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Early postnatal skeletal myofibre formation in piglets of low birth weight is stimulated by L-carnitine supplementation during suckling

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Abstract
To study the effect of L-carnitine supplementation to suckling piglets on early postnatal myofibre formation, muscle growth, and body composition, 48 piglets of low (LW) and middle (MW) birth weight from 9 German Landrace gilts received 400 mg L-carnitine (n=25) or a placebo (n=23) once daily from d 7 to 27 of age and were slaughtered on day 28 of age (weaning).
The proportions of perirenal (P=0.10) and intramuscular fat (P=0.05) were lower in carnitine-treated piglets. Circulating glucose concentration tended to be increased in treated LW piglets (P=0.13). In female carnitine-treated piglets, the proportion of esterified carnitine in semitendinosus (ST) muscle was increased (P<0.001). The ratio of lactate dehydrogenase to isocitrate dehydrogenase tended to be smaller in ST muscle of treated piglets (P=0.12) indicating a more oxidative muscle metabolism. The total number of ST myofibres was increased by 13% (P=0.02) in treated LW piglets thereby reaching the unchanged level of MW littermates. In addition, treated LW piglets displayed a 2.4-fold mRNA expression of the gene encoding the embryonic isoform of the myosin heavy chain in ST muscle than control piglets (P=0.05). L-carnitine-treated piglets exhibited a higher DNA:protein ratio (P=0.02) in ST muscle, which resulted from a higher DNA concentration (P=0.04). However, because myofibre size, creatine kinase activity, and protein concentration remained unchanged, the ST muscle of treated piglets was not less mature. It seems that intensified fatty acid oxidation improved energy balance and stimulated myogenic proliferation, which in LW piglets may have contributed to a compensatory increase in myofibre number. Thus, particularly in LW piglets an early postnatal L-carnitine supplementation may attenuate the negative consequences of low birth weight on body composition and meat quality at market weight.

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; 2001a,b; 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

A total of 48 piglets of low (LW; ≤ 1.16 kg) and middle (MW; > 1.16-1.38 kg) birth weight (each within one third of frequency distribution of all piglets born) from 9 German Landrace gilts were supplemented orally once daily with 400 mg carnitine (n=25) or a placebo (n=23) from days 7 to 27 of age. At slaughter on d 28 (weaning), 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), isocitrate dehydrogenase (ICDH). In addition, in ST of LW piglets, mRNA abundance of the embryonic isoform of myosin heavy chain (MYHCembr) was quantified by RT-PCR. 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 and white glycolytic fibres. In ST sections of LW piglets, fibres expressing MYHCembr were detected using the anti-human monoclonal antibody F1.652. 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, birth weight group, sex, replicate and corresponding interactions as fixed factors and the sow as a random factor. Data are presented as least squares means ± SE. Differences were considered significant if $P < 0.05$ and were considered as tendencies if $0.05 < P \leq 0.13$.

Results and Discussion

Birth weight was not significantly different between control and carnitine piglets (**Table 1**). At weaning, carnitine-treated piglets tended to be lighter than piglets of the control group. The carnitine group deposited less fat than the controls as indicated by a lower percentage of perirenal fat ($P = 0.10$) and a smaller lipid percentage ($P = 0.05$).

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

	Control	L-Carnitine	*P*
Birth weight, kg	1.17 ± 0.03	1.15 ± 0.03	0.59
Live weight on d 28, kg	7.09 ± 0.23	6.66 ± 0.18	0.12
Dissection			
Lean meat, %[a]	56.6 ± 0.54	56.7 ± 0.49	0.87
Subcutaneous fat, %[a]	14.0 ± 0.52	13.8 ± 0.47	0.67
Internal organs, %[b]	14.5 ± 0.45	14.4 ± 0.42	0.88
Perirenal fat, %[b]	0.60 ± 0.05	0.54 ± 0.04	0.10
Omental fat, %[b]	0.93 ± 0.06	0.94 ± 0.06	0.82
Chemical analysis			
Whole empty body			
Protein, %	16.2 ± 0.28	16.2 ± 0.27	0.65
Lipid, %	14.3 ± 0.59	13.8 ± 0.54	0.19
Dry matter, %			
Meat			
Protein, %	16.9 ± 0.20	16.9 ± 0.19	0.98
Lipid, %	10.4 ± 0.36	9.6 ± 0.30	0.05
Dry matter, %	28.3 ± 0.27	27.6 ± 0.21	0.03

[a] Calculated from left half carcass
[b] Related to whole empty body

These results are consistent with several studies on the effects of carnitine-supplementation on body composition (Owen et al., 1996; 2001a,b; Heo et al., 2000a) and reflect the stimulation of fatty acid oxidation by carnitine. According to the glucose-fatty acid cycle, an increase in fatty acid oxidation would also have an glucose-sparing effect and would increase the capacity for gluconeogenesis. For blood glucose, the effect of carnitine was not significant (data not shown). However, a tendency of an interaction ($P = 0.09$) between treatment and birth weight group occurred for glucose with carnitine-treated LW piglets tending to higher concentrations than the LW control (control, LW: 8.24 ± 0.93 mmol/L; carnitine, LW: 9.50 ± 0.75 mmol/L; $P = 0.13$), whereas there was no difference in MW piglets. The concentrations of IGF-I, urea and non-esterified fatty acids in blood plasma were not affected by carnitine (data not shown). Carnitine supplementation increased the concentrations of free (1.9-fold) and esterified (2.8-fold) carnitine ($P < 0.001$). The greater increase in esterified carnitine than in free carnitine is most likely a result of increased β-oxidation. The LDH:ICDH ratio (**Table 2**) tended to be smaller in response to L-carnitine with LDH being a marker for the anaerobic energy production and ICDH being a marker for oxidative energy generation via the citric acid cycle. This indicates that ST muscle metabolism tended to be more oxidative in response to L-carnitine supplementation and may be due to the role of carnitine in maintaining a pool of free coenzyme A

and thus stimulating citric acid cycle activity. These marginal changes in muscle metabolism were not reflected by metabolic fibre type distribution (data not shown) which was not affected by carnitine.

Table 2. ST muscle characteristics of 28 d old piglets supplemented with L-carnitine compared with a control group (LSMeans ± SE)

	Control	L-Carnitine	*P*
ST muscle characteristics			
Free carnitine, µg/g wet tissue	92 ± 8	178 ± 7	< 0.001
Esterified carnitine, µg/g wet tissue	39 ± 8	109 ± 6	< 0.001
Weight, g	25.3 ± 1.24	24.1 ± 0.99	0.38
Cross-sectional area, mm²	583 ± 25	578 ± 20	0.86
Total fibre number, thousands[a]	766 ± 23	797 ± 18	0.22
Mean fibre area, µm²	665 ± 32	639 ± 26	0.49
Protein, mg/g	189 ± 6	181 ± 4	0.20
DNA, mg/g	1.39 ± 0.03	1.47 ± 0.03	0.04
DNA:protein ratio, µg/g [b]	7.34 ± 0.31	8.20 ± 0.23	0.02
CK, IU/mg protein	19.9 ± 0.9	20.3 ± 0.7	0.68
LDH, IU/g protein	1,655 ± 72	1,570 ± 53	0.30
ICDH, IU/g protein	34.4 ± 2.1	37.2 ± 1.6	0.19
LDH:ICDH ratio	49.4 ± 3.2	43.8 ± 2.5	0.12

[a] Treatment x birth weight interaction ($P = 0.02$), increased in LW
[b] Treatment x sex interaction ($P = 0.05$), increased in males

Carnitine supplementation had no effect on the weight and MCSA of ST (**Table 2**). For ST total fibre number (**Figure 1**) an interaction occurred between treatment and birth weight ($P = 0.02$) with untreated LW piglets exhibiting a considerably smaller total fibre number than untreated MW piglets (712 ± 35 x 10³ vs. 820 ± 22 x 10³). Interestingly, carnitine treatment increased total fibre number in LW piglets (control: 712 ± 35 x 10³, carnitine: 801 ± 25 x 10³; $P = 0.02$) so that their fibre number equals to MW piglets. This was accompanied by a 2.4-fold higher MYHCembr mRNA expression in ST muscle of carnitine-treated compared with control piglets ($P = 0.05$; **Figure 2**), whereas the number of fibres immuno-reactive for MYHCembr was not affected (data not shown). In response to carnitine supplementation, DNA concentration was significantly increased which resulted in an increased DNA:protein ratio ($P = 0.02$).

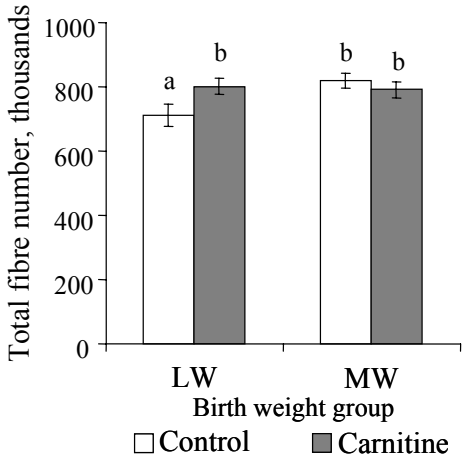

Figure 1. Total number of muscle fibres in the *semitendinosus* muscle of 28 d old piglets of low (LW) and medium (MW) birth weight supplemented with L-carnitine compared with a control group. Different letters indicate significant differences ($P < 0.05$).

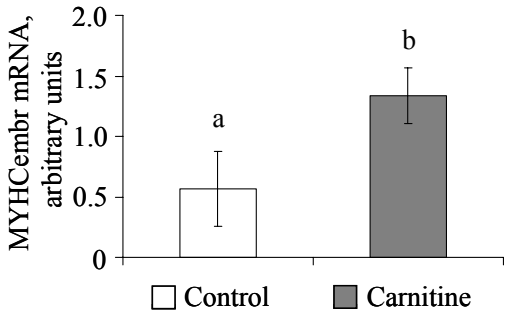

Figure 2. Real-time PCR analysis of the mRNA expression of the gene encoding the embryonic isoform of myosin heavy chain (MYHCembr) in the semitendinosus muscle of 28 d old piglets of low birth weight supplemented with L-carnitine compared with a control group. Data are expressed in arbitrary units after normalization to TATA box binding protein (TBP) mRNA expression. Different letters indicate a significant difference ($P = 0.05$).

Piglets of low birth weight exhibit a lower total number of skeletal myofibres at birth and throughout life compared with piglets of middle and heavy birth weight (Rehfeldt & Kuhn, 2006). This finding was also supported by the present study in 28-day old control piglets. L-carnitine obviously stimulated the early postnatal increase in total fibre number in these disadvantaged LW piglets and thereby compensated the initial differences to MW piglets. In the pig an increase in fibre number has been observed in ST and psoas major muscles between birth and 5-7 weeks of life (Rehfeldt et al., 2008b) which could be explained by the appearance of a third generation of very small fibres that express developmental myosin isoforms (Mascarello et al., 1992; Lefaucheur et al., 1995). The greater abundance of MYHCembr mRNA in carnitine-treated LW piglets may thus be related to the stimulation of early postnatal fibre formation. In addition, the greater DNA concentration and DNA:protein ratio in response to carnitine treatment indicate an intensified myogenic proliferation. Commonly, a higher DNA:protein ratio is indicative of a less differentiated state. This is not the case in the present study because myofibre size, CK activity as a marker for myogenic differentiation, and protein concentration remained unchanged by carnitine.

Conclusions

Supplementing piglets of low birth weight with L-carnitine during the suckling period increased the total fibre number in ST muscle. This may be the result of intensified myogenic proliferation, although the detailed mechanisms require further research. We suggest that L-carnitine stimulated energy generation from β-oxidation of fatty acids and thus affected whole body and muscle metabolism. As a consequence myogenic proliferation appears to be stimulated in LW piglets which enabled compensatory myofibre formation. It remains to be investigated, whether the elevated myofibre number in L-carnitine-treated piglets of low birth weight, which we observed at four weeks of age, maintains through to adulthood and whether this will attenuate the negative consequences of low birth weight on body composition and meat quality at market weight.

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