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# Effects of L-carnitine supplementation on body weight losses and metabolic profile in obese and insulin-resistant ponies during a 14-week body weight reduction programme

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ABSTRACT

There is growing awareness of obesity and insulin resistance in ponies; therefore nutritional management strategies need to be developed. The present study was conducted to investigate the effects of L-carnitine supplementation on body weight (BW) losses, insulin sensitivity and selected metabolic parameters in obese and insulin-resistant ponies during a body weight reduction programme (BWRP).

Sixteen obese ponies (mean body condition score of $7.8 \pm 1.5$ on a scale of 1–9; and a mean cresty neck score (CNS) of $3.6 \pm 1.0$ on a scale of 0–5) were assigned to a randomized, double-blind, placebo-controlled study. During a BWRP lasting 14 weeks, the ponies were fed 1.0–1.2 kg hay/100 kg body weight (BW) daily and 100 g grass meal (estimated energy intake: 7.0–8.3 MJ/DE/100 kg BW) and moderately exercised for 45 min 6 days a week. Eight ponies received L-carnitine supplementation (1.3 g/100 kg BW, twice a day), and 8 ponies received a placebo supplement. A frequently sampled intravenous glucose tolerance test was used to assess insulin sensitivity. Routine blood samples were collected for analysis of glucose, insulin, leptin, urea, beta-hydroxybutyrate, free fatty acids (FFA) and triglycerides (TG). Ponies lost approximately 1% BW per week over the BWRP (time: $P < 0.01$, L-carnitine supplementation: $P = 0.79$). BW losses were accompanied by a significant improvement in insulin sensitivity (time: $P < 0.01$, L-carnitine supplementation: $P = 0.39$). Significant time-related decreases were observed for plasma glucose, serum insulin, and leptin, while significant increases were observed for serum urea, FFA, and TG during the BWRP, without any further improvement by L-carnitine supplementation. Energy intake of 7 MJ/100 kg BW results in BW losses, thereby improving insulin sensitivity and glucose metabolism. L-carnitine supplementation does not further improve glucose or fat metabolism.



## 1. Introduction

Obesity is a common problem in horses, especially in the so-called easy keepers. Local or general adiposity and insulin resistance, as well as a predisposition to laminitis, have been recently described as the equine metabolic syndrome (Frank et al., 2009). In the insulin-resistant state, tissues like skeletal muscle or adipose tissue do not properly respond to insulin, thereby causing a reactive response in insulin secretion by pancreatic ß-cells (Kahn 1978). In obesity, over-expressed adipocytokines such as leptin may impair insulin signalling and upregulate inflammatory cytokines, which further contribute to impaired insulin

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signalling and endothelial dysfunction (Radin et al., 2009). Strategies for nutritional intervention primarily rely on energy restriction, resulting in improved glucose tolerance (Freestone et al., 1992; Van Weyenberg et al., 2008) or at least a reduction in hyperinsulinaemia (resting insulin $< 20$ μU/mL) (Dugdale et al., 2010). In addition to energy restriction, nutraceuticals like L-carnitine, which may facilitate body weight loss or improve insulin sensitivity, are of particular interest. L-Carnitine is well known for its role in transporting activated long-chain fatty acyl groups across the inner mitochondrial membrane and into the mitochondrial matrix for subsequent ß-oxidation (Zeyner and Harmeyer, 1999). It also shuttles accumulated acetyl-CoA molecules out of the mitochondria (Zeyner and Harmeyer, 1999). This buffering function of L-carnitine is of special interest because intracellular accumulation of acetyl-CoA derivates by glucose oxidation has been implicated in the development of insulin resistance. L-carnitine reduces the acyl-CoA/CoA ratio in mitochondria, which in turn increases the activity of pyruvate dehydrogenase, thereby facilitating glucose oxidation (Power et al., 2007). Furthermore, the role of L-carnitine supplementation in the altered expression of glycolytic and gluconeogenic enzymes and the altered expression of enzymes in the insulin cascade has been the focus of interest in recent studies (Keller et al., 2011; Ringseis et al., 2012). In humans, L-carnitine supplementation improves glucose tolerance and in part facilitates body weight loss, in particular during insulin-resistant states (Ringseis et al., 2012; Mingrone, 2004; Mingorance et al., 2011; Molfino et al., 2010). In healthy ponies, L-carnitine supplementation reduced postprandial glycaemic and insulinaemic profiles and increased postprandial plasma leptin responses (Van Weyenberg et al., 2009). However, studies of L-carnitine supplementation in obese and insulin-resistant ponies undergoing a body weight reduction programme (BWRP) are lacking. The aim of this study was to investigate the effects of L-carnitine supplementation on body weight reduction, insulin sensitivity, and selected metabolic parameters in obese and insulin-resistant ponies during a BWRP lasting several weeks. We hypothesized that L-carnitine supplementation in obese ponies will improve metabolic situation, in particular insulin sensitivity, in addition to promoting the beneficial effects of body weight reduction.

## 2. Materials and methods

### 2.1. Animals

Sixteen mature ponies (11 mares and 5 geldings; Shetland ponies or mixed-breed ponies) with a mean age of $10.6 \pm 5.1$ years; mean body weight of $199 \pm 64$ kg; mean body condition score (BCS) of $7.8 \pm 1.5$ on a scale of 1–9 (Henneke et al., 1983); and a mean cresty neck score (CNS) of $3.6 \pm 1.0$ on a scale of 0–5 (Carter et al., 2009) were individually housed in box stalls (see Supplementary Table S1), bedded on rubber blankets, and turned out onto a dry lot for 5 h/d. The ponies had free access to water at all times. The project was approved by the Ethics Committee for Animal Rights Protection of the Leipzig District government (No A06/10), in accordance with German legislation for animal rights and welfare.

### 2.2. Body weight reduction programme (BWRP)

Four weeks before the onset of the BWRP, ponies were provided hay *ad libitum*.

During the 14-week BWRP (September–December 2010), ponies were fed hay [dry matter (DM) intake 0.9–1.1% of BW, same batch throughout the study, see Supplementary Table S2] and 50 g of a commercial mineral mixture (Horsal Basis®, Schauman, Pinneberg, Germany) mixed in 50 g of grass meal, resulting in a calculated daily energy intake between 7 (BWRP weeks 5–6 and 9–14) and 8.4 MJ/100 kg BW (BWRP weeks 0–4 and 7–8). The hay was offered in 2 equal portions twice a day in hay nets. During the whole observation period, there were no feed refusals.

During BWRP ponies were exercised according to a low-intensity protocol 6 days a week (25 min walk and 15 min trot through the countryside).

### 2.3. L-carnitine supplementation

Eight ponies were randomly chosen to receive L-carnitine supplementation (Carnifeed®, Lonza, Basel, Switzerland; 1.3 g/100 kg BW, twice a day), and 8 ponies received a placebo supplement (silicic acid, 1.3 g/100 kg BW, twice a day) during the 14 weeks of the BWRP. The amount of L-carnitine or placebo was weekly adjusted to BW using a scale system precise to 0.01 g. L-carnitine or placebo was mixed into 50 g grass meal, and feed intake was controlled. During the whole observation period, there were no feed refusals.

### 2.4. Physical measurements

Ponies were weighed weekly after 12 h of feed restriction by using an electronic scale system for large animals (scaling precision: 0.5 kg).

BCS and CNS were recorded weekly by the same 2 observers throughout the study, who were blinded to previous measures.

### 2.5. Frequently sampled intravenous glucose tolerance test

Frequently sampled intravenous glucose tolerance test (FSIGTT) was performed before and one day after the BWRP at 0730 h following a 12 h complete overnight fast. At 0700 h an indwelling catheter (1.8 by 2.35 mm/12 G, Braun Melsungen AG, Melsungen, Germany) was inserted into the jugular vein. Blood was initially collected for baseline measurements of plasma glucose and serum insulin, followed by intravenous (IV) administration of 100 mg glucose/kg BW (40% dextrose). Blood was collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16 and 19 min after glucose administration. An IV dose of insulin (20 μU/kg BW, Insuman Rapid®, Sanofi-Aventis, Frankfurt a. Main, Germany) was injected at 20 min, and additional blood samples were collected at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150 and 180 min after glucose infusion. The test