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ORIGINAL ARTICLE

# Carnitine status of pregnant women: effect of carnitine supplementation and correlation between iron status and plasma carnitine concentration

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**Background/Objectives:** It has been shown that plasma carnitine concentrations markedly decline during gestation in women. The reason for this, however, is unknown. One objective of this study was to investigate the effect of carnitine supplementation on plasma carnitine concentrations in pregnant women. The second objective was to investigate the hypothesis that reduced plasma carnitine concentrations during gestation are caused by a reduced carnitine synthesis because of a diminished iron status.
**Subjects/Methods:** Healthy pregnant women ($n=26$) were randomly assigned in two groups receiving either a L-carnitine supplement (500 mg L-carnitine per day as L-carnitine L-tartrate) ($n=13$) or placebo ($n=13$) from the 13th week of gestation to term.
**Results:** In the control group, there was a marked reduction of plasma carnitine concentration from the 12th week of gestation to term. This reduction was prevented by the supplementation of carnitine. In the control group, there was a positive relationship between the parameters of iron status (mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and ferritin) and plasma concentration of carnitine ($P<0.05$). Moreover, there were inverse correlations between the concentrations of ferritin and the carnitine precursor γ-butyrobetaine in plasma, and between γ-butyrobetaine and carnitine in plasma ($P<0.05$).
**Conclusions:** This study confirms that plasma carnitine concentrations decline in the course of pregnancy, an effect that can be prevented by the supplementation of carnitine. Data of this study, moreover, suggest that the decline of plasma carnitine concentration during pregnancy could be caused by a reduced rate of carnitine biosynthesis, possibly because of an inadequate iron status.
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**Keywords:** pregnancy; carnitine status; carnitine supplement; iron status; ferritin

## Introduction

Carnitine (L-3-hydroxy-4-*N*-*N*-*N*-trimethylaminobutyrate) is an essential metabolite, which has a number of indispensable functions in intermediary metabolism. The most important function lies in its role in the transport of activated long-chain fatty acids from the cytosol to the mitochondrial matrix, where β-oxidation takes place. The other functions of carnitine, include the transfer of products of peroxisomal β-oxidation to the mitochondria for oxidation in the citrate cycle, the modulation of the acyl-coenzyme A (CoA)/CoA-ratio and the storage of energy as acetylcarnitine (McGarry and Brown, 1997; Rebouche and Seim, 1998; Steiber *et al*., 2004). All those tissues that use fatty acids as a fuel source need carnitine for their normal function. Carnitine is derived from dietary sources and endogenous biosynthesis. Carnitine biosynthesis involves a complex series of reactions involving several tissues. Lysine provides the carbon

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backbone of carnitine. Lysine in protein peptide linkages undergoes methylation of the ε-amino group to yield trimethyllysine, which is released on protein degradation. The released trimethyllysine is further oxidized to γ-butyrobetaine by the action of trimethyllysine dioxygenase, 3-hydroxy-*N*-trimethyllysine aldolase and 4-*N*-trimethylaminobutyraldehyde dehydrogenase. γ-Butyrobetaine is hydroxylated by γ-butyrobetaine dioxygenase to form carnitine. In humans, this last reaction occurs primarily in the liver and the kidney (Vaz and Wanders, 2002). Two of the enzymes involved in carnitine synthesis, namely trimethyllysine dioxygenase and γ-butyrobetaine dioxygenase, contain iron with a catalytic function (Lindstedt and Lindstedt, 1970; Vaz *et al*., 2001). Accordingly, inadequate iron supply has been shown to lower plasma carnitine concentrations in children (Cemeroglu *et al*., 2001; Tanzer *et al*., 2001; Citak *et al*., 2006; Böhles *et al*., 1991) or rat pups (Bartholmey and Sherman, 1985, 1986), probably because of a reduced carnitine biosynthesis rate.

Gestation is a physiological state that leads to several metabolic changes. It can be viewed as an anabolic state with an increase in maternal fat and protein stores (Lain and Catalano, 2007). Plasma carnitine concentrations, however, decrease in women during gestation. At the time of delivery, plasma carnitine concentrations decrease to about half of the concentrations of non-pregnant women (Bargen-Lockner *et al*., 1981; Cederblad *et al*., 1985, 1986; Genger *et al*., 1988; Marzo *et al*., 1994; Schoderbeck *et al*., 1995), even though dietary carnitine intake increase, as gestation proceeds (Cho *et al*., 2003). Similar low carnitine concentrations are only found in patients with carnitine deficiency (Duran *et al*., 1990). It is unclear whether such low carnitine concentrations in pregnant women have adverse effects on their metabolism or on the metabolism of the fetus. Nevertheless, because of the important functions of carnitine, it is possible that a higher carnitine status could have beneficial effects on pregnant women and their fetuses. In healthy adult persons, it has been shown that dietary supplementation of carnitine is able to increase plasma carnitine concentration, although most of the additional carnitine is renally excreted (Mitchell and Snyder, 1991; Volek *et al*., 2002; Spiering *et al*., 2007). Therefore, it is expected that the supplementation of carnitine may also increase plasma carnitine concentrations in pregnant women. However, to our knowledge, less is known about the effect of carnitine supplementation on the carnitine status in pregnant women. Therefore, one aim of this study was to test the hypothesis that carnitine supplementation increases plasma carnitine concentrations in pregnant women relative to non-supplemented pregnant women, and thus prevents the decline of plasma carnitine during pregnancy. As carnitine can cross the placenta (Schmidt-Sommerfeld *et al*., 1985; Grube *et al*., 2005), and therefore enters the fetal blood, it is likely that supplementation of mothers with carnitine also leads to an increase of the carnitine concentration in the fetal blood. To test these hypotheses, we supplemented pregnant women with carnitine in a physiological dose (500 mg/day) from the 13th week of pregnancy until delivery, and determined concentrations of carnitine in maternal plasma and plasma from the umbilical vein.

The second aim of this study was to elucidate a possible reason for the reduction of plasma carnitine concentrations during gestation. Epidemiologic studies have disclosed that fertile, non-pregnant women, often have a low iron status (Milman, 2006). Pregnant women have even a particular high risk of iron deficiency because of the high iron demands of the developing fetus (Harvey *et al*., 2007). Owing to the requirement of iron for carnitine biosynthesis, we hypothesized that an inadequate iron status could lead to a reduced endogenous carnitine biosynthesis in pregnant women, which could be an explanation for the low plasma carnitine concentrations. To test this hypothesis we examined the relationship between the parameters of iron status and plasma concentrations of carnitine, and the carnitine precursors γ-butyrobetaine and 6-*N*-trimethyllysine.

## Subjects and methods

*Subjects*

Twenty-six apparently healthy, pregnant women aged 22–40 years and in the 12th week of gestation were recruited for the study through medical practitioners of Department of Obstetrics and Gynecology of the University Hospital. They were divided into the following two groups: L-carnitine supplementation vs placebo ($n = 13$ for each group). A sample size of five in each group will be sufficient to detect a clinically relevant increase of 8.5 μmol/l of free carnitine in the plasma, using a two-tailed *t*-test of difference between means, a power of 90% and a significance level of 5%. The calculation is based on the assumption that plasma carnitine concentrations are normally distributed with a s.d. of 3.5 μmol/l (Günter *et al*., 2002). This number was increased to 13 per group, total of 26, to allow for a possible high drop-out rate.

The characteristics of the subjects and their neonates are given in Table 1. Before being accepted for the study, each woman completed a health questionnaire. Metabolic diseases, therapy with anticonvulsive, high-risk pregnancy, disorder in carnitine biosynthesis and β-oxidation, counted among exclusion criteria. The study was approved by the Local Research Ethics Committee. In addition to receiving standard obstetric care from their own clinician during the course of study, all subjects were monitored by a gynecologist at the Department of Obstetrics and Gynecology, University Hospital.

*Study design*

The study was a randomized, single-blind, placebo-controlled study investigating the effects of supplementation with 500 mg L-carnitine per day (as L-carnitine L-tartrate) in

**Table 1** Characteristics of pregnant women and their neonates

| | *Placebo* | *+ L-carnitine* | P |
|---|---|---|---|
| Mothers (*n*) | 13 | 13 | |
| Maternal age at conception (years) | 31 ± 5 | 30 ± 4 | NS |
| Body weight[a] | 63.0 ± 12.0 | 69.2 ± 16.9 | NS |
| Height (cm)[a] | 163 ± 8 | 167 ± 6 | NS |
| Body mass index (kg/m$^2$)[a] | 23.6 ± 3.3 | 24.8 ± 5.6 | NS |
| Neonates (*n*) | 13 | 13 | |
| Gestational age (week) | 40 ± 1 | 40 ± 2 | NS |
| Neonatal weight (g) | 3488 ± 545 | 3566 ± 598 | NS |
| Neonatal height (cm) | 50.7 ± 2.7 | 50.8 ± 2.0 | NS |

Abbreviation: NS, non significant.
All values are $\bar{x} \pm$ s.d.
[a]12 wk of gestation.

pregnant women. Supplements and placebo tablets were manufactured and donated by LONZA (Basel, Swiss) and Flash Biolab GmbH (Gelsenkirchen, Germany), respectively. Placebo tablets were identical in color, size and shape to the L-carnitine supplements, and all tablets were supplied in coded opaque bottles, such that subjects were blinded to their study group. Women were asked to take two tablets at the same time each day from the 13th week of gestation until delivery. Compliance with the protocol could not be controlled. Maternal blood samples were taken every trimester (12th, 20th, 32nd weeks of gestation), delivery and lactation (2, 14 day, 1 month). In addition, samples of the umbilical cord blood and amniotic fluid were taken at the time of delivery. Plasma and serum were separated from whole blood by centrifugation at 1500*g* for 10 min at 4 °C. Samples were stored at −80 °C.

*Sample analysis*
Plasma (20 μl) and amniotic fluid (20 μl) were added with methanol containing the internal standard. An 1100-er series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a Kromasil 100 column (5 μm particle size, 125 mm length, 2 mm internal diameter, CS-Chromatographie Service, Langerwehe, Germany) and an API 2000 LC-MS/MS-System (Applied Biosystems, Darmstadt, Germany) were used for the quantification of free carnitine, acetyl carnitine, propionyl carnitine, γ-butyrobetaine and 6-*N*-trimethyllysine. For detection, the analytes were ionized by positive ion (5500 V) electrospray. As eluents, methanol and a methanol:water:acetonitrile mixture (50:45:5) were used (Ringseis *et al*., 2007). A whole blood count (XE-2100; Sysmex GmbH, Norderstedt, Germany), including leukocyte, erythrocyte, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and thrombocyte, was carried out at central labor Halle University Hospital. Serum ferritin was determined with commercial enzyme linked immune sorbent assay kit (IBL, Hamburg, Germany), intra- and inter-assay coefficients of variation being 7.8% ($n = 20$) and 7.9% ($n = 7$), respectively.

*Statistical methods*
Evidence for a difference between the two groups was tested by Student's *t*-test using the Minitab Statistical Software (Release 13, Minitab Inc., State College, PA, USA). For evaluation of differences between the stages of gestation and lactation, one-factorial analysis of variance was used; for significant *F*-values ($P < 0.05$), means were compared by Fisher's multiple range test. For correlation analysis, the Pearson product moment correlation coefficient was calculated. A significance level of 0.05 was used for all statistical tests.

## Results

Significant differences in maternal and neonatal characteristics were not observed between the two groups (Table 1), namely, maternal age ($P = 0.70$), gestational age ($P = 0.60$), neonatal weight ($P = 0.73$) and neonatal height ($P = 0.33$).

In the placebo group, a continuous decrease of the concentration of free and total carnitine from the 12th week of gestation until delivery was observed (Table 2). The supplementation of L-carnitine caused an increase of plasma carnitine concentration compared to the placebo group, and thus prevented the decrease of carnitine concentration during pregnancy. At delivery, concentrations of free carnitine, carnitine esters and total carnitine in plasma were significantly higher in women supplemented with carnitine than in women of the placebo group ($P < 0.05$, Table 2). During lactation, concentrations of free carnitine, carnitine esters and total carnitine in plasma were markedly higher than those at delivery ($P < 0.05$, Table 2). During lactation, there was, however, no difference in the concentration of free, esterified and total carnitine between the women supplemented with carnitine during pregnancy and those of the placebo group (Table 2).

The plasma concentrations of the carnitine precursors γ-butyrobetaine and 6-*N*-trimethyllysine in plasma were similar in both groups during gestation (Table 3). Concentrations of these two metabolites in plasma, were however, significantly lower during pregnancy than during the subsequent lactation period ($P < 0.05$, Table 3).

Women supplemented with L-carnitine had higher concentrations of esterified and total carnitine in the umbilical cord plasma than women of the placebo group ($P < 0.05$), and there was also a tendency towards increased concentrations of free carnitine in women supplemented with L-carnitine ($P = 0.06$, Table 4). In contrast, carnitine concentrations in amniotic fluid were not significantly different between both groups of women (Table 4). Concentrations of the carnitine precursors γ-butyrobetaine and 6-*N*-trimethyl-

**Table 2** Concentration of total carnitine, free carnitine and carnitine esters in plasma of women receiving either a L-carnitine supplement (500 mg per day) or a placebo from the 12th week of gestation until delivery at various time points

| | *Total carnitine (μmol/l)* | | | *Free carnitine (μmol/l)* | | | *Carnitine esters (μmol/l)* | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Placebo* | *+ L-carnitine* | | *Placebo* | *+ L-carnitine* | | *Placebo* | *+ L-carnitine* | |
| | *(n = 13)* | *(n = 13)* | P | *(n = 13)* | *(n = 13)* | P | *(n = 13)* | *(n = 13)* | P |
| *Gestation (weeks)* | | | | | | | | | |
| 12 | $21.5 \pm 4.61^{b}$ | $22.8 \pm 6.39^{a}$ | NS | $18.4 \pm 4.21^{b}$ | $20.2 \pm 4.44^{b}$ | NS | $2.82 \pm 0.56^{a}$ | $3.52 \pm 1.43^{a}$ | NS |
| 20 | $16.9 \pm 3.04^{a,b}$ | $22.3 \pm 1.66^{a}$ | $<0.01$ | $15.2 \pm 3.94^{a,b}$ | $17.5 \pm 2.14^{a,b}$ | NS | $2.84 \pm 1.03^{a}$ | $3.64 \pm 0.51^{a}$ | 0.10 |
| 32 | $15.8 \pm 2.65^{a}$ | $18.3 \pm 4.65^{a}$ | NS | $12.7 \pm 2.26^{a}$ | $15.2 \pm 4.33^{a,b}$ | 0.15 | $3.05 \pm 0.93^{a}$ | $3.26 \pm 0.34^{a}$ | NS |
| Delivery | $13.9 \pm 3.25^{a}$ | $20.0 \pm 5.45^{a}$ | $<0.01$ | $10.7 \pm 4.56^{a}$ | $14.9 \pm 5.23^{a}$ | $<0.05$ | $3.18 \pm 1.05^{a}$ | $5.09 \pm 1.34^{b}$ | $<0.0011$ |
| *Lactation (days)* | | | | | | | | | |
| 2 | $21.1 \pm 4.74^{b}$ | $23.0 \pm 5.59^{a}$ | NS | $17.9 \pm 3.58^{b}$ | $19.8 \pm 5.02^{b}$ | NS | $2.78 \pm 0.821^{a}$ | $3.23 \pm 0.937^{a}$ | NS |
| 14 | $30.3 \pm 6.89^{c}$ | $32.4 \pm 4.88^{b}$ | NS | $25.8 \pm 6.14^{c}$ | $28.0 \pm 9.67^{c}$ | NS | $4.48 \pm 1.78^{b}$ | $4.00 \pm 1.10^{a,b}$ | NS |
| 28 | $33.4 \pm 6.72^{c,d}$ | $36.1 \pm 6.32^{b,c}$ | NS | $29.7 \pm 7.16^{d}$ | $32.5 \pm 7.06^{c,d}$ | NS | $4.77 \pm 1.44^{b}$ | $4.83 \pm 0.46^{b}$ | NS |

Abbreviation: NS, non significant.
All values are $\bar{x} \pm$ s.d.
Means sharing not the same superscript letter ($^{a,b,c,d}$) within one column are significantly different ($P<0.05$) by Fisher's multiple range test.

**Table 3** Concentration of γ-butyrobetaine and 6-*N*-trimethyllysine in plasma of women receiving either a L-carnitine supplement (500 mg per day) or a placebo from the 12th week of gestation until delivery at various time points

| | *γ-Butyrobetaine (μmol/l)* | | | *6-N-Trimethyllysine (μmol/l)* | | |
|---|---|---|---|---|---|---|
| | *Placebo (n = 13)* | *+ L-carnitine (n = 13)* | P | *Placebo (n = 13)* | *+ L-carnitine (n = 13)* | P |
| *Gestation (weeks)* | | | | | | |
| 12 | $0.352 \pm 0.076^{a}$ | $0.355 \pm 0.119^{a}$ | NS | $0.133 \pm 0.027$ | $0.128 \pm 0.021^{a,b}$ | NS |
| 20 | $0.325 \pm 0.086^{a}$ | $0.324 \pm 0.036^{a}$ | NS | $0.150 \pm 0.055$ | $0.119 \pm 0.016^{a}$ | 0.12 |
| 32 | $0.365 \pm 0.037^{a}$ | $0.316 \pm 0.083^{a}$ | 0.15 | $0.147 \pm 0.023$ | $0.140 \pm 0.009^{a,b}$ | NS |
| Delivery | $0.310 \pm 0.060^{a}$ | $0.360 \pm 0.089^{a}$ | 0.14 | $0.154 \pm 0.049$ | $0.184 \pm 0.059^{b}$ | NS |
| *Lactation (days)* | | | | | | |
| 2 | $0.424 \pm 0.097^{a}$ | $0.287 \pm 0.104^{a}$ | 0.02 | $0.162 \pm 0.020$ | $0.180 \pm 0.038^{b}$ | NS |
| 14 | $0.485 \pm 0.058^{b}$ | $0.505 \pm 0.111^{b,c}$ | NS | $0.221 \pm 0.043$ | $0.213 \pm 0.040^{c}$ | NS |
| 28 | $0.534 \pm 0.092^{b}$ | $0.523 \pm 0.096^{b}$ | NS | $0.226 \pm 0.031$ | $0.255 \pm 0.060^{c}$ | 0.16 |

Abbreviation: NS, non significant.
All values are $\bar{x} \pm$ s.d.
Means sharing not the same superscript letter ($^{a,b,c,d}$) within one column are significantly different ($P<0.05$) by Fisher's multiple range test.

lysine in the umbilical cord plasma and amniotic fluid were also not different between both groups (Table 4).

The whole blood count at delivery, including leukocyte ($P=0.42$), erythrocyte ($P=0.82$), hemoglobin ($P=0.95$), hematocrit ($P=0.81$), MCV ($P=0.96$), MCH ($P=0.8$), mean corpuscular hemoglobin concentration ($P=0.45$), thrombocyte ($P=0.68$) and ferritin ($P=0.79$), did not differ between both groups (Table 5).

Correlation analysis showed positive linear correlations in the subjects of the placebo group between plasma concentrations of free or total carnitine and MCV ($r=0.79$, $P<0.01$; $r=0.84$, $P<0.01$), MCH ($r=0.68$, $P<0.01$; $r=0.72$, $P<0.01$) or serum ferritin ($r=0.68$, $P<0.05$; $r=0.62$, $P=0.06$) (Figure 1). Moreover, there was an inverse linear correlation between the plasma concentration of free carnitine and that of γ-butyrobetaine in the placebo group ($P<0.1$, Figure 2). In the group supplemented with L-carnitine these correlations were not observed. In both groups of women, there was also an inverse correlation between plasma concentration of γ-butyrobetaine and serum ferritin concentration ($P<0.05$, Figure 2).

## Discussion

The present study agrees with other studies ((Bargen-Lockner *et al*., 1981; Cederblad *et al*., 1985, 1986; Genger *et al*., 1988; Marzo *et al*., 1994; Schoderbeck *et al*., 1995; Cho *et al*., 2003) in showing that plasma carnitine concentrations in women markedly decline during pregnancy. In our study, plasma

**Table 4** Concentration of total carnitine, free carnitine, carnitine esters, γ-butyrobetaine and 6-*N*-trimethyllysine in the umbilical cord plasma and amniotic fluid at delivery in women receiving either a L-carnitine supplement (500 mg per day) or a placebo from the 12th week of gestation until delivery

| | *Umbilical cord plasma* | | | *Amniotic fluid* | | |
|---|---|---|---|---|---|---|
| | *Placebo* | *+ L-Carnitine* | | *Placebo* | *+ L-Carnitine* | |
| | *(μmol/l)* | | | *(μmol/l)* | | |
| | *(n = 13)* | *(n = 11)* | P | *(n = 9)* | *(n = 8)* | P |
| Total carnitine | 18.5 ± 3.22 | 22.6 ± 4.84 | <0.05 | 11.8 ± 3.32 | 15.5 ± 4.83 | NS |
| Free carnitine | 14.1 ± 2.65 | 17.0 ± 3.64 | NS | 8.69 ± 2.61 | 11.0 ± 3.21 | NS |
| Carnitine esters | 4.39 ± 4.39 | 5.64 ± 5.64 | <0.05 | 3.12 ± 0.89 | 4.47 ± 2.34 | NS |
| γ-Butyrobetaine | 0.400 ± 0.159 | 0.440 ± 0.081 | NS | 0.301 ± 0.125 | 0.201 ± 0.068 | NS |
| 6-*N*-Trimethyllysine | 0.242 ± 0.049 | 0.267 ± 0.064 | NS | 0.584 ± 0.131 | 0.594 ± 0.098 | NS |

Abbreviation: NS, non significant.
All values are $\bar{x} \pm$ s.d.

**Table 5** Blood count and serum concentration of ferritin at delivery in women receiving either a L-carnitine supplement or a placebo from the 12th week of gestation until delivery

| | *Placebo* *(n = 13)* | *+ L-carnitine* *(n = 13)* | P |
|---|---|---|---|
| Leukocyte (Gpt/l) | 11.7 ± 3.4 | 12.9 ± 3.8 | NS |
| Erythrocyte (Tpt/l) | 4.13 ± 0.47 | 4.17 ± 0.34 | NS |
| Hemoglobin (mmol/l) | 7.75 ± 0.91 | 7.78 ± 0.67 | NS |
| Hematocrit | 0.36 ± 0.04 | 0.37 ± 0.03 | NS |
| MCV (fl) | 87.9 ± 3.4 | 88.0 ± 4.2 | NS |
| MCH (fmol) | 1.88 ± 0.10 | 1.87 ± 0.11 | NS |
| MCHC (mmol/l) | 21.4 ± 0.5 | 21.2 ± 0.4 | NS |
| Thrombocyte (Gpt/l) | 217 ± 47 | 209 ± 53 | NS |
| Ferritin (μg/l) | 19.8 ± 13.4 | 18.3 ± 12.6 | NS |

Abbreviations: MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; NS, non significant.
All values are $\bar{x} \pm$ s.d.

carnitine concentration at delivery in the placebo group was approximately 40% lower than that at week 12 of pregnancy. Plasma carnitine concentrations at delivery in the placebo group, moreover, were approximately 60% lower than that during the subsequent lactation. These findings confirm that pregnant women have normally a very low carnitine status at delivery. Plasma carnitine concentrations below 20 μmol/l have even been considered as a marker of carnitine deficiency (Böhles *et al*., 1994), although it is unknown whether these low carnitine concentrations have adverse effects on the metabolism of women or their fetus. The present study shows that the supplementation with a moderate amount of L-carnitine (500 mg per day), starting at week 13 of pregnancy, prevents the decline of plasma carnitine concentration from week 12 to delivery. As it has been demonstrated that carnitine is transported through the placenta by novel organic cation transporters (Schmidt-Sommerfeld *et al*., 1985; Grube *et al*., 2005), it was not surprising that women supplemented with carnitine had a higher concentration of carnitine in the umbilical cord plasma, which represents plasma from the newborn infant than women of the placebo. It remains unknown whether increased carnitine concentrations offer beneficial effects to the newborn infant. However, L-carnitine plays an important role in energy production immediately after birth. During the intrauterine phase the supply of the fetus with amino acids, glucose, minerals and fatty acids from the mother via the placenta is essential for its development. The rate of fatty acid oxidation in the fetus is low (Novak *et al*., 1981). Immediately after birth, oxidation of fatty acids becomes important because of the discontinuation in the glucose supply and the rapid exhaustion of glycogen stores (Warshaw and Curry, 1980). Sufficient concentrations of L-carnitine in tissues are needed for the utilization of fatty acids for energy production. L-carnitine is required for both, the release of fatty acids from adipose tissue and fatty acid utilization (Novak *et al*., 1975a, b; Hahn, 1982). Therefore, it is possible that newborn infants of mothers supplemented with carnitine during pregnancy are able, because of their higher carnitine concentrations, to switch on fatty acid oxidation faster than those of mothers not supplemented with carnitine.

To our knowledge, the reason for the marked decline of plasma carnitine concentration in the course of pregnancy has not yet been clarified. Physiologically, plasma carnitine concentrations are strongly controlled by the renal excretion of carnitine (Rebouche and Seim, 1998). It has been found that urinary excretion of carnitine even declines during pregnancy and paralleled plasma carnitine concentrations (Cederblad *et al*., 1986; Marzo *et al*., 1994). Therefore, an increased renal excretion of carnitine can be ruled out as a possible reason for the low plasma carnitine concentrations during late pregnancy. In this study, we observed that concentrations of the carnitine precursors 6-*N*-trimethyllysine and γ-butyrobetaine, and in plasma are markedly lower during pregnancy than during the subsequent lactation. 6-*N*-trimethyllysine is released from proteins on protein degradation, and is converted subsequently into γ-butyrobetaine. This reaction occurs in almost all tissues; however,

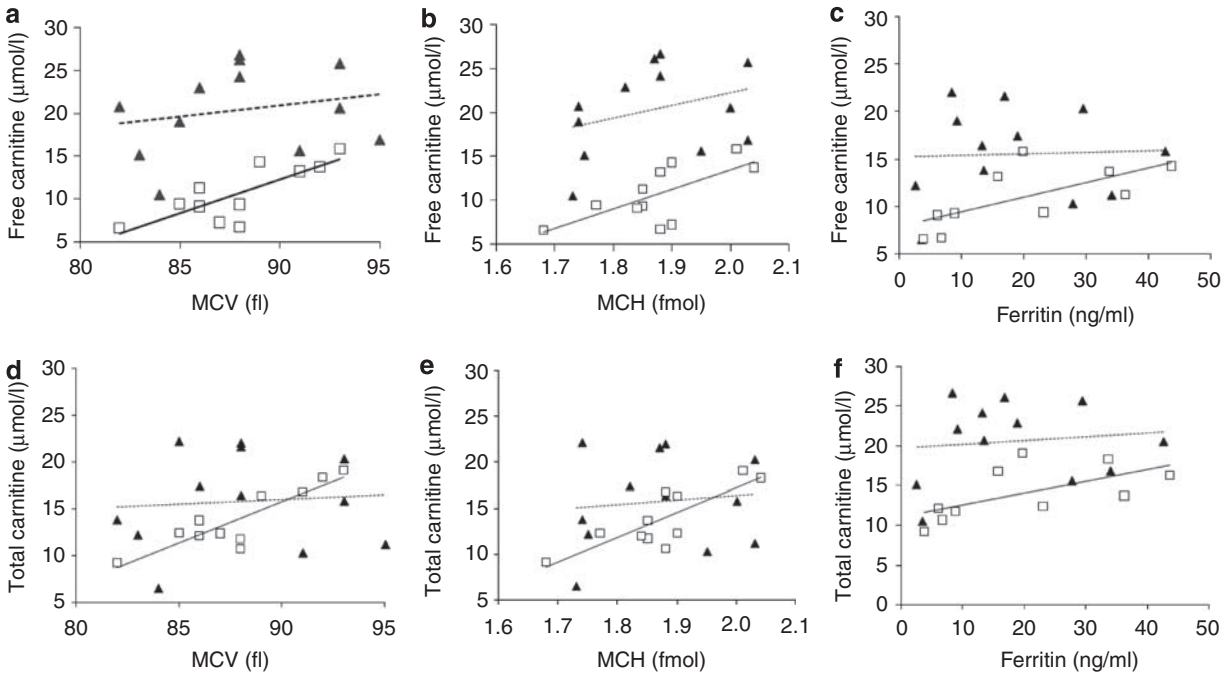


**Figure 1** Relationships between (**a**) plasma concentration of free carnitine and mean corpuscular volume (MCV), (**b**) plasma concentration of free carnitine and mean corpuscular hemoglobin (MCH), (**c**) plasma concentration of free carnitine and serum ferritin, (**d**) plasma concentration of total carnitine and MCV, (**e**) plasma concentration of total carnitine and MCH and (**f**) plasma concentration of total carnitine and serum ferritin (▲ + L-carnitine group, □ placebo group).

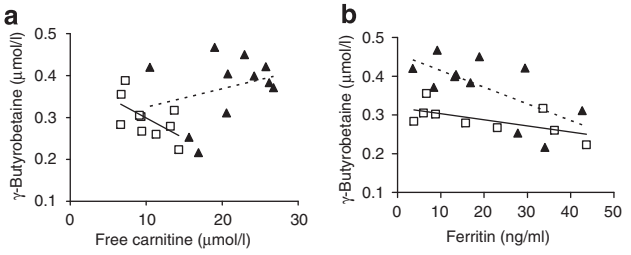


**Figure 2** Relationship between (**a**) plasma concentration of γ-butyrobetaine and free carnitine and (**b**) plasma concentration of γ-butyrobetaine and serum ferritin (▲ + L-carnitine group, □ placebo group).

most of the γ-butyrobetaine is produced in the skeletal muscle (Vaz and Wanders, 2002). γ-Butyrobetaine is secreted from tissues into blood and taken up into the liver and the kidney, where it is converted into carnitine by γ-butyrobetaine dioxygenase (Vaz and Wanders, 2002). Pregnancy represents an anabolic state, in which the rate of protein degradation in the body is reduced compared to the non-pregnant organism (Lain and Catalano, 2007). It is possible that concentrations of 6-*N*-trimethyllysine and γ-butyrobetaine were simply lower during pregnancy than lactation because of a reduced release of 6-*N*-trimethyllysine from body protein. It has been suggested that the availability of γ-butyrobetaine in the liver and the kidney is rate-limiting for carnitine synthesis (Rebouche *et al*., 1989). The finding of a reduced γ-butyrobetaine concentration in plasma therefore, could indicate that the production of carnitine might have been reduced in the liver and the kidney during pregnancy because of a reduced delivery of γ-butyrobetaine into these tissues. We are aware that this study contains a relatively small number of subjects, but nevertheless the finding that there was not a positive, but rather an inverse correlation between plasma γ-butyrobetaine and carnitine concentrations, however, argues against the suggestion that plasma carnitine concentration was reduced because of a reduced availability of γ-butyrobetaine. The finding of positive correlations between the parameters of iron status (MCV, MCH, serum ferritin concentration) and the concentrations of free and total carnitine in women receiving the placebo rather indicates that reduced plasma carnitine concentrations in pregnant women are because of a low iron status. The inverse relationship between γ-butyrobetaine and plasma ferritin concentration, which was observed in both supplemented and un-supplemented women, indicates that the conversion of γ-butyrobetaine into carnitine by γ-butyrobetaine dioxygenase was impaired in women with a low iron status. The inverse correlation between plasma γ-butyrobetaine and carnitine also indicates that an impaired conversion of γ-butyrobetaine into carnitine could have been responsible for low plasma carnitine concentrations. The activity of γ-butyrobetaine dioxygenase, the enzyme which catalyses the conversion of γ-butyrobetaine into carnitine, depends on the availability of iron (Bartholmey

and Sherman, 1986). We assume that the activity of this enzyme was reduced in pregnant women because of their low iron status, which in turn leads to a reduced carnitine biosynthesis. This could provide an explanation for the low plasma carnitine concentrations in pregnant women. In iron-deficient children, positive linear correlations between serum carnitine and ferritin concentrations have also been found (Böhles *et al*., 1991; Cemeroglu *et al*., 2001; Tanzer *et al*., 2001; Citak *et al*., 2006). Those studies also revealed a significant role of the iron supply for the carnitine status.

In conclusion, this study confirms that carnitine plasma concentrations decline during the course of pregnancy. Moreover, it is shown that carnitine supplementation prevents the marked decrease of plasma carnitine concentration from week 12 of pregnancy to delivery. Positive correlations between concentrations of free and total carnitine, and parameters of iron status (MCV, MCH, serum ferritin concentration) and in women receiving the placebo suggest that the reduced plasma carnitine concentrations in pregnant women are due to their low iron status. Perhaps, an inadequate iron status impairs carnitine synthesis, which in turn provides an explanation for the low plasma carnitine concentrations observed in pregnant women.

## Conflict of interest

The authors declare no conflict of interest.

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