Changes in structural and functional properties of skeletal muscle by L-carnitine supplementation to suckling piglets

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Abstract

To investigate, whether L-carnitine is effective in stimulating the early postnatal increase in the number of myofibres, 30 piglets of low (LW) and middle (MW) birth weight from 6 German Landrace sows were supplemented once daily with 400 mg L-carnitine (n=16) or a placebo (n=14) from days 7 to 28 of age (weaning and slaughtering). Carnitine tended to decrease the perirenal fat percentage, and supplemented females exhibited a significantly lower percentage of lipid in the carcass. Carnitine concentration in *semitendinosus* muscle (ST) was more than doubled in response to treatment. The total number of ST myofibres was increased by 19% in treated LW pigs thereby reaching the unchanged level of MW littermates. Myofibres tended to be smaller and protein concentration and protein/DNA ratio were significantly lower in treated pigs. Muscular isocitrate dehydrogenase activity as a marker of oxidative metabolism tended to be increased, whereas lactate dehydrogenase and creatine kinase activities, and fibre type composition were not influenced by carnitine. The results suggest that carnitine supplementation during the suckling period stimulates the early postnatal increase in myofibre number in piglets of low birth weight. This might compensate for the far-reaching consequences of low birth weight on growth, carcass and meat quality.

Introduction

Piglets of low birth weight exhibit a lower total number of skeletal muscle fibres at birth and throughout life compared with piglets of middle and heavy birth weight (Rehfeldt & Kuhn, 2006). These pigs have a limited potential for muscular lean accretion, and therefore deposit more fat resulting in a lower carcass quality at market weight. Moreover, due to the highly hypertrophied fibres, meat quality is poor, as indicated by higher drip loss, lower pH45, lower impedance values and higher content of heat-stable collagen (Gondret *et al*., 2006; Rehfeldt *et al*., 2008). Consequently, an increase in the number of muscle fibres could contribute to improve carcass and meat quality. The majority of muscle fibres is formed prenatally (e.g. Rehfeldt *et al*., 2000). However, some increase in the total fibre number has also been observed in pig *semitendinosus* muscle shortly after birth (Rehfeldt *et al*., 2000) and could be associated with the appearance of a third generation of small fibres during the first two postnatal weeks (Lefaucheur *et al*., 1995). L-carnitine has been shown to stimulate prenatal myofibre formation (Musser *et al*., 2001). In growing and finishing pigs, carnitine increased protein accretion and percentage of lean and muscle, but decreased fat deposition (Owen *et al*., 1996; 2001; Heo *et al*., 2000). Therefore, the objective of this study was to investigate whether carnitine has the potential to affect early postnatal muscle growth and body composition of suckling piglets.

Material and methods

Thirty piglets of low (LW) and middle (MW) birth weight (each within one third of frequency distribution of all piglets born) from 6 German Landrace sows were supplemented orally once daily with 400 mg carnitine (n=16) or a placebo (n=14) from days 7 to 28 (weaning) of age. At slaughter on d 28, blood samples were collected for the analysis of IGF-I, glucose, urea and non-esterified fatty acids. Samples from *semitendinosus* (ST) muscle were collected for analysis of DNA, protein, carnitine, activities of creatine kinase (CK), lactate dehydrogenase (LDH) and isocitrate dehydrogenase (ICDH). For histological examination, one sample each was collected from the dark (deep), bright (superficial) and central portion of the mid-belly. Serial transverse sections were stained with eosin, and for NADH-tetrazolium reductase which enables classification into red oxidative, intermediate oxidative and white glycolytic fibres. Fibre type distribution and fibre cross sectional area (FCSA) were determined on 900 muscle fibres (300 in each portion) by image analysis (TEMA v1.00, Scan Beam APS, Hadsund, Denmark). The number of fibres per unit area was used to estimate the total number of fibres by multiplication with the ST muscle cross-sectional area (MCSA). Body composition was determined by dissection and chemical analysis.

For statistical analysis, all data were subjected to analysis of variance, using the Mixed Model procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC, USA) with treatment, sex, birth weight group and

corresponding interactions as fixed factors and the sow as a random factor. Significance of differences was concluded at $P<0.05$.

Results and discussion

Birth weight was not significantly different between control and carnitine piglets (Table 1). Live weight at weaning (d 28) was numerically, but not significantly lower in the carnitine group. The percentage of perirenal fat tended to be lower in carnitine supplemented piglets ($P=0.10$). Moreover, carnitine supplemented females tended to have a lower percentage subcutaneous fat (control: 14.2%; carnitine: 13.3%; $P=0.12$) and had a lower percentage analysis-derived lipid in the carcass (control: 14.6%; carnitine: 13.1%; $P<0.05$; Figure 1). The concentrations of IGF-I, glucose, urea and non-esterified fatty acids in blood plasma were not affected by carnitine (data not shown) indicating that the metabolic status of the carnitine group remained unchanged. Carnitine supplementation led to an increase of total carnitine concentration by 245% ($P<0.001$), but had no effect on the weight and MCSA of ST. In accordance with the results of Rehfeldt & Kuhn (2006), the total number of muscle fibres was considerably lower in LW (718 x 10^3) than in MW (816 x 10^3) piglets ($P<0.05$). Carnitine tended to increase the total number of muscle fibres ($P=0.12$). An interaction occurred between treatment and birth weight ($P<0.05$) with an increase in fibre number by 19% in LW piglets ($P<0.05$), whereas there was no effect in MW piglets (Figure 2). Consequently, carnitine seems to prolong the period of fibre hyperplasia in LW piglets so that their fibre number equals to MW piglets.

Table 1. Body weight, body composition, and *semitendinosus* (ST) muscle characteristics of 28 d old piglets supplemented with L-carnitine compared with a control group (LSMeans ± SE)

	Control	L-Carnitine	*P*
Birth weight (kg)	1.16 ± 0.04	1.17 ± 0.03	0.86
Live weight on d 28 (kg)	7.05 ± 0.49	6.50 ± 0.32	0.30
Weight of ST (g)	24.47 ± 2.11	23.45 ± 1.29	0.68
Body composition			
Lean meat (%)[a]	56.35 ± 0.88	56.36 ± 0.68	1.00
Subcutaneous fat (%)[a]	13.91 ± 0.76	14.17 ± 0.61	0.67
Internal organs (%)[b]	14.15 ± 0.53	14.48 ± 0.36	0.55
Perirenal fat (%)[b]	0.68 ± 0.07	0.59 ± 0.05	0.10
Omental fat (%)[b]	0.90 ± 0.06	0.95 ± 0.05	0.37
Protein (%)[c]	16.2 ± 0.18	16.2 ± 0.11	0.80
Lipid (%)[c]	14.1 ± 0.76	14.0 ± 0.57	0.95
ST muscle characteristics			
MCSA (mm^2)	584 ± 44	565 ± 29	0.68
Total carnitine (µg/g)	115 ± 20	282 ± 14	< 0.001
Total fibre number (x 10^3)	741 ± 35	793 ± 26	0.12
FSCA ($µm^2$)	726 ± 52	632 ± 33	0.13
Protein (mg/g)	217 ± 15.4	185 ± 10.8	< 0.05
DNA (mg/g)	1.35 ± 0.13	1.43 ± 0.11	0.39
DNA/protein (µg/g)	6.28 ± 0.84	7.83 ± 0.65	< 0.05
CK (IU/mg protein)	18.5 ± 1.47	20.6 ± 0.87	0.24
LDH (IU/g protein)	1310 ± 242	1410 ± 203	0.59
ICDH (IU/g protein)	29.8 ± 3.4	36.7 ± 2.3	0.06
LDH/ICDH (IU/g)	45.4 ± 5.0	39.0 ± 3.3	0.22

[a] Lean meat and fat percentage of half carcass derived from dissection

[b] Related to final live weight

[c] Protein and lipid percentage of half carcass derived from chemical analysis

The higher fibre number was associated with a smaller mean FCSA in the carnitine group ($P=0.13$). Protein concentration in carnitine piglets was lower ($P<0.05$) and this in turn resulted in a higher DNA/protein ratio ($P<0.05$). In combination with lower fibre size, this suggests a relative immaturity of skeletal muscle in carnitine-treated piglets. The specific activity of ICDH, as an indicator for the oxidative metabolic pathway, tended to be higher in the carnitine group whereas LDH (marker for the anaerobic-glycolytic pathway) and CK as well as the LDH/ICDH ratio were not different. The marginal differences in enzyme activities are reflected by the fibre type distribution (data not shown) which was not affected by carnitine.

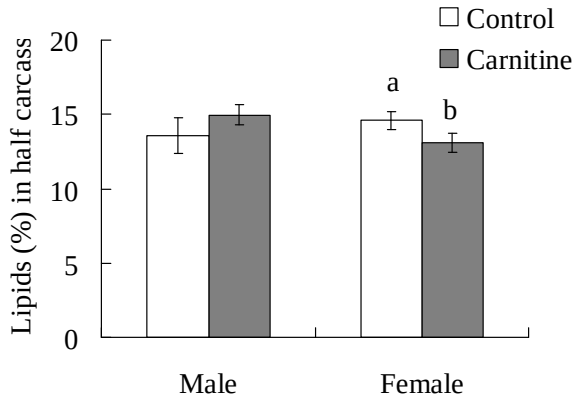

Figure 1. Percentage of lipids in the half carcass of 28 d old male and female piglets supplemented with L-carnitine compared with a control group. Columns represent LSMeans ± SE. Different letters indicate significant differences (P<0.05).

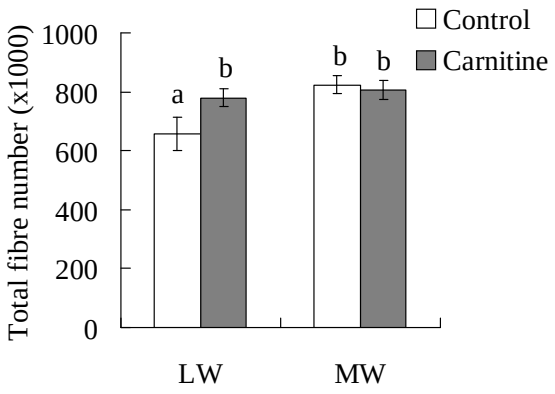

Figure 2. Total number of muscle fibres in the *semitendinosus* muscle of 28 d old piglets of low (LW) and medium (MW) birth weight supplemented with L-carnitine compared with a control group. Columns represent LSMeans ± SE. Different letters indicate significant differences (P<0.05).

Conclusions

Carnitine supplementation during the suckling period stimulates the early postnatal increase in myofibre number in piglets of low birth weight in association with delayed myofibre protein accretion. This might compensate for the far-reaching consequences of low birth weight on growth, carcass and meat quality. As indicated by an increase in muscular ICDH activity and a lower body fat content, a possible mechanism of carnitine action is the stimulation of fatty acid oxidation thereby improving the energy balance in muscle tissue.

References

Gondret, F., Lefaucheur, L., Juin, H., Louveau, I. & Lebret, B. 2006. Low birth weight is associated with enlarged muscle fiber area and impaired meat tenderness of the longissimus muscle in pigs. J. Anim Sci. 84, 93-103.

Heo, K., Odle, J., Han, I.K., Cho, W., Seo, S., van Heugten, E. & Pilkington, D.H. 2000. Dietary l-carnitine improves nitrogen utilization in growing pigs fed low energy, fat-containing diets. J. Nutr. 130, 1809-1814.

Lefaucheur, L., Edom, F., Ecolan, P. & Butler-Browne, G.S. 1995. Pattern of muscle fiber type formation in the pig. Dev. Dyn. 203, 27-41.

Musser, R.E., Goodband, R.D., Owen, K.Q., Davis, D.L., Tokach, M.D., Dritz, S.S. & Nelssen, J.L. 2001. Determining the effect of increasing l-carnitine additions on sow performance and muscle fibre development of the offspring. J. Anim. Sci. 79 (Suppl. 2), 65 (Abstr.).

Owen, K.Q., Nelssen, J.L., Goodband, R.D., Weeden, T.L. & Blum, S.A. 1996. Effect of l-carnitine and soybean oil on growth performance and body composition of early-weaned pigs. J. Anim Sci. 74, 1612-1619.

Owen, K.Q., Nelssen, J.L., Goodband, R.D., Tokach, M.D. & Friesen, K.G. 2001. Effect of dietary l-carnitine on growth performance and body composition in nursery and growing-finishing pigs. J. Anim Sci. 79, 1509-1515.

Rehfeldt, C., Fiedler, I., Dietl, G. & Ender, K. 2000. Myogenesis and postnatal skeletal muscle cell growth as influenced by selection. Livest. Prod. Sci. 66, 177-188.

Rehfeldt, C. & Kuhn, G. 2006. Consequences of birth weight for postnatal growth performance and carcass quality in pigs as related to myogenesis. J. Anim. Sci. 84, Suppl: E113-E123.

Rehfeldt, C., Tuchscherer, A., Hartung, M. & Kuhn, G. 2008. A second look at the influence of birth weight on carcass and meat quality in pigs. Meat Sci. 78, 170-175.