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Tissue Carnitine Concentrations after Total Parenteral Nutrition with and without L-Carnitine Supplementation

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ABSTRACT The concentrations (μmoles/g dry weight) of total carnitine (TC), free carnitine (FC) and acylcarnitine (AC) were determined in skeletal muscle, heart, liver, kidney and brain cortex of male mini pigs (4000-5000 g) after seven days of total parenteral nutrition (TPN) with amino acids 5% (3.0g/kg/d), glucose (25g/kg/d) and lipids 20% (4g/kg/d). This regime was administered with L-carnitine supplementation (1.5 mg/kg/d; n = 7) (group 1) and without it (n = 5) (group 2). Orally alimented animals (n = 5) served as controls (group 3). (Average carnitine intake: 3 mg/d) Carnitine free TPN affected only the concentrations in muscle. TC was markedly reduced (3.6 ± 0.8) when compared with oral controls (5.8 ± 0.7) ($p<0.01$). This decrease was exclusively due to AC, whereas FC concentrations remained almost unchanged. In group 1 the concentrations of TC in skeletal muscle, heart and brain cortex were higher than in both the other groups. The increase was mainly due to AC and FC remained unchanged in heart and brain. The concentrations in liver and kidney were not affected by either carnitine free or carnitine supplemented TPN. AC, determined as described, consists almost entirely of acid soluble acetyl-carnitine, the major product of fatty acid oxidation. Since the AC concentrations were almost exclusively altered by the two TPN-regimes we conclude that the observed changes reflect regulatory changes of fatty acid oxidation. Thus the decrease of muscle TC in group 2 is considered a consequence of an insulin induced down regulation (plasma insulin: mean 20 μU/ml; maximum: 60 μU/ml) of fatty acid oxidation in consequence of high glucose intake (25 g/kg/d). The increased TC concentrations after carnitine supplemented TPN are discussed to reflect an enhancement of oxidative degradation of fatty acids as a pharmacological effect of L-carnitine.

INTRODUCTION

Carnitine is essential for the transport of long chain fatty acids from the cytosol to the site of ß-oxidation in the mitochondria [1]. Carnitine is maintained in most body tissues at markedly higher concentrations than those found in plasma [2]. This especially applies to skeletal muscle (red fiber type) and the heart [3, 4], which use fatty acids as major source of energy. Tissues are dependent on carnitine supply either by de novo synthesis in the liver, originating from trimethyllysine, or by dietary intake. Decreased plasma [5] and tissue [6] carnitine concentrations have been reported during carnitine free total parenteral nutrition (TPN). Plasma levels of carnitine are difficult to interpret because carnitine is distributed between different tissues. In order to investigate the influence of carnitine substitution during TPN on its tissue concentration, we compared the effect of carnitine free and carnitine supplemented TPN.

METHODS

Male mini pigs (Göttingen, FRG,) underwent TPN during a seven day period. The nutrients (amino acids 5%: 3.0 g/kg/day; glucose 25 g/kg/day; lipid emulsion 20%: 4.0 g/kg/day; minerals and vitamins) were administered through a central venous catheter, which was inserted into the external jugular vein as described previously [17].

The animals were divided into three groups:

Group 1: n = 7 ($\bar{x}$ = 4150 g) Supplementation with 1.5 mg L-carnitine/kg/day.

Group 2: n = 5 ($\bar{x}$ = 4900 g) Without L-carnitine supplementation.

Group 3: n = 5 ($\bar{x}$ = 4050 g) served as a control group and was alimented orally with a special pig chow (Muskator®). The total carnitine content of this diet was analyzed and an average oral carnitine intake of 1.5-3.0 mg/day was estimated.

Seven days after starting the experiment the animals

were killed in deep Ketanest®-anesthesia by aspiration of whole blood. The tissues (skeletal muscle, heart, kidney, liver and brain cortex) were removed immediately, cut into two pieces and weighed. One was dried at 100°C to constant weight; the other was homogenized in 5% trichloracetic acid (1: 10 / w : v) and centrifuged. Muscle tissue was excised from soleus muscle which is predominantly a red fiber tissue [8]. Carnitine was measured in the supernatant according to McGarry and Foster [7]. It was determined before (free carnitine) and after alkaline hydrolysis (total carnitine). Acyl carnitine was calculated from the difference of total and free carnitine. This acid soluble acyl carnitine was considered to contain mainly acetyl carnitine [9, 10]. Concentrations were expressed as mean ± 1 SD. The statistical comparison of the means was evaluated by Student's t-test for unpaired data.

RESULTS

The results are listed in Table 1. The carnitine levels of various organs were differently influenced by TPN. Carnitine free TPN lead to a marked reduction of TC in skeletal muscle when compared to oral controls. This decrease was mainly due to AC, whereas FC concentrations remained almost unchanged. The TC concentrations in the other tissues were not affected by carnitine free TPN.

In the carnitine supplemented group the concentrations of TC in skeletal muscle were higher than in oral controls. AC concentrations were similar in both these groups, FC however was elevated when compared to oral and carnitine free controls. The concentrations of TC, AC and FC in liver and kidney were not affected by either carnitine free or carnitine supplemented TPN. In contrast to muscle carnitine, the levels of heart and brain did not decrease during carnitine free TPN but showed an increase after carnitine supplementation. This increase was mainly due to AC.

DISCUSSION

In our study with pigs the largest amounts of carnitine were found in muscle and heart, which is in agreement with data in rats [11] and humans [4]. This is not unexpected because soleus muscle is a red fiber type muscle which is known to derive its energy primarily from the utilization of fat [8, 12] as does the heart [13]. In humans Cederblad *et al.* [4] measured a lower carnitine concentration in heart than in skeletal muscle. We confirm this for the mini pig. Our values also correspond to the lower range of the carnitine concentrations of muscle and heart determined by the same authors in men [4]. In contrast in rodents the concentration of carnitine in heart is about twice as high as in skeletal muscle [11, 14]. In human muscle the carnitine concentration is 5-10 times higher than in rats [11] and mice [14] and lower than in the sheep [15]. On the basis of this species difference we therefore suppose that the pig is an adequate animal model to be compared with this metabolic situation in humans.

Decreased tissue concentrations of TC were reported from premature infants fed parenterally for longer than 15 days. The lowest TC concentrations were observed in liver

Table 1 Tissue carnitine concentrations of different organs after TPN with and without L-Carnitine supplementation (1.5 mg/kg/day). (μmoles/gram dry weight)

ORGANS	*Total Carnitine*		*Acylcarnitine*		*Free Carnitine*	
Skeletal Muscle						
Oral Controls		5.8 ± 0.7		4.6 ± 0.6		1.1 ± 0.3
With Carnitine	p<0.05	6.5 ± 0.4 } p<0.01	n.s.	4.2 ± 0.6 } p<0.01	p<0.05	2.3 ± 0.9 } n.s.
Without Carnitine	p<0.001	3.6 ± 0.8	p<0.02	2.4 ± 1.2	n.s.	1.3 ± 0.7
Heart						
Oral Controls		4.8 ± 0.5		3.7 ± 0.6		1.2 ± 0.3
With Carnitine	p<0.005	6.7 ± 0.7 } n.s.	p<0.05	4.9 ± 0.9 } n.s.	n.s.	1.8 ± 0.6 } n.s.
Without Carnitine	p<0.01	4.8 ± 1.1	p<0.05	3.3 ± 1.3	n.s.	1.4 ± 0.5
Brain Cortex						
Oral Controls		4.1 ± 0.5		3.6 ± 0.5		0.5 ± 0.1
With Carnitine	p<0.005	5.8 ± 0.8 } n.s.	p<0.005	5.3 ± 0.7 } n.s.	n.s.	0.6 ± 0.1 } n.s.
Without Carnitine	p<0.02	4.1 ± 0.09	p<0.05	3.5 ± 1.1	n.s.	0.5 ± 0.2
Liver						
Oral Controls		2.5 ± 0.4		2.1 ± 0.3		0.4 ± 0.03
With Carnitine	n.s.	2.8 ± 0.6 } n.s.	n.s.	2.4 ± 0.5 } n.s.	n.s.	0.4 ± 0.1 } n.s.
Without Carnitine	n.s.	3.0 ± 0.4	n.s.	2.4 ± 0.4	n.s.	0.5 ± 0.03
Kidney						
Oral Controls		4.6 ± 0.2		3.9 ± 0.2		0.7 ± 0.2
With Carnitine	n.s.	4.9 ± 0.4 } n.s.	n.s.	4.1 ± 0.4 } n.s.	n.s.	0.9 ± 0.2 } n.s.
Without Carnitine	n.s.	4.4 ± 0.7	n.s.	3.6 ± 0.7	n.s.	0.8 ± 0.1

and heart followed by skeletal muscle [6]. It was suggested that carnitine synthesis from its precursors was not adequate to maintain tissue stores. Because these data only referred to TC concentrations they do not allow further conclusions. On the other hand however the decrease of total serum carnitine concentrations during TPN of prematures was reported to be evenly distributed between free- and acylcarnitine [18]. The reduced TC concentration that we found in the skeletal muscle was exclusively due to a decrease of AC. Our analysis covers only the acid soluble form of AC which is mainly acetyl carnitine [9, 10], the major product of fatty acid oxidation. Thus the reduced TC concentration observed in our experiment seemed to be mainly the result of a decreased fatty acid oxidation. Insulin may be the common denominator for a decreased fatty acid oxidation and decreased muscle carnitine concentrations. It is known from studies in diabetic rats that the elevated carnitine content of soleus muscle is effectively reduced after the administration of insulin [16]. The plasma insulin concentrations that we determined during TPN had a mean of 20 μU/ml and reached individual values as high as 60 μU/ml. This reflects the high glucose intake (25 g/kg/day) during TPN and a hormonal negative feed back on fatty acid oxidation. Therefore we do not conclude that the reduced carnitine concentrations in our study reflect a beginning deficiency of carnitine, but are the consequence of metabolic regulation. From this point of view it is interesting to see that the carnitine concentrations in muscle, heart and brain were found increased after L-carnitine supplemented TPN. Because the increment is exclusively due to acid soluble AC (acetylcarnitine) we conclude that the oxidative degradation of fatty acids has been stimulated as a consequence of L-carnitine supplementation. This is in accordance to our previous data which showed that L-carnitine supplementation during TPN leads to metabolic changes conclusive for an enhanced fatty acid oxidation [17].

The described effects on tissue carnitine concentrations show that an intravenous L-carnitine supplementation as small as 1.5 mg/kg/day is effective. The present data make it worthwhile to continue studies on the influence of exogenous L-carnitine on the regulation of fatty acid oxidation.

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