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Selective regulation of myofiber differentiation by energy status during postnatal development

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Harrison, A. P., A. M. Rowlerson, and M. J. Dauncey. Selective regulation of myofiber differentiation by energy status during postnatal development. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R667–R674, 1996.—The role of energy status in postnatal regulation of porcine skeletal muscle development has been determined in littermate animals kept for 3–4 wk on a high (H) or low (L) energy intake (H = 2L), at a thermally neutral [26°C (26H and 26L, respectively)] or low [10°C (10H and 10L, respectively)] environmental temperature. A variety of skeletal muscles was assessed at 7 wk of age for changes in myofiber hypertrophy and differentiation. In contrast with findings in adult humans and rats, there was no selective preservation of type I slow-oxidative fiber size during energy restriction. However, differentiation between mature skeletal myosin heavy-chain isoforms was markedly affected by energy status, and in rhomboideus there were particularly striking effects of both nutrition and temperature: proportions of type I fibers from the four groups 26H, 26L, 10H, and 10L were 34 ± 2, 50 ± 4, 73 ± 2, and 72 ± 3 ($P < 0.005$ for diet at 26°C; $P < 0.001$ for temperature). These changes may have been induced by alterations in both thyroid status and contractile activity. They support the hypothesis of a key role for rhomboideus muscle in thermoregulation and demonstrate the plasticity of skeletal muscle differentiation to environmental change during postnatal life.

differentiation; myosin isoforms; nutrition; temperature; thyroid hormones; skeletal muscle

ENERGY STATUS can be modified by alterations in energy intake and energy expenditure, and the postnatal development of skeletal muscle is susceptible to both these factors (7, 9, 17). Furthermore, changes in the properties of skeletal muscle due to energy status may in turn have both immediate and long-term consequences for locomotor, postural, and thermogenic function.

The major effect of undernutrition on skeletal muscle fibers is a reduction in fiber size (29), and in general fast-twitch fibers appear to atrophy more than slow-twitch fibers. Thus an investigation into the response of the triceps brachii and quadriceps femoris muscles to endurance training in undernourished men revealed a reduction in the size of fast- but not slow-twitch fibers (28). A similar finding of selective preservation of slow fiber size has been observed in malnourished human patients (27), and food restriction in the rat also tends to affect fast fiber size selectively, especially in the parasternal intercostal and extensor digitorum longus muscles (25). It has been hypothesized that this selective atrophy of fast-twitch fiber mass would be advantageous in terms of energy conservation (14, 31) because slow-twitch muscles use less ATP per unit isometric tension developed than do fast-twitch muscles. It is possible, however, that this mechanism of adaptation to undernutrition occurs only in mature muscles and may not be used during early postnatal development, a period of particularly dynamic myofiber hypertrophy.

A low environmental temperature markedly increases energy expenditure by either nonshivering or shivering thermogenesis. In many large mammals, such as humans after the neonatal period and pigs, this increase in heat production is predominantly via the asynchronous muscular contraction associated with shivering (13, 17). In view of the marked influence of contractile activity in determining myofiber type (24), it is possible that a low environmental temperature may itself influence differentiation between mature myosin isoforms. It has been shown previously that the proportion of oxidative fibers is greater in a wide variety of skeletal muscles from young pigs in a cold compared with a warm environment, under conditions of controlled food intake (9). Moreover, in pigs acclimated to cold from weaning until 100 kg body wt there is a greater proportion of type I slow-twitch fibers in semispinalis, a deep cervical/interscapular neck muscle, but not in longissimus dorsi, a dorsal muscle of the thoracic/lumbar region, compared with animals at thermal neutrality (20). However, these latter findings are complicated by the fact that food intake was not controlled, because cold acclimation increases food intake, and this can itself directly affect thyroid hormone status (6), which in turn plays an important role in determining the transition between myosin heavy-chain (MyHC) isoforms (5, 22). An increase in proportion of type I fibers would not only increase fatigue resistance of muscle, but could also enhance its thermogenic properties because type I fibers exhibit a more intense energetic metabolism over a long-term period than do type II fast-twitch fibers (30).

The present study has therefore tested the hypotheses that during postnatal development *1*) energy restriction selectively preserves the area of type I slow fibers and hence acts as a means of energy conservation, and *2*) environmental temperature exerts a muscle-specific effect on differentiation of myofibers and hence on the thermogenic function of skeletal muscle. Parts of this article have been the subject of preliminary communications (10, 11).

METHODS

Animals. The pig was chosen as the most appropriate animal model for assessing the role of early postnatal changes

in energy status on skeletal muscle development. This species allows careful control of food intake and environmental temperature, and interactions with genetic factors can be minimized by making comparisons within litters. Furthermore, it is biologically similar to the human infant in that it is relatively mature at birth and has similar critical temperature and zone of thermal neutrality (17).

Twenty-four male pigs of the Large White breed comprising six litters each of four animals were weaned at 3 wk of age. Their energy status was modified by alterations in energy intake and environmental temperature. Littermates were selected to be of similar body weight, and they were allocated to one of four treatment groups at random. They were paired for the first 24 h and kept in pens inside a temperature-controlled room maintained close to thermal neutrality (26°C). For two of the littermates, the temperature was kept at 26°C, whereas for the two other littermates it was decreased gradually over the next 1–2 wk to 10°C, a temperature that results in an approximate doubling of metabolic rate in young pigs (17). At each temperature, animals were provided with either a high (H) or low (L) level of food intake, where H = 2L, thus establishing four treatment groups per litter: 26H, 26L, 10H, and 10L, with six animals in each group. The food (Ultrawean, Dalgety, Bristol, UK) provided 14 kJ gross energy/g wet wt and contained (in %) 32 carbohydrate, 22.5 protein, 5.5 fat, 3.5 fiber, and 6 ash, with added vitamins and minerals. Food was provided on an ad libitum basis for the first 2 days of housing after which time a set amount was provided daily at 0915. This was increased gradually as the animals grew (12, 17), and by 7 wk of age the daily intakes of 700 and 350 g provided 9.8 and 4.9 MJ for the H and L diets, respectively. Water was freely available, and lighting was on from 0800 to 2000 daily.

Tissue sampling. Muscles selected for analysis were longissimus dorsi (white, dorsal), rhomboideus (red, interscapular), and soleus (red, hindleg). At 7 wk of age, with body weights of between 6 and 13 kg, depending on treatment, animals were sedated 20–24 h after the last meal by an intramuscular injection of ketamine hydrochloride (1.0 ml Vetalar, 100 mg/ml; Parke-Davis Veterinary, Pontypool, UK) and killed with a 0.7 ml/kg body wt intracardiac injection of (20% wt/vol) pentobarbitone sodium (Duphar). Plasma and liver samples were stored at −40°C. Muscle samples of ~1 cm^3 for histochemistry and immunocytochemistry were dissected rapidly, mounted on cork blocks, coated in embedding compound (Cryo-M-Bed, Bright Instrument, Huntingdon, Cambridgeshire, UK), frozen by 30-s immersion in isopentane cooled in liquid nitrogen, and stored at −70°C. Muscle samples of ~10 g for assessment of myosin isoforms by gel electrophoresis were frozen rapidly in liquid nitrogen and stored at −40°C. Care was taken to ensure that muscles from each animal were sampled at the same relative point in relation to both depth and distance from origin.

Histochemical analysis. Serial sections of muscle were cut on a cryostat (Bright Instrument) at −22°C and a thickness of 10 μm. Myosin adenosinetriphosphatase (mATPase) activity was assessed using the method of Brooke and Kaiser (4). After acid (pH 4.10, 4.35, 4.45, or 4.50) or alkali preincubation (pH 9.4), sections were rinsed in (in mmol/l) 10 tris(hydroxymethyl)aminomethane (Tris) and 25 $CaCl_2$ (pH 7.8), transferred to a buffer containing (in mmol/l) 50 2-amino-2-methyl-1,3-propanediol ("2213"), 18 $CaCl_2$, 50 KCl, and 2.7 ATP (pH 9.4), and incubated at 37°C for 20 min. Sections were then rinsed in 1% wt/vol $CaCl_2$, immersed in 2% wt/vol $CoCl_2$ for 3 min, washed in 2213 buffer and 40% acetone (pH 8.6), and immersed in 1% vol/vol $(NH_4)_2S$, before they were finally rinsed in water and dehydrated. Succinate dehydrogenase (SDH) activity was determined on serial sections using the method of Bancroft and Stevens (2) as described previously (9).

By combining results from the two procedures above, muscle fibers were classified on the basis of their histochemical staining and hence according to *1*) their contractile properties, as either type I slow (acid-stable and alkali-labile mATPase), type II fast (acid-labile and alkali-stable mATPase), or type IIC (acid- and alkali-stable mATPase); and *2*) their metabolic properties, as either oxidative (SDH activity = high or intermediate) or glycolytic (SDH activity = low or none). The definition of glycolytic activity was confirmed subsequently using menadione-linked α-glycerophosphate dehydrogenase staining (P. White and M. J. Dauncey, unpublished observations) according to the method of Bancroft and Stevens (2). On the basis of this histochemical classification, individual fibers have been defined as slow oxidative (SO), fast oxidative glycolytic (FOG), and fast glycolytic (FG). The FOG fibers probably included types IIA and IIX, whereas the FG probably comprised the IIB type (26). The IIC fibers probably represented fibers in transition between the slow and fast MyHC isoforms (3); very small embryonic/neonatal fibers were excluded from this category.

Immunocytochemistry. To verify the accuracy of the histochemically based fiber type classification for distinguishing between type I slow and type II fast fibers, serial sections were taken for indirect immunoperoxidase staining with type-specific antibodies. The anti-I serum raised in rabbits reacts only with slow (type I) myosin (21), and the monoclonal anti-fast (Sigma clone M432) reacts with most fast MyHC isoforms, including the adult IIA, IIX, and IIB forms. Comparison between the histochemical mATPase fiber type classification and the immunoperoxidase staining showed that there was good agreement between these two methods for assessing a fiber as either slow or fast twitch. Quantitative estimates of myofiber type and size were therefore based on the histochemical staining.

Fiber type distribution and size. The relative distribution of fiber types was assessed in a standard field of 279,400 $μm^2$, and fiber typing involved between 500 and 1,300 fibers counted over three fields for each muscle sample. Fiber type cross-sectional area was determined in a standard field area of 111,900 $μm^2$, and mean fiber area of each of the four fiber types was determined on 20–90 fibers of each type per muscle sample. Quantitation was with a Seescan AO10 research-grade image analysis system (Seescan, Cambridge, UK).

MyHC isoforms. Frozen muscle samples of 0.5 g wet wt were smashed in an anvil press that had been cooled to −70°C. The shattered tissue was added to an eight times weight per volume solution containing (in mM) 300 KCl, 100 Na_2HPO_4, 1 $MgCl_2$, and 0.1% β-mercaptoethanol, pH 6.5. The muscle solution was homogenized for 5 s and stirred at 4°C for 30 min before centrifugation for 5 min at 6,000 revolutions per minute. The supernatant, which consisted of a crude MyHC extract, was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, 5% polyacrylamide running gels were used with a 3% stacking gel. A 10-μl aliquot of crude myosin extract, ~2.5 ng protein, was electrophoresed for 3–4 h at 80 V and 0.01 A constant current. Protein bands were detected by silver staining, according to the procedure of Morrissey (23). The optical density (OD) of MyHC bands within SDS gels was determined using a Seescan image analyzer, and a histogram of the OD measurements taken for each of the protein bands was plotted. The height of each histogram peak, corresponding to either a fast or slow MyHC isoform, was measured, and the

values were used to give a ratio of fast-to-slow MyHC isoforms for each muscle sample.

Thyroid hormone status. Plasma was analyzed for total 3,5,3′-triiodothyronine (T_3) concentration by radioimmunoassay (RIA) using a Coat-A-Count Kit (Diagnostic Products, Los Angeles, CA). Hepatic 5′-deiodinase activity was determined as described previously (7). Briefly, 2.5 g liver was homogenized in a buffer of the following composition (in mmol/l) 50 Tris·HCl, 250 sucrose, 5 EDTA, pH 7.2, and a 200-μl aliquot of supernatant was incubated at 37°C for 30 min in the presence of 100 μl thyroxine (12.8 μmol/l). The reaction was stopped by addition of 2 ml ice-cold 95% vol/vol ethanol, and the concentration of T_3 was determined using RIA. The activity of 5′-deiodinase was calculated as the concentration of T_3 produced in picomoles per minute expressed per gram protein.

Statistical analysis. Differences between means and interactions between energy intake and environmental temperature were tested for statistical significance by analysis of variance. A randomized block design was used, where blocks were litters and the treatments were energy intake and environmental temperature. Data are presented as mean values ± SE. Results were considered statistically significant at the 5, 1, or 0.1% levels.

RESULTS

Growth rates and thyroid status. Growth rates of animals in the four treatment groups differed markedly during the 4-wk period of study (Fig. 1), reflecting the large differences in energy status between the groups. Animals fed a high energy intake at thermal neutrality (26H) attained the fastest rate of growth, whereas those in the cold and on a low intake (10L) used a large proportion of their dietary energy for regulatory thermogenesis and thus grew most slowly. The 26L and 10H treatment groups grew at rates between these two extremes, illustrating the close interaction between diet and temperature in determining the energy status of the individual. There were highly significant effects of both diet and temperature on growth rate between 4 and 7 wk of age ($P < 0.001$ for both comparisons) and no interaction between the two variables. Body weights at 7 wk of age (means ± SE) were 12.6 ± 0.3, 8.7 ± 0.6, 9.8 ± 0.3, and 6.3 ± 0.4 kg for the 26H, 26L, 10H, and 10L treatment groups, respectively.

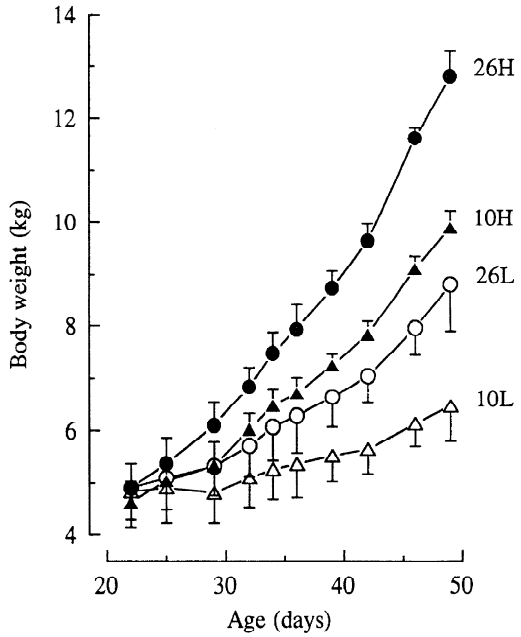

Fig. 1. Growth rates of animals in the 4 treatment groups: littermate pigs living at 26 or 10°C on a high (H) or a low (L) food intake (where H = 2L). Rate of growth is related to energy status and hence the balance between energy intake and energy expenditure. Results are means ± SE (n = 6 in each group). Effects of both nutrition and temperature on rate of growth between 4 and 7 wk of age were significant ($P < 0.001$ for both comparisons).

Table 1. *Influence of energy status on plasma concentration of T_3 and hepatic 5′-deiodinase activity*

Measurement	Treatment Group 26H	26L	10H	10L
Plasma T_3, nmol/l	1.59 ± 0.10	1.14 ± 0.09	1.49 ± 0.16	0.99 ± 0.10
Deiodinase activity, pmol $T_3 \cdot min^{-1} \cdot g$ protein^{-1}	412 ± 22	347 ± 23	431 ± 28	279 ± 34

Values are means ± SE for 6 litters, each of 4 animals, at 7 wk of age: littermates had been living at 26 or 10°C on a high (H) or low (L) energy intake (where H = 2L). Samples were obtained 20–24 h after the last meal. Analysis of variance showed that the effect of energy intake was significant for both plasma T_3 and deiodinase activity ($P < 0.001$). There was no significant effect of temperature and no interaction between the effects of energy intake and temperature.

Thyroid status of the animals was assessed from estimates of plasma T_3 concentration and hepatic deiodinase activity. Results indicated a highly significant effect of energy intake on both these parameters (Table 1): animals on the high food intake had a greater circulating level of T_3 and hepatic deiodinase activity ($P < 0.001$ for both comparisons) than those on the low food intake, whether they were at 26 or 10°C. In contrast, there was no effect of environmental temperature on either of these parameters.

Myofiber hypertrophy. Results for cross-sectional areas of the four different fiber types are presented in Fig. 2. For longissimus dorsi and soleus muscles, the size of all fibers was markedly affected by energy status, and the results were closely related to growth rate. Thus fiber cross-sectional area was greatest in animals with the highest energy status (26H) and smallest in those with the lowest energy status (10L). All fiber types were affected similarly, and there was no selective preservation of size for any of the four types of fiber. Analysis of variance showed that the effects of both nutrition and temperature were highly significant ($P < 0.001$), and there was no interaction between these two variables.

The results for rhomboideus were not as clear-cut as those for the two other muscles, and there was greater variability between animals. Nevertheless, the results tended to show the same trend as those for longissimus dorsi and soleus, with fiber cross-sectional area being greatest in the 26H and least in the 10L animals. The effects of nutrition were on the borderline of significance for type I fibers ($P = 0.06$); for types II FOG and FG the effects of nutrition were significant ($P < 0.02$

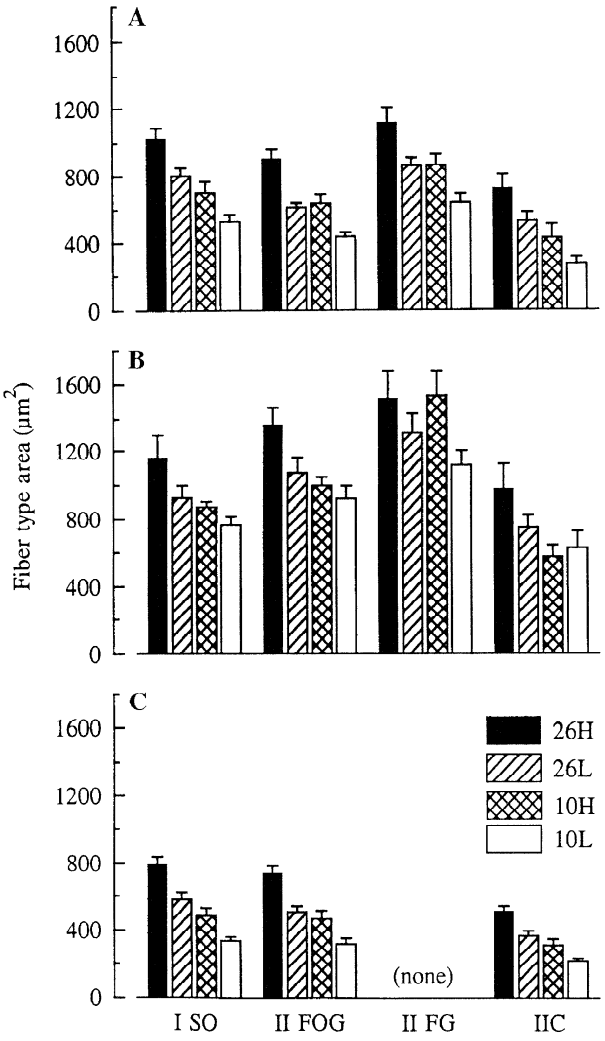

Fig. 2. Cross-sectional areas of myofibers in longissimus dorsi (*A*), rhomboideus (*B*), and soleus (*C*) muscles from 7-wk-old pigs that had been living at 26 or 10°C on a high (H) or low (L) energy intake. Fibers were classified as type I slow oxidative (I SO), type II fast oxidative glycolytic (II FOG), type II fast glycolytic (II FG), or type IIC (IIC). In soleus muscle, all fibers were found to be oxidative, hence no II FG fibers are reported. Results are mean values ± SE (n = 6 in each group). For longissimus dorsi and soleus, all fiber types were significantly affected by both nutrition and temperature ($P < 0.001$). For rhomboideus, statistical significance of results was not as clear-cut, and results of analyses of variance are presented in text.

and $P < 0.002$, respectively) with the low energy intake resulting in smaller fibers, but there was no effect on the size of type IIC fibers ($P > 0.4$). Similarly for temperature, three of the four fiber types were significantly smaller at 10°C than at 26°C: type I SO ($P < 0.02$), type II FOG ($P < 0.002$), type IIC ($P < 0.03$), and there were no interactions between temperature and diet. The size of type II FG fibers was, however, unaffected by environmental temperature ($P > 0.2$).

In general, therefore, myofiber hypertrophy in early postnatal life was impaired when energy availability for growth was restricted because of either a low energy intake or a high thermoregulatory demand. This response was observed in all fiber types, with the only marked exception being that the size of type II FG fibers in rhomboideus was preserved at the low temperature, especially in animals on the high intake diet (Fig. 2).

Myofiber differentiation. As expected there were marked differences in fiber type distribution between the three muscles, with the white longissimus dorsi containing a very high proportion of type II fast fibers and the red rhomboideus and soleus having a high proportion of type I slow fibers and being predominantly oxidative. For example, in the 26H group the percentages of type I fibers (means ± SE) were 8 ± 1, 37 ± 3, and 34 ± 2 in longissimus dorsi, soleus, and rhomboideus muscles, respectively, and percentage values for oxidative fibers (types I + II FOG + II C) were 47 ± 3 (longissimus dorsi), 100 (soleus), and 80 ± 3 (rhomboideus).

There was found to be a striking effect of energy status on myofiber differentiation of rhomboideus muscle; an example of results from 26H and 10L animals is presented in Fig. 3. This shows that the proportion of type I fibers was much greater in the animal at 10°C on a low food intake compared with its littermate at 26°C on a high intake. Mean values for the four treatment groups are given in Fig. 4. This shows that at 7 wk postnatally, after only 3–4 wk of treatment, energy restriction at thermal neutrality (26H vs. 26L) resulted in a much higher proportion of type I SO fibers ($P < 0.005$) and a lower proportion of type II FG fibers ($P < 0.001$) in rhomboideus muscle. In contrast, there was no overall effect of energy intake on the proportions of fiber types in longissimus dorsi or soleus muscles.

An elevated level of energy expenditure for 3–4 wk, induced by a low environmental temperature, also resulted in a very much greater proportion of type I fibers and reduction in proportions of type II fibers in rhomboideus muscle (26 vs. 10°C, $P < 0.001$). Similar but much smaller effects were observed in longissimus dorsi, in which there was a greater proportion of type I SO ($P < 0.05$) and lower proportion of type II FG ($P < 0.005$) fibers after cold acclimation. In soleus, by contrast, there was no significant effect of environmental temperature on fiber type distribution.

Thus the extent to which energy status modified fiber type proportions during the first 2 mo postnatally was not related simply to the overall contractile or metabolic characteristics of the muscle examined; fiber type distribution of the red rhomboideus was affected to a much greater extent than either the white longissimus dorsi or red soleus muscles.

MyHC isoforms. Changes in energy status during postnatal life were found to alter the levels of slow and fast MyHC isoforms in a manner similar to those induced in fiber type distribution (Fig. 5). Thus, in rhomboideus, where marked changes in fiber type proportion were observed with cold acclimation and energy restriction, the ratio of fast to slow MyHC, determined with the use of SDS-PAGE, closely resembled the ratio of fast to slow fiber type proportions determined by enzyme histochemistry. In soleus muscle, in which there were no overall effects of either nutrition or temperature on fiber type proportions, there was

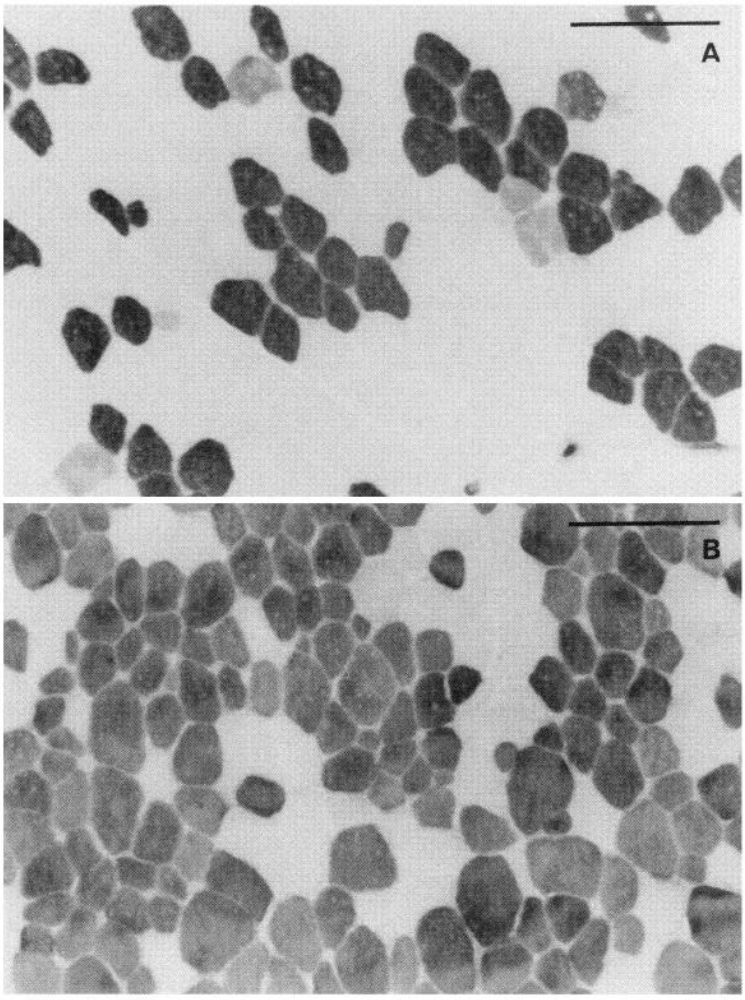

Fig. 3. Proportions of myofiber types in rhomboideus muscle of littermate animals at 26°C on a high energy intake (*A*) or at 10°C on a low energy intake (*B*). Muscle sections were stained for myosin ATPase activity after preincubation at pH 4.35; dark fibers = type I slow twitch. Scale bar = 100 μm. Mean values ± SE for all animals in each of the 4 treatment groups are given in Fig. 4.

also no significant change in the ratio of fast to slow MyHC for three of the four treatment groups. Only in soleus muscle from the 26H group was there a significant difference between the ratio of fast to slow fiber proportions determined by the two methods ($P < 0.05$).

Where very slight changes in fiber type proportion were detected by means of mATPase histochemistry, as in the case of longissimus dorsi muscle, an accurate assessment of fast-to-slow MyHC ratio could not be made because the very low concentration of slow MyHC tended to be masked by the very strong band obtained from the high concentration of fast MyHC.

DISCUSSION

A major finding of the present study is that the phenotype of mature myofibers can be modified significantly in postnatal life by alterations in both energy intake and environmental temperature. Furthermore, results are muscle specific and are not related simply to the contractile or metabolic properties of the muscle but rather to its physiological function. In addition, it has been shown that during postnatal development a period of energy restriction has no fiber type-selective effects on hypertrophic growth.

Myosin heavy-chain isoforms. Muscle fibers are commonly classified as fast or slow twitch on the basis of their histochemical reaction for mATPase (4). This approach is based on the assumption that the histochemical reaction is due to mATPase activity and that it reflects the biochemical properties of myosin that make it fast or slow. The present study has shown that marked changes in fiber type proportion assessed by histochemistry are confirmed not only by immunocytochemistry but also by gel electrophoresis of crude muscle extracts. Thus the differences in fiber type induced by changes in energy status reflect changes in the concentration of contractile proteins within specific muscles and hence in the functional properties of these muscles.

Myofiber hypertrophy. The present study has shown that energy restriction in the young pig does not result in selective preservation of the size of type I SO fibers. Instead, the cross-sectional areas of all fiber types in longissimus dorsi and soleus muscles were smaller in animals on a low compared with a high energy intake, and fiber area tended to be affected in a similar manner in rhomboideus. This result contrasts with findings in adult humans in which energy deficiency over a 2-wk

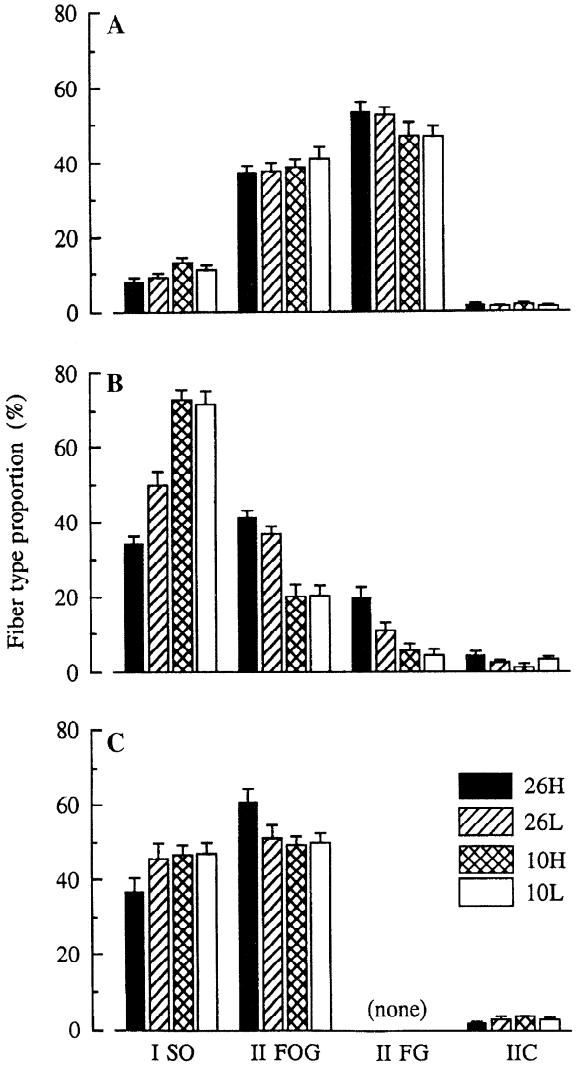

Fig. 4. Proportions of myofiber types in longissimus dorsi (*A*), rhomboideus (*B*), and soleus (*C*) muscles from 7-wk-old pigs that had been living at 26 or 10°C on a high (H) or low (L) energy intake. Fibers were classified as I SO, II FOG, II FG, or IIC. In soleus muscle, all fibers were found to be oxidative, hence no II FG fibers are reported. Results are means ± SE (n = 6 in each group). There were major effects of energy status on rhomboideus, and results from analyses of variance are given in text.

period resulted in smaller fast-twitch but not slow-twitch fibers in triceps brachii and quadriceps femoris muscles (28). Moreover, food restriction in the adult rat also tends to affect the size of fast fibers selectively (25). Because the nutritional physiology and pattern of development of humans is much more similar to that of the pig than the rat, it is probable that the contrast between the present findings and those in adults are related to differences in age rather than to species differences. Selective preservation of slow-twitch fiber size during a period of reduced energy availability would be energetically advantageous because the energy expenditure per unit tension developed is lower in slow-twitch fibers than in fast-twitch fibers. It is probable, therefore, that selective preservation of fiber size during undernutrition is used as a means of energy conservation only when myofibers are fully mature, whereas energy conservation during early postnatal development is effected by reducing the rate of hypertrophy of all fibers.

The observation that the cross-sectional area of type II FG fibers in rhomboideus was unaffected in animals at a cold compared with a thermally neutral temperature is intriguing. One possible explanation is that small type II FG fibers are converted to type II FOG or even type I SO fibers, because it was observed that the proportion of type II FG fibers was lower in the cold, whereas the proportions of types II FOG and I SO were greater. Such a response would ensure that rhomboideus muscle retained a limited capability for fast contraction and may also serve to facilitate rapid transition of fiber type once an animal is no longer in a cold environment.

Myofiber differentiation. Changes in energy status were found to induce marked differences in fiber type proportions within rhomboideus and small but significant differences in fiber type proportions of longissimus dorsi muscle. Thus in rhomboideus the proportion of type I SO fibers was 47% greater in animals on a low compared with a high energy intake at thermal neutrality, and the value was a further 45% greater in animals at 10°C than at 26°C on the low intake. Two dominant

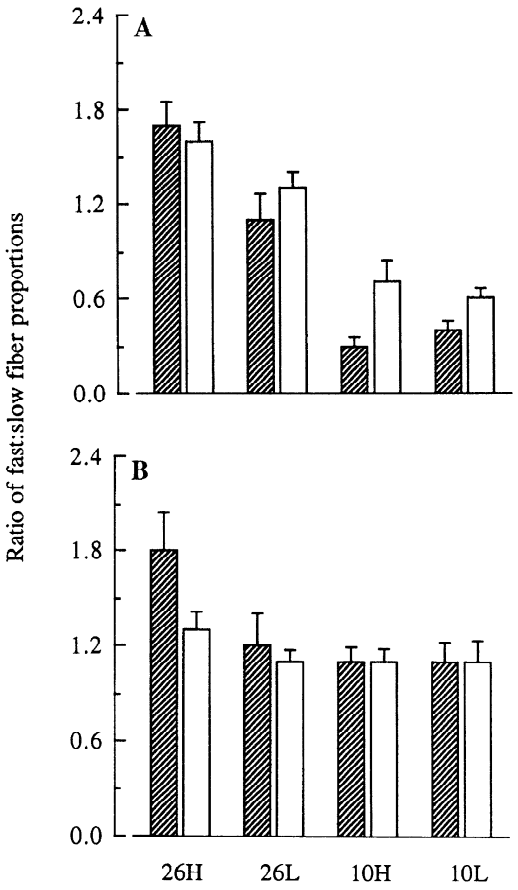

Fig. 5. Comparison between ratio of fast to slow fiber type proportions determined from myosin ATPase activity using enzyme cytochemistry (hatched bars) and the ratio of fast to slow myosin heavy-chain isoforms determined by gel electrophoresis (open bars). Results are means ± SE for animals in each of the 4 treatment groups [n = 3 in each group for rhomboideus (*A*), n = 6 in each group for soleus (*B*)].

regulators of muscle fiber differentiation are thyroid status and contractile activity. The greater proportion of type I fibers in the 26L compared with the 26H group (50% compared with 34%, respectively) may have been due to the low concentration of circulating T_3 and reduction in hepatic 5′-deiodinase activity in these animals. Not only do thyroid hormones regulate the transition from neonatal to adult myosin isoforms during normal development, they also enhance conversion from slow- to fast-twitch fibers postnatally in a muscle-specific fashion (5). There are several subtypes of nuclear receptors for thyroid hormone that can either activate or repress transcription of T_3-inducible genes (19). The possibility is that selective changes in these subtypes could be involved in the muscle-specific regulation of MyHC gene expression by energy status.

Although the most well-studied hormone with respect to muscle development is thyroid hormone (5), other hormonal factors that may have been involved in determining the differences in fiber type due to changes in energy status include growth hormones and glucocorticoids. Not only has growth hormone been implicated in increasing the oxidative capacity and proportion of type I fibers in muscle (1, 18), but growth hormone receptor gene expression in porcine mucle is elevated in young animals on a low compared with a high energy intake at 10°C compared with 26°C (7). Furthermore, cortisone can induce changes in MyHC distribution in rat muscles (25), and circulating levels of cortisol are markedly elevated in young pigs at a low compared with a high environmental temperature, particularly when given a low energy intake (8). The precise roles of growth hormone and cortisol in regulating the expression of MyHC isoforms during development clearly require further investigation.

Although level of energy intake had a marked effect on thyroid status, no such effect was observed as a result of change in environmental temperature. This agrees with previous reports in which it has been shown that an elevation in circulating concentrations of thyroid hormones and their nuclear receptors in muscle can be maintained in a cold environment only if animals are allowed to feed ad libitum (6). Therefore, the markedly greater proportion of type I fibers in cold-acclimated animals cannot be attributed to differences in thyroid status. Instead, it was probably due to the increased contractile activity associated with shivering because electromyographic recordings indicate that at 10°C young pigs shiver for most of the time (13). Furthermore, under conditions of long-term electrical stimulation, a situation that can be compared to some extent with continuous shivering, a transition from fast to slow fiber type occurs (24). The very high proportion of type I fibers in rhomboideus muscle at 10°C compared with 26°C would not only improve fatigue resistance and facilitate the continuous contractile activity required for shivering, but it would also contribute directly to regulatory heat production. This latter effect is of particular significance because rhomboideus muscle is located near to the thermosensitive neurons of the upper spinal cord. The present finding of marked changes in fiber type proportion after cold acclimation, combined with increases in subsarcolemmal mitochondrial mass (15) and loose-coupling of subsarcolemmal mitochondria (16) of rhomboideus, suggests a key role for this muscle in thermoregulation. Moreover, the probability is that this function is shared with other muscles in the interscapular region (20).

Perspectives

The postnatal period is a particularly dynamic phase of skeletal muscle development in terms of fiber hypertrophy. In adults, it has been shown previously that during energy restriction there is selective preservation of type I slow-twitch fiber size, which could act as a mechanism for energy conservation. We have now shown that in developing muscles no such mechanism operates but rather that changes in proportion of fiber types and contractile proteins occur in early postnatal life. These changes are muscle specific and related to anatomic location and function rather than to fiber type composition of the muscle per se. Postnatal changes in myofiber differentiation between mature myosin heavy-chain isoforms will affect not only the contractile properties of muscle but also its ability to play a key role as a thermogenic organ.

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