

## Original Studies

### Plasma Carnitine Levels of Pregnant Adolescents in Labor

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**Abstract.** *Study Objective:* To determine the concentration of plasma carnitine (total, free, and acylcarnitine) during the delivery of uncomplicated pregnancies of adolescent women. To investigate the relationship between maternal and neonatal levels of carnitine and to compare these carnitine levels between pregnant and nonpregnant adolescents.

*Design:* Samples of maternal and umbilical blood were taken at the time of delivery and examined for the determination of the carnitine—total, free, and acylcarnitine—concentration by the use of an enzymatic-radioisotope method. Twenty-two cases of uncomplicated adolescent pregnancies with a normal labor and without perinatal complications were examined. The plasma level of carnitine was also examined in 17 healthy nonpregnant adolescent women, which constituted the control group.

*Results:* The concentrations of plasma carnitine in adolescent pregnancies at the time of delivery were calculated at  $19.6 \pm 2.15$   $\mu\text{Mol/L}$  (total),  $12.62 \pm 1.31$   $\mu\text{Mol/L}$  (free), and  $6.98 \pm 1.55$   $\mu\text{Mol/L}$  (acylcarnitine). The corresponding mean values in umbilical plasma were  $30.31 \pm 2.06$   $\mu\text{Mol/L}$ ,  $22.39 \pm 1.64$   $\mu\text{Mol/L}$ , and  $7.92 \pm .96$   $\mu\text{Mol/L}$ . There is a statistically significant difference between the mean values in maternal and umbilical plasma ( $P < .0001$  for total and free carnitine and  $P < .012$  for acylcarnitine). The correlations between adolescent pregnant women and their infants as regards total, free, and acylcarnitine were 0.137, 0.018, and 0.33, respectively. Neither of these parameters was statistically significant. The corresponding mean values of carnitine in nonpregnant adolescent women were statistically significantly higher than in adolescent pregnant women (total carnitine:  $41.61 \pm 3.09$   $\mu\text{Mol/L}$ , free:  $31.39 \pm 2.81$   $\mu\text{Mol/L}$ , acylcarnitine:  $10.22 \pm 1.88$   $\mu\text{Mol/L}$ ,  $P < .0001$ ).

*Conclusions:* The concentration of plasma carnitine at the end of adolescent pregnancy is low compared to the levels of umbilical carnitine at birth and that found in nonpregnant adolescent women. It may not have an obvious impact on the utilization of fatty acids in an uncomplicated full-term pregnancy; however, it suggests the potential risk for neonatal fatty-acid oxidation in a preterm or complicated pregnancy.

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**Key Words.** Carnitine—Adolescent pregnancy—Acylcarnitine—Total carnitine—Free carnitine

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#### Introduction

Carnitine (3-hydroxy-4-N-trimethylaminobutyric acid) is essential for the intramitochondrial translocation of fatty acids<sup>1</sup> and branched chain keto acids<sup>2</sup> as well as the exit of excess acyl groups from inside the mitochondrial matrix.<sup>3</sup> The body's supply of carnitine is derived in part from food and in part by endogenous synthesis from lysine and methionine.<sup>4</sup> The final reaction of hydroxylation to  $\gamma$ -butyrobetaine occurs in the liver and kidneys of humans.<sup>5</sup> Several other tissues can synthesize the immediate precursor of carnitine ( $\gamma$ -butyrobetaine) but lack the last biosynthetic enzyme that hydroxylates  $\gamma$ -butyrobetaine to form carnitine.<sup>5</sup> Since cardiac muscle and skeletal muscle cannot synthesize carnitine, it must be transported into these tissues through the blood stream. Total carnitine consists of free carnitine and its esters. In the plasma, most of the esterified carnitine is present as acetylcarnitine. The total number of esters of carnitine in plasma is referred to as acylcarnitine. When the rate of oxidation of fatty acids is increased, as in starvation or when a high-fat diet is consumed, the level of acylcarnitine in the blood rises<sup>6</sup> and a significant positive correlation is found between plasma levels of ketones and acylcarnitine.<sup>6,7</sup>

Sufficient concentrations of carnitine must be present in tissues for the efficient utilization of fatty acids.<sup>8</sup> This is especially important in the neonate who relies heavily on fatty-acid oxidation for energy.<sup>9,10</sup> Development of the capacity to utilize substrates other than glucose for fuel is an important postnatal adaptation. Hepatic glycogen is rapidly depleted within the

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first 24 hours after birth, with levels falling to 10% of peak term values. Lipolysis is very important to the maintenance of energy homeostasis when the oral intake of energy is low, such as in the newborn straight after birth. L-carnitine enhances glycerol release from newborn subcutaneous adipose tissue fragments. The neonate may have a limited supply of carnitine for a variety of reasons.<sup>5,11–15</sup> Some evidence suggests that the ability of neonates to synthesize carnitine is limited by immature hepatic- $\gamma$ -butyrobetaine hydroxylase activity.<sup>16</sup> Rebouche reported that at 3 months of age  $\gamma$ -butyrobetaine hydroxylase activity represents about 12% of that found in adults and in 2- to 5-year-old children reached only 30%.<sup>16</sup>

Very little is known about the transport of carnitine across the placenta and the ability of the human fetus to synthesize carnitine. Discrepant results have been obtained concerning the total-carnitine levels in maternal and neonatal plasma. No difference in total carnitine was found in one study<sup>17</sup> and the total-carnitine concentration in others was higher in umbilical venous blood than in maternal blood.<sup>18,19</sup> However, carnitine concentration in the blood, serum, or plasma of pregnant women is reported to decrease as gestation proceeds.<sup>19,20</sup> In contrast, other researchers have found that only the serum-free carnitine is decreased and that the concentration of esterified carnitine, which ordinarily makes up about 20% of the total, is increased in pregnant women.<sup>18</sup> The total-carnitine concentrations of plasma, heart, and skeletal muscle of the rat show significant increases after birth.<sup>21</sup> Similar results have been found in humans using autopsy and biopsy tissue.<sup>22</sup> It is reported that the level of carnitine in plasma and tissues is elevated from newborns to adults.<sup>16,21–23</sup> However, there are no reports about carnitine concentrations during adolescence. It is possible that changes such as the beginning of menstrual cycles and the rapid growth of the body (especially the skeletal muscles and bones) lead to some differentiation in the distribution of carnitine between serum and tissues, and increased metabolic demands. Moreover, adolescent pregnancy has been accepted as high-risk, with an increased risk of some complications such as preeclampsia and low-birthweight infants. Bone mineral content, iron stores, and caloric intake are often reduced among adolescent girls, and iron-deficiency anemia is frequently found.

The aim of this study is to determine the plasma concentrations of carnitine and its derivatives at delivery in uncomplicated adolescent pregnancies and to compare this with the plasma concentration of carnitine in nonpregnant adolescents. Moreover, the study aims to investigate the relationship between the carnitine plasma concentration in mothers and their neonates when the perinatal period is uncomplicated.

## Materials and Methods

### Subjects

Two groups of adolescents were studied. The first group was composed of 22 pregnant adolescents. They had a mean age of 17.1 yr, range 15–18. The duration of pregnancies ranged from 258 to 282 days. All the mothers were healthy and had no complications during the period of observation. They did not use any medication during pregnancy except iron and vitamin supplements and had an uneventful delivery. The control group comprised 17 healthy, nonpregnant women of adolescent age. Their mean age was 16.5 yr, range 15–18. They did not use illicit drugs and there were not statistically significant differences with regard to nicotine and alcohol use, lifestyle, and diet between them and the group of pregnant adolescents. The body mass of nonpregnant and pregnant (prior to pregnancy) adolescents was within normal limits for their age. The neonates seemed to be healthy after delivery except for two with mild respiratory distress. They were apparently free of anatomical abnormalities. Otherwise, all maternal-fetal pairs had a normal perinatal clinical course. Infant crown-heel length, head circumference, and subcutaneous fat was measured in regular conditions with standard equipment.<sup>24</sup>

### Design

During labor, which lasted 6–18 hrs, the women had an intravenous infusion of normal saline (no more than 60 mL/h) and epidural analgesia was not used. Some of these took pethidine 50 mg intramuscularly, many hours before delivery. Samples of maternal blood were taken from the antecubital vein, which was not used for infusion, within 30 minutes before or during delivery. Umbilical blood was taken in a heparinized tube kept on ice. The plasma was separated within 30 minutes following sampling and kept at  $-20^{\circ}\text{C}$  until analyzed. All determinations were done in duplicate.

### Chemical Procedures

Carnitine was assayed according to the method of McGarry and Foster<sup>25</sup> with modifications by Evageliou.<sup>26</sup> This is an enzymatic radioisotope method. Acylcarnitine was calculated as the difference between total carnitine, obtained after alkaline hydrolysis, and free carnitine.

### Statistical Analysis

The nonparametric Wilcoxon's rank sum test for paired samples was used for the comparison of the means of total, free, and acylcarnitine between pregnant adolescents and their neonates. The nonparametric Mann-Whitney's rank test for unpaired samples was used for the comparison of the means of total,

free, and acylcarnitine between pregnant and nonpregnant adolescent women.

## Results

Plasma-carnitine values are shown in Table 1. Carnitine levels at the end of gestation in adolescent women are decreased compared to nonpregnant controls. A significant difference ( $P < .0001$ ) was found in the mean values of total, free, and acylcarnitine levels between the pregnant and nonpregnant adolescents (Table 2).

The mothers (Table 3) had lower values of total and free carnitine than their infants at birth ( $P < .0001$ ) as well as of acylcarnitine ( $P < .012$ ). A positive correlation was not found between the maternal and infant plasma carnitine values at delivery. The values of total, free, and acylcarnitine between mothers and their offspring presented correlations of .137, .018, and .33 respectively, but none of these parameters had a statistical significance.

There were no correlations between maternal carnitine concentrations and the maternal age of some pregnancies' parameters (e.g., height, weight, weight gain, hemoglobin). No relationships were found between plasma carnitine and the infant's measurements at birth, such as head circumference, weight, and height. Correlations were not found between carnitine value and infant's hematological parameters, such as hematocrit, hemoglobin, white blood count, and platelets at birth.

## Discussion

Several authors<sup>19,20,27</sup> have reported that plasma-free and total-carnitine levels at delivery are decreased to about half the concentration seen in nonpregnant women. It is remarkable that the major decrease in plasma carnitine occurred during the first half of the pregnancy.<sup>28</sup> In contrast, other workers have found that only the serum-free carnitine is decreased and the concentration of esterified carnitine is elevated in pregnant

women.<sup>18</sup> The elevation of fatty-acid oxidation, which takes place toward the end of gestation and during delivery,<sup>29</sup> may be associated with the conversion of free carnitine to carnitine esters.<sup>18</sup> However, we found that there is a significant decrease of total-carnitine levels in the blood plasma as a result of a decrease of both free carnitine and acylcarnitine levels. The concentration of total carnitine in nonpregnant women has been evaluated to be  $38 \pm 1 \mu\text{Mol/L}$ <sup>20</sup> to  $57.5 \pm 8.3 \mu\text{Mol/L}$ .<sup>19</sup> In the same reports, the corresponding values of total carnitine in the pregnant women at delivery were  $18 \pm 2 \mu\text{Mol/L}$  and  $17.95 \pm 1 \mu\text{Mol/L}$ . Differences in methodology or specificity of procedures to determine the total, free, and esterified forms of carnitine probably resulted in differences found in concentrations in blood plasma. The concentrations of total carnitine found in the present study for adolescent women at birth and nonpregnant adolescents are  $19.6 \pm 2.15 \mu\text{Mol/L}$  and  $41.61 \pm 3.09 \mu\text{Mol/L}$ , respectively. Their difference is statistically significant ( $P < .0001$ ). However, our study shows that there are no significant differences between the concentrations of total carnitine in pregnant adolescents and pregnant women in adulthood, as they are referred to in some reports in the literature.<sup>19,20</sup> In comparison to some of these reports, we did not find any statistically significant difference between total-carnitine concentrations in nonpregnant adolescents and nonpregnant adult women.<sup>20,27</sup> There are no other published reports on plasma-carnitine levels in pregnant and nonpregnant adolescent women for a comparison of our results.

This observed decrease in plasma-carnitine concentration during gestation may be due to a plethora of agents. The increase in total body water is one of these. However, by the 20<sup>th</sup> week of pregnancy, levels have already fallen to 50% of prepregnancy values.<sup>28</sup> An increase in the renal clearance of carnitine is possible. Carnitine facilitates the removal of excess and potentially toxic acyl groups from the cell, which are excreted as acylcarnitine into urine.<sup>30</sup> It is possible that there is an increased need of carnitine during pregnancy to perform this metabolic function. If so, it would decrease the free-carnitine levels and increase

**Table 1.** Plasma Concentrations of Total Carnitine, Free Carnitine, and Acylcarnitine in Maternal and Umbilical Blood During Delivery of Adolescent Women and the Corresponding Values in the Plasma of Nonpregnant Adolescent Women

	Adolescents: during labor n = 22	Neonates: umbilical cord n = 22	Nonpregnant adolescents n = 17
Total carnitine	$19.6 \pm 2.15$ (16.3–23.9)	$30.31 \pm 2.06$ (27.2–34.8)	$41.61 \pm 3.09$ (37.3–47.1)
Free carnitine	$12.62 \pm 1.31$ (9.9–14.6)	$22.39 \pm 1.64$ (19–25.9)	$31.39 \pm 2.81$ (25.1–35)
Acylcarnitine	$6.98 \pm 1.55$ (4.3–10.4)	$7.92 \pm 0.96$ (6.3–9.9)	$10.22 \pm 1.88$ (7.2–13.3)

All values are means in  $\mu\text{Mol/L} \pm \text{SD}$ . The range of the values is given in parentheses. n = number of cases.

**Table 2.** Comparison of the Mean Values of the Plasma Concentrations of Total Carnitine, Free Carnitine, and Acylcarnitine between Pregnant Adolescents during Labor and Nonpregnant Adolescents

	Adolescents: during labor n = 22	Nonpregnant adolescents n = 17	Statistical significance
Total carnitine	19.6 ± 2.15	41.61 ± 3.09	<i>P</i> < .0001
Free carnitine	12.62 ± 1.31	31.39 ± 2.81	<i>P</i> < .0001
Acylcarnitine	6.98 ± 1.55	10.22 ± 1.88	<i>P</i> < .0001

All values are means in  $\mu\text{Mol/L} \pm \text{SD}$ . n = number of cases.

the clearance of acylcarnitine.<sup>28</sup> It has been shown that plasma-carnitine levels in rats were influenced by androgens and estrogens.<sup>31</sup> It may be that hormonal changes during pregnancy are influential factors on plasma carnitine in the human. Although the plasma concentration of carnitine is most often used as the parameter for the determination of carnitine availability, muscle-carnitine levels have not been determined in pregnant women, and it is not known whether a decrease in muscle-carnitine concentration (representing over 90% of total body carnitine) also occurs. It is recognized that plasma-carnitine levels may not truly reflect the tissue-carnitine status.<sup>32</sup> It is not known if uterine contractions during labor may contribute to further decrease of carnitine levels at the end of labor. However, the carnitine concentration in maternal blood just prior to the initiation of contractions has not been determined, although it is expected to be low.<sup>19,20</sup>

The most important question in all relevant reports is whether the developing fetus contributes to this decrease of maternal carnitine levels and what is the relation between maternal and fetal carnitine levels. The mechanism by which carnitine is supplied to the human fetus is not understood. Some authors believe that carnitine passes freely across the placenta via passive infusion.<sup>33</sup> Conflicting results have been reported regarding total-carnitine levels in maternal and neonatal plasma at the time of delivery. Similar values<sup>17</sup> and even higher values<sup>18,19</sup> have been found in the umbilical vein. The perinatal conditions, the manner of delivery, and the gestational age are factors influencing plasma-carnitine levels.<sup>27</sup> Women with abnormal pregnancies may also have significantly different levels.<sup>19</sup> There are no reports about the levels of carnitine in the mother and fetus in an adolescent's pregnancy.

Most authors agree that despite decreasing carnitine levels in maternal plasma at the end of gestation, the fetal carnitine level is low in comparison with that in adults or in children of 8–10 years of age ( $38.3 \pm 1.2 \mu\text{Mol/L}$ ).<sup>23</sup> In the present study the total-carnitine level of infants at birth was found to be  $30.31 \pm 2.06 \mu\text{Mol/L}$ . It is significantly higher than the mean value in maternal plasma (*P* < .0001) but lower than the value of carnitine levels in nonpregnant adults or children as shown above. A significantly positive correlation has been found between maternal and fetal plasma carnitine at delivery,<sup>18</sup> and maternal carnitine levels may be the most important factor influencing the concentration of plasma carnitine in the infant.<sup>27</sup> However, the positive correlation has not been found in the present study, which indicates that the initial total-carnitine levels after birth may not depend on the maternal levels of carnitine.

It is not clear if low carnitine levels in adolescents' labor have a correlation or an impact on the increased fatty-acid oxidation toward the end of the gestation. The higher levels of carnitine in the umbilical plasma than in maternal plasma may be the result of a placental transfer of carnitine to the fetus during pregnancy. These higher levels may also indicate that carnitine is synthesized to some degree by the fetus, at least during the advanced stages of pregnancy, which probably explains the absence of a positive correlation between maternal and umbilical carnitine levels in our study. However, the umbilical plasma-carnitine level is also low, probably with no obvious effect on the metabolism of the full-term, healthy neonates of this study. Nevertheless, under different conditions, and given that fat is the main source of energy in the postnatal period, the lower carnitine depots in addition to the in-

**Table 3.** Comparison of the Mean Values of the Plasma Concentrations of Total Carnitine, Free Carnitine, and Acylcarnitine between Maternal and Umbilical Blood during Delivery of Adolescent Women

	Adolescents: during labor n = 22	Neonates: umbilical cord n = 22	Statistical significance
Total carnitine	19.6 ± 2.15	30.31 ± 2.06	<i>P</i> < .0001
Free carnitine	12.62 ± 1.31	22.39 ± 1.64	<i>P</i> < .0001
Acylcarnitine	6.98 ± 1.55	7.92 ± 0.96	<i>P</i> < .012

All values are means in  $\mu\text{Mol/L} \pm \text{SD}$ . n = number of cases.

fant's limited capacity for carnitine biosynthesis may be critical in the maintenance of the neonate's energy homeostasis or even to the survival of premature infants—without exogenous carnitine intake. It is possible that pathological conditions of pregnancy, such as toxemia, placental dysfunction, or diabetes mellitus, not uncommon in adolescent pregnancy, may lead to limited supply of carnitine to the fetus and/or to the birth of a preterm/low-birth weight neonate with evident impaired ability to utilize lipid emulsions, due to the lower rate of carnitine biosynthesis.

Our study demonstrates the low plasma-carnitine levels in uncomplicated, full-term adolescent pregnancy and indicates the potential risk for the neonates born prematurely or after a complicated pregnancy, which, however, needs further investigation.

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