Restricted Feed Intake during Fattening Reduces Intramuscular Lipid Deposition without Modifying Muscle Fiber Characteristics in Rabbits[1]

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ABSTRACT The present study was conducted to determine the effects of feed restriction during fattening on muscle fiber characteristics and intramuscular lipid traits. From 11 wk of age onward, rabbits were given free access to feed (control group), or received 70% of the control feed intake (restricted group). At the same weight at slaughter, restricted-fed rabbits were 3 wk older than controls (18 vs. 15 wk). The longissimus lumborum (LL, white loin), biceps femoris (BF, white thigh) and semimembranosus proprius (SMP, red thigh) muscles were then removed, and biochemical and histochemical assays were performed. In the three muscles, there was no effect of feed restriction on mean fiber size or percentage of the different fiber types. Restricted vs. control feeding resulted in a significant reduction ($P < 0.001$) in total lipid content in all three muscles. This reduction was paralleled by a decline ($P < 0.001$) in the activities of the malic enzyme and glucose-6 phosphate dehydrogenase (G6PDH), generating NADPH for the support of fatty acid synthesis. The diet-induced variations in lipid concentration and enzyme activities were larger ($P < 0.05$) in the pure oxidative SMP muscle than in the predominantly fast-twitch glycolytic LL and BF muscles. Whatever feeding status, the ratio of malic enzyme to G6PDH activities was sharply lower ($P < 0.001$) in SMP than in BF and LL muscles (averaging 1.5 vs. 9 and 15, respectively). These data indicate that nutritional status regulates intramuscular lipid deposition, without changing fiber-type composition. Further studies are necessary to determine the role of G6PDH in the lipogenic process of oxidative vs. glycolytic muscles. J. Nutr. 130: 228–233, 2000.

KEY WORDS: • *food restriction* • *myofiber* • *intramuscular fat* • *lipogenesis* • *rabbits*

Various factors influence the postnatal development of skeletal muscles, such as physical activity, endocrine status, and nutrition. Numerous studies performed in different species, excluding rabbits, have demonstrated that restriction of feed intake is able to modify morphological, physiological or biochemical characteristics of muscles, and eventually meat quality.

Restricted feeding alters the size of the myofibers (Harrison et al. 1996, Solomon et al. 1988 in pigs, Yambayamba and Price 1991 in beef) and favors the oxidative metabolism pathway, as evidenced by the higher percentage of oxidative fibers in muscles of feed-restricted compared to well-nourished animals (Seideman and Crouse 1986 in beef, Solomon and Lynch 1988 in lambs, Solomon et al. 1988 in pigs). But conflicting data are often reported depending on species, muscle considered (Brandstetter et al. 1998), age of the animal (Harrison et al. 1996) and whether the effect of feed restriction was studied at similar age or at similar body weight (Candek-Potokar et al. 1999).

Restricting feed allowance may also result in a reduction of muscle lipid concentration, as evidenced in the longissimus muscle of pigs slaughtered at the same weight as their well-nourished counterparts (Candek-Potokar et al. 1998, Wood and Warris 1990). However, the mechanisms whereby restricted feed intake impairs intramuscular fat level are not well understood. In subcutaneous and internal adipose tissues (e.g., Ingle et al. 1973 in sheep, Mills et al. 1989 in steers, Mersmann et al. 1981, Steele and Frobish 1976, in pigs), feed restriction and deprivation depress the rate of de novo lipogenesis, as well as the activities of numerous enzymes involved in the synthesis of fatty acids (acetyl-CoA-carboxylase and fatty acid synthase) or responsible for generating NADPH for the support of lipogenesis [malic enzyme and glucose 6 phosphate dehydrogenase (G6PDH)[3]]. However, it is not known whether moderate feed restriction also affects lipogenic rate and enzyme activities in muscles of locomotion. To our knowledge, there is only one study that demonstrates that severe feed deprivation has no effect on the acetyl-CoA-carboxylase activity in a glycolytic hindlimb muscle of rats (Winder et al. 1995). As a whole, little information is available on the regulation of the different lipogenic enzymes in the muscle.

Muscles which differ in their metabolic properties are not homogeneous in final lipid content. Variation between muscle

[1] Presented in part in the 3rd French-British Meeting on Nutrition, September 30, October 1–2, 1998, Nancy, Fr. [Gondret, F., Lebas, F. & Bonneau, M. (1998) Effects of feed restriction during fattening on muscle lipid traits in the rabbit.]

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[3] Abbreviations used: ACC, acetyl-CoA-carboxylase; BF, biceps femoris; G6PDH, glucose-6P dehydrogenase; LL, longissimus lumborum; NZW, New Zealand White; SDH, succinate dehydrogenase; SMP, semimembranosus proprius.

Manuscript received 29 April 1999. Initial review completed 26 July 1999. Revision accepted 18 October 1999.

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types in intramuscular fat is primarily related to differences in the accumulation of adipocytes along fiber fasciculi; however, the precise relationships between metabolic type and intramuscular fat remain rather controversial (Candek-Potokar et al. 1999, Gondret et al. 1998). Moreover, the relationship between lipogenic enzyme activities and muscle carbohydrate metabolism is mainly unknown.

Therefore, the present study was undertaken to examine the effects of a 30% food-intake restriction on morphological, metabolic and biochemical characteristics of three rabbit muscles. Moderate but long restriction of feed intake during fattening was chosen because such feeding system is sometimes carried out in rabbit meat industry (Ouhayoun et al. 1986). We measured de novo lipogenic enzyme activities in muscle homogenates in order to study the cellular mechanism for putative differences in lipid content between restricted and well-nourished rabbits. In addition, the experiment investigates some aspects of intramuscular lipid deposition in relation to muscle energy metabolism.

MATERIALS AND METHODS

Animals and sample collection. Rabbits were reared and slaughtered in compliance with national regulations for humane care and use of animals in research (certificate of authorization to experiment of living animals n°02376 delivered by the French Department of Agriculture to F. Lebas). Male White New Zealand rabbits (NZW) from the strain INRA 1077 were purchased from the Animal Breeding Unit (Institut National de la Recherche Agronomique, Castanet Tolosan, France). From weaning (4 wk) to 11 wk of age (beginning of the period of accelerated fat deposition), rabbits were reared collectively (n = 6 per cage), and had free access to water and feed. At 11 wk of age, 60 rabbits of average body weight (2377 ± 21 g) were allotted to one of two dietary groups. They were given free access to feed (control, n = 30) or received 70% of the control feed intake (restricted, n = 30). During the subsequent experimental period, the rabbits were housed in individual cages. They had free access to water and were fed the same pelleted diet based on alfalfa, wheat, beet pulp, soybean and sunflower meals. The diet contained 14.7% cellulose, 16.5% crude protein, 2.8% fat and 10.3 MJ/kg digestible energy. Feed was distributed three times a wk (2/7, 2/7 and 3/7 of the weekly allowance; Ouhayoun et al. 1986). The amount of feed allocated to restricted rabbits at each distribution was calculated on the basis of data of the weekly spontaneous feed consumption obtained in a preliminary study carried out on 20 NZW rabbits reared under the same conditions. Feed refusals did not occur in the restricted group, whereas they were removed and measured just before each distribution in the control group. Live body weight was recorded weekly during the experiment.

At 15 wk of age (2905 ± 31 g body weight), 15 rabbits were selected from the control group, so that their weight was representative of the mean body weight of their counterparts. They were then feed-deprived overnight, and slaughtered the following morning by electric stunning and exsanguination. Restricted rabbits were maintained on the above regimen until they reached the average weight of 2905 g, at which time 15 rabbits were selected on the basis of their weight, and slaughtered under the same conditions. Carcasses were prepared as described by Blasco et al. (1993), by removing the skin, feet, paws, genital organs, urinary bladder and digestive tracts. Carcass, liver, and perirenal fat were weighed, and dressing out percentage (carcass weight/body weight, a commonly used indicator of rabbit carcass quality) was determined. Immediately after slaughter, samples from the left and right longissimus lumborum (LL, white dorsal) at the level of the 3–7th lumbar vertebra, biceps femoris (BF, white thigh), and semimembranosus proprius (SMP, red thigh) muscles were excised from the carcass. These three muscles were chosen because of their importance for rabbit meat industry and their differences in contractile and metabolic characteristics. Muscles were carefully trimmed from external fat and epimysium. The left sample was immediately frozen in liquid nitrogen for biochemical measurements. The right sample was restrained on flat sticks and frozen in isopentane (cooled by liquid nitrogen) for subsequent histological examinations. All muscle samples were stored at −80°C until analyses.

Determination of muscle fiber characteristics: morphometrics, contractile and oxidative activities. From the right part of each muscle sample, serial cross-sections (10 μm) were cut on a cryostat (2800 Frigocut Reichert-Jung, Francheville, France). All sections were taken at a comparable relative position in each muscle. One section was stained with azorubin, and mean fiber cross-sectional area (μm^2) was measured with a projection microscope (Visopan Reichert, Wien, Austria) and a programmable planimeter (Hitachi Siko, Tokyo, Japan), using 200 fibers counted over three fields per each section. The second section was processed for the actomyosin ATPase activity (Guth and Samaha 1969), and myofibers were classified on the basis on their contractile properties as type I (slow-twitch), type IIA or type IIB + IIX (fast-twitch). The third section was stained for succinate dehydrogenase (SDH, an enzyme specific of the aerobic oxidative pathway) activity in order to evaluate the oxidative capacity of the myofibers (Nachlas et al. 1957). Myofibers have been defined as either oxidative (SDH activity = high) or nonoxidative (SDH activity = low or none). Proportions of the different fiber types were evaluated on 900–1300 fibers counted over three fields for each muscle section.

Determination of muscle lipid traits: biochemical content and lipogenesis. Total lipid contents were extracted according to Folch et al (1957), using a 10-g sample of the left part in each LL and BF muscles, and a 1-g sample in each SMP muscle. Total lipid contents were expressed as g/100 g of fresh tissue. The activities of enzymes considered as rate-controlling steps in the process of lipogenesis (acetyl-CoA carboxylase ACC; EC 6.4.1.2), or related to NADPH production for fatty acid synthesis (G6PDH, EC 1.1.1.49; and malic enzyme, EC 1.1.1.40) were measured on muscle homogenates. Briefly, a weighed quantity of tissue (1.5 g for BF and LL, and 0.5 g for SMP) was homogenized in 2.5 mL of saccharose (0.25 mol/L) ice-cold buffer. After centrifugation at 30,000 × g at 4°C for 40 min, the resulting supernatant fraction was collected and used for enzyme assays. The activity of muscle ACC was determined by the $H^{14}CO_3^-$-fixation method (Chang et al. 1967). The activities of malic enzyme and G6PDH were assayed as described by Hsu and Lardy (1969) and Ficht et al. (1959), respectively, using spectrophotometric absorbance at 340 nm. Lipogenic enzyme activities were expressed as unit/min/g of fresh tissue, and were also normalized using cytosol protein content (Lowry et al. 1951).

Statistical analyses. Growth performances and slaughtering data were compared for the effect of feeding level, using the one-way ANOVA (General Linear Model procedure of SAS, 1990). Muscle histological and biochemical data were analyzed by multifactor ANOVA, including the main effects of feeding level (F), muscle (M) and their interaction (F × M). Where applicable, multiple comparison of means was performed, using the LSMEANS statement of the GLM procedure. Data are presented as the means of each feeding group within each muscle, and pooled SEM together with the significance levels of the main effects and interactions. A further analysis was performed using Student's t test to compare restricted to control data within each muscle.

RESULTS

Growth rates, food consumption and carcass data. Growth performance and carcass data of the 30 experimental rabbits are given in **Table 1.** The overall level of restriction achieved was 29.8% in the restricted group compared to control rabbits. From 11 wk to slaughter age, growth rate was 53% lower ($P < 0.001$) and the gain/feed ratio was 36% lower ($P < 0.001$) in feed-restricted rabbits than in controls. At the same slaughter weight, restricted rabbits were 3 wk older than controls. Carcass weight, dressing out percentage, as well as amount of perirenal fat, were significantly reduced ($P < 0.001$) in restricted rabbits compared with controls. In contrast, liver

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TABLE 1

Growth performance and carcass data of control and feed-restricted rabbits slaughtered at the same weight[1]

	Feeding group	
	Control	Restricted
Growth performance		
Weight at slaughter, *g*	2905 ± 31	2933 ± 33
Age at slaughter, *wk*	15	18
Growth rate, *g/d*	18.4 ± 0.9	8.6 ± 0.6*
Feed intake, *g/d*	131.2 ± 3.1	92.0 ± 0.8*
g gain/g feed	0.14 ± 0.003	0.09 ± 0.001*
Carcass data		
Carcass weight, *g*	1910 ± 25	1726 ± 17*
Dressing out percentage, *g/100 g*	65.7 ± 0.4	58.9 ± 0.5*
Liver weight, *g*	80.3 ± 2.5	73.2 ± 2.4
Perirenal fat weight, *g*	43.8 ± 5.4	11.7 ± 1.4*

[1] Values are means ± SEM; n = 15. *Significantly different from control, $P \leq 0.05$.

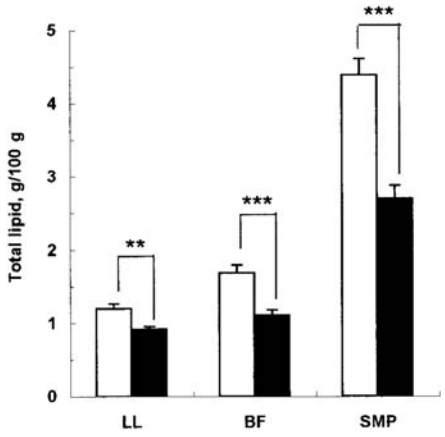

FIGURE 1 Total lipid concentration (g/100 g of fresh muscle) in longissimus lumborum (LL), biceps femoris (BF) and semimembranosus proprius (SMP) in control (□) and feed-restricted (■) rabbits slaughtered at the same weight. Values are means ± SEM, n = 15. Within each defined muscle, restricted values are significantly different from control data at $^{**}P < 0.01$ or $^{***}P < 0.001$ (Student t test). Data were also compared using a two-way ANOVA with the main effects of feeding group (F), muscle type (M) and their interaction (F × M). Significant *P*-values were found for feeding ($P = 0.0001$), muscle ($P = 0.0001$) and their interaction ($P = 0.0001$).

weight was not significantly different ($P > 0.05$) between the two groups.

Myofiber characteristics. No difference was observed for mean cross-sectional area of fibers between restricted and control rabbits compared at the same weight at slaughter. Mean comparisons revealed that, irrespective of the feeding status, mean fiber size was higher in SMP than in LL, and it was the lowest in BF (**Table 2**) None of the muscles studied showed evidence for an effect of feed restriction on contractile fiber-type composition (Table 2). As expected, there were marked differences in fiber-type composition between the three muscles with SMP being a pure slow-twitch type I muscle, whereas LL and BF were mixed fast-twitch muscles. LL had the lowest proportion of type I and the highest proportion of type IIB + IIX fibers, whereas BF displayed the highest proportion of type-IIA fibers. A muscle-specific response to restriction was observed ($P < 0.01$ for F × M) for the percentage of oxidative fibers which was not significantly affected by feed restriction in SMP and BF muscles whereas it was lowered ($P < 0.001$) in LL muscle (Table 2).

Intramuscular lipid traits. Restricted vs. control feeding resulted in a significantly lower total lipid content in all three muscles (**Fig. 1**) A significant effect of interaction (F × M) was observed ($P < 0.001$), in which the relative reduction induced by feed restriction was larger in SMP (−39%) than in BF (−31%), whereas it was the smallest in LL (−25%). Irrespective of feeding group, total lipid content was higher in SMP than in either of the other two muscles ($P < 0.0001$). It was slightly lower ($P < 0.01$) in LL than in BF in control rabbits, whereas LL and BF muscles of restricted rabbits had lipid contents that did not differ ($P = 0.3$).

Muscle metabolic capacity for de novo lipogenesis was assessed by in vitro measurements of enzyme activities in muscle homogenates (**Table 3**). Whatever the muscles, there was no significant difference between feeding groups for acetyl-CoA carboxylase (ACC) activity. Malic enzyme and glucose-6-phosphate dehydrogenase (G6PDH) were slightly lower ($P < 0.05$) in feed-restricted rabbits than in controls. The relative reductions in malic enzyme and G6PDH activities were greater ($P < 0.05$) in SMP than in BF or LL muscles.

The effect of muscle type on enzyme activity levels was dependent on the method of expressing the activities. What-

TABLE 2

Histological myofiber characteristics of control and feed-restricted rabbits slaughtered at the same weight[1,2]

	LL		BF		SMP			*P*-values[3]		
	Control	Restricted	Control	Restricted	Control	Restricted	SEM	Feeding	Muscle	F × M
Mean cross-section area, μm^2	2698[a]	2694[a]	2526[b]	2452[b]	3106[c]	3088[c]	222	NS	0.0001	NS
Contractile fiber-type composition, %										
Type I	1.4[a]	1.3[a]	5.0[b]	4.5[b]	100.0[c]	100.0[c]	1.0	NS	0.0001	NS
Type IIA	4.6[a]	4.2[a]	6.0[b]	6.5[b]	0.0[c]	0.0[c]	1.5	NS	0.0001	NS
Type IIB + IIX	94.0[a]	94.5[a]	89.0[b]	89.0[b]	0.0[c]	0.0[c]	2.0	NS	0.0001	NS
Proportion of oxidative fibers, %	16.9[b]	11.8[a]	21.0[c]	20.0[c]	100.0[d]	100.0[d]	1.7	0.0001	0.0001	0.0001

[1] Values are means, n = 15 for each muscle and in each treatment. Means in the same row with different superscript letters differ ($P \leq 0.05$).
[2] BF, biceps femoris muscle; LL, longissimus lumborum muscle; SMP, semimembranosus proprius muscle; NS, $P > 0.05$.
[3] Two-way ANOVA: feeding, significant influence of feeding group; muscle, significant influence of muscle type; F × M, interaction.

TABLE 3

Lipogenic enzyme activities in muscles of control and feed-restricted rabbits slaughtered at the same weight[1,2]

	LL		BF		SMP			*P*-values[4]		
Enzyme activity	Control	Restricted	Control	Restricted	Control	Restricted	SEM	Feeding	Muscle	F × M
Acetyl-CoA carboxylase										
U/g tissue[3]	0.15	0.13	0.16	0.15	1.26	1.16	0.70	NS	0.0001	NS
10^3 U/mg protein	1.96	2.02	2.41	2.28	41.9	38.8	8.5	NS	0.0001	NS
Malic enzyme										
U/g tissue	1498	1322[b5]	1552	1358[b]	1728	1255[c]	218	0.0001	NS	0.02
U/mg protein	22.0	19.1[a]	28.4	22.8[b]	52.5	38.6[c]	5.5	0.0001	0.0001	0.0001
G6PDH										
U/g tissue	100	81[a]	190	152[a]	1066	761[c]	107	0.0001	0.0001	0.0001
U/mg protein	1.46	1.16[a]	3.26	2.52[a]	34.7	25.4[c]	2.8	0.0001	0.0001	0.0001

[1,2] See Table 2.
[3] One unit (U) of activity is defined as 1.0 nmol bicarbonate incorporated [acetyl-CoA-carboxylase] or as 1.0 nmol of reduced nucleotides [malic enzyme and glucose-6 phosphate dehydrogenase (G6PDH)] per min.
[4] Two-way ANOVA: feeding, significant influence of feeding group; muscle, significant influence of muscle type; F × M, interaction.
[5] Within each defined muscle and in a row, restricted values differ from control at $^aP \leq 0.05$, $^bP < 0.01$, $^cP < 0.001$ (Student's t test).

ever the feeding status of the rabbits, all three enzyme activities were markedly higher ($P < 0.001$) in SMP than in both BF and LL muscles, when expressed on a soluble protein content basis. However, when the activities were expressed on a gram tissue basis, the results for malic enzyme were not as clear-cut as those for G6PDH and ACC (Table 3). Indeed, malic enzyme level was higher in SMP than in the other two muscles ($P < 0.05$) in control rabbits, whereas it was not significantly different from that in BF and LL muscles of restricted rabbits. The reason for this discrepancy was mainly due to differences in the cytosol protein content, which was much higher in LL and BF than in SMP (data not shown). No clear-cut differences in enzyme activities were found for between the two fast-twitch glycolytic BF and LL muscles (Table 3). Moreover, muscle type markedly affected ($P < 0.001$) the ratio of malic enzyme to G6PDH. Indeed, whatever the feeding group, the malic enzyme level was about 9- and 15-fold higher than that of G6PDH in BF and LL, respectively, whereas the ratio of malic enzyme to G6PDH was only about 1.5 in SMP muscle.

DISCUSSION

In contrast with all the studies performed to date in rabbits that focused on growth performances and carcass characteristics (Ledin 1984, Ouhayoun et al. 1986), we have investigated for the first time the effects of moderate feed restriction during fattening on morphological, biochemical and metabolic characteristics of three different skeletal muscles. Furthermore, the parallel study of muscle fiber-type composition, lipid concentration and lipogenic enzyme activities provided new insight in the regulation of lipid deposition in skeletal muscles, in rabbits as in other species.

Effects of feed restriction on muscle characteristics. In the present work, rabbits were subjected to a 30% restriction of feed intake during fattening period, which resulted in a 53% decrease in growth rate. When slaughtered at the same body weight (2.9 kg), but at different ages (18 vs. 15 wk), restricted and control rabbits displayed similar mean myofiber cross-sectional areas. This suggests that, in rabbits, enlargement of muscle fibers is correlated with body weight rather than age. In the three muscles, the lack of differences in contractile fiber-type composition between the two groups is consistent with the fact that the muscle contractile differentiation is mostly completed at 2 mos of age in rabbits (Gondret et al. 1996). The present study has also shown that the percentage of oxidative fibers was not altered in SMP and BF muscles of restricted compared with control rabbits. In other species, feed restriction has either no effect on muscle metabolism (Brandstetter et al. 1998 in cattle, Candek-Potokar et al. 1999 in pigs, Maxwell et al. 1992 in rats), or increases the percentage of oxidative fibers (Harrison et al. 1996 in pigs, Seideman and Crouse 1986 in cattle, Solomon and Lynch 1988 in lambs). In contrast to these latter results, we found a markedly lower proportion of oxidative fibers in the LL muscles of feed-restricted rabbits compared to control rabbits. However, the observed variation in the metabolic activity of LL muscles probably reflected differences in age rather than in nutritional status. Indeed, in rabbits, it has been reported that age-related changes toward a less oxidative pathway continue at least until 12 wk of age in the LL muscle, whereas metabolic myofiber differentiation is already finished before 9 wk of age in the other muscles (Dalle-Zotte and Ouhayoun 1995).

Previous studies in rabbits (Gondret et al. 1997, 1998) have shown that the muscle fat content markedly increases from 15 wk onward. In contrast, data from the current investigation demonstrate that, in the three muscles studied, intramuscular fat contents were much lower in the 18 wk-old restricted rabbits than in the 15 wk-old controls. The lower muscle fat content of feed-restricted rabbits, slaughtered at the same weight as their well-nourished counterparts, is consistent with results in pigs (Candek-Potokar et al. 1998, Wood and Warris 1990). The precise mechanisms by which accumulation of intramuscular lipids is impaired by food restriction are not completely understood. Marbling may be due to de novo lipogenesis in the intramuscular adipose tissue (Chakrabarty and Romans 1972, Gondret et al. 1997 in rabbits, Lee and Kauffman 1974 in pigs, May et al. 1995 in cattle) and/or occurs as a result of an uptake of fatty acids via lipoprotein lipase activity (Haugebak et al. 1974 in sheep). Complete feed deprivation depresses the rate of de novo lipogenesis in extramuscular adipose tissues of various species (e.g., Ingle et al. 1973), however data are lacking on the variations of lipogenic enzyme activities in skeletal muscles of animals adapted to feed intake below ad libitum. The current results show that both slow- and fast-twitch muscles of restricted rabbits exhibited lower activity levels of G6PDH and malic enzyme than those

found in controls. The hexose monophosphate pathway and the malic enzyme activity generate NADPH for fatty acid synthesis in extra- and intra-muscular adipose tissues (e.g., Ingle et al. 1973, Lee and Kauffman 1974). Furthermore, we previously reported (Gondret et al. 1997) that changes with age in lipid and triglyceride contents of rabbit longissimus muscle are closely associated with the age-related patterns of malic enzyme and G6PDH activities. Therefore, it is reasonable to speculate that when carbohydrate availability is reduced in the food-restricted rabbits, the enzymatic machinery of the intramuscular adipocyte has adapted in such a way as to reduce the capacity for NADPH generation for fatty acid synthesis, thereby facilitating the use of C-substrates toward maintenance processes and skeletal muscle fiber size preservation. However, the measured activities of the NADPH-producing enzymes reflected metabolic capacities and not in vivo actual rates. Furthermore, a precise temporal association between variations in these enzyme activities and changes in the muscle lipid concentration is lacking. Therefore, further investigations, combining the use of C^{14}-labeled substrates, determination of tissue NADPH concentrations and isolation of intramuscular adipocytes, are needed to draw such a conclusion.

The finding that the activity of ACC in rabbit muscles was not affected by dietary manipulations (long-term feed restriction) is in accordance with the results of Winder et al. (1995) in a glycolytic muscle of starved/refed rats. These observations suggest that there is no clear association between muscle ACC activity and intramuscular total lipid content. ACC has been postulated as the rate-limiting enzyme in the process of de novo fatty acid synthesis in liver and extramuscular adipose tissue. However, in nonlipogenic tissues such as cardiac and skeletal muscles, a 280 kDa isoform of ACC, distinct from the 265 kDa species found in the main lipogenic sites, is predominantly expressed (Bianchi et al. 1990, Trumble et al. 1995). This isoenzyme of ACC very likely plays an important role in governing the rate of fatty acid oxidation during muscle contraction (Trumble et al. 1995, Winder et al. 1995), rather than controlling de novo lipogenesis.

As a whole, the results reported herein demonstrate that feed restriction during fattening affects intramuscular lipid deposition without any modification of fiber characteristics.

Muscle-dependent variations in lipid deposition. This study had the secondary objective of documenting aspects of intramuscular lipogenesis in relation to muscle energy metabolism. Significant interactions between feeding level and muscle metabolism were observed, in which the effects of feed restriction on lipid concentrations and enzyme activities were more acute in the pure oxidative SMP than in the two glycolytic BF and LL muscles. The great differences in triglyceride content among SMP, BF and LL muscles in normal-fed rabbits (Gondret et al. 1998) have very likely accounted for the different degrees of response to undernutrition of these muscles.

Muscle lipid content as well as enzyme activities (expressed on a soluble protein basis) were higher in SMP than in BF or LL muscles. The highest ACC activity found in SMP is in agreement with the highest ability of the pure oxidative muscles to oxidize fatty acids during muscle contraction (Goodpaster and Kelley 1998 for review). The higher activities of G6PDH and malic enzyme found in SMP compared to BF and LL may be related to the greater capacity of the former muscle to accumulate lipids in the interfascicular adipocytes (Gondret et al. 1998). The much lower activity of G6PDH compared to that of malic enzyme observed in the two glycolytic muscles confirms previous studies on rabbit longissimus muscle (Gondret et al. 1997, 1998). But surprisingly, the ratio of malic enzyme to G6PDH activities was markedly ($P < 0.0001$) lower in SMP (about 1.5) than in the other two muscles (about 9 and 15 in BF and LL, respectively). The reason for such a lower activity ratio in the oxidative muscle than in the two glycolytic muscles is unclear. Studies on isolated intramuscular porcine adipocytes show that less than half of G6PDH activity is present in muscle adipocytes, whereas more than 80% of malic enzyme activity measured on homogenates could be ascribed to the intramuscular adipose tissue (Mourot and Kouba 1998). Furthermore, porcine myofibers have been shown to react positively for G6PDH (Allen et al. 1967), the pattern of G6PDH stain being almost identical to that of SDH positive fibers, indicative of the oxidative capacity of the fibers. These findings suggest that some portion of the G6PDH activity might be restricted to an oxidative pathway of carbohydrate metabolism within the myofibers, as suggested by Lee and Kauffman (1974) in pigs. The fact that a same rank order of SMP > BF > LL was observed for the ratio of G6PDH to malic enzyme activities and for the proportion of oxidative fibers supports this hypothesis. However, other authors have detected very little G6PDH activity in the myofibers of the rats (Glock and McLean 1954), mice, pigs and rabbits (Ogata and Mori 1964). Therefore, further investigations are needed to characterize the role of G6PDH in the energy metabolism of individual muscle fibers in the rabbits, and to better understand the pattern of intramuscular lipid deposition in oxidative compared to glycolytic muscles.

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