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Original article

Changes in muscle fiber populations and muscle enzyme activities in the primiparous lactating sow

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Summary — An experiment involving 12 primiparous Large White sows was conducted to investigate changes in contractile and metabolic characteristics of skeletal muscle during the first 3 weeks of lactation. The sows lost 19.7 ± 6.6 kg of body weight. No change in DNA concentration was observed in the longissimus dorsi (LD), a fast-twitch glycolytic muscle, and the trapezius (T), a mainly slow-twitch oxidative muscle during lactation. The percentage of type I fibers increased ($P < 0.05$) in LD, but not in T. The muscle fiber cross sectional area (CSA) of IIB fibers, which represents about 78% of the total number of LD fibers, decreased by 18% ($P < 0.01$) by lactation; the CSAs of I and IIA fibers were not significantly affected. Marker enzyme activities for oxidative and glycolytic metabolisms decreased in both muscles during lactation. The decrease in oxidative enzyme activities was particularly dramatic in T ($P < 0.001$). No significant relationship was observed between sow weight loss and changes in muscle fiber CSA or enzyme activities. The extent to which the results could be related to a negative nutritional balance or to changes in hormonal status is discussed.

swine / lactation / skeletal muscle / muscle fiber / metabolism

Résumé — Modifications des types de fibres et des activités enzymatiques du muscle chez la truie primipare en lactation. *Une expérience portant sur 12 truies primipares de race Large White a été réalisée afin d'étudier les modifications de certaines caractéristiques contractiles et métaboliques du muscle squelettique pendant les 3 premières semaines de lactation. Les truies ont perdu 19,7 ± 6,6 kg de poids vif. Aucune modification de la concentration en ADN des muscles longissimus (LD) (un muscle à contraction rapide et métabolisme glycolytique) et trapézius (T), (un muscle essentiellement à contraction lente et métabolisme oxydatif) n'a été observée. Le pourcentage de fibres de type I n'a augmenté que dans le LD (P < 0,05). L'aire de section transversale (AST) des fibres IIB, qui représentent 78% environ du nombre total de fibres du muscle LD, a diminué de 18% (P < 0,01) pendant la lactation; l'AST des fibres I et IIA n'a pas été significativement modifiée. Les activités d'enzymes marqueurs des métabolismes oxydatif et glycolytique ont diminué dans les 2 muscles au cours de la lactation. La décroissance du métabolisme oxydatif a été particulièrement importante dans le muscle T (P < 0,001). Aucune relation n'a été observée entre la perte de poids vif des truies et la réduction de l'AST des fibres du LD ou des activités enzymatiques. La possibilité d'interpréter les résultats en fonction de la balance nutritionnelle des animaux ou de leur état hormonal est discutée.*

porc / lactation / muscle squelettique / fibre musculaire / métabolisme

INTRODUCTION

More attention has been paid to the adequacy of muscle metabolism in relation to growth or to under-nutrition than in relation to lactation. However, the sow is under extensive nutritional stress during early lactation, particularly when feed intake does not meet the nutrient requirement for milk production and maintenance. During the first weeks post-partum, tissue catabolism results in loss of body fat (Whittemore *et al,* 1980), negative nitrogen balance (Elsley and MacPherson, 1972) and loss of muscle tissue (Etienne *et al,* 1985). The latter authors showed that primiparous sows lost about 10 kg of muscle during the first 3 weeks of lactation, whatever their energetic level (14.2 or 10.3 Mcal ME/d). Muscle weight loss during lactation is now well known, but, to our knowledge, very little attention has been paid to the possible alteration of tissular metabolism particularly skeletal muscle during lactation in the sow.

Since skeletal muscles are generally composed of different fibers demonstrating various biochemical, histochemical and physiological properties, we wished to determine whether early lactation in sows induced specific changes in the biochemical and histochemical properties of the main populations of fibers.

The objective of the present study therefore was to investigate the changes in some biochemical and histochemical characteristics of muscles of different contractile and metabolic types during early lactation in primiparous sows.

MATERIALS AND METHODS

Animals

During pregnancy 12 Large White primiparous sows were fed 2.2 kg/d of a standard diet containing 13% crude protein and 3 000 kcal ME/kg. After farrowing, they received a diet containing 14.6% crude protein and 3 200 kcal ME/kg. The feeding level was progressively increased from 2 to 5 kg/d between d 0 and 5 post-partum, and was then maintained at 5 kg/d until weaning at 21 d. The sows were fed twice daily and water was available *ad libitum.* Mean energy intake over the 21 d of lactation was 14.8 Mcal ME/d. The daily supply of nutrients (protein, amino acids, vitamins and minerals) met or exceeded INRA recommendations (1984). The composition and characteristics of the diets were defined previously (Noblet and Etienne, 1986). Litter size was standardized to 10 piglets on the day following farrowing. The piglets did not receive any feed or water supplement from birth to 3 weeks of age.

The weight of the sow and the litter was recorded on the days of farrowing (D_0) and weaning (D_{+21}); backfat thickness of the sows was ultrasonically determined at shoulder and last rib levels, 50 mm from the mid-line, on each side of the back. Backfat measurements were averaged within and between sites.

Muscle measurements

Muscle biopsy

A general anaesthesia, starting with an iv injection of pentothal, was maintained for 20 min through inhalation of a mixture of halothane and oxygen. Biopsies of the *longissimus dorsi* (LD), a glycolytic muscle, and of the trapezius (T), an oxidative muscle, were performed 6 d before farrowing (D_{-6}) on the left side and after 21 d of lactation (D_{+21}) on the right side. Muscle samples weighing about 1 g were obtained *via* an incision of the skin and subcutaneous adipose tissue. The biopsy was kept on flat sticks, promptly frozen in isopentane cooled by liquid nitrogen (–160 °C) and stored at –80 °C until metabolic enzyme analysis and histological examination.

Muscle cellularity

DNA concentration was measured fluorometrically using Hoescht 33258 according to West *et al* (1985). The method, developed for cultured

mammalian cells, was adapted to 40 μm thick muscle slices.

Histological examination

Ten-μm thick muscle sections were obtained with a cryostat at –20 °C. They were processed according to the myosin ATPase technique following pre-incubation at pH 4.10, 4.35 and 10.4 (Padykula and Herman, 1955; Guth and Samaha, 1969). Myofibers were classified as type I, IIA and IIB according to the terminology of Brooke and Kaiser (1970). Type IIC fibers were not considered because very few of them could be observed at this stage of development (Suzuki and Cassens, 1980). For each sample, percentages of the 3 types were determined on about 1 500 fibers, and mean cross sectional areas were estimated by planimetry on at least 100 fibers of each type.

Enzyme analysis

Metabolic enzyme activities were measured from frozen tissue. Muscle tissue was thawed and homogenized in ice-chilled .1 mol/l phosphate buffer (pH 7.5) containing 2 mmol/l EDTA. The homogenate was centrifuged (1 500 g for 10 min at 4 °C) and the supernatant collected to measure the following enzyme activities: citrate synthase (CS) (Srere, 1969), isocitrate dehydrogenase (ICDH) (Bernt and Bergmeyer, 1974), 3-hydroxy-acyl-CoA dehydrogenase (HAD) and lactate deshydrogenase (LDH) (Bass *et al,* 1969) in order to characterize the citric acid cycle activity (CS and ICDH), lipid oxidation (HAD) and glycolytic capacity (LDH). Enzyme activities were determined at 30 °C using spectrophotometric techniques and expressed as mmol (substrate degraded)•min^{-1}•g^{-1} (fresh tissue).

Statistical analysis

Differences between means were tested for statistical significance by the *t*-test for paired observations, each sow being its own control (Snedecor and Cochran, 1957).

RESULTS AND DISCUSSION

Growth performance

All sows nursed 10 piglets from farrowing to weaning 3 wk later. No health problem was noted during the experimental period. During the 3 wk of lactation, the average daily feed intake of the sows was 4.6 ± 0.4 kg; litter weight gain was 40.1 ± 4.7 kg and the sows lost 19.7 ± 6.6 kg of body weight ($P < 0.001$), *ie* 10% of their initial body weight (198 kg). A 2.9 mm decrease ($P < 0.001$) in dorsal fat thickness was observed, indicating fat mobilization. Using the equations calculated by Etienne *et al* (1989), muscle loss was estimated as 11.1 kg in the present experiment. These results are equivalent to those reported by Etienne *et al* (1985) in primiparous sows maintained in similar experimental conditions.

Muscle DNA concentration

No change in the DNA concentration of either muscle was observed during lactation. DNA concentrations were 0.35 ± 0.02 and 0.34 ± 0.03 mg/g of fresh muscle in LD muscle and 0.76 ± 0.09 and 0.75 ± 0.18 in T muscle 6 d before farrowing and after 21 d of lactation, respectively. These values are in the same range as in muscles of pigs of 100 kg live weight (Powell and Aberle, 1975; Harbison *et al,* 1976). The higher concentration of DNA in T than in LD accords with previous results obtained in laboratory animals (Schmalbruch and Hellhammer, 1977). Because skeletal muscle fibers are multinucleated, DNA concentration should not be equated with muscle fiber number but be considered as an esti-

mate of the cytoplasmic volume associated with each nucleus. Thus, the size of the nucleoplasmic units was not modified during lactation. In consequence, if we assume that muscle weight decreases during lactation (Etienne *et al,* 1985), this suggests that the amount of muscular DNA decreased during lactation. Taking into account Moss' results (1968) showing the constancy of the ratio between the number of nuclei and the mean cross sectional area of myofibers, a decrease in the latter could be predicted; this is reported later on. The absence of change in LD muscle DNA concentration during lactation, whereas the fiber cross sectional area decreased, shows that DNA concentration is not a good estimate of muscle fiber size.

Percentages of muscle fibers of each type

In this study, we have selected 2 skeletal muscles presenting various contractile and metabolic characteristics. The proportions of type I, IIA and IIB fibers were 11.7, 10.0, 78.3 and 59.2, 21.9, 18.9 in LD and T muscle, respectively. No significant change was noticed in trapezius, whereas the percentage of type I increased ($P < 0.05$) in LD muscle during lactation (fig 1). Since the total number ot muscle fibers is determined before birth in pigs (Staun, 1963; Stickland and Goldspink, 1973), the increase in the proportion of type I fibers in longissimus was generated by a transformation of fibers from type IIA, or type IIB

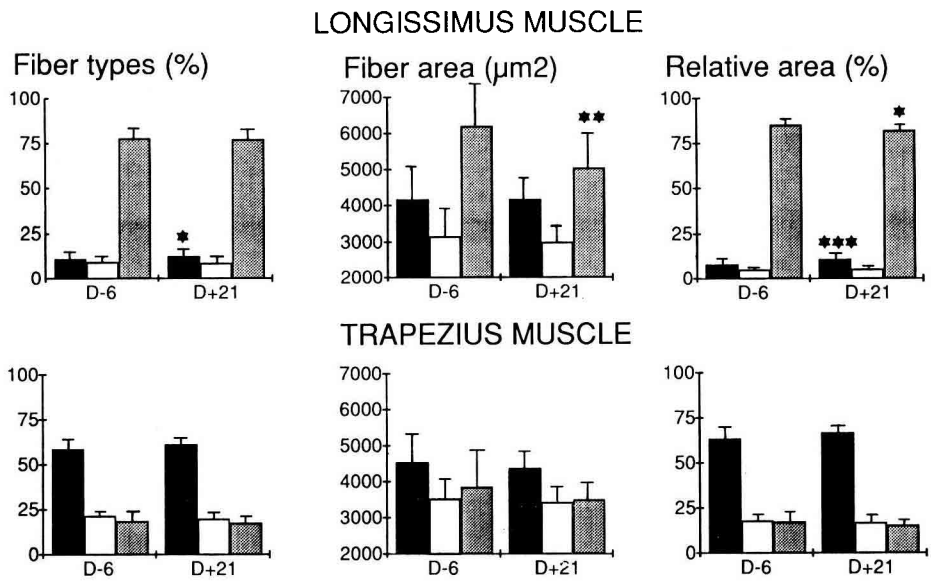

Fig 1. Changes in muscle histological characteristics between 6 d before farrowing (D_{-6}) and the 21st d of lactation (D_{+21}) in the primiparous sow. Type I ■, type IIA □ and type IIB ▨ fibers (Brooke and Kaiser, 1970). The mean values are indicated with the SEM. Compared with D_{-6}: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

via IIA, to type I fibers. This effect is similar to that observed in fast muscles after hypothyroidism in laboratory animals (Nwoye and Mommaerts, 1981). The lower concentrations of serum T_4 during lactation as compared to gestation in the primiparous sow (Brendemuhl *et al,* 1987) might explain the increase in type I fibers in LD muscle, but direct evidence is still lacking. Moreover, other studies are necessary to establish whether the lower T_4 concentration observed during lactation was due to a decrease in secretion and/or to an increase in peripheric utilization, *ie* tissues and milk. Thus, a real hypothyroidal status during lactation is still hypothetical and should be studied at a muscular level.

Muscle fiber area

Muscle fiber cross sectional areas (CSA) were not significantly affected by lactation, with the exception of type IIB fibers in LD muscle ($P < 0.01$; fig 1). Reid *et al* (1980) previously reported a decrease in mean fiber diameter in T muscle of early lactating cows. However, they only studied T muscle and did not take into account fiber types. In the sow, the LD, a white muscle, seemed to be more sensitive to the atrophic action of lactation, and within this muscle, mainly the type IIB fibers (about 78% of the total number of fibers) were affected. Since the total number of muscle fibers did not change, the decrease in fiber cross sectional areas in LD muscle (– 18.2%) suggests a decrease in the weight of this muscle. Muscles may be affected contrary to their development since LD muscle, a late developing muscle, is more affected than T, an earlier developing muscle (Davies, 1974; Dickerson and McCance, 1960).

These changes are very similar to those observed in laboratory animals after a period of starvation, restricted feeding or glucocorticoid treatment that induces a decrease in the size of fast twitch fibers, particularly glycolytic fibers, while slow-twitch fibers are much more resistant to atrophy (Goldberg and Goodman, 1969; Vignos and Greene, 1973; Kelly and Goldspink, 1982; Seene and Viru, 1982; Kelly *et al,* 1986). Activity and/or hormone responsiveness could generate the difference in response between muscles. Within a muscle, the activation threshold increases from type I to IIA and IIB fibers (Henneman *et al,* 1974; Vollestad and Blom, 1985). The continuous activity of T muscle in the maintenance of body posture and in movements of a slow, repetitive nature, may make this muscle somewhat resistant to atrophy. In contrast, the longissimus muscle, a fast-twitch glycolytic muscle, is less frequently recruited and is clearly more susceptible to atrophy.

The higher resistance of T muscle to atrophy may also be due to its more intense metabolism since it has been shown in laboratory animals that RNA concentration and protein synthesis (Flaim *et al,* 1980), protein turnover (Lewis *et al,* 1984), incorporation of glucose into glycogen (Bar and Blanchaer, 1965; Beatty and Bocek, 1970), turnover of glycogen (Villa-Moruzzi *et al,* 1979), insulin receptor concentration (Lefaucheur *et al,* 1986) and the sensitivity of glucose uptake to insulin (Bonen *et al,* 1981; Hom and Goodner, 1984) are higher in slow red than in fast white muscles.

Insulin and glucocorticoids might be involved in the higher sensitivity of IIB fibers to atrophy. Indeed, insulin is known to stimulate protein synthesis and inhibit protein degradation in muscle (Goldberg, 1979; Tischler, 1981; Etherton, 1982), and it may be that the lower number of insulin receptors in fast white fibers (Lefaucheur *et al,* 1986) makes them more sensitive to the reduction of plasma insulin level occur-

ring after farrowing (Atinmo *et al,* 1976; Armstrong *et al,* 1986). It has been shown that the catabolism induced by starvation is triggered, at least in part, by glucocorticoids (Goldberg *et al,* 1980) and that IIB fibers are more susceptible to the atrophying action of glucocorticoids than I and IIA fibers (Kelly *et al,* 1986). Nevertheless, other hormonal changes occur during early lactation *vs* gestation. For instance, higher growth hormone levels have been demonstrated in dairy cows during early lactation (Kunz *et al,* 1985) but this hormone is probably not involved in mechanisms leading to fiber hypotrophy; it would rather contribute, like hypothyroidism, to spare muscular proteins. Further investigations are needed to understand the hormonal mechanisms involved.

Enzyme activities

Oxidative (CS, ICDH, HAD) and glycolytic (LDH) enzyme activities decreased in LD and T muscles during lactation (fig 2); oxidative activities were particularly reduced in the red T muscle. Thus, in the lactating sow, energy and protein deficits (Etienne *et al,* 1989) seem to be related to a general decrease in both muscular oxidative and glycolytic metabolisms. Energy and/or protein restriction also induce a decline in oxidative metabolism in rats, sheep and monkeys (Taskar and Tulpule, 1964; Goldspink and Waterson, 1971; Howarth and Baldwin, 1971; Suzuki, 1972; Oldfors *et al,* 1983; Mehta *et al,* 1987) but do not modify or even increase glycolytic enzyme activities in the muscle of rats and rhesus mon-

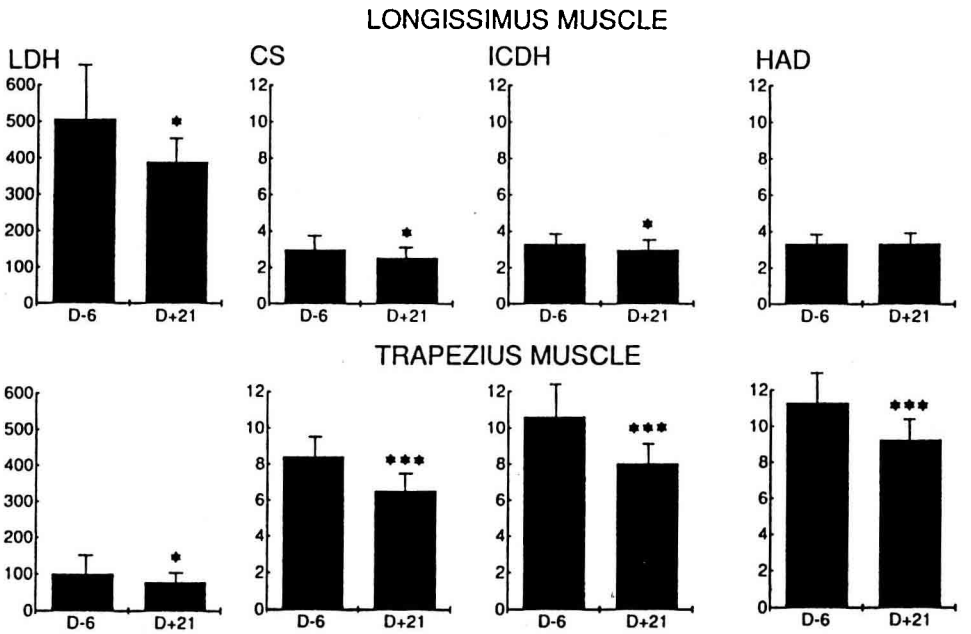

Fig 2. Changes in muscle enzyme activities between 6 d before farrowing (D_{-6}) and the 21st d of lactation (D_{+21}) in the primiparous sow. LDH: lactate dehydrogenase; CS: citrate synthase; ICDH: isocitrate dehydrogenase; HAD: beta-hydroxyacyl coenzyme-A dehydrogenase. The mean values are indicated with SEM. Compared with D_{-6}: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

keys (Taskar and Tulpule, 1964; Mehta *et al*, 1987). If muscle metabolic response to starvation or feed restriction is similar in swine and in these species, lactation and a low plane of nutrition could distinctly affect glycolytic metabolism.

A general reduction in muscular glycolytic and oxidative metabolisms has already been reported in hypothyroidism in laboratory animals (Johnson and Turnbull, 1984). Since T_4 is decreased during lactation in the sow (Brendemuhl *et al*, 1987), it might be involved in the overall reduction in muscle energy metabolism during early lactation *via* a decrease in T_4 uptake by muscle.

CONCLUSION

The present study shows that histochemical and biochemical changes occur in muscle of primiparous sows during lactation. It appears that IIB fibers, mainly of the glycolytic type, are selectively affected during early lactation when food intake does not meet nutrient demand for milk production. As the musculature represents about 40% of the body mass after farrowing and is predominantly made up of fast glycolytic muscles, the fast glycolytic skeletal muscles probably constitute a substantial part of the labile protein reserve. The observed reduction in muscle energy metabolism, characterized by a decrease in oxidative and glycolytic enzyme activities, and the increase of type I fibers of LD muscle could be related to a reduction in thyroxine levels in sows during late gestation and lactation. However, further research is needed to demonstrate this hypothesis.

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