported previously as a correlated response to high-growth selection [38, 39]. Our results suggest that this is caused by a reduction in satellite cell activity as myogenic cell proliferation was only 50 % compared with unselected controls. Interestingly, the differences in muscle mass decreased with increasing age, and at 1 year of age, muscle mass fell even below that of the

unselected control strain suggesting sarcopenia and early senescence. This is consistent with the severe reduction in lifespan in this strain [40] and may also be associated with the restricted locomotory activity observed with increasing age. Higher rates of both anaerobic glycolysis and oxidative metabolism in response to selection were clearly visible at day 44 and 181 of age. However, no shift to one of these pathways was detectable by enzyme activities, although the percentage of white glycolytic myofibres was increased at day 44 of age suggesting no strong relationship in the changes of enzyme activities and fibre types. At day 396 of age, only oxidative rate was higher in the DU6 compared with the DUK strain. Results suggest that selection for high body mass increased metabolic rate, which has been shown previously for overall protein turnover in DU6 mice [37].

Selection for high endurance running (DUhLB) had no effect on body mass, except a decrease at day 181, but resulted in lower skeletal muscle mass in terms of *Rectus femoris* muscle at day 23, 181 and 396 of age. This suggests that this genotype experiences energy deficit that does not allow muscle accretion comparable with unselected controls. The lower muscle mass seems to result from diminished growth of myofibres in size, but not from a reduction in fibre number. A higher myofibre number as observed previously in response to endurance treadmill exercise or selection for an index of endurance fitness and body mass [41] was not apparent in this strain. The results on enzyme activities suggest that these mice exhibit higher metabolic rate in terms of energy generation by oxidative and glycolytic pathways until day 44 of age in comparison with unselected control mice. Obviously, both enzyme systems involved in energy generation have adapted to meet the demands of the higher running performance, which was 172 % (3,600 vs. 1,020 m) of the control strain in generation 58 of selection [42]. Likewise, analyses of metabolic markers in blood plasma of 70-day-old DUhLB mice in generation 54 of selection revealed changes in metabolism associated with enhanced demands for energy generation [27]. On the other hand, decreased CK activities suggest that the storage of energy in terms of creatine phosphate and its release plays an inferior role compared with unselected control mice.

High-protein/low-carbohydrate intake during pregnancy

Despite signs of intrauterine growth retardation in terms of birth weight or litter weight [8, 12], maternal HP exposure during pregnancy did not affect postnatal growth and skeletal muscle properties recorded in offspring at day 23 and 44 of age in all genotypes of mice examined. Obviously, any changes caused by protein excess or carbohydrate deficiency during pregnancy and appearing at birth [8, 43] were compensated until weaning, when the offspring were suckled by a dam receiving a standard diet. These results are consistent with those obtained in an experiment with pigs [9]. Despite lower body weight at birth, neither signs of retarded muscle growth nor changes in muscle metabolism were apparent at weaning, after the piglets have been cross-fostered to sows fed an adequate control diet. Altogether, it appears that excess protein together with low-carbohydrate intakes during pregnancy only has very little effect on the foetal programming of skeletal muscle phenotype when offspring is reared by dams on adequate control diets.

Controlled animal studies investigating effects of excessive intake of energy or selected macronutrients during pregnancy on offspring phenotypes are scarce. Nevertheless, some studies have investigated the effects of maternal energy overnutrition by increasing the overall energy intake without changing the composition of the diet. In pigs, such additional feed allowance during pregnancy had no beneficial effects on the progeny, and in some cases, early postnatal body and muscle growth was even slowed down and more adipose tissue was deposited (for review see [44]). However, the investigated planes of nutrition providing excess protein but simultaneously excess energy are not comparable with excess protein at unchanged energy level but carbohydrate deficiency. High-protein/low-carbohydrate diets as used as in the present study cause lipolysis and energy deficit in the maternal organism [22, 45].

High-protein/low-carbohydrate intake during lactation

Body and muscle growth

In contrast to effects of the high-protein/low-carbohydrate diet during pregnancy, the high-protein diet fed during lactation as represented by C–HP and HP–HP groups substantially retarded postnatal muscle growth during the suckling period, which was still apparent after weaning, when mouse offspring was fed a standard diet. The proportion of parenchyma in the mammary gland was reduced, milk lactose content was decreased, and the mRNA expression of lactogenic genes examined in DUK mice was also reduced in response to the maternal HP diet during lactation [8]. Thus, the experimental HP diet very likely caused a state of maternal energy deficit leading to the production of less nutritious milk. At weaning, skeletal muscle growth was more reduced than remaining body constituents, which underlines the high dependency of this tissue on whole-body energy expenditure [46].

During postnatal growth, muscle growth is achieved mainly by an increase in muscle fibre size but not by an

increase in muscle fibre number [47]. Myofibre growth in turn is mediated through the proliferative activity of satellite cells [48, 49] and muscle-specific protein accretion [50]. Our results reveal that skeletal muscle growth retardation was caused by reduced growth of the myofibres in size, but not by reduced myofibre hyperplasia. This is important, because in rodents the number of myofibres is still increasing shortly after birth [51–53]. Given that high-protein/low-carbohydrate dietary intakes reduced milk quality, our results may be comparable with those obtained in response to maternal food restriction during lactation. Thus, Glore and Layman [54] did not find any decrease in fibre number in rat offspring when dams were undernourished during lactation at about 50 % of feed intake. Consistent with our results, decreases in muscle mass were associated with smaller myofibre diameters as well as reduced DNA and protein contents. In addition, there were no differences in myofibre number when mice were raised in litters of different size of 4, 8 or 12 pups [55, 56], even though growth rate was clearly reduced. Decreased muscular DNA accumulation during lactation is suggested to result from less proliferative activity of satellite cells, which has been observed in rat and mouse models showing restricted muscle growth [36, 57]. Proliferation of satellite cells should be intensified again after weaning, when the mice were withdrawn from the suboptimal dietary conditions and exposed to an adequate standard diet. Actually, we saw a twofold greater proliferative activity of satellite cells in C–HP offspring as early as on 2nd day after switch to the standard diet, which is indicative of a fast onset of compensatory muscle growth. Restriction in muscle growth, however, was only partly compensated at day 44 and 181 of age and almost completely as late as at 1 year of age. Similarly, nutrient restriction during lactation via differing litter size was not completely compensated until day 42 of age [56].

An important question of our study was, whether mice of specific body conditions representing an obese phenotype on the one hand and a phenotype with improved physical fitness on the other hand would respond differently to the suboptimal maternal HP diet. Our results show that body and muscle growth at weaning was similarly reduced in DU6 mice selected for high body mass compared with unselected DUK mice in response to the HP diet during lactation. However, an important difference was that muscular DNA accumulation was impaired in growth-selected but not in unselected control mice that responded only with a decrease in muscle protein accretion. Assuming that milk composition was similarly changed in mice of both strains, the process of myogenic proliferation seems to be more sensitive to malnutrition in DU6 than in DUK mice. Consistently, myoblast cultures derived from the same mouse strains in an earlier generation were more responsive to withdrawal of serum and growth factors in terms of DNA synthesis rate or apoptosis [39, 58], which is another evidence for the higher sensitivity of DU6 muscle cells to nutritional insult. This is presumably caused by their higher nutritional requirements of DU6 mice.

At day 44 and 181 of age, strain-specific responses of DU6 to the HP diet fed to dams during lactation were only seen in terms of decreased body weight, but not with respect to muscle accretion, total DNA and protein contents. However, a specific response to the HP diet during lactation became apparent again at 1 year of age, when muscle mass was significantly reduced only in the obese DU6 mice. Thus, results reveal a lower capacity of satellite cell proliferation and protein accretion, when the early reduction in muscle mass was associated with a substantial decrease in myonuclei. It seems that not only a reduction in myofibre number, but also an early postnatal reduction in muscular DNA in response to nutrient restriction may have long-lasting consequences on the muscle phenotype at old age and enhance sarcopenia (see also [14, 18]). With the exception of a higher reduction in body weight at weaning, the high-running performance genotype DUhLB showed responses in body and muscle growth to the HP diet during lactation similar to the unselected control strain. The comparison of the genotypes per se has shown that especially the DU6hLB strain is highly dependent on nutritional energy and does less likely revert to energy stores, which is indicated by less CK activity and may also explain the high acute response of body weight to malnutrition.

Muscle metabolism

Changes in overall metabolism of mouse offspring suckled by dams receiving a high-protein/low-carbohydrate diet during lactation were found previously by hepatic transcriptome analysis that revealed the largest numbers of affected genes mapped to metabolic pathways with a clear focus on hexose and pyruvate metabolism, fatty acid metabolism as well as protein/amino acid metabolism [43]. In skeletal muscle, the activities of LDH or ICDH and the distribution of red, intermediate and white fibre types are characteristic of the predominant metabolic pathway providing the energy for fibre contraction. The RF muscle examined in this study is highly oxidative at birth and develops predominantly glycolytic features during postnatal growth through differentiation into fibre types of different metabolic features [51].

The exposure to HP pre-weaning diet led to a shift to oxidative muscle metabolism at the expense of anaerobic glycolytic metabolism in terms of increased ICDH activity and decreased LDH activity in young mouse offspring at weaning without changing fibre type composition. We conclude that skeletal muscle adapted to the suboptimal

nutritional supply by a higher efficacy of energy generation via intensifying the oxidative pathway within individual fibre types. The mechanism by which the oxidative pathway is stimulated is suggested to be via AMP-activated kinase (AMPK) as a key sensor of fuel and energy status in skeletal muscle. Increases in fatty acid oxidation, number of mitochondria and mitochondrial enzymes as well as decreased protein synthesis are down-stream effects of AMPK activation [59, 60]. The decrease in LDH activity in response to the HP diet during lactation indicates a reduction of glycolytic anaerobic performance of skeletal muscle in the offspring. This decline is in agreement with results obtained previously in response to various protocols of protein malnutrition in animal models or in humans and has found to be associated with a loss in muscle strength [61, 62]. It is under discussion that decreased circulating plasma IGF-1 in response to protein malnutrition may be responsible for the decline in LDH activity [62]. Because of inverse or positive correlations of ICDH and LDH activities, respectively, with FCSA, we suggest the preference for the oxidative over the anaerobic glycolytic pathway of muscle energy metabolism in response to malnutrition to be also a direct function of reduced myofibre size. Given that fusion distances within fibres become shorter with smaller fibre girth and relative capillary supply improved, there is less need for anaerobic compared to oxidative energy generation.

Conclusion

Our results show that a maternal high-protein/low-carbohydrate diet fed during lactation, but not during pregnancy, negatively affects skeletal muscle growth in the offspring, which is particularly pronounced in a genotype with a high growth rate compared with a genotype exhibiting high physical endurance fitness or an unselected control genotype. Moreover, the high-protein/low-carbohydrate diet fed to dams during lactation causes a transient adaptive shift towards oxidative at the expense of anaerobic glycolytic muscle metabolism.

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Conflict of interest The authors declare that they have no conflict of interest.

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