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REVIEW

Role of carnitine in the regulation of glucose homeostasis and insulin sensitivity: evidence from in vivo and in vitro studies with carnitine supplementation and carnitine deficiency

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Abstract
Background Although carnitine is best known for its role in the import of long-chain fatty acids (acyl groups) into the mitochondrial matrix for subsequent β-oxidation, carnitine is also necessary for the efflux of acyl groups out of the mitochondria. Since intracellular accumulation of acyl-CoA derivatives has been implicated in the development of insulin resistance, carnitine supplementation has gained attention as a tool for the treatment of insulin resistance. More recent studies even point toward a causative role for carnitine insufficiency in developing insulin resistance during states of chronic metabolic stress, such as obesity, which can be reversed by carnitine supplementation.
Methods The present review provides an overview about data from both animal and human studies reporting effects of either carnitine supplementation or carnitine deficiency on parameters of glucose homeostasis and insulin sensitivity in order to establish the less well-recognized role of carnitine in regulating glucose homeostasis.
Results Carnitine supplementation studies in both humans and animals demonstrate an improvement of glucose tolerance, in particular during insulin-resistant states. In contrast, less consistent results are available from animal studies investigating the association between carnitine deficiency and glucose intolerance. The majority of studies dealing with this question could either find no association or even reported that carnitine deficiency lowers blood glucose and improves insulin sensitivity.
Conclusions In view of the abovementioned beneficial effect of carnitine supplementation on glucose tolerance during insulin-resistant states, carnitine supplementation might be an effective tool for improvement of glucose utilization in obese type 2 diabetic patients. However, further studies are necessary to explain the conflicting observations from studies dealing with carnitine deficiency.

Keywords Carnitine · Glucose homeostasis · Insulin sensitivity · Type 2 diabetes

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Introduction

Carnitine is a water soluble quaternary amine (3-hydroxy-4-*N*,*N*,*N*-trimethylaminobutyric acid), which is essential for normal function of all tissues. Dietary sources of carnitine include mainly products of animal origin, such as meat and dairy products [1]. Through an omnivorous diet, approximately 0.3–1.9 mg carnitine is provided per kg body weight and day, whereas vegetarians consume less than 0.02 mg per kg body weight and day [2]. Nonetheless, vegetarians maintain normal carnitine levels indicating that humans also effectively synthesize carnitine, which was estimated to be 0.19 mg carnitine per kg body weight and day [3]. This implies that endogenous synthesis provides 90% of total body carnitine in strict vegetarians and about 25% in omnivores [4].

Endogenous carnitine synthesis starts with the release of trimethyllysine (TML) from lysosomal protein breakdown, which is subsequently converted to γ-butyrobetaine by a series of enzymatic reactions involving trimethyllysine

dioxygenase, 3-hydroxy-*N*-trimethyllysine aldolase, and 4-*N*-trimethylaminobutyraldehyde dehydrogenase (TMABA-DH). Finally, γ-butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase (BBD) to form carnitine [5]. At least in rats, it was demonstrated that carnitine supplementation at levels exceeding the demand decreases carnitine biosynthesis through depressing the activity of BBD [6]. In humans, the major sites of carnitine synthesis are the liver and the kidneys because these are the only tissues with a considerable activity of BBD [5]. From extrahepatic tissues, γ-butyrobetaine is excreted and transported via the circulation to the liver, where it is converted into carnitine [5]. Since carnitine synthesis is dependent on the availability of several co-factors including the micronutrients vitamin C, vitamin B_6, and iron, a deficiency in these nutrients can lead to carnitine deficiency (reduced plasma carnitine concentration compared to normal (25–50 μmol/L [7])), in particular when carnitine is not provided from the diet in sufficient amounts.

All tissues that are incapable of producing carnitine are highly dependent on active carnitine uptake from blood. Delivery of carnitine from plasma into cells is catalyzed by novel organic cation transporters (OCTNs), from which the OCTN2 isoform is the physiologically most important carnitine transporter [8, 9]. OCTN2 is of great importance for maintaining normal carnitine levels in serum because it is also responsible for tubular reabsorption of carnitine in the kidney where approximately 99% of all free carnitine filtered is reabsorbed when plasma free carnitine concentration is in its normal range. Only when plasma carnitine concentration exceeds the normal range (supraphysiologic levels), the excess carnitine is rapidly eliminated due to saturation of the reabsorption mechanism [7, 10], which explains that the ability to maintain supra-physiologic plasma carnitine concentrations is limited [11, 12]. Patients carrying a mutation in the OCTN2 gene develop primary systemic carnitine deficiency with markedly reduced serum carnitine levels (0–5 μmol/L) because most of the filtered carnitine is lost in the urine [13].

The most documented function of carnitine in intermediary metabolism is its role in fatty acid catabolism by facilitating the translocation of long-chain fatty acids (acyl groups) from the cytosol into the mitochondrial matrix for subsequent β-oxidation. This complicated translocation process is catalyzed by the action of three carnitine-dependent enzymes that together represent the carnitine shuttle system [14]. Carnitine palmitoyltransferase I (CPT I) is responsible for the initial step in the carnitine shuttle system by catalyzing the reversible transesterification of long-chain acyl-CoA with carnitine. The resulting long-chain acylcarnitine is then transported through the inner mitochondrial membrane via carnitine/acylcarnitine translocase and then regenerated to acyl-CoA by the action of CPT II at the matrix side of the mitochondrial membrane where β-oxidation takes place. Besides its role in permitting mitochondrial oxidation of long-chain fatty acids, carnitine is also necessary for the efflux of acetyl and acyl groups out of the mitochondria in the case that substrate oxidation exceeds the energy demand, and therefore, accumulating acyl-CoA intermediates are converted back to acylcarnitines that are transported out of mitochondria and cells. This latter function of carnitine is less well documented. However, since intracellular accumulation of acyl-CoA derivatives has been implicated in the development of insulin resistance in skeletal muscle and heart, carnitine supplementation has gained attention as a tool for the treatment or prevention of insulin resistance and type 2 diabetes mellitus [15]. In addition, more recent studies even point toward a causative role for carnitine deficiency in developing mitochondrial dysfunction and insulin resistance during states of chronic metabolic stress such as obesity and aging [16–18], which can be reversed by oral carnitine supplementation [18].

The purpose of this review is, first, to provide an overview about data from both animal and human studies reporting effects of either carnitine supplementation or carnitine deficiency on parameters of glucose homeostasis and insulin sensitivity in order to establish the less well-recognized role of carnitine in regulating glucose homeostasis and, second, to summarize the mechanisms of action underlying the regulation of glucose homeostasis by carnitine.

Effect of carnitine supplementation on glucose homeostasis and insulin sensitivity

Human studies

Human studies with subjects with metabolic disorders

A total of sixteen human studies with subjects with metabolic disorders reporting an effect of carnitine supplementation on parameters of glucose tolerance and insulin sensitivity were identified using the PubMed database (key words used in different combinations were as follows: carnitine supplementation, carnitine infusion, glucose metabolism, insulin resistance, human study, randomized control trial; time frame: no limitation). Table 1 provides an overview about design, subject characteristics, carnitine dose and formulation, treatment duration, and outcome of these studies. As evident from this overview, the majority of these studies (11 out of 16) revealed an improvement of parameters of glucose tolerance, like fasting plasma glucose, fasting plasma insulin, area under the curve for glucose (AUC_{GLC}), area under the curve for insulin (AUC_{INS}),

Table 1 Effect of carnitine supplementation on glucose tolerance in human studies with subjects with metabolic disorders

Study design	Subjects and characteristics	Diet	Dosage and formulation of carnitine/day	Route of application	Treatment duration	Carnitine effects on glucose tolerance	Overall changes in glucose tolerance	Refs.
RCT	16 patients with DM-2 or IFG (12M/4F), no anti-diabetic therapy, aged 49.7–81.7 years, BMI $\leq$ 35.4 kg/m^2	Hypocaloric (1,200 kcal/day F, 1,400 kcal/M; 55% carbohydrates, 25% lipids, 20% proteins)	4 g LC	Oral	2 weeks	$AUC_{GLC}\leftrightarrow$ $GLC_{fasting}\leftrightarrow$ $INS_{fasting}\downarrow$ HOMA-IR$\downarrow$	$\uparrow$	[32]
RCT, double-blind	94 patients with newly diagnosed DM-2 (47M/47F), aged 43–58 years	n.m.	2 g LC	Oral	24 weeks	$GLC_{fasting}\leftrightarrow$	$\uparrow$	[33]
RCT, double-blind	35 caucasian patients with DM-2 (22M/13F), aged 51.3 $\pm$ 3.7 years, disease duration of 12.3 $\pm$ 3.4 years, BMI $<$ 30 kg/m^2, medication with oral anti-diabetic, no insulin or lipid lowering medication	n.m.	3 g LC	Oral	12 weeks	$GLC_{fasting}\downarrow$	$\uparrow$	[31]
RCT, double-blind	74 patients with NASH (40M/34F), aged 28–60 years, lowest possible therapeutic dosage	Hypocaloric (1,600 kcal/d); 30 min home-based whole body stretching routine 3 times/week	2 g LC	Oral	24 weeks	$GLC_{fasting}\downarrow$ $INS_{fasting}\downarrow$ HOMA-IR$\downarrow$	$\uparrow$	[26]
RCT, double-blind	258 caucasian DM-2 patients (126 orlistat group 62M/64F; 132 orlistat + LC group 65M/67F), aged 47–59 years, BMI $\geq$ 30 kg/m^2	Hypocaloric (near 600 kcal daily deficit, 50% carbohydrates, 30% lipids, 20% proteins); walking or cycling for 20–30 min 3–5 times/week	2 g LC combined with orlistat	Oral	48 weeks	$GLC_{fasting}\downarrow$ $INS_{fasting}\downarrow$ (vs. baseline) HOMA-IR$\downarrow$	$\uparrow$	[19]
RCT, double-blind	254 caucasian DM-2 patients (125 63M/62F sibutramin group, 129 65M/64F in sibutramin + LC group) aged $\geq$ 18 years, BMI $\geq$ 30 kg/m^2	Hypocaloric (near 600 kcal daily deficit, 50% carbohydrates, 30% lipids, 20% proteins); walking or cycling for 20–30 min 3–5 times/week	2 g LC combined with sibutramin	Oral	48 weeks	$GLC_{fasting}\downarrow$ (vs. baseline) $INS_{fasting}\downarrow$ HOMA-IR$\downarrow$	$\uparrow$	[20]
Uncontrolled, longitudinal, study	32 subjects with decreased insulin sensitivity, aged 21–58 years	No changes in dietary intake	2 g ALC	Oral	24 weeks	$AUC_{GLC}\downarrow$ $AUC_{INS}\downarrow$	$\uparrow$	[102]
RCT, single-blind	8 overweight/obese male participants, subjects studied twice, aged 19–36 years	No changes in dietary intake	3 g LC-tartrate	Oral	2 weeks	$AUC_{INS}\uparrow$ $AUC_{GLC}\uparrow$ HOMA-IR$\leftrightarrow$	$\leftrightarrow$	[21]
RCT, double-blind	12 patients with DM-2 of $<$5 years (6M/6F), aged 30–60 years, BMI $\leq$ 30 kg/m^2	Isocaloric diet, containing more than 250 g carbohydrates/day for 3 days before the study	3 g LC	Oral	4 weeks	$GLC_{fasting}\leftrightarrow$	$\leftrightarrow$	[25]

Table 1 continued

Study design	Subjects and characteristics	Diet	Dosage and formulation of carnitine/day	Route of application	Treatment duration	Carnitine effects on glucose tolerance	Overall changes in glucose tolerance	Refs.
RCT, double-blind	29 pre-diabetic subjects, aged 20–55 years, BMI $\leq$ 40 kg/m^2, no medication	No changes in dietary intake	1.35 g ALC	Oral	8 weeks	AUC_{GLC}↔↓ HOMA-IR↔↓	↔	[22]
RCT, double-blind	46 non-insulin-dependent diabetic patients (16M/30F; $n = 23$/group), aged 55–61 years, BMI $\leq$ 30 kg/m^2	No changes in dietary intake, daily live and activities	3 g LC	Oral	12 weeks	$GLC_{fasting}$↔ $INS_{fasting}$↔	↔	[23]
RCT	15 patients with DM-2 (8M/7F), aged 39.3–55.3 years BW 59–77.3 kg, each subject studied twice	Weight-maintaining, consisting of 250 g carbohydrates/day for 1 week before the study	45 μg/kg BW/min LC	i.v.	3 h	M↑ GOX↑	↑	[27]
RCT, double-blind	18 DM-2 patients (8M/10F), aged 52.3 ± 12 years, BMI $\leq$ 32 kg/m^2, medication with oral anti-diabetic, each subject studied twice	Weight-maintaining, consisting of 250 g carbohydrates/day for 1 week before the study	Priming bolus of 5 mg/kg BW ALC afterward at a rate of 0.025–1 mg/kg BW during EHC	i.v.	180 min	M↑ dose-dependent M/I↑ dose-independent $INS_{fasting}$↓ GOX↔	↑	[96]
RCT	9 non-insulin-dependent diabetic patients (7M/2F), aged 39–64 years, BMI $\leq$ 33 kg/m^2, medication with oral anti-diabetic, each subject studied twice	Isocaloric (containing 200 g carbohydrates)	3 mmol LC during EHC	i.v.	3 h	$GLC_{fasting}$↔ $INS_{fasting}$↔ M↑	↑	[122]
Placebo controlled trial	13 hemodialysed patients with chronic renal failure (LC group $n = 7$, 3M/4F; control group $n = 6$, 2M/4F), aged 38–51 years	n.m.	1 g LC	i.v.	One bolus	K_{itt}↑	↑	[123]
Case–control study	4 person on long-term HPN	HPN formula	66 μg/kg BW/min LC for 20 min and 33 μg/kg BW/min LC + 1 g LC/day	i.v.	180 min + 4 weeks	$GLC_{fasting}$↔ $INS_{fasting}$↔ GOX↔	↔	[24]

RCT randomized controlled trial, *DM-2* type 2 diabetes mellitus, *M/F* male/female, *BW* body weight, *BMI* body mass index, $GLC_{fasting}$ fasting plasma glucose, $INS_{fasting}$ fasting plasma insulin, *IFG* impaired fasting glucose, *i.v.* intravenous, AUC_{GLC} area under the curve for glucose, AUC_{INS} area under the curve for insulin, *HOMA-IR* homeostasis model assessment of insulin resistance, =fasting plasma insulin × fasting serum glucose/405, *NASH* non-alcoholic steatohepatitis, *EHC* euglycemic hyperinsulinemic clamp, *M* glucose tissue uptake rate at steady state (mg/min × kg), *M/I* ratio of end-clamp glucose tissue uptake and end-clamp insulinemia [(mg/kg BW/min)/(μUI/mL)], *GOX* glucose oxidation rate, K_{itt} fractional disappearance rate of insulin, *ALC* acetyl-L-carnitine, *LC* L-carnitine, *HPN* home parenteral nutrition, *n.m.* not mentioned

glucose oxidation rate (GOX), and/or homeostasis model assessment of insulin resistance (HOMA-IR), by carnitine supplementation. It is obvious, moreover, that the improvement of glucose tolerance following carnitine supplementation in these studies was independent of the route of carnitine administration (i.v. vs. oral), the treatment duration, the metabolic disorder of the subjects [pre-diabetic, diabetic, overweight/obese, or patients with nonalcoholic steatohepatitis (NASH), chronic renal failure, or home parenteral nutrition (HPN)], the carnitine formulation [carnitine vs. acetyl-L-carnitine (ALC) or carnitine-tartrate], or the carnitine dosage. However, it has to be

considered that in two of the studies with a positive outcome, carnitine supplementation (2 g oral carnitine) was studied in combination with an anti-obesity drug (either orlistat [19] or sibutramin [20]) compared to monotherapy with the anti-obesity drugs. A treatment group receiving carnitine alone was not included in these two studies with diabetic subjects. Thus, it remains unresolved from these long-term studies (48 weeks) whether the observed reductions of fasting glucose, fasting insulin, and HOMA-IR in the groups receiving carnitine plus orlistat [19] or carnitine plus sibutramin [20] were due to carnitine supplementation or due to an interaction between carnitine and the anti-obesity drugs.

The oral carnitine dosages shown to be effective were in the range between 2–4 g carnitine and 2 g ALC, respectively. These have to be considered as supra-physiologic considering that carnitine intake from an omnivorous diet was estimated to be only up to 1.9 mg carnitine/kg body weight and day corresponding to approximately 150 mg carnitine/day for a 80 kg person. Since lower carnitine dosages than 2 g carnitine/ALC were not tested in either of these studies, the possibility cannot be ruled out that lower dosages might have been sufficient to positively impact glucose tolerance. Therefore, future studies testing a broad range of different carnitine dosages, from physiologic to supra-physiologic, are necessary to establish the minimum effective carnitine dosage for improving glucose tolerance.

Only in five studies considered in this review, parameters of glucose homeostasis or insulin sensitivity were not altered compared to control/placebo treatment [21–25]. Since in two of these five studies, the treatment duration was quite long (8 weeks [22] and 12 weeks [23]), the lack of a significant effect of carnitine in these studies cannot be explained by an inadequate treatment duration. However, it is worth mentioning that in contrast to the studies of González-Ortiz et al. [25] and Liang et al. [23], which did not find any effect of 3 g oral carnitine on either fasting plasma glucose, HbA1c, serum insulin, and/or glucose disposal constant, Bloomer et al. [22] observed at least a slight improvement of glucose tolerance in response to 1.35 g oral ALC, as evidenced by minor decreases in blood glucose, insulin, and HbA1c. A too low carnitine dosage as argued by Liang et al. [23], which used 3 g oral carnitine, also cannot explain the failure of carnitine treatment considering that glucose tolerance was improved by carnitine even at lower dosages in three other studies (2 g oral carnitine) [19, 20, 26]. Great differences in the basal carnitine intake and the intake of certain micronutrients (vitamin C, vitamin B_6, and iron), which required as co-factors for endogenous carnitine synthesis, from the diet between subjects from studies with no carnitine effect and those with a positive outcome are also unlikely to be responsible for the different outcome, because in three studies with no carnitine effect, the subjects were not restricted in their dietary habits and maintained on their normal omnivorous diet. Moreover, regarding that different carnitine supplementation studies revealed either no effect or a positive impact on glucose tolerance in subjects with the same metabolic disorder (e.g., type 2 diabetes), indicates that the influence of carnitine supplementation is not dependent on the type of metabolic disorder of the study subjects. However, one issue that is probably of great importance when trying to explain differences in the outcome between studies is whether the carnitine status of the subjects was within or below the physiologic range (normal serum carnitine concentration: 25–50 μmol/L) before starting with supplementation. This question, however, cannot be adequately answered because, with the exception of only one study [24], all other studies did not report serum carnitine concentrations of the study subjects. Therefore, further carnitine supplementation studies, in which plasma carnitine concentrations of the study subjects before and after the supplementation period are taken into account, should help to resolve the inconsistent observations from different studies.

Human studies with healthy subjects

Only six human studies with healthy subjects reporting an effect of carnitine supplementation on parameters of glucose tolerance and insulin sensitivity were identified using the PubMed database (same search criteria as for subjects with metabolic disorders; Table 2). Five of these studies showed an improvement of parameters of glucose tolerance in response to carnitine supplementation, whereas the remaining study did not reveal an improved glucose tolerance as evidenced by unaltered fasting glucose and fasting insulin levels [24]. The lack of effect of i.v. carnitine supplementation on glucose tolerance in this study probably cannot be attributed to an insufficient carnitine dosage because in another study with a positive outcome [27], the i.v. carnitine dosage was markedly lower (45 vs. 66 μg/kg body weight; [27] and [24], resp.). Other reasons such as differences in the diet composition (carnitine concentration, micronutrients such as vitamin C, vitamin B_6, and iron) between subjects of this study and those with a positive outcome could not be evaluated because the diet of the subjects was not reported [24]. Even though the reason for the lack of a carnitine effect in the above-mentioned study remains a matter of speculation, the importance of this study should not be overstated considering the very small number of subjects included ($n = 4$). In the studies reporting an improved

Table 2 Effect of carnitine supplementation on glucose tolerance in human studies with healthy subjects

Study design	Subjects and characteristics	Diet	Dosage and formulation of carnitine/day	Route of application	Treatment duration	Carnitine effects on glucose tolerance	Overall change in glucose tolerance	Refs.
RCT, single-blind	8 lean, male participants subjects studied twice, aged 19–36 years	No changes in dietary intake	3 g LC-tartrate	Oral	2 weeks	$AUC_{GLC}\downarrow$ $AUC_{INS}\leftrightarrow$ HOMA-IR$\leftrightarrow$	↑	[21]
RCT	20 healthy volunteers (11M/9F), aged 39.3–55.3 years BW 59–77.3 kg, each subject studied twice	Weight-maintaining, consisting of 250 g carbohydrates/day for 1 week before the study	45 μg/kg BW/min LC	i.v.	3 h	M↑	↑	[27]
Cross-over design	47 healthy volunteers	n.m.	6 g LC	i.v.	3 h	$GLC_{plasma}\downarrow$ $INS_{plasma}\leftrightarrow$	↑	[29]
RCT	2 parallel studies of 40 and 20 healthy volunteers	n.m.	4 g and 2 g LC, resp.	i.v.	n.m.	$GLC_{plasma}\downarrow$ $INS_{plasma}\leftrightarrow$	↑	[30]
RCT	14 healthy volunteers (7M/7F), aged 33.8 ± 10.9 years, BMI 23.6 ± 2.7 kg/m^2, each subject studied twice	Standard composition (55% carbohydrates, 30% lipids, 15% proteins) ad libitum, with at least 250 g carbohydrates/day	80 mg LC/g glucose	i.v.	One bolus	$AUC_{GLC}\downarrow$ ISI$\leftrightarrow$	↑	[28]
Case–control study	4 healthy volunteers	n.m.	66 μg/kg BW/min LC for 20 min and 33 μg/kg BW/min LC	i.v.	180 min	$GLC_{fasting}\leftrightarrow$ $INS_{fasting}\leftrightarrow$ GOX$\leftrightarrow$	↔	[24]

n.m. not mentioned, *RCT* randomized controlled trial, *M/F* male/female, *BW* body weight, *BMI* body mass index, GLC_{plasma} plasma glucose, $GLC_{fasting}$ fasting plasma glucose, INS_{plasma} plasma insulin, $INS_{fasting}$ fasting plasma insulin, *IFG* impaired fasting glucose, AUC_{GLC} area under the curve for glucose, AUC_{INS} area under the curve for insulin, *i.v.* intravenous, *HOMA-IR* homeostasis model assessment of insulin resistance, =fasting plasma insulin × fasting serum glucose/405, *ISI* insulin sensitivity index, *M* glucose tissue uptake rate at steady state (mg/min × kg), *GOX* glucose oxidation rate, *LC* L-carnitine

glucose tolerance in healthy subjects [21, 27–30], the beneficial response to carnitine supplementation occurred independent of the route of carnitine administration (i.v. vs. oral), the treatment duration, the carnitine formulation (carnitine vs. carnitine-tartrate), and the carnitine dosage, which varied between 3 g oral carnitine-tartrate and 2–6 g i.v. carnitine. Due to the lack of appropriate titration studies testing the effect of carnitine at different dosages, future studies are required to establish the minimum effective carnitine dosage in healthy subjects. As mentioned above, these future studies should also consider the carnitine status of the subjects before and after the supplementation.

Interestingly, in one of the studies with a positive outcome [21], the effect of 3 g oral carnitine-tartrate supplementation was directly compared in both healthy (normal weight) and overweight/obese subjects to elucidate whether the response of glucose tolerance is different between these two groups [21]. This study indeed revealed that carnitine-tartrate reduced the AUC_{GLC} following an oral glucose tolerance test only in the normal-weight subjects, but not in the overweight/obese subjects indicating that carnitine supplementation is not useful in subjects with metabolic disorders. However, considering the great number of studies [27, 31–33] reporting beneficial effects of carnitine supplementation in obese or diabetic subjects, it cannot be deduced from the study of Galloway et al. [21] that the effect of carnitine on glucose tolerance is dependent on the health/metabolic status. Therefore, future studies are necessary to clarify these inconsistencies.

To summarize, the majority of human studies dealing with carnitine supplementation in either healthy subjects or subjects with metabolic disorders shows an improvement of

Table 3 Effect of carnitine supplementation on glucose tolerance in different animal species (in vivo)

Species	Study design	Daily dosage and formulation of carnitine	Route of application	Treatment duration	Carnitine effects on glucose tolerance	Overall change in glucose tolerance	Refs.
Mouse	3 experiments: 1. obese diabetic transgenic mouse model (BAP agouti) with insulin resistance ($n = 15$/group) 2. out-bred mice on a Black Swiss/129 background with glucose intolerance fed a diet with moderate fat content ($n = 8$/group) 3. C57BL/6J mice fed a high-fat diet for 8 weeks ($n = 20$/group) each mouse strain (±LC)	0.5% LC of diet	Oral	3–6 weeks washout 1 week (exp. 1) 1 week (exp. 2) 8 weeks (exp. 3)	$GLC_{fasting}\downarrow$	↑	[35]
Mouse	KK-A^y mice with DM-2	30 mmol LC as Zn-complex	Oral	2 weeks	$GLC_{fasting}\leftrightarrow\downarrow$ $AUC_{GLC}\downarrow$	↑	[42]
Rat	OZR and LZR ($n = 10$/group) LZR (±PLC), OZR (±PLC); $n = 5$/group	200 mg PLC/kg BW	Oral	20 weeks	*OZR*: $GLC_{fasting}\leftrightarrow$ $AUC_{GLC}\downarrow$ $INS_{fasting}\downarrow$ HOMA-IR↓	↑	[38]
Mouse	Female mice WT (±LC), L-SACC1 (±LC); $n = 5$–9/group	1.5 g LC/kg BW	i.p.	1 week	*L-SACC1*: $INS_{fasting}\downarrow$ *WT*: $INS_{fasting}\leftrightarrow$	↑	[36]
Rat	Adult male Wistar rats Control (±LC), high fructose (±LC); $n = 6$/group	300 mg LC/kg BW	i.p.	9 weeks	*High fructose*: $GLC_{fasting}\downarrow$ *Control*: $GLC_{fasting}\leftrightarrow$	↑	[40]
Rat	Adult male Wistar rats Control (±LC), high fructose (±LC); $n = 6$/group	300 mg LC/kg BW	i.p.	4 weeks	*High fructose*: $GLC_{fasting}\downarrow$ $INS_{fasting}\downarrow$ HOMA-IR↓ $AUC_{GLC}\downarrow$ $AUC_{INS}\downarrow$	↑	[39]
Rat	Control (± LC), STZ-D (± LC)	3 g LC/kg BW	i.p.	6 weeks	*STZ-D*: $GLC_{plasma}\downarrow$	↑	[37]
Rat	Control (±LC), STZ-D (±LC)	3 g LC/kg BW	i.p.	2 weeks	*STZ-D*: $GLC_{fasting}\downarrow$	↑	[34]
Rat	Control (±LC), high fructose (±LC); $n = 6$/group	300 mg LC/kg BW	i.p.	3 weeks	*High fructose*: $GLC_{fasting}\downarrow$ $INS_{fasting}\downarrow$ HOMA-IR↔	↑	[41]

AUC_{GLC} area under the curve for glucose, AUC_{INS} area under the curve for insulin, *BW* body weight, *i.p.* intraperitoneal, *HOMA-IR* homeostasis model assessment of insulin resistance, =fasting plasma insulin × fasting serum glucose/405, $GLC_{fasting}$ fasting plasma glucose, $INS_{fasting}$ fasting plasma insulin, *STZ-D* streptozotocin-induced diabetic, *LC* L-carnitine, *PLC* propionyl-L-carnitine, *DM-2* type 2 diabetes mellitus, *OZR* obese Zucker rat, *LZR* lean Zucker rat, *WT* wild type

glucose tolerance and/or insulin sensitivity. Nevertheless, inconsistent observations with regard to the effect of carnitine in subjects with similar metabolic disorders or dietary habits require further well-designed clinical studies, which have to take into account the carnitine status before and after the supplementation period. The fact that none of the studies considered carnitine supplementation caused an impairment of glucose tolerance or caused other adverse effects indicates that safety concerns with carnitine supplementation are unfounded.

Animal studies

In vivo studies

Nine animal studies reporting an effect of carnitine supplementation on parameters of glucose tolerance and insulin sensitivity could be considered for this review using PubMed database (key words used in different combinations were as follows: L-carnitine, carnitine supplementation, carnitine infusion, glucose metabolism, insulin resistance, animal study; time frame: no limitation). In Table 3, an overview is given about the most important characteristics of these animal experiments (species, design, carnitine dose and formulation, treatment duration, outcome), which evaluated the effect of either i.p. or oral supplementation of carnitine or its derivative propionyl-L-carnitine (PLC) on parameters associated with glucose homeostasis [34–41]. In one study, which evaluated anti-diabetic and insulinomimetic effects of Zn(II) complexes, carnitine was administrated orally as a Zn(II) complex [42]. It is obvious from Table 3 that, in contrast to the human studies, all animal studies showed an improvement of glucose tolerance in response to carnitine supplementation, as evidenced by reduced fasting glucose and fasting insulin levels and/or reduced AUC_{GLC} and AUC_{INS} on glucose tolerance test [34–42]. Differences between studies with respect to the route of carnitine administration (i.p. vs. oral), the treatment duration (ranging between 1 and 20 weeks), the animal model (rat vs. mouse), and the carnitine formulation (carnitine vs. PLC) had no effect on the outcome. However, it could be clearly shown that the effect of carnitine and PLC, respectively, on glucose tolerance was dependent on the insulin status of the animals. Only in diabetic rats, induced either by fructose [39–41] or high-fat diet feeding [35], streptozotocin injection [34, 37] or genetically as in the Zucker rat [38], the BAP agouti mouse [35], or the KK-A^y mouse [42], carnitine supplementation reduced fasting glucose and/or insulin levels. In one study [40], in which also non-diabetic rats were used, i.p. carnitine administration at a dosage of 300 mg/kg body weight failed to exhibit an effect on glucose tolerance. Similarly, i.p. carnitine administration at a high dosage of 1.5 g/kg body weight improved insulin sensitivity only in insulin-resistant liver-specific CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1)-deficient mice but not in wild-type mice [36]. One reason for the different response of diabetic and non-diabetic animals to carnitine supplementation might be that the diabetic state leads to a reduction of plasma and tissue carnitine concentrations [18], which is restored to normal levels by carnitine supplementation, whereas in the non-diabetic animals, the carnitine levels in plasma and tissues are within the normal range even without supplementation.

To sum up, the results from studies with experimental animals consistently demonstrate that carnitine supplementation improves glucose tolerance during insulin-resistant states, such as diabetes or obesity.

Studies with isolated rat hearts

A total of ten studies evaluating an effect of carnitine [43–46] or PLC [47–52] on glucose utilization in the perfused isolated working rat heart were identified via PubMed database search (key words used in different combinations were as follows: L-carnitine, carnitine supplementation, glucose metabolism, isolated rat heart; time frame: no limitation). These studies and their experimental design are summarized in Table 4. The heart was of special interest in these studies because myocardial dysfunction, such as diastolic dysfunction and impairment of relaxation, is typically associated with diabetes and pressure-overload myocardial hypertrophy. Under normal conditions, fatty acid oxidation contributes to 60–80% of total ATP production in the myocardium, whereas the remaining ATP is generated from oxidation of glucose and lactate [53]. In contrast, in the diabetic heart, ATP production is almost completely derived from fatty acid oxidation [54], and this increase in fatty acid oxidation and the parallel decrease in glucose oxidation have been made responsible for the development of heart dysfunction in diabetes [55]. Therefore, therapeutic strategies aiming to overcome the inhibitory effect of fatty acids on glucose metabolism in the heart are of benefit in diabetic animals [45, 54, 56]. Noteworthy, the above-mentioned studies with isolated working rat hearts consistently demonstrated that carnitine perfusion increased glucose oxidation and its contribution to total ATP production and improved cardiac function, irrespective of the rat model used (healthy, ischemic, diabetic, carnitine-deficient, or pressure overload-induced heart hypertrophy).

Collectively, the results from studies with isolated working rat hearts consistently demonstrate that carnitine perfusion stimulates glucose oxidation and improves cardiac function.

Effect of carnitine deficiency on glucose homeostasis and insulin sensitivity

Human and animal studies with carnitine deficiency

A total of ten studies were considered as suitable to evaluate the relationship between carnitine deficiency and glucose tolerance in humans and animals (key words used in different combinations were as follows: carnitine deficiency, glucose metabolism, valproic acid, JVS mice, pivalate, diabetes, mildronate; time frame: no limitation). As shown in Table 5, only four of these studies revealed that carnitine deficiency is associated with an impaired

Table 4 Effect of carnitine supplementation on glucose utilization in isolated rat hearts (in vitro)

Isolated heart model	Feeding period	Dose and formulation of carnitine in perfusion buffer	Perfusion duration	Carnitine effects on glucose utilization	Refs.
Ischemic diabetic hearts (streptozotocin-induced)	–	10 mmol/L LC	2.5 h	*Control*: GOX↑ *STZ-D*: GOX↑ Glycolysis rates↑	[45]
Ischemic hearts	–	10 mmol/L LC	1 h	GOX↑ Glycolysis rates↔	[44]
Intact hearts	–	10 mmol/L LC + palmitate	1 h	GOX↑ Glycolysis rates↑	[43]
Intact hearts	–	5 mmol/L LC	1 h	GOX↑ Glycolysis rates↑	[46]
Hypertrophied hearts	–	1 mmol/L PLC	1 h	GOX ↑ Glycolysis rate↔	[47]
Hypertrophied hearts	8 weeks, 60 mg PLC/kg BW and day via drinking water	–	45 min	*Control*: GOX↑ Glycolysis rate↔	[48]
Ischemic diabetic hearts (streptozotocin-induced)	6 weeks, 1 g PLC/L via drinking water ad libitum	–	30 min	*STZ-D*: GLC_{plasma}↔ GOX↑	[49]
Diabetic heart (streptozotocin-induced)	6 weeks, 1 g PLC/l via drinking water ad libitum	–	30 min	*STZ-D*: GOX↑	[51]
Reperfused diabetic heart (streptozotocin-induced)	6 weeks, 1 g PLC/l via drinking water ad libitum	–	30 min	*STZ-D*: GLC_{plasma}↓ GOX↑	[52]
Diabetic heart (streptozotocin-induced)	6 weeks, 1 g PLC/l via drinking water ad libitum	–	60 min	*STZ-D*: GLC_{plasma}↔ GOX↑	[50]

BW body weight, *STZ-D* streptozotocin-induced diabetic, *LC* L-carnitine, *PLC* propionyl-L-carnitine, GLC_{plasma} plasma glucose, *GOX* glucose oxidation rate

glucose tolerance [18, 57–59], whereas the remaining six studies could not establish this association [60–65].

In humans, secondary carnitine deficiency is observed during treatment with valproic acid [66], a broad-spectrum anti-epileptic drug that is now used commonly for several other neurological and psychiatric indications. Valproic acid contributes to carnitine deficiency through decreasing the concentration of α-ketoglutarate that is required for de novo biosynthesis of carnitine. Evidence exist that serious complications occurring in some patients receiving valproic acid chronically, such as hepatotoxicity and hyperammonemic encephalopathy, are promoted by carnitine deficiency [67], wherefore carnitine supplementation during valproic acid therapy in high risk patients, such as children, is generally recommended. In one study in 23 psychiatric patients [59] with chronic valproic acid treatment and documented hypocarnitinemia, increased fasting plasma glucose levels were reported indicating that an impaired carnitine status contributes to glucose intolerance in humans.

In two studies [57, 60], the juvenile visceral steatosis (JVS) mouse was used as a model for carnitine deficiency. JVS mice develop severe systemic carnitine deficiency due to a mutation in the gene encoding the novel organic cation transporter 2 (OCTN2), which operates on renal reabsorption, intestinal absorption, and tissue distribution of carnitine [68]. Using this genetic carnitine deficiency model, it was shown that glucose and insulin homeostasis is significantly disturbed during carnitine deficiency, as evidenced by hyperinsulinemia, and that carnitine administration restores these perturbations [57]. In contrast, no effect on parameters of glucose (plasma glucose) and insulin homeostasis was observed in another study using the JVS mouse model of carnitine deficiency [60]. One important reason that may explain the different outcomes between these two studies might be that in the latter study [60] plasma glucose was

Table 5 Effect of carnitine deficiency on glucose tolerance in humans and different animal models

Species	Type of carnitine deficiency	Study design	Effects of carnitine deficiency on glucose tolerance	Overall change in glucose tolerance	Refs.
Human	SCD induced by valproic acid	23 psychiatric patients	$GLC_{fasting}\uparrow$	↓	[59]
Mouse	SCD induced by high-fat diet feeding	Feeding either a standard diet or high-fat diet ad libitum for 10 weeks	$AUC_{GLC}\uparrow$	↓	[58]
Mouse	JVS mouse model of PCD	Control (+/+) versus JVS (JVS/JVS)	$GLC_{fasting}\downarrow$ $INS_{fasting}\uparrow$	↓	[57]
Mouse	JVS mouse model of PCD	Control (+/+) versus JVS (JVS/JVS) under fed and fasting conditions/	$GLC_{fasting}\leftrightarrow$	↔	[60]
Rat	SCD induced by high-fat diet feeding	Feeding either a standard or high-fat diet for 12 months	$GLC_{fasting}\uparrow$ $INS_{fasting}\uparrow$ HOMA-IR↑	↓	[18]
Rat	SCD induced by sodium pivalate	Drinking water with either sodium pivalate or not for 28 weeks afterward hearts were excised and perfused	$GLC_{fasting}\leftrightarrow$ GOX↔	↔	[62]
Rat	SCD induced by sodium pivalate	Drinking water with either sodium pivalate or not for 8 weeks	$GLC_{fasting}\leftrightarrow$	↔	[61]
Mouse	SCD induced by mildronate	Mildronate (i.p., 200 mg/kg BW) daily for 20 days	$GLC_{fed}\downarrow$	↑	[63]
Rat	SCD induced by mildronate	Mildronate (p.o., 100 and 200 mg/kg BW) daily for 8 weeks	$GLC_{fasting}\downarrow$ $GLC_{fed}\downarrow$	↑	[64]
Rat	SCD induced by mildronate	Mildronate (p.o., 200 mg/kg BW) daily for 4 weeks	$GLC_{fasting}\downarrow$ $GLC_{fed}\downarrow$	↑	[65]

BW body weight, *JVS* juvenile visceral steatosis, *PCD* primary carnitine deficiency, *SCD* secondary carnitine deficiency, AUC_{GLC} area under the curve for glucose, *HOMA-IR* homeostasis model assessment of insulin resistance, =fasting plasma insulin × fasting serum glucose/405, $GLC_{fasting}$ fasting plasma glucose, GLC_{fed} feeding plasma glucose, $INS_{fasting}$ fasting plasma insulin, *GOX* glucose oxidation rate

determined in the fed state, but in the fasted state in the other study [57].

In two other studies [61, 62], pivalate was administrated to rats to induce carnitine deficiency. Pivalate administration causes induction of secondary carnitine deficiency due to excessive loss of carnitine via the urine through formation of pivaloylcarnitine [69]. In both studies using the pivalate model of carnitine deficiency, glucose tolerance was not significantly impaired by pivalate treatment. At least in one of the two studies, the plasma glucose concentration was numerically lowered (about 20%) by pivalate treatment [61]. Probably, this effect was not significant due to the little number of animals (four in the control group and six in the pivalate group) used in this study. In the other study [62], however, the pivalate-induced carnitine deficiency had not any impact on parameters of glucose tolerance. Since pivalate administration caused severe carnitine deficiency in rats of both studies as evidenced by a marked reduction in plasma free carnitine concentration (from 35 to <10 μmol/L after 1 week of pivalate administration), the lack of a significant effect cannot be attributed to an inadequate impairment of the carnitine status [61]. Future studies with a greater number of animals should help to clarify the inconsistent observations from studies using the pivalate model of carnitine deficiency.

The authors of two further studies investigating the relationship between carnitine deficiency and glucose tolerance [18, 58] used a high-fat diet to induce carnitine deficiency. Long-term feeding of a high-fat diet impairs whole body carnitine status due to compromising the capacity of the liver to synthesize and take up carnitine. As the underlying mechanism of high-fat diet administration, reduction of transcript levels of genes responsible for carnitine synthesis, such as BBD and TMABA-DH, and carnitine uptake (OCTNs) has been identified [18]. It has been postulated that the reduced transcript levels of the respective genes is due to disruption of peroxisome proliferator-activated receptor α (PPARα) function in response to high-fat diet administration, because PPARα has been identified as a critical transcriptional regulator of BBD, TMABA-DH, and OCTN2 in mice, rats, and pigs [70–76]. Using the high-fat diet-induced model of carnitine deficiency, Noland et al. [18] clearly showed that carnitine insufficiency contributes to mitochondrial dysfunction and insulin resistance. The same relationship could be observed in several rat models of genetic obesity [18]. Importantly, oral carnitine supplementation reversed these perturbations in concert with an

improved glucose tolerance [18] underscoring the beneficial role of carnitine for glucose tolerance. A more recent study from our own group confirmed the findings from Noland et al. [18] in that we also observed that feeding a high-fat diet to sedentary mice reduced total carnitine status (plasma: from 19.5 to 12.9 μmol/L; liver: from 150 to 122 nmol/g) and expression of BBD, TMABA-DH, and OCTN2 in the liver [58]. Interestingly, when mice on the high-fat diet were regularly trained on a motorized treadmill (35 min, 5 times a week) for 10 weeks, the impaired carnitine status (reduced liver carnitine concentration) could be completely restored to normal level found in sedentary mice fed a standard diet [58]. On the molecular level, we found that regular endurance exercise caused a significant increase in the hepatic expression of BBD, TMABA-DH, and OCTN2 in mice fed the high-fat diet indicating that endurance exercise is capable of completely reversing the high-fat diet-induced impairment of hepatic carnitine status by stimulating carnitine synthesis and uptake.

Another approach to induce carnitine deficiency is administration of the pharmacological agent mildronate, which reduces carnitine levels in plasma and heart via inhibiting BBD and OCTN2. Mildronate is a cardioprotective drug whose mechanism of action is based on reducing the availability of carnitine [77]. In sharp contrast to the abovementioned studies that reported either no effect or an impairment of glucose tolerance in response to carnitine deficiency, three studies from the same group demonstrated in different animal models (ICR mice [63], Goto-Kakizaki rats [64], obese Zucker rats [65]) that mildronate, by reducing plasma and heart carnitine concentrations, lowers plasma glucose and insulin concentration and/or protects against diabetic complications. This surprising effect of mildronate-induced carnitine deficiency on glucose tolerance has been explained by a partial inhibition of fatty acid oxidation through reduction of CPT I-dependent long-chain fatty acid import into the mitochondria [63, 78–80] and a concomitant increase in glucose uptake and oxidation in the heart [64, 65]. Based on their results and taking into account the reported beneficial effects of carnitine supplementation in animal models of diabetes, Liepinsh et al. [64] concluded that both mildronate-induced reduction in carnitine availability and pharmacological supplementation with carnitine can stimulate glucose metabolism and that both of these treatments could be effective for diabetes therapy. Future studies are necessary to explain the contrasting observations that carnitine deficiency induced by mildronate improves glucose tolerance whereas carnitine deficiency in JVS mice is accompanied by an impaired glucose tolerance [57].

Approaches to cause a moderate carnitine deficiency, such as feeding a lysine deficient diet, have not been studied in the context of glucose homeostasis. However, since feeding a lysine deficient diet causes only minor decreases of carnitine levels in plasma, heart, and skeletal muscle [81], it is questionable whether feeding a lysine deficient diet has an impact on glucose tolerance.

To summarize, results from animal studies investigating the possible association between carnitine deficiency and glucose intolerance are conflicting. Only four studies revealed a direct relationship between carnitine deficiency and glucose intolerance, whereas the majority of studies dealing with this question could not establish this relationship. Further studies are necessary to explain the apparently conflicting observation that both carnitine deficiency induced by mildronate and pharmacological supplementation with carnitine can stimulate glucose metabolism.

Carnitine status in humans with diabetes

Since mechanistic studies in experimental animals point toward a causative role for carnitine deficiency in the development of insulin resistance [18, 82], we carried out a PubMed search for studies reporting on carnitine concentrations in diabetic subjects. According to this, eight studies could be identified (key words used in different combinations were as follows: free carnitine, carnitine status, diabetes, humans; time frame: no limitation). With the exception of two studies [83, 84], all other studies considered reported that diabetic subjects have reduced plasma free carnitine concentrations [[85–90], Table 6]. For instance, in one study with adult diabetic patients with pronounced hyperglycemia, approximately 40% lower plasma free carnitine concentrations were observed when compared to healthy subjects [85]. Similarly, markedly reduced serum carnitine levels (<20 μmol/L) were observed in children with type 1 diabetes mellitus [86]. Moreover, reduced serum carnitine levels were also reported for type 2 diabetic women with hyperglycemia and diabetic complications (retinopathy, hyperlipidemia, and neuropathy) compared to type 2 diabetic women without complications (39.6 μmol/L vs. 53.4 μmol/L serum free carnitine) [87]. Although it is not possible to draw any conclusions from these human studies with diabetic subjects on a causal relationship between an impaired carnitine status and diabetes development, these observations provide at least indication for an association between impaired carnitine status and glucose intolerance. However, regarding that plasma free carnitine concentrations were still within or only slightly below the physiological range reported for healthy subjects (25–50 μmol/L), it cannot be deduced from these studies that diabetes is associated with carnitine deficiency.

Mechanisms of action of carnitine on glucose metabolism

Several mechanisms of action of carnitine have been proposed to be causative for its beneficial effect on glucose tolerance

and insulin sensitivity. One important mechanism by which carnitine improves insulin sensitivity represents enhancement of mitochondrial oxidation of long-chain acyl-CoAs. Accumulation of long-chain acyl-CoAs and other fatty acid metabolites impair insulin signaling and therefore contribute to the development of insulin resistance in skeletal muscle and heart. Since this effect of carnitine is well documented, the reader is referred to the literature with regard to this [14, 91, 92]. Other mechanisms of action of carnitine on glucose homeostasis and insulin sensitivity that will be discussed in this chapter include (1) modulating the intramitochondrial acetyl-CoA/CoA ratio and the activity of the pyruvate dehydrogenase complex (PDHC), (2) altering expression of glycolytic and gluconeogenic enzymes, (3) altering expression of genes of the insulin signaling cascade, and (4) stimulation of the IGF-1 axis and IGF-1 signaling cascade. The mechanisms of carnitine in improving glucose utilization in the heart (5) and the specific role of PLC (6) in this regard will be discussed separately at the end of this chapter.

Modulating the intramitochondrial acetyl-CoA/CoA ratio and the activity of the PDHC

A large body of evidence suggests that carnitine and its derivatives ALC and PLC enhance glucose utilization by stimulating the activity of PDHC [93], which is a key enzymatic complex in glucose oxidation, because intramitochondrial acetyl-CoA can be converted with carnitine into ALC via the carnitine acetyltransferase that is then transported out of the mitochondria. Therefore, carnitine strongly reduces intramitochondrial acetyl-CoA levels resulting in a 10- to 20-fold decrease in the acetyl-CoA/CoA ratio [43, 94, 95]. This mechanism of action of carnitine and ALC is supposed to be responsible for the enhancement of glucose utilization in both healthy and type 2 diabetic subjects [27, 28, 33, 96]. However, Mingrone et al. [27] suggested that stimulation of oxidative glucose utilization following carnitine infusion occurs only in diabetic patients, in which PDHC activity is depressed and thus restored to normal by carnitine administration. In contrast, in healthy subjects where PDHC activity is normal, the increased glucose uptake following carnitine administration is supposed to cause glycogen accumulation in the cells [27]. The latter assumption from Mingrone et al. [27] is supported by findings from Stephens et al. [97], which also observed that i.v. carnitine administration during euglycemic hyperinsulinemic clamp (EHC) conditions in healthy young men elevated muscle glucose uptake and non-oxidative glucose disposal (as indicated by an elevated muscle glycogen content) but inhibited glucose oxidation at the level of PDHC, probably due to a carnitine-mediated increase in fatty acid oxidation. Evidence for a preferred use of fatty acids and a decreased use of glucose as energy source in carnitine-supplemented subjects was also provided from another study with healthy young male adults [98]. Moreover, indications for a stimulation of non-

Table 6 Human studies reporting on the carnitine status in diabetic subjects

Design	Carnitine status (plasma free carnitine)	Refs.
Diabetic patients versus healthy volunteers (control)	Control: 35 μmol/L	[85]
	Diabetic patients: 21 μmol/L ($P < 0.05$ vs. control)	
Type 1 diabetic children versus control	Reduced ($P < 0.05$ vs. control)	[86]
Type 2 diabetic women		[87]
Without (control; $n = 18$)	Control: 53.4 μmol/L	
With retinopathy	Retinopathy: 39.0 μmol/L ($P < 0.05$ vs. control)	
With hyperlipidemia	Hyperlipidemia: 39.6 μmol/L ($P < 0.05$ vs. control)	
With neuropathy	Neuropathy: 40.4 μmol/L ($P < 0.05$ vs. control)	
Type 2 diabetic patients		[88]
Without complications (control, $n = 15$)	Control: 58.1 μmol/L	
With retinopathy ($n = 20$)	Retinopathy: 34.2 μmol/L ($P < 0.05$ vs. control)	
With hyperlipidemia ($n = 13$)	Hyperlipidemia: 34.9 μmol/L ($P < 0.05$ vs. control)	
With polyneuropathy ($n = 20$)	Polyneuropathy: 35.7 μmol/L ($P < 0.05$ vs. control)	
Type 1 diabetic patients versus healthy controls	Reduced ($P < 0.05$ vs. control)	[89]
Type 2 diabetic patients	Not different from control	[83]
Type 1 diabetic subjects versus normal subjects (controls)	Not different from control (48.2 vs. 48.7 μmol/L)	[84]
Diabetic children		[90]
Without ketosis (control)	Control: 50.0 μmol/L	
With ketosis	With ketosis: 29.7 μmol/L	
With ketoacidosis	With ketoacidosis; 24.6 μmol/L	

oxidative glucose disposal in response to i.v. carnitine during an EHC in healthy adults were also provided from another group [99]. An important difference between studies reporting a stimulation of muscle PDHC activity following carnitine administration and the study of Stephens et al. [97] is that in the latter study, a combined infusion regime with both carnitine and insulin was applied to increase muscle carnitine content. According to a recent study from the same group [100], i.v. carnitine administration for 5 h failed to increase muscle carnitine content in healthy subjects, even though plasma carnitine concentration was maintained at a supra-physiological concentration of about 500 μmol/L by carnitine infusion. This observation casts the contention into doubt that oral or i.v. carnitine supplementation can increase muscle carnitine content in healthy subjects. Stephens et al. [100] clearly showed that the muscle carnitine content could be elevated only when the carnitine infusion-mediated hypercarnitemia was maintained in the presence of hyperinsulinemia. Based on the findings of Stephens et al. [100], a mechanism involving hyperinsulinemia-induced stimulation of OCTN2 gene transcription in skeletal muscle that is secondary to the action of insulin on increasing sarcolemmal Na^+/K^+-ATPase pump activity, and thus intracellular Na^+ flux [101], is responsible for the elevation of muscle carnitine content. To sum up, modulation of PDHC activity is probably an important mechanisms through which carnitine exerts an effect on whole body glucose homeostasis. However, whether carnitine has a stimulatory or inhibitory effect on PDHC activity is probably dependent on several factors including the metabolic and health status (e.g., normo-insulinemic vs. hyperinsulinemic) of the subjects.

Altering expression of glycolytic and gluconeogenic enzymes

A further mechanism explaining improvement of glucose tolerance with carnitine supplementation is modulation of expression of glycolytic and gluconeogenic enzymes. Ruggenenti et al. [102] suggested from their findings in insulin-resistant type 2 diabetic patients that oral ALC supplementation corrects an inappropriate shift in substrate use from carbohydrates to lipids, which is common in type 2 diabetic patients [103], through modulating the expression of glycolytic and gluconeogenic enzymes. Indeed, an earlier study from Hotta et al. [57] using the JVS mouse model of primary carnitine deficiency revealed that hepatic transcript levels of glycolytic enzymes such as glucokinase and pyruvate kinase are reduced, whereas hepatic transcript level of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PCK1) is increased in this genetic model of carnitine deficiency. Since carnitine administration restored these alterations in gene expression to normal levels [57], these findings are indeed supportive of an effect of carnitine on expression of genes involved in glucose metabolism. Further support for this assumption is given by a very recent study of our group using whole genome DNA microarray analysis to explore gene regulatory effects of carnitine in the liver of pigs [104]. This study revealed that carnitine supplementation increased expression of genes involved in glucose transport (e.g., GLUT8), conversion of glucose into glucose 6-phosphate (hexokinase D), and glycolysis (e.g., glycerol-3-phosphate dehydrogenase) and leads to increased glucose oxidation. The DNA microarray analysis further revealed that genes involved in gluconeogenesis (e.g., PCK1, FBP2) were down-regulated in pig liver by carnitine supplementation [104]. This indicates that suppression of hepatic gluconeogenesis also contributes to the positive effect of carnitine on glucose utilization. In line with this, a recent study in rats showed that carnitine is capable of restoring an increase in the activity of the gluconeogenic enzymes PCK1 and fructose-1,6-bisphosphatase isozyme 2 (FBP2) by feeding fructose [105], which increases the availability of the gluconeogenic substrates pyruvate, lactate, and glycerol.

Altering expression of genes of the insulin signaling cascade

It has been suggested recently that the inhibitory effect of carnitine on gluconeogenic enzyme activities is the consequence of an improvement in the action of insulin [27, 106], which is a known repressor of expression of gluconeogenic genes. In line with this, the abovementioned DNA microarray analysis in liver of pigs [104] revealed that carnitine supplementation alters the expression of genes involved in the insulin signaling cascade, like insulin receptor substrate-2, phosphatidylinositol 3-kinase regulatory alpha subunit, and receptor protein-tyrosine kinase erbB-3 precursor [104]. In addition, Galloway et al. [21] proposed a direct insulin-like effect of carnitine, based on their findings that oral carnitine administration caused an early and low peak glucose concentration in lean subjects during an oral glucose tolerance test. However, the same authors also suggested that carnitine supplementation enhances insulin secretion through maintenance of glucose or fatty acid oxidation in the pancreatic β-cell by buffering acyl groups or through generation of glutamate for maintenance of ATP/ADP ratio [107]. The role of carnitine as a stimulator of insulin action is confirmed by the observation that carnitine administration in healthy volunteers leads to a stimulation of glucose disposal and oxidation [28]. These authors suggested that carnitine could exert its action on glucose metabolism at the insulin receptor level, by increasing transmembrane glucose transport or at the post-receptor level, namely the insulin signaling cascade.

Stimulation of the IGF-1 axis and IGF-1 signaling cascade

Besides stimulating glucose metabolism through altering the insulin signaling cascade, Molfino et al. [32] suggested that carnitine may be beneficial to glucose metabolism by enhancing the regeneration of the endocrine pancreas by modulating the insulin-like growth factors (IGFs) and IGF binding proteins. This assumption was based on the observation that in streptozotocin-induced diabetic rats, liver IGF-1 mRNA expression is reduced but is restored by carnitine supplementation [108]. Support for the mechanism proposed by Molfino et al. [32] is given by results from another DNA microarray experiment performed in skeletal muscle of piglets [109]. This study showed that carnitine supplementation alters expression of genes dealing with IGF binding, such as IGF-1 receptor, and 3-phosphoinositide-dependent protein kinase-1. In agreement with this, recent studies in animals and humans revealed that carnitine influences the IGF axis by increasing plasma concentrations of IGF-1 and IGF-2 [108, 110–112]. The increase in plasma levels of especially IGF-1 is considered to be responsible for the phenomenon that carnitine increases birth weights of piglets born to sows fed carnitine [113–115], because IGF-1 is a key hormone favouring placenta development and intra-uterine nutrition [116, 117]. These findings therefore strongly suggest that carnitine activates the IGF-1 signaling pathway and that the latter is possibly linked to the improvement of glucose tolerance.

Mechanisms of carnitine in improving glucose utilization in the heart

There is convincing evidence that carnitine exerts a key role in the regulation of glucose metabolism in the heart, through binding of acetyl groups, which leads to a decrease in the intramitochondrial acetyl-CoA/CoA ratio and a stimulation of PDHC activity [45]. The physiological significance of carnitine in the heart is evident from clinical studies demonstrating that myocardial carnitine deficiency is associated with cardiomyopathy [118, 119] and that carnitine supplementation is beneficial in restoring tissue levels of carnitine and improving heart function [118, 120]. Interestingly, improvement of cardiac function of diabetic or carnitine-deficient hearts is not due to an improvement in fatty acid oxidation rates but an increase in overall glucose utilization [43–46]. The observation that myocardial fatty acid oxidation rates are not stimulated with carnitine has been explained by a mechanism involving malonyl-CoA inhibition of fatty acid oxidation in the heart [46]. The latter is explained by the increase in cytosolic acetyl-CoA in response to carnitine that in turn increases malonyl-CoA. Because malonyl-CoA is a potent allosteric inhibitor of CPT I, carnitine indirectly decreases fatty acid β-oxidation through reducing fatty acid entry into the mitochondria. In line with this, Schönekess et al. [47] observed that increasing carnitine levels in hypertrophied hearts leads to an elevation of malonyl-CoA levels but not to a stimulation of fatty acid oxidation.

Specific mechanisms of PLC in improving glucose utilization in the heart

Additional mechanisms explaining the enhancement of glucose utilization in the heart are probably unique to the carnitine derivative PLC. Among others, Broderick et al. [49] suggested that PLC modulates energy metabolism in the heart, due to its ability to act as an anaplerotic substrate with the propionyl group entering the tricarboxylic acid cycle as succinyl-CoA, which thereby stimulates the tricarboxylic acid cycle flux. The carnitine moiety released from this reaction is available as buffer for the intramitochondrial acetyl/CoA ratio that in turn could increase the PDHC activity, thereby, resulting in an improvement of glucose oxidation and insulin sensitivity in the heart. In addition, the succinyl-CoA that can enter the tricarboxylic acid cycle stimulates the utilization of acetyl-CoA derived from PDHC activity [121] Furthermore, the released carnitine moiety can also enhance fatty acid oxidation via stimulation of the carnitine shuttle system [49], thus, preventing the accumulation of lipid intermediates and improving insulin sensitivity. Collectively, these observations strongly suggest that PLC can increase both glucose and fatty acid oxidation in the heart. Interestingly, these beneficial metabolic changes induced by chronic PLC supplementation partially prevent a generalized depression in mitochondrial respiratory function of the diabetic rat heart [50]. The subsequently enhanced conversion of pyruvate to acetyl-CoA may limit the degree of lactacidosis during ischemia and stimulate glucose oxidation in the perfused heart—an effect that is supposed to be important during states of impaired glucose utilization, such as in the ischemic diabetic heart.

Conclusions and future perspectives

Carnitine supplementation studies in both humans and animals demonstrate an improvement of glucose tolerance and/or insulin sensitivity, in particular during insulin-resistant states. Moreover, the majority of human studies in diabetic subjects reported diminished plasma free carnitine concentrations, even though the levels were still within or only slightly below the physiological range reported for healthy

subjects. In contrast, less consistent results are available from animal studies investigating the association between carnitine deficiency and glucose intolerance. The majority of studies dealing with this question could either find no association or even reported that mildronate-induced carnitine deficiency lowers blood glucose, improves insulin sensitivity, and protects from diabetic complications. Further studies are therefore necessary to explain the apparently conflicting observation that carnitine supplementation but also mildronate-induced carnitine deficiency improves glucose homeostasis. In view of the abovementioned beneficial effect of carnitine supplementation on glucose tolerance during insulin-resistant states, carnitine supplementation might be an effective tool for improvement of glucose utilization in obese type 2 diabetic patients. The fact that none of the carnitine supplementation studies in humans reported any adverse effects even at very high dosages (e.g., 4 g oral carnitine) indicates that safety concerns with carnitine supplementation are unfounded. Nonetheless, more well-designed (double-blinded, randomized, placebo-controlled) and larger-scale clinical trials are required to establish the efficacy of carnitine supplementation as an adjunctive treatment for type 2 diabetes.

References

1. Rebouche CJ, Seim H (1998) Carnitine metabolism and its regulation in microorganisms and mammals. Annu Rev Nutr 18:39–61
2. Rebouche CJ, Bosch EP, Chenard CA, Schabold KJ, Nelson SE (1989) Utilization of dietary precursors for carnitine synthesis in human adults. J Nutr 119:1907–1913
3. Lombard KA, Olson AL, Nelson SE, Rebouche CJ (1989) Carnitine status of lactoovovegetarians and strict vegetarian adults and children. Am J Clin Nutr 50:301–306
4. Rebouche CJ (1992) Carnitine function and requirements during the life cycle. FASEB J 6:3379–3386
5. Vaz FM, Wanders RJ (2002) Carnitine biosynthesis in mammals. Biochem J 361:417–429
6. Davis AT (1999) Alterations in carnitine biosynthetic enzyme activities in carnitine deficient and carnitine supplemented rats. Experimental Biology'99, Washington, DC (Program addendum, abstract LB208)
7. Rebouche CJ, Chenard CA (1991) Metabolic fate of dietary carnitine in human adults: identification and quantification of urinary and fecal metabolites. J Nutr 121:539–546
8. Tamai I, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A (1998) Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. J Biol Chem 273:20378–20382
9. Tamai I, Ohashi R, Nezu JI, Sai Y, Kobayashi D, Oku A, Shimane M, Tsuji A (2000) Molecular and functional characterization of organic cation/carnitine transporter family in mice. J Biol Chem 275:40064–40072
10. Engel AG, Rebouche CJ, Wilson DM, Glasgow AM, Romshe CA, Cruse RP (1981) Primary systemic carnitine deficiency. II. Renal handling of carnitine. Neurology 31:819–825
11. Harper P, Elwin CE, Cederblad G (1988) Pharmacokinetics of intravenous and oral bolus doses of L-carnitine in healthy subjects. Eur J Clin Pharmacol 35:555–562
12. Sahajwalla CG, Helton ED, Purich ED, Hoppel CL, Cabana BE (1995) Multiple-dose pharmacokinetics and bioequivalence of L-carnitine 330-mg tablet versus 1-g chewable tablet versus enteral solution in healthy adult male volunteers. J Pharm Sci 84:627–633
13. Scaglia F, Wang Y, Longo N (1999) Functional characterization of the carnitine transporter defective in primary carnitine deficiency. Arch Biochem Biophys 364:99–106
14. Kerner J, Hoppel C (2000) Fatty acid import into mitochondria. Biochim Biophys Acta 1486:1–17
15. Mingorance C, Rodríguez-Rodríguez R, Justo ML, Alvarez de Sotomayor M, Herrera MD (2011) Critical update for the clinical use of L-carnitine analogs in cardiometabolic disorders. Vasc Health Risk Manag 7:169–176
16. Koves TR, Ussher JR, Noland RC, Slentz D, Mosedale M, Ilkayeva O, Bain J, Stevens R, Dyck JR, Newgard CB, Lopaschuk GD, Muoio DM (2008) Mitochondrial overload and incomplete fatty acid oxidation contribute to skeletal muscle insulin resistance. Cell Metab 7:45–56
17. An J, Muoio DM, Shiota M, Fujimoto Y, Cline GW, Shulman GI, Koves TR, Stevens R, Millington D, Newgard CB (2004) Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. Nat Med 10:268–274
18. Noland RC, Koves TR, Seiler SE, Lum H, Lust RM, Ilkayeva O, Stevens RD, Hegardt FG, Muoio DM (2009) Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. J Biol Chem 284:22840–22852
19. Derosa G, Maffioli P, Ferrari I, D'Angelo A, Fogari E, Palumbo I, Randazzo S, Cicero AF (2010) Orlistat and L-carnitine compared to orlistat alone on insulin resistance in obese diabetic patients. Endocr J 57:777–786
20. Derosa G, Maffioli P, Salvadeo SA, Ferrari I, Gravina A, Mereu R, D'Angelo A, Palumbo I, Randazzo S, Cicero AF (2010) Sibutramine and L-carnitine compared to sibutramine alone on insulin resistance in diabetic patients. Intern Med 49:1717–1725
21. Galloway SD, Craig TP, Cleland SJ (2011) Effects of oral L-carnitine supplementation on insulin sensitivity indices in response to glucose feeding in lean and overweight/obese males. Amino Acids 41:507–515
22. Bloomer RJ, Fisher-Wellman KH, Tucker PS (2009) Effect of oral acetyl L-carnitine arginate on resting and postprandial blood biomarkers in pre-diabetics. Nutr Metab (Lond) 6:25
23. Liang Y, Yanbing L, Shan J, Yu B, Ho Z (1998) The effects of oral L-carnitine treatment on blood lipid metabolism and the body fat content in the diabetic patient. Asia Pac J Clin Nutr 7:192–195
24. Bowyer BA, Fleming CR, Haymond MW, Miles JM (1989) L-Carnitine: effect of intravenous administration on fuel homeostasis in normal subjects and home-parenteral-nutrition patients with low plasma carnitine concentrations. Am J Clin Nutr 49:618–623
25. González-Ortiz M, Hernández-González SO, Hernández-Salazar E, Martínez-Abundis E (2008) Effect of oral L-carnitine administration on insulin sensitivity and lipid profile in type 2 diabetes mellitus patients. Ann Nutr Metab 52:335–338
26. Malaguarnera M, Gargante MP, Russo C, Antic T, Vacante M, Malaguarnera M, Avitabile T, Li Volti G, Galvano F (2010) L-Carnitine supplementation to diet: a new tool in treatment of nonalcoholic steatohepatitis—a randomized and controlled clinical trial. Am J Gastroenterol 105:1338–1345

27. Mingrone G, Greco AV, Capristo E, Benedetti G, Giancaterini A, De Gaetano A, Gasbarrini G (1999) L-Carnitine improves glucose disposal in type 2 diabetic patients. J Am Coll Nutr 18:77–82
28. De Gaetano A, Mingrone G, Castagneto M, Calvani M (1999) Carnitine increases glucose disposal in humans. J Am Coll Nutr 18:289–295
29. Angelini A, Imparato L, Landi C, Porfido FA, Ciarimboli M, Marro A (1993) Variation in levels of glycaemia and insulin after infusion of glucose solutions with or without added L-carnitine. Drugs Exp Clin Res 19:219–222
30. Negro P, Gossetti F, La Pinta M, Mariani P, Carboni M (1994) The effect of L-carnitine, administered through intravenous infusion of glucose, on both glucose and insulin levels in healthy subjects. Drugs Exp Clin Res 20:257–262
31. Rahbar AR, Shakerhosseini R, Saadat N, Taleban F, Pordal A, Gollestan B (2005) Effect of L-carnitine on plasma glycemic and lipidemic profile in patients with type 2 diabetes mellitus. Eur J Clin Nutr 59:592–596
32. Molfino A, Cascino A, Conte C, Ramaccini C, Rossi Fanelli F, Laviano A (2010) Caloric restriction and L-carnitine administration improves insulin sensitivity in patients with impaired glucose metabolism. JPEN J Parenter Enteral Nutr 34:295–299
33. Derosa G, Cicero AF, Gaddi A, Mugellini A, Ciccarelli L, Fogari R (2003) The effect of L-carnitine on plasma lipoprotein(a) levels in hypercholesterolemic patients with type 2 diabetes mellitus. Clin Ther 25:1429–1439
34. Paulson DJ, Schmidt MJ, Traxler JS, Ramacci MT, Shug AL (1984) Improvement of myocardial function in diabetic rats after treatment with L-carnitine. Metabolism 33:358–363
35. Power RA, Hulver MW, Zhang JY, Dubois J, Marchand RM, Ilkayeva O, Muoio DM, Mynatt RL (2007) Carnitine revisited: potential use as adjunctive treatment in diabetes. Diabetologia 50:824–832
36. Dai T, Abou-Rjaily GA, Al-Share' QY, Yang Y, Fernström MA, Deangelis AM, Lee AD, Sweetman L, Amato A, Pasquali M, Lopaschuk GD, Erickson SK, Najjar SM (2004) Interaction between altered insulin and lipid metabolism in CEACAM1-inactive transgenic mice. J Biol Chem 279:45155–45161
37. Rodrigues B, Xiang H, McNeill JH (1988) Effect of L-carnitine treatment on lipid metabolism and cardiac performance in chronically diabetic rats. Diabetes 37:1358–1364
38. Mingorance C, Gonzalez del Pozo M, Dolores Herrera M, Alvarez de Sotomayor M (2009) Oral supplementation of propionyl-L-carnitine reduces body weight and hyperinsulinaemia in obese Zucker rats. Br J Nutr 102:1145–1153
39. Rajasekar P, Kaviarasan S, Anuradha CV (2005) L-Carnitine administration prevents oxidative stress in high fructose-fed insulin resistant rats. Diabetol Croat 1:21–28
40. Rajasekar P, Anuradha CV (2007) L-Carnitine inhibits protein glycation in vitro and in vivo: evidence for a role in diabetic management. Acta Diabetol 44:83–90
41. Rajasekar P, Viswanathan P, Anuradha CV (2008) Renoprotective action of L-carnitine in fructose-induced metabolic syndrome. Diabetes Obes Metab 10:171–180
42. Yoshikawa Y, Ueda E, Sakurai H, Kojima Y (2003) Anti-diabetes effect of Zn(II)/carnitine complex by oral administration. Chem Pharm Bull (Tokyo) 51:230–231
43. Broderick TL, Quinney HA, Lopaschuk GD (1992) Carnitine stimulation of glucose oxidation in the fatty acid perfused isolated working rat heart. J Biol Chem 267:3758–3763
44. Broderick TL, Quinney HA, Barker CC, Lopaschuk GD (1993) Beneficial effect of carnitine on mechanical recovery of rat hearts reperfused after a transient period of global ischemia is accompanied by a stimulation of glucose oxidation. Circulation 87:972–981
45. Broderick TL, Quinney HA, Lopaschuk GD (1995) L-Carnitine increases glucose metabolism and mechanical function following ischaemia in diabetic rat heart. Cardiovasc Res 29:373–378
46. Broderick TL, Panagakis G, DiDomenico D, Gamble J, Lopaschuk GD, Shug AL, Paulson DJ (1995) L-Carnitine improvement of cardiac function is associated with a stimulation in glucose but not fatty acid metabolism in carnitine-deficient hearts. Cardiovasc Res 30:815–820
47. Schönekess BO, Allard MF, Lopaschuk GD (1995) Propionyl L-carnitine improvement of hypertrophied heart function is accompanied by an increase in carbohydrate oxidation. Circ Res 77:726–734
48. Schönekess BO, Allard MF, Lopaschuk GD (1995) Propionyl L-carnitine improvement of hypertrophied rat heart function is associated with an increase in cardiac efficiency. Eur J Pharmacol 286:155–166
49. Broderick TL, Driedzic W, Paulson DJ (2000) Propionyl-L-carnitine effects on postischemic recovery of heart function and substrate oxidation in the diabetic rat. Mol Cell Biochem 206:151–157
50. Broderick TL, Haloftis G, Paulson DJ (1996) L-Propionylcarnitine enhancement of substrate oxidation and mitochondrial respiration in the diabetic rat heart. J Mol Cell Cardiol 28:331–340
51. Broderick TL (2008) ATP production and TCA activity are stimulated by propionyl-L-carnitine in the diabetic rat heart. Drugs R D 9:83–91
52. Felix C, Gillis M, Driedzic WR, Paulson DJ, Broderick TL (2001) Effects of propionyl-L-carnitine on isolated mitochondrial function in the reperfused diabetic rat heart. Diabetes Res Clin Pract 53:17–24
53. Neely JR, Morgan HE (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Annu Rev Physiol 36:413–459
54. Wall SR, Lopaschuk GD (1989) Glucose oxidation rates in fatty acid-perfused isolated working hearts from diabetic rats. Biochim Biophys Acta 1006:97–103
55. Chatham JC, Forder JR (1997) Relationship between cardiac function and substrate oxidation in hearts of diabetic rats. Am J Physiol 273:H52–H58
56. Nicholl TA, Lopaschuk GD, McNeill JH (1991) Effects of free fatty acids and dichloroacetate on isolated working diabetic rat heart. Am J Physiol 261:H1053–H1059
57. Hotta K, Kuwajima M, Ono A, Nakajima H, Horikawa Y, Miyagawa J, Namba M, Hanafusa T, Horiuchi M, Nikaido H, Hayakawa J, Saheki T, Kono N, Noguchi T, Matsuzawa Y (1996) Disordered expression of glycolytic and gluconeogenic liver enzymes of juvenile visceral steatosis mice with systemic carnitine deficiency. Diabetes Res Clin Pract 32:117–123
58. Ringseis R, Mooren FC, Keller J, Couturier A, Wen G, Hirche F, Stangl GI, Eder K, Krüger K (2011) Regular endurance exercise improves the diminished hepatic carnitine status in mice fed a high fat diet. Mol Nutr Food Res 55:S193–S202
59. Cuturic M, Abramson RK, Moran RR, Hardin JW (2011) Carnitine and metabolic correlates in hospitalized psychiatric patients: a follow-through report. J Psychiatr Pract 17:35–40
60. Ushikai M, Horiuchi M, Kobayashi K, Matuda S, Inui A, Takeuchi T, Saheki T (2011) Induction of PDK4 in the heart muscle of JVS mice, an animal model of systemic carnitine deficiency, does not appear to reduce glucose utilization by the heart. Mol Genet Metab 102:349–355
61. Nakajima H, Kodo N, Inoue F, Kizaki Z, Nukina S, Kinugasa A, Sawada T (1996) Pivalate affects carnitine status but causes no severe metabolic changes in rat liver. J Nutr 126:1683–1687
62. Broderick TL, Christos SC, Wolf BA, DiDomenico D, Shug AL, Paulson DJ (1995) Fatty acid oxidation and cardiac function in

the sodium pivalate model of secondary carnitine deficiency. Metabolism 44:499–505
63. Liepinsh E, Vilskersts R, Skapare E, Svalbe B, Kuka J, Cirule H, Pugovics O, Kalvinsh I, Dambrova M (2008) Mildronate decreases carnitine availability and up-regulates glucose uptake and related gene expression in the mouse heart. Life Sci 83:613–619
64. Liepinsh E, Vilskersts R, Zvejniece L, Svalbe B, Skapare E, Kuka J, Cirule H, Grinberga S, Kalvinsh I, Dambrova M (2009) Protective effects of mildronate in an experimental model of type 2 diabetes in Goto-Kakizaki rats. Br J Pharmacol 157:1549–1556
65. Liepinsh E, Skapare E, Svalbe B, Makrecka M, Cirule H, Dambrova M (2011) Anti-diabetic effects of mildronate alone or in combination with metformin in obese Zucker rats. Eur J Pharmacol 658:277–283
66. Krähenbühl S, Mang G, Kupferschmidt H, Meier PJ, Krause M (1995) Plasma and hepatic carnitine and coenzyme A pools in a patient with fatal, valproate induced hepatotoxicity. Gut 37:140–143
67. Lheureux PE, Penaloza A, Zahir S, Gris M (2005) Science review: carnitine in the treatment of valproic acid-induced toxicity—what is the evidence? Crit Care 9:431–440
68. Horiuchi M, Kobayashi K, Yamaguchi S, Shimizu N, Koizumi T, Nikaido H, Hayakawa J, Kuwajima M, Saheki T (1994) Primary defect of juvenile visceral steatosis (jvs) mouse with systemic carnitine deficiency is probably in renal carnitine transport system. Biochim Biophys Acta 1226:25–30
69. Holme E, Greter J, Jacobson CE, Lindstedt S, Nordin I, Kristiansson B, Jodal U (1989) Carnitine deficiency induced by pivampicillin and pivmecillinam therapy. Lancet 2:469–473
70. Luci S, Geissler S, König B, Koch A, Stangl GI, Hirche F, Eder K (2006) PPARalpha agonists up-regulate organic cation transporters in rat liver cells. Biochem Biophys Res Commun 350:704–708
71. Van Vlies N, Ferdinandusse S, Turkenburg M, Wanders RJ, Vaz FM (2007) PPAR alpha-activation results in enhanced carnitine biosynthesis and OCTN2-mediated hepatic carnitine accumulation. Biochim Biophys Acta 1767:1134–1142
72. Koch A, König B, Stangl GI, Eder K (2008) PPAR alpha mediates transcriptional upregulation of novel organic cation transporters-2 and -3 and enzymes involved in hepatic carnitine synthesis. Exp Biol Med (Maywood) 233:356–365
73. Ringseis R, Eder K (2009) Influence of pharmacological PPARα activators on carnitine homeostasis in proliferating and non-proliferating species. Pharmacol Res 60:179–184
74. Ringseis R, Wege N, Wen G, Rauer C, Hirche F, Kluge H, Eder K (2009) Carnitine synthesis and uptake into cells are stimulated by fasting in pigs as a model of nonproliferating species. J Nutr Biochem 20:840–847
75. Wen G, Ringseis R, Eder K (2010) Mouse OCTN2 is directly regulated by peroxisome proliferator-activated receptor α (PPARα) via a PPRE located in the first intron. Biochem Pharmacol 79:768–776
76. Wen G, Kühne H, Rauer C, Ringseis R, Eder K (2011) Mouse γ-butyrobetaine dioxygenase is regulated by peroxisome proliferator-activated receptor α through a PPRE located in the proximal promoter. Biochem Pharmacol 82:175–183
77. Dambrova M, Liepinsh E, Kalvinsh I (2002) Mildronate: cardioprotective action through carnitine-lowering effect. Trends Cardiovasc Med 12:275–279
78. Asaka N, Muranaka Y, Kirimoto T, Miyake H (1998) Cardioprotective profile of MET-88, an inhibitor of carnitine synthesis, and insulin during hypoxia in isolated perfused rat hearts. Fundam Clin Pharmacol 12:158–163
79. Spaniol M, Kaufmann P, Beier K, Wüthrich J, Török M, Scharnagl H, März W, Krähenbühl S (2003) Mechanisms of liver steatosis in rats with systemic carnitine deficiency due to treatment with trimethylhydraziniumpropionate. J Lipid Res 44:144–153
80. Kuka J, Vilskersts R, Cirule H, Makrecka M, Pugovics O, Kalvinsh I, Dambrova M, Liepinsh E (2011) The cardioprotective effect of mildronate is diminished after co-treatment with L-carnitine. J Cardiovasc Pharmacol Ther. doi:10.1177/1074248411419502
81. Borum PR, Broquist HP (1977) Lysine deficiency and carnitine in male and female rats. J Nutr 107:1209–1215
82. Cave MC, Hurt RT, Frazier TH, Matheson PJ, Garrison RN, McClain CJ, McClave SA (2008) Obesity, inflammation, and the potential application of pharmaconutrition. Nutr Clin Pract 23:16–34
83. Pregant P, Kaiser E, Schernthaner G (1993) No effect of insulin treatment or glycemic improvement on plasma carnitine levels in type 2 diabetic patients. Clin Investig 71:610–612
84. Okuda Y, Kawai K, Murayama Y, Yamashita K (1987) Postprandial changes in plasma ketone body and carnitine levels in normal and non-insulin-dependent diabetic subjects. Endocrinol Jpn 34:415–422
85. De Palo E, Gatti R, Sicolo N, Padovan D, Vettor R, Federspil G (1981) Plasma and urine free L-carnitine in human diabetes mellitus. Acta Diabetol Lat 18:91–95
86. Winter SC, Simon M, Zorn EM, Szabo-Aczel S, Vance WH, O'Hara T, Higashi L (1989) Relative carnitine insufficiency in children with type I diabetes mellitus. Am J Dis Child 143:1337–1339
87. Poorabbas A, Fallah F, Bagdadchi J, Mahdavi R, Aliasgarzadeh A, Asadi Y, Koushavar H, Vahed Jabbari M (2007) Determination of free L-carnitine levels in type II diabetic women with and without complications. Eur J Clin Nutr 61:892–895
88. Tamamoğullari N, Siliğ Y, Içağasioğlu S, Atalay A (1999) Carnitine deficiency in diabetes mellitus complications. J Diabetes Complicat 13:251–253
89. Pregant P, Schernthaner G, Legenstein E, Lienhart L, Bruck S, Schnack C, Kaiser E (1991) Decreased plasma carnitine in type I diabetes mellitus. Klin Wochenschr 69:511–516
90. Soltész G, Melegh B, Sándor A (1983) The relationship between carnitine and ketone body levels in diabetic children. Acta Paediatr Scand 72:511–515
91. McGarry JD, Brown NF (1997) The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 244:1–14
92. Steiber A, Kerner J, Hoppel CL (2004) Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 25:455–473
93. Uziel G, Garavaglia B, Di Donato S (1988) Carnitine stimulation of pyruvate dehydrogenase complex (PDHC) in isolated human skeletal muscle mitochondria. Muscle Nerve 11:720–724
94. Lysiak W, Lilly K, DiLisa F, Toth PP, Bieber LL (1988) Quantitation of the effect of L-carnitine on the levels of acid-soluble short-chain acyl-CoA and CoASH in rat heart and liver mitochondria. J Biol Chem 263:1151–1156
95. Ramsay RR, Zammit VA (2004) Carnitine acyltransferases and their influence on CoA pools in health and disease. Mol Aspects Med 25:475–493
96. Giancaterini A, De Gaetano A, Mingrone G, Gniuli D, Liverani E, Capristo E, Greco AV (2000) Acetyl-L-carnitine infusion increases glucose disposal in type 2 diabetic patients. Metabolism 49:704–708
97. Stephens FB, Constantin-Teodosiu D, Laithwaite D, Simpson EJ, Greenhaff PL (2006) An acute increase in skeletal muscle carnitine content alters fuel metabolism in resting human skeletal muscle. J Clin Endocrinol Metab 91:5013–5018

98. Wyss V, Ganzit GP, Rienzi A (1990) Effects of L-carnitine administration on VO2max and the aerobic-anaerobic threshold in normoxia and acute hypoxia. Eur J Appl Physiol Occup Physiol 60:1–6
99. Ferrannini E, Buzzigoli G, Bevilacqua S, Boni C, Del Chiaro D, Oleggini M, Brandi L, Maccari F (1988) Interaction of carnitine with insulin-stimulated glucose metabolism in humans. Am J Physiol 255:E946–E952
100. Stephens FB, Constantin-Teodosiu D, Laithwaite D, Simpson EJ, Greenhaff PL (2005) Insulin stimulates L-carnitine accumulation in human skeletal muscle. FASEB J 20:377–379
101. Clausen T (2003) Na+–K+ pump regulation and skeletal muscle contractility. Physiol Rev 83:1269–1324
102. Ruggenenti P, Cattaneo D, Loriga G, Ledda F, Motterlini N, Gherardi G, Orisio S, Remuzzi G (2009) Ameliorating hypertension and insulin resistance in subjects at increased cardiovascular risk: effects of acetyl-L-carnitine therapy. Hypertension 54:567–574
103. Zhou YP, Ling ZC, Grill VE (1996) Inhibitory effects of fatty acids on glucose-regulated B-cell function: association with increased islet triglyceride stores and altered effect of fatty acid oxidation on glucose metabolism. Metabolism 45:981–986
104. Keller K, Ringseis R, Priebe S, Guthke R, Kluge H, Eder K (2011) Transcript profiling in the liver of piglets fed L-carnitine. Nutr Metab (Lond) 8:76
105. Rajasekar P, Anuradha CV (2007) Fructose-induced hepatic gluconeogenesis: effect of L-carnitine. Life Sci 80:1176–1183
106. Proulx F, Lacroix J, Qureshi IA, Nadeau D, Gauthier M, Lambert M (1997) Acquired carnitine abnormalities in critically ill children. Eur J Pediatr 156:864–869
107. Newsholme P, Brennan L, Rubi B, Maechler P (2005) New insights into amino acid metabolism, beta-cell function and diabetes. Clin Sci (Lond) 108:185–194
108. Heo YR, Kang CW, Cha YS (2001) L-Carnitine changes the levels of insulin-like growth factors (IGFs) and IGF binding proteins in streptozotocin-induced diabetic rat. J Nutr Sci Vitaminol (Tokyo) 47:329–334
109. Keller J, Ringseis R, Priebe S, Guthke R, Kluge H, Eder K (2011) Dietary L-carnitine alters gene expression in skeletal muscle of piglets. Mol Nutr Food Res 55:419–429
110. Kita K, Kato S, Amanyaman M, Okumura J, Yokota H (2002) Dietary L-carnitine increases plasma insulin-like growth factor-I concentration in chicks fed a diet with adequate dietary protein level. Br Poult Sci 43:117–121
111. Doberenz J, Birkenfeld C, Kluge H, Eder K (2006) Effects of L-carnitine supplementation in pregnant sows on plasma concentrations of insulin-like growth factors, various hormones and metabolites and chorion characteristics. J Anim Physiol Anim Nutr 90:487–499
112. Di Marzio L, Moretti S, D'Alò S, Zazzeroni F, Marcellini S, Smacchia C, Alesse E, Cifone MG, De Simone C (1999) Acetyl-L-carnitine administration increases insulin-like growth factor 1 levels in asymptomatic HIV-1-infected subjects: correlation with its suppressive effect on lymphocyte apoptosis and ceramide generation. Clin Immunol 92:103–110
113. Musser RE, Goodband RD, Tokach MD, Owen KQ, Nelssen JL, Blum SA, Campbell RG, Smits R, Dritz SS, Civis CA (1999) Effects of L-carnitine fed during lactation on sow and litter performance. J Anim Sci 77:3289–3295
114. Ramanau A, Kluge H, Spilke J, Eder K (2002) Reproductive performance of sows supplemented with dietary L-carnitine over three reproductive cycles. Arch Anim Nutr 56:287–296
115. Ramanau A, Kluge H, Spilke J, Eder K (2004) Supplementation of sows with L-carnitine during pregnancy and lactation improves growth of the piglets during the suckling period through increased milk production. J Nutr 134:86–92
116. Kelley RL, Jungst SB, Spencer TE, Owsley WF, Rahe CH, Mulvaney DR (1995) Maternal treatment with somatotropin alters embryonic development and early postnatal growth of pigs. Domest Anim Endocrinol 12:83–94
117. Baumann MU, Deborde S, Illsley NP (2002) Placental glucose transfer and fetal growth. Endocrine 19:13–22
118. Waber LJ, Valle D, Neill C, DiMauro S, Shug A (1982) Carnitine deficiency presenting as familial cardiomyopathy: a treatable defect in carnitine transport. J Pediatr 101:700–705
119. Glasgow AM, Engel AG, Bier DM, Perry LW, Dickie M, Todaro J, Brown BI, Utter MF (1983) Hypoglycemia, hepatic dysfunction, muscle weakness, cardiomyopathy, free carnitine deficiency and long-chain acylcarnitine excess responsive to medium chain triglyceride diet. Pediatr Res 17:319–326
120. Tripp ME, Katcher ML, Peters HA, Gilbert EF, Arya S, Hodach RJ, Shug AL (1981) Systemic carnitine deficiency presenting as familial endocardial fibroelastosis: a treatable cardiomyopathy. N Engl J Med 305:385–390
121. Tassani V, Cattapan F, Magnanimi L, Peschechera A (1994) Anaplerotic effect of propionyl carnitine in rat heart mitochondria. Biochem Biophys Res Commun 199:949–953
122. Capaldo B, Napoli R, Di Bonito P, Albano G, Saccà L (1991) Carnitine improves peripheral glucose disposal in non-insulin-dependent diabetic patients. Diabetes Res Clin Pract 14:191–195
123. Günal AI, Celiker H, Dönder E, Günal SY (1999) The effect of L-carnitine on insulin resistance in hemodialysed patients with chronic renal failure. J Nephrol 12:38–40