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Original article

Study of the influence of age and weaning on the contractile and metabolic characteristics of bovine muscle

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Summary — Weaning is an interesting period for the study of the nutritional regulation of muscle energy metabolism, since during this stage the nature of the substrates supplied to the muscle and their energy balance are profoundly changed. The aim of this study was to determine the effect of these modifications on the contractile and metabolic characteristics of bovine muscle. Two similar groups of 7 male Montbéliard calves were used with the same age and weight, and with the same energy intake. One group consisted of milk-fed calves, the other of weaned animals. The latter were progressively weaned over a period between 107 and 128 d. The average age at slaughter in the 2 groups was 170 d. Biopsy specimens of semitendinosus (ST) muscle were taken at the ages of 66 d, 94 d (before the beginning of weaning) and 136 d (at the end of weaning) to follow the evolution of muscle characteristics. Samples of longissimus thoracis (LT) muscle were taken 24 h after slaughter and used to study the changes in protein and DNA content. The proportion and area of the different types of fiber, I (slow, oxidative), IIA (fast, oxido-glycolytic), IIB (fast, glycolytic) and IIC (fast/slow, oxidoglycolytic) were measured by immunohistochemistry and image analysis. The metabolism of the muscles was determined by studying isocitrate dehydrogenase (ICDH, oxidative) and lactate dehydrogenase (LDH, glycolytic) activity. The results obtained between 2 and 6 months of life showed an overall increase in the area of the fibers (I, IIA, IIB and IIC) and a conversion of type IIA fibers into type IIB accompanied by a shift in the energy metabolism towards a glycolytic type. Weaning caused temporary stress, whose main consequences were to decrease overall muscle fiber area and the percentage of type IIB fibers, and increase the proportion of type IIC fibers in weaned animals. These effects may have been due to the nutritional and behavioral disturbances that accompany weaning, because 42 d after the end of weaning there was no difference in the size of ST and LT fibers between the 2 groups whereas the proportion of type IIA fibers was still higher in weaned animals.

bovine / muscle fiber / weaning / age

Résumé — Étude de l'influence de l'âge et du sevrage sur les caractéristiques contractiles et métaboliques des muscles de Bovins. *Le sevrage constitue un modèle intéressant d'étude de la régulation nutritionnelle du métabolisme énergétique musculaire puisque au cours de cette période la nature et l'équilibre énergétique des substrats arrivant au muscle sont profondément modifiés. L'ex-*

périence décrite a pour objectif d'étudier l'effet de ces modifications sur les caractéristiques contractiles et métaboliques des fibres musculaires. Elle a porté sur 2 lots homogènes de 7 veaux mâles Montbéliards de même âge, même poids et ayant consommé la même quantité d'énergie : un lot allaité et un lot sevré. Les animaux sevrés ont subi un sevrage progressif entre 107 et 128 j. Les animaux des 2 lots ont été abattus à un âge moyen de 170 j. Sur ces animaux, des biopsies musculaires du muscle Semitendinosus *(ST) ont été effectuées à 66 j, 94 j (avant le début du sevrage) et à 136 j (à la fin du sevrage) afin de suivre l'évolution des caractéristiques de ce muscle. Des échantillons des muscles ST et* Longissimus thoracis *(LT) ont été prélevés 24 h après l'abattage des animaux. Sur ces muscles, nous avons suivi l'évolution des teneurs en protéines et ADN. La proportion et la surface des différents types de fibres ont été évaluées par immunohistochimie et analyse d'images : I (lente, oxydative), IIA (rapide, oxydo-glycolytique), IIB (rapide, glycolytique) et IIC (rapide et lente, oxydo-glycolytique). Le métabolisme des muscles a été caractérisé en étudiant l'activité des enzymes isocitrate déshydrogénase (ICDH, oxydative) et lactate déshydrogénase (LDH, glycolytique). Les résultats obtenus mettent en évidence, entre 2 et 6 mois, une augmentation de la surface de l'ensemble des fibres (I, IIA, IIB et IIC) et une conversion des fibres de type IIA en fibres de type IIB qui s'accompagne d'une orientation du métabolisme énergétique vers un type glycolytique. Le sevrage entraîne une perturbation ponctuelle qui se traduit principalement par des surfaces de l'ensemble des fibres musculaires ainsi qu'un pourcentage de fibres de type IIB plus faible et un pourcentage en fibres de type IIC plus élevé chez les animaux sevrés. Ces résultats pourraient être la conséquence d'un stress à la fois nutritionnel et physiologique qui accompagne le sevrage. En effet, si on observe les muscles ST et LT 42 j après la fin du sevrage, il ne subsiste plus de différences dans la taille des fibres entre les animaux sevrés et allaités ; en revanche, la proportion de fibres de type IIA reste supérieure chez les animaux sevrés.*

Bovin / fibre musculaire / sevrage / âge

INTRODUCTION

Skeletal muscle consists of different types of fibers, classified according to their contractile and metabolic activity (Brooke and Kaiser, 1970). While the number of bovine muscle fibers is definitively determined at birth (Robelin *et al*, 1991), the contractile and metabolic differentiation of the fibers continues thereafter. During animal growth, particularly at the end of the fetal period and at the beginning of postnatal life, muscle undergoes profound and rapid changes which are partly governed by nutritional factors.

Muscle can adjust their metabolism in response to changes in diet, especially at weaning, when the nature and equilibrium of the energy substrates are acutely modified. The weaning period is doubly important in cattle since it is the stage at which the animal changes from a monogastric into a ruminant. Young ruminants require a period of several weeks to adapt to solid feed and during this time they are in a state of under-nutrition (Kouame *et al*, 1984). The energy deficit triggers an adaptive stress response (Lapeyronie, 1990). To offset the nutritional loss resulting from the withdrawal of milk from its diet, the animal must be able to rapidly increase its feed intake. The adjustment is not immediate and occurs during a phase of transition that is characterized by a retardation of growth, whose extent and duration depend on various factors (Lapeyronie, 1990). Weaning is therefore a period of duress for the animal and entails nutritional and behavioral disturbances whose effects on contractile and metabolic characteristics are unknown.

The aim of this study was to determine the influence of age and weaning on the contractile and metabolic characteristics of the muscle fibers of Montbéliard calves that were progressively weaned from the age of 107 d.

MATERIALS AND METHODS

Animal management

Two similar groups of 7 male Montbéliard calves with an average weight of 119 kg were formed on the basis of average daily weight gain, average nutritional efficiency and live weight at the age of 88 d. From age 107 d, the weaned group (W) received a diet with a maximum volatile-fatty-acid content. The other group (M) received milk feed that contained as much glucose as possible. Both groups received the same energy for an equivalent weight. Until the age of 107 d, all animals were fed with milk (Univor energie) of 21% fat content to achieve an average daily weight gain of 1 000 g. From 107 to 128 d of life, group W calves were progressively weaned with a concentrate composed of dehydrated lucerne 18 (30%), beet pulp (40%), barley (14%), soyabean cake 48 (11%) and mineral compound (5%). The concentrate contained 16.2% protein and had a 1.8% fat content. They were also given 1 kg hay to avoid the risk of tympanites. From the age of 107 d, group M calves were fed with special weaning milk (Univor) comprising an energy supply of 40% glucides, 35% lipids and 25% proteins. The animals were weighed daily throughout the experimental period. They were slaughtered between 159 and 180 d of life at a mean age of 173 ± 5 d in group W and 170 ± 8 d in group M with respective mean liveweights of 225.2 ± 2.2 and 208 ± 4.17 kg and empty live/weight of 192.8 ± 2.8 and 194.7 ± 6.8 kg.

Samplings

Biopsy samples of the semitendinosus (ST) muscle were taken in all animals at 66 d when their average live-weight was 92 kg. Further samples were taken in 6 animals at age 94 d, just before weaning (average weight of 146 kg), and at 136 d, just after weaning (average weight of 170 kg). Samples of ST were taken in the central part of the muscle and samples of longissimus thoracis (LT) muscle at the sixth rib 24 h after slaughter. Samples used for biochemical measurements were directly frozen in liquid nitrogen, stored at –80°C and then ground. Specimens used for histological investigations were frozen progressively in isopentane, then in liquid nitrogen and were stored at –80°C until use.

Chemical analyses

Determination of protein/DNA ratio

Total muscle protein content, expressed in mg/g of muscle, was determined according to the method of Lowry *et al* (1951) with bovine albumin serum as standard. DNA content, expressed in μg/g of muscle, of samples taken at slaughter was measured by the method of Labarca and Paigen (1980). The protein/DNA ratio was expressed in mg of protein per mg of DNA.

Determination of contractile type of muscle fibers

Histological, immunohistochemical and histochemical studies

Serial sections 10 μm thick were cut perpendicular to the muscle fibers with a cryotome at –25°C. Three types of staining were performed on the sections to determine the size of the fibers, their contractile property and their metabolic activity.

The muscle fibers were treated with azorubine, which stains all fibers irrespective of contractile or metabolic type. It shows the general histological architecture of the muscle and thereby allows determination of the number and diameter of the fibers. Slow (I) and fast (II) contractile types were determined by immunohistochemistry according to the immunofluorescence technique described by Pons *et al* (1986), who used monoclonal antibodies specific to myosin heavy chains (MHC). Their production, purification and characterization have been described by Léger *et al* (1985). Their reactivity in cattle has been tested by Robelin *et al* (1993). Type IIA (oxidoglycolytic) fibers were distinguished from type IIB (glycolytic) by succinate dehydrogenase (SDH) activity according to the method of Pearse (1968). This enzyme, which is present in the cell wall of mitochondria, is characteristic of an oxidative metabolism. Fibers of this metabolic type (I, IIA) were stained dark-blue in the presence of nitrobluetetrazolium, which is reduced by the action of the enzyme, whereas glycolytic fibers (IIB) were stained pale-blue. A fourth type of fiber, IIC, was evidenced. The cells were recognized by both anti-MHC1 (slow) and anti-MCH2 (fast) antibodies and had an oxidoglycolytic metabolism.

The proportion of each type of fiber and their average area were determined in 2 different sites on each serial section. Between 100 and 200 fibers were analyzed by an image-analysis computer program (Visilog).

ELISA: enzyme-linked immunosorbent assay

This technique was used to quantify the proportion of slow isoform MHC1. It was based on the method of Winkelmann *et al* (1983) modified by Picard *et al* (1994).

Determination of muscle metabolic type

The metabolic type of the muscles studied was determined by measuring the enzyme activities of ICDH according to the method of Briand *et al* (1981), and of lactate dehydrogenase (LDH) by the method of Ansay (1974). ICDH activity was measured only on samples taken at slaughter since the biopsy specimens were not large enough for all assays to be performed.

Analysis of results

The results were subjected to variance analysis SAS package (1985), to study the effect of age (by comparing biopsy samples taken at different stages of growth) or that of weaning (by a comparison of specimens sampled at the same age in milk-fed and weaned animals).

RESULTS

Influence of age

The influence of age was studied in group M animals. As the amount of muscle in the biopsy specimens was not large enough to allow all biochemical assays, DNA content was measured solely in samples taken at slaughter. Hence, only total muscle protein content was measured at all stages. This content remained fairly stable with age.

At all ages, ST muscle contained the 4 types of muscle fiber I, IIA, IIB and IIC (fig 1a). There was a smaller proportion of type I fibers than of fast type IIA and IIB fibers. The proportion of type I fibers increased significantly ($P < 0.05$) from 7.6 to 15.2% between days 66 and 170 whereas that of type IIA decreased. The proportion of type IIA fibers remained not significantly different between 66 and 94 d of life and then decreased significantly ($P < 0.001$), to reach 13.2% at 170 d. The percentage of type IIB fibers increased significantly ($P < 0.05$) from 68.2% at 66 d to 75.8 at 136 d and then decreased to 69.8% at 170 d. The proportion of type IIC fibers was low (< 3.5%) and varied with age, but not significantly, between 2.2 and 1.2%.

There was a significant increase ($P < 0.001$) in the total average area of the muscle fibers from 1 582 to 2 345 μm^2 between the ages of 66 and 170 d (fig 1b). Type IIC fibers were the smallest in area of the 4 types (603 μm^2 at 66 d and 1 063 μm^2 at 170 d) and type IIB the largest (1 888 μm^2 at 66 d and 2 664 μm^2 at 170 d). Type I and IIA fibers had comparable areas of an intermediate size. The area of type I and IIB fibers increased significantly ($P < 0.05$ and $P < 0.001$, respectively) between 66 and 170 d of life. The area of type IIA and IIC fibers also increased during the same period but not significantly.

The percentage of MHC1, as determined by ELISA, increased significantly ($P < 0.05$) between days 66 and 170 from 23.4 to 35.1% (fig 2a); the same trend was shown by immunohistochemistry for type I fibers. LDH activity, which is indicative of glycolytic metabolism, remained stable at 0.6 nkat/mg of proteins between 66 and 94 days of life and then significantly increased ($P < 0.005$) to reach 0.69 nkat/mg of proteins at 170 d (fig 2b).

Influence of weaning

The influence of weaning on the contractile and metabolic types of muscle was studied

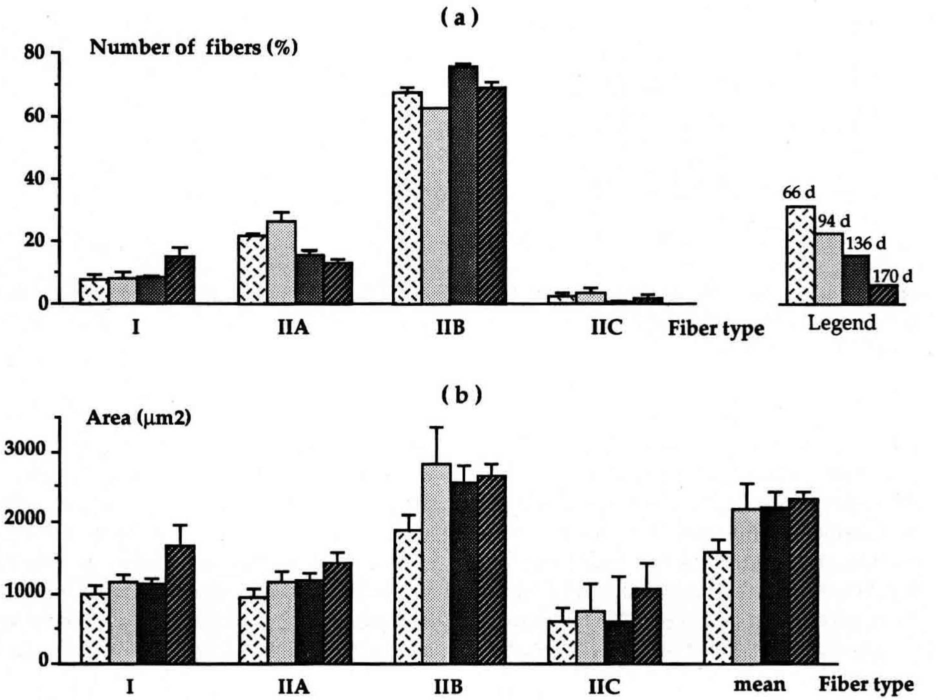

Fig 1. Evolution with age of the histological characteristics of ST muscle. **(a)** Evolution of the number (in %) of the fiber types I, IIA, IIB and IIC; and **(b)** evolution of the area of the different types of fiber and the average area of all the fibers.

on biopsy specimens of ST sampled just before and at the end of weaning, and on samples of ST and LT muscles taken at slaughter (42 d after weaning). To clearly determine their evolution during the period of weaning, muscle characteristics were studied short-term, between the beginning and end of weaning, and long-term, between the end of weaning and slaughter.

Short-term influence of weaning on ST musle

There was no significant difference between groups M and W at 136 d of life, *ie* 8 d after the end of weaning (figs 3 and 4). However, group W animals had a slightly lower percentage of type IIB fibers and a higher percentage of type IIA and IIC fibers (fig 3a). Moreover, the overall area of the different types of fibers was smaller in group W (fig 3 b) and the glycolytic activity (LDH) lower (fig 4).

Long-term influence of weaning on muscles sampled at slaugher

Forty-two days after the end of weaning there was no significant difference between the 2 groups of animals in total DNA and protein contents of ST and LT muscles. The protein/DNA ratio was not significantly dif-

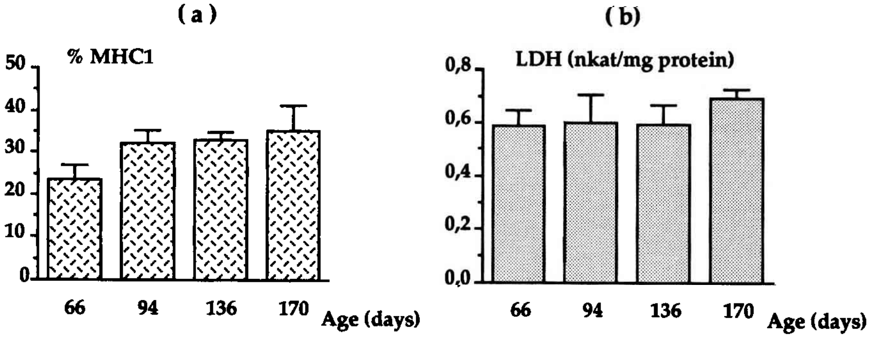

Fig 2. Evolution with age of the biochemical characteristics of ST muscle. **(a)** Evolution of the percentage of type I myosin heavy chains (MHC1) measured by ELISA; and **(b)** evolution of lactate dehydrogenase (LDH) activity.

ferent between the 2 groups. Weaned animals had a significantly ($P < 0.05$) higher proportion (21.4%) of type IIA fibers in the LT muscle (fig 5a) than milk-fed animals. In ST muscle, the difference observed was not significant (fig 6a). The average area of the fibers overall and that of type IIB fibers were not significantly higher in animals of group W (figs 5b and 6b). The area of type I fibers was lower in weaned animals in the 2 muscles. Study of the contractile type of the fibers, made by measuring the percentage of MHC1 by ELISA technique, showed no significant difference in either of the muscles between the 2 groups (fig 7a). However, group W animals tended to have a lower

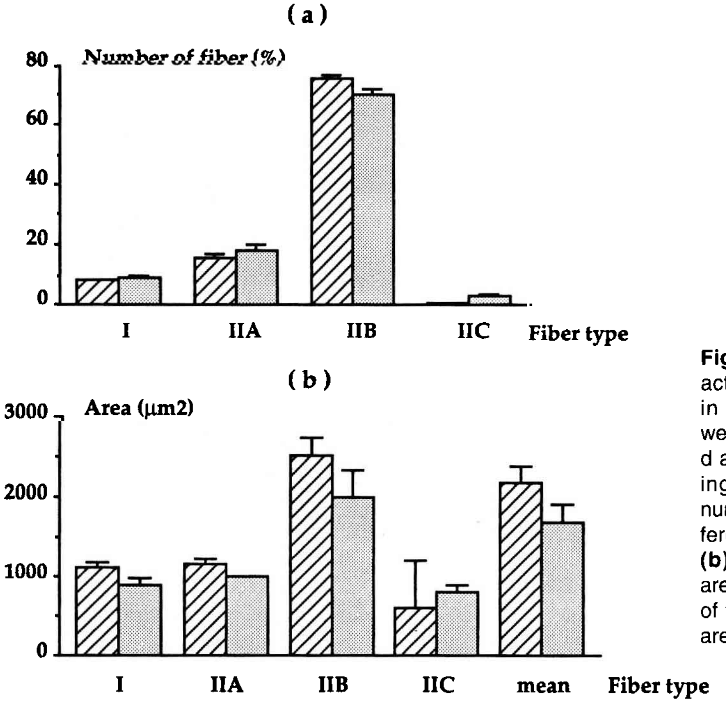

Fig 3. Histological characteristics of ST muscle in milk-fed (M, ▨) and weaned (W, ▩) animals 8 d after the end of weaning. **(a)** Comparison of number (in %) of the different types of fiber; and **(b)** comparison of the area of the different types of fiber and the average area of all the fibers.

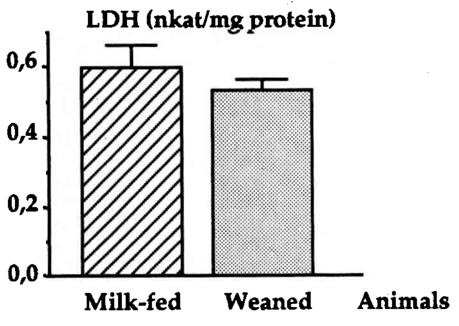

Fig 4. LDH activity of ST muscle in milk-fed animals (M) and weaned animals (W), observed 8 d after the end of weaning.

percentage of MCH1 (fig 7a). There was no significant difference between groups W and M in the oxidative and glycolytic activities of ST and LT muscles (figs 7b and 7c) but the metabolism of muscles in group W evolved towards a more glycolytic type than that of group M animals.

Evolution of characteristics during the period of weaning

Between 94 d (beginning of weaning) and 136 d of life (end of weaning), all the features studied had a similar evolution in both milk-fed and weaned animals (figs 8 and 9) with no significant difference between the 2 groups. However, the percentage of MHC1 decreased in W animals and was more stable in M animals, but the difference was not significant (fig 8a). LDH activity (fig 8b) increased in group M but decreased in group W. On the contrary, the percentage of type IIC fibers increased in group W and decreased in group M (fig 9a).

Between 136 and 170 d, glycolytic activity significantly increased in group W animals (fig 8b). In group M animals LDH activity continued to increase but there was no significant difference between this period and the previous one in the evolution of the other parameters measured. The area of type IIB and IIA fibers and the mean area

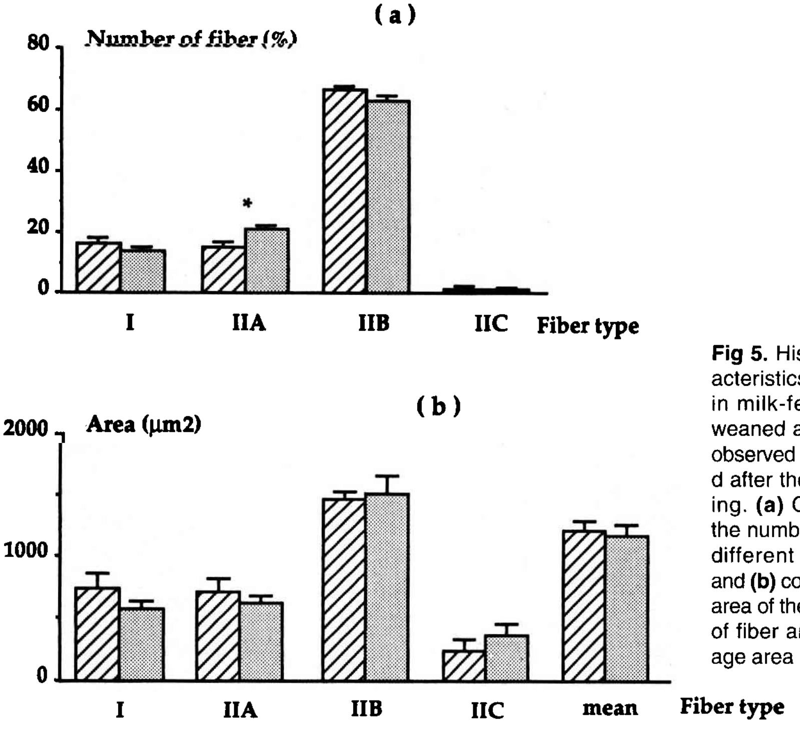

Fig 5. Histological characteristics of LT muscle in milk-fed (M, ▨) and weaned animals (W, ▩) observed at slaughter, 42 d after the end of weaning. **(a)** Comparison of the number (in %) of the different types of fiber; and **(b)** comparison of the area of the different types of fiber and of the average area of all the fibers.

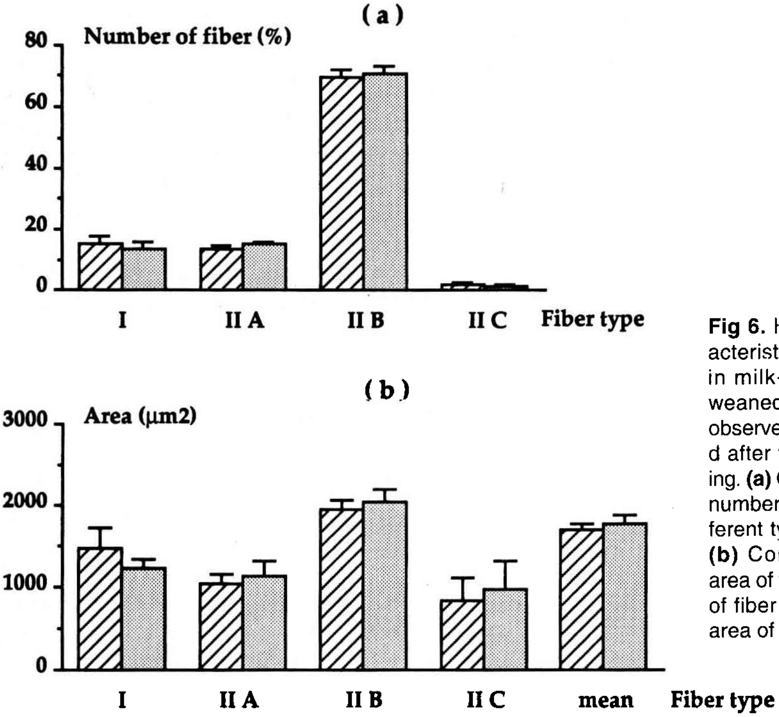

Fig 6. Histological characteristics of ST muscle in milk-fed (M, ▨) and weaned animals (W, ▩) observed at slaughter, 42 d after the end of weaning. **(a)** Comparison of the number (in %) of the different types of fiber; and **(b)** Comparison of the area of the different types of fiber and the average area of all the fibers.

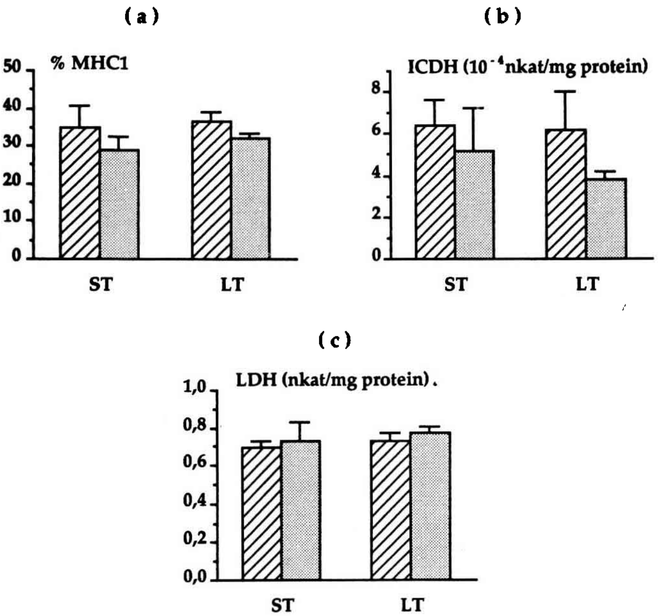

Fig 7. Biochemical characteristics of ST and LT muscles of milk-fed (M,▨) and weaned animals (W,▩) observed at slaughter, 42 d after the end of weaning. **(a)** Comparison of the percentage of type 1 myosin heavy chains (MHC1) measured by ELISA; **(b)** comparison of isocitrate dehydrogenase (ICDH) activity; and **(c)** comparison of lactate dehydrogenase (LDH) activity.

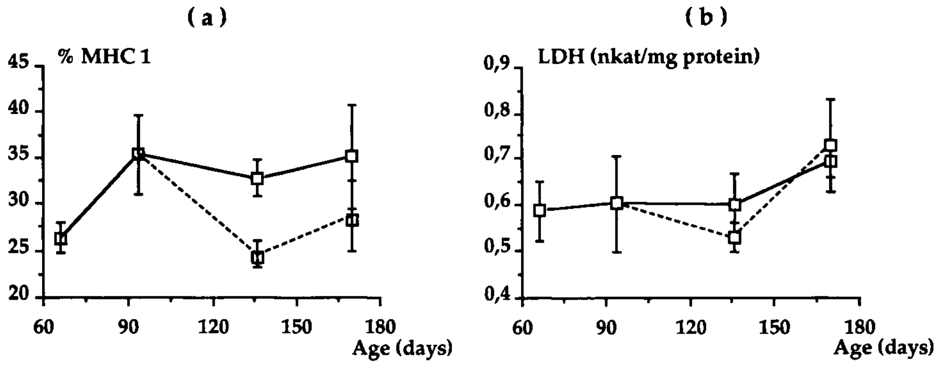

Fig 8. Evolution of the biochemical characteristics of ST muscle in milk-fed (M, —) and weaned animals (W, ---) during the period of weaning. **(a)** Evolution of the percentage of type 1 myosin heavy chains (MHC1) measured by ELISA; and **(b)** evolution of lactate dehydrogenase (LDH) activity.

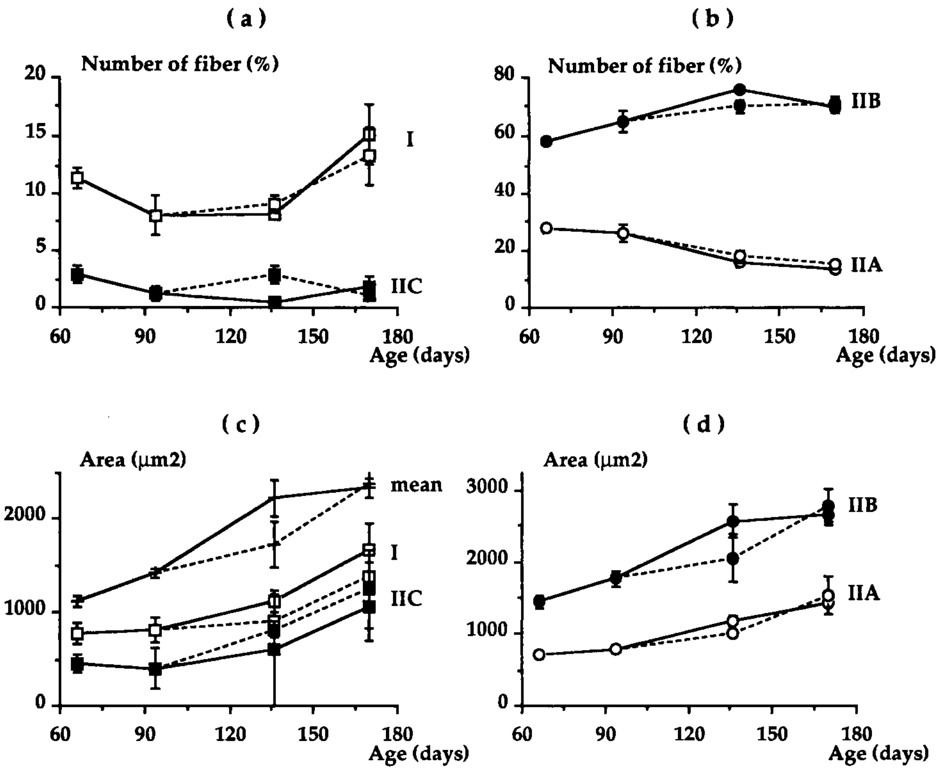

Fig 9. Evolution of the histological characteristics of ST muscle in milk-fed (M, —) and weaned animals (W, ---) during the period of weaning. **(a)** Evolution of the number (in %) of type I and IIC fibers; (b) evolution of the number (in %) of type IIA and IIB fibers; **(c)** evolution of the area of type I and IIC fibers and of the average area of all the fibers; and **(d)** evolution of the area of type IIA and IIB fibers.

of all the fibers also significantly increased in group W (figs 9c and 9d).

DISCUSSION

Influence of age

Our findings are novel in that they yield information on the evolution of the contractile and metabolic characteristics of bovine muscle during an early phase of development, between 1and 6 months of life, a period that has not been widely studied.

As the number of muscle fibers does not increase beyond birth (Robelin *et al*, 1991), postnatal muscle growth is chiefly characterized by an increase in the size of the fibers and changes in the proportion of the different types (Jurie *et al*, 1994). Our results showed that muscle protein content was fairly stable (about 180 mg/g) between 1 and 6 months and are in agreement with the findings of Jurie *et al* (1994) in Limousin calves aged 1–12 months. Hence, muscle protein content, which rises sharply during fetal life (Robelin *et al*, 1991), is stable from birth until the age of about 12–16 months (Jurie *et al*, 1994).

Between 66 and 170 d of life, the mean area of the fibers increased 1.5-fold. This increase in fiber size confirms the findings of other authors including Jurie *et al* (1994), who showed that hypertrophy plays an important role in postnatal muscle growth, particularly until the age of 12 months.

The percentage of type I fibers, measured by histochemistry or by an ELISA test for MHC1, rose significantly between 66 and 170 d of life. The same rise has been observed in Limousin calves between 1 and 6 months (Jurie *et al*, 1994). An increase in the proportion of type I fibers in the postnatal period has already been reported in species that are relatively immature at birth such as sheep (Suzuki and Cassens, 1983; Finkelstein *et al*, 1992) and pigs (Lefaucheur and Vigneron, 1986). In cattle, which are more fully developed at birth, this increase probably occurs at the end of the fetal period (Robelin *et al*, 1991) and seems to continue after birth.

The conversion of IIA fibers into IIB fibers has been observed in numerous species including cattle (Ashmore *et al*, 1972; Dreyer *et al*, 1977; Spindler *et al*, 1980; Seideman and Crouse, 1986; Solomon *et al*, 1986; Jurie *et al*, 1994). The new evidence yielded by our findings is that the conversion begins very soon after birth. In species whose stage of development is less advanced at birth this transformation is characteristic of the postnatal period whereas perinatal growth is characterized by an increase in the proportion of type I fibers.

We observed type IIC fibers in ST muscle at all the stages studied. Their proportion decreased with age, falling from 2.2% at 66 d to 1.1% at 170 d. In Limousin calves, Jurie *et al* (1994) observed proportions of 2.7 and 1.5% at 1 and 6 months, respectively. A decrease in the percentage of type IIC fibers with age, or their disappearance, has been reported in rats and mice in the first few days after birth (D'albis *et al*, 1989), in sheep and mice during the first weeks of postnatal life (Suzuki and Cassens, 1983; Lefaucheur and Vigneron, 1986), and in human infants until the age of 12 months. These cells, which simultaneously express slow and fast MHC, occur in the fetus from 220 d of gestation onwards. At birth, type IIC fibers account for 4% of the total in ST muscle (Robelin *et al*, 1993). Thereafter, their percentage decreases and in adult bovine ST they are no longer present (Johnston *et al*, 1975). However, in 2 studies, that of Young and Bass (1984) and Totland and Kryvi (1991), the presence of about 1% of type IIC fibers was reported in adult cattle. These fibers could have resulted from the fusion of satellite cells (Feldman and Stockdale, 1991) or may represent a transitional form occurring during the transformation of type I into type II fibers (Billeter *et al*, 1981).

LDH activity, which was stable until the age of 94 d, increased significantly between 94 and 170 d. A rise in glycolytic activity with age has been observed in several species including piglets (Lefaucheur and Vigneron, 1986), rabbits (Briand *et al*, 1994) and Limousin calves (Jurie *et al*, 1994). The rise may be caused by an adaptation of the metabolism to the increase in body area, which then leads to decreased oxygen diffusion to the cells.

Muscle growth in young cattle aged between 2 and 6 months is thus characterized by an increase in the proportion of type I fibers, the conversion of type IIA fibers into type IIB, a rise in glycolytic activity and an increase in fiber area.

Influence of weaning

Weaning is a critical phase in animal development. In ruminants, this period is accompanied by functional and metabolic changes in the digestive process. The novel contribution of this study is to show how these modifications affect the contractile and metabolic characteristics of muscles.

Short-term influence on ST muscle

Twenty-nine days after the beginning of weaning, the muscles of group W animals contained fewer type IIB fibers and more type IIA fibers than those of their milk-fed counterparts, their metabolism was more oxidative and the overall surface of their muscle fibers smaller. While these results are not significant, they nevertheless indicate a slowing down of muscle growth. As stated earlier, in cattle, as in numerous other species, there is a conversion of type IIA fibers into type IIB with age, associated with an increase in fiber area and in glycolytic metabolism. In weaned animals, the pace of these developmental trends seems to be slower. Weaned animals also have a higher proportion of type IIC fibers than milk-fed animals, which is further evidence of a slowing down of muscle growth.

At weaning, cattle change from a diet rich in long fatty acids and glucose to one rich in volatile fatty acids. This change in nutritional intake results in a temporary fall in glycemia (1 ot 2 d after weaning) and an increase in volatile fatty-acid production by rumen fermentation (Kouame *et al*, 1984). Young ruminants need a period of adaptation to solid feed that lasts a few weeks, during which they are in a state of undernutrition (Kouame *et al*, 1984). The energy deficit triggers an adaptive stress response which is both nutritional and behavioral (Lapeyronie, 1990). A consequence of the nutritional deficit is an activation of the neuroendocrine, adrenal medulla and sympathetic nervous system, which affects muscle metabolism. The corticotropic axis is also activated as a result of psychological stimulation caused by the unpleasantness of the experience. The withdrawal of the feeding bucket represents an environmental impoverishment and the loss of an important point of reference and creates a feeling of frustration that results in an increased production of plasma corticosteroids (Dantzer *et al*, 1980). In lambs that were milk-fed, as were the calves in our experiment, it was shown that the corticotropic axis is hyperactive (Lapeyronie, 1990). As this hyperactivity may be caused by the mode of artificial feeding (twice-daily distribution of ration, morning and evening), the corticotropic axis must be stimulated during this transitional period to maintain normal glycemia. In addition, Mobert and Wood (1981) showed that corticotropic axis response is heightened in animals that are separated from their mother at birth, as were the animals in our trial.

Several authors have reported that energy undernutrition slows down the 'maturing process' of muscle fibers (Lanz *et al*, 1992; Ward and Stickland, 1993). Postnatal energy restriction results in a decrease in the proportion of type IIB fibers and an increase in that of type IIA fibers (Seideman and Crouse, 1986; Beverly *et al*, 1991; Lanz *et al*, 1992). These changes are accompanied by decreased glycolytic metabolism (Raju, 1975; Howells *et al*, 1978). Another principal effect of energy restriction is a reduction in the size of fibers (Goldspink and Ward 1979; Bedi *et al*, 1982). Our results are therefore entirely consistent with previously reported findings. These changes in muscle characteristics are due to the multiple hormonal disturbances related to energy restriction and are known to be accompanied by a decrease in the circulating levels of thyroid hormones and in IGF I, and by an increase in GH and insulin levels. These different factors have a regulatory effect on muscle growth (Bacou and Vigneron, 1988). In addition, an increase in circulating glucocorticosteroids results in an increased catabolism of glycolytic fibers (Bacou and Vigneron, 1988), which again is consistent with our results.

Long-term influence on ST and LT muscles

Weaning elicited a similar response in ST and LT muscles but its effect on the latter was greater. At 170 d of life, no significant effect of weaning had been observed on ST whereas in LT the percentage of type IIA fibers was significantly higher in weaned calves than in milk-fed animals.

Forty-two days after the end of weaning there was no difference in the proportion of types I and IIB fibers between groups W and M but the percentage of type IIA fibers was higher in weaned animals, particularly in LT muscle. It is also noteworthy that the mean area of the fibers in group W animals was smaller 8 d after weaning but significantly greater after 42 d. The same trend was observed for type IIB fibers, and only the area of type I fibers was lower in weaned animals.

The digestive behavior of milk-fed animals is in fact similar to that of monogastrics; because of the closure reflex of the esophageal groove, the feed ingested does not pass in transit through the reticulo-rumen but goes to the abomasum. The reflex continues all the time that milk is fed. The ingestion of milk governs the pulsatility of GH (Orskov *et al*, 1970). GH has an anabolic effect on type I fibers (Bacou and Vigneron, 1988), which could explain the differences in size of type I fibers between weaned and milk-fed animals.

Evolution between the beginning of weaning and slaughter

The main evolution between the end of weaning and slaughter was a significant increase in LDH activity in weaned animals, together with an increase in the mean area of fibers, particularly in that of type IIB fibers. During the same period no significant difference was observed in milk-fed animals. It is possible that weaned animals had compensatory growth at this time. Drew and Reid (1975) showed that the major event in this phase is rehydration of the muscle accompanied by a rapid increase in the size of muscle fibers, as from the first days of compensation. This could explain why there was no longer any difference in fiber size at 170 d of life between weaned and milk-fed animals. However, the compensatory growth was not sufficient to make up the delay in the transformation of type IIA into type IIB fibers caused by energy restriction.

CONCLUSION

This study shows the evolution of muscle fiber characteristics in cattle during the early period of 2–6 months of life, an age at which no comparable studies have been made. Growth during this period was characterized by an increase in fiber size and by conversion of type IIA fibers into type IIB. These changes were correlated with a shift in the metabolism of ST muscle towards a more glycolytic type.

This work has also shown that the nutritional and behavioral consequences of the stress caused by weaning affect muscle characteristics, creating temporary disturbances. Forty-two days after the end of weaning there was no difference in muscle fiber size between weaned and milk-fed animals. However, in weaned animals the transformation of type IIA into type IIB fibers was still progressing at a slower rate.

Further studies dealing specifically with the expression of key enzymes in energy metabolism are needed to have a fuller understanding of the mechanisms involved during the period of weaning.

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