The Iraqi J. Vet. Med. 36 (1): 68 – 74; 2012

Effect of L-carnitine administration to pregnant mice on some reproductive hormones and organs

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Accepted: 13/ 11/ 2011

Summary

Carnitine is quaternary ammonium compound and required for the transport of fatty acids from the cytosol into the mitochondria for the generation of metabolic energy. The aims of the present study were to assess the effects of L-carnitine administration to pregnant mice on some parameters of reproductive performance and pregnancy outcome.

One hundred and five pregnant female mice Swiss albino strain mice age: 12-14 weeks were used in this study. Pregnant mice were divided randomly into three equal groups including control group (administered distilled water; DW), low dose group (T1) administered 0.5 mg/Kg L-carnitine and high dose group (T2) administered 1 mg/Kg L-carnitine. Daily administration of D.W. or L-carnitine was continued from day 1 (day post-sexual mating) until parturition. Hormone assay involving follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E_2), litter size, percentage of female sex, weight of the reproductive system and endometrial thickness were assessed.

Assessment of levels of serum reproductive hormones appeared that the FSH and LH and E_2 for both treated groups were increased significantly ($P<0.05$) as compared to the control group. Moreover, significant increment ($P<0.05$) in the weight of reproductive system, litter sizes and a significant increment ($P<0.05$) in the thickness of endometrium for both treated groups was observed as compared to the control group.

Conclusion: administration of 0.5 mg/Kg L-carnitine to pregnant mice had beneficial effects on pregnancy and offspring outcomes.

Key words: L-carnitine, mice, reproduction, pregnancy, FSH, LH, Estrogen.

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تاثير اعطاء مادة ال-كارنيتين الى حوامل الفئران على بعض صفات الكفاءة التناسلية

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الخلاصة

يعرف ال-كارنيتين على انه مركب رباعي المونيوم وهو ضروري لانتاج الطاقة الايضية من خلال نقل الاحماض الدهنيه من سيتوبلازم الخلية الى بيوت الطاقة. لذلك تهدف الدراسة الحالة الى معرفة تأثيراعطاء ال-كارنيتين للأناث الفئران الحوامل على بعض الصفات التكاثرية وناتج الحمل.استخدمت الدراسة مئة وخمسة أنثى فأر نوع الابرص السويسري عمر:12-14 اسبوع، والتي قسمت عشوائيا الى ثلاث مجاميع متساوية و تتضمن مجموعة السيطرة (عوملت بالماء المقطر فقط)، مجموعة الجرعة المنخفضة (T1):عوملت بـ 0,5 ملغم/كلغم ال- كارنيتين ومجموعة الجرعة العالية (T2):عوملت بـ 1 ملغم/كلغم الل -كارنيتين خلال فترة الحمل. وبعد الولادة تمت دراسة مستوى الهرمونات لكل من FSH و LH وE_2 وحجم الولادة ووزن الاعضاء التكاثرية بالاضافة الى قياس سمك طبقة بطانة الرحم للامهات ونسبة الاناث في المواليد.

اظهرت الدراسة الحالية زيادة معنوية ($P<0.05$) في كل من مستوى الهرمونات التناسلية في مصل الدم المقاسة (FSH و LH و E_2) ووزن اعضاء التناسلية وحجم المواليد وكذلك سمك بطانة الرحم لكلا مجموعتي

المعاملة بالمقارنه مع مجموعة السيطرة. نستنتج من نتائج الدراسة الحالية بأن التجريع بالجرعة المنخفضة لمادة أل-كارنيتين للامهات الحوامل له تاثير مفيد للحمل ولناتج المواليد.

كلمات مفتاحية:- كارنيتين , حوامل, فئران, التناسلية, استروجين.

Introduction

Carnitine, or 3-hydroxy-4-N-trimethylaminobutyrate, is a ubiquitous molecule within mammalian tissues, which was first discovered in the skeletal muscle extracts in the early twentieth century (1). The crucial role of L-carnitine in metabolism was not elucidated until 1955, and its deficiency was not described until 1972 (2). Carnitine was certified as an essential nutrient of multifunction for the body (3). A trimethylated amino acid, roughly similar in structure to choline, L-carnitine is a cofactor required for transformation of free long-chain fatty acids into acylcarnitines, and for their subsequent transport into the mitochondrial matrix, where they undergo beta-oxidation for cellular energy production (4).

In normal animals, the excretion of unchanged carnitine in urine seems to be the main pathway of loss. This excretion is increased in thyrotoxic and decreased in hypothyroid patients (5). Also, in normal animals carnitine is lost mainly by excretion in the urine (6). The absorption and deposition of dietary carnitine in human found that carnitine absorption is dependent on the intake amount. Approximately 54-87% of dietary carnitine is absorbed in the intestine and enters the bloodstream of rats and human being (7 and 8). Carnitine uptake from blood into tissues takes place via an active transport process against concentration gradient. Furthermore, tissue carnitine concentration is 20-50 folds higher than in plasma (9 and 10). Carnitine biosynthesis accounts for one third to one half of the total carnitine sources when omnivorous diet is consumed (11).

After oral administration of radioactive-labeled carnitine in rats, labeled trimethylamine N-oxide and butyrobetaine were found in urine and feces, respectively (12). Carnitine degradation in mammals was restricted to the non-absorbed carnitine in the intestinal tract, whereas absorbed or intravenously administered carnitine and endogenous carnitine were mostly eliminated in urine (13), and also excreted in milk (14). The European Food Safety Authority has made an extensive safety evaluation and concluded that up to 2 g L-carnitine or the equivalent 3 g L-carnitine tartrate are regarded safe for daily consumption (15).

Animal studies had revealed no harm to the fetus but that no adequate studies in pregnant women had been conducted. L-carnitine had been given to pregnant women late in pregnancy with resulting positive outcomes (16). Therefore, the aims of the present study were to assess the effects of L-carnitine administration to pregnant mice throughout all gestation days on some reproductive hormones and pregnancy outcome.

Materials and Methods

One hundred and five mature Swiss albino strain female mice age: 12-14 weeks; weight 25-28 g were used which obtained from animal house at Institute of Embryo Research and Infertility Treatment/Al-Nahrain University. Each female in the metestrus phase was caged with mature healthy male mouse, and the occurrence of vaginal plug was considered as the first day of pregnancy. The pregnant females were isolated in the cages alone. Pregnant mice were divided randomly into three equal groups (each group contains 35 pregnant mice) including control group (administered distilled water), low dose group (T1) administered 0.5 mg/Kg L-carnitine and high dose group (T2) administered 1 mg/Kg L-carnitine. Daily administration of D.W. or L-carnitine was continued from day 1 (day post-sexual mating) until parturition.

Low and high doses of L-carnitine were prepared by dissolving one crushed tablet (1000 mg tablet; Harbin Yeekong Herb Inc.; Australia) in 100 mL and 50 mL of distilled water; respectively. Each pregnant mouse was orally administered 0.05 mL from one of previous two solutions throughout pregnancy period.

At end of gestation period, 105 pregnant mice were delivered. Litter size and percentages of the female to male new born pups were determined. From 30 delivered mice, blood samples were taken under light anesthesia using diethyl ether (Fluka; Germany) by heart puncture using 2 mL syringe attached to 21-gauge needle and put in 1.5 mL tube and left for 10 minutes. serum were separated from blood using centrifugation for 2500 RPM for 8 minutes and preserved in refrigerator freezer at -20 ^{o}C untill the time of the hormone analyses (FSH, LH and E_2) using radioimmunoassay (RIA) technique at Biochemical tests laboratory, Institute of Embryo Research and Infertility Treatment.

Reproductive organs consisting ovaries, uterine horns and vagina were taken and cleared from attached adipose tissues. Weight of whole reproductive system was assessed using sensitive balance (BL-2105; Germany). Then, tissue of uterine horn was fixed and processed for histological sectioning to measure thickness of the endometrium according to procedure was mentioned by (17).

4. Statistics:

Data analyses were conducted using Statistical Analysis Package for Social Sciences (SPSS, version 14). All values were presented as mean and standard error of mean (Mean ± S.E.M). To compare among means of three groups, multiple analysis of variance (MANOVA) analysis and student t-test were used. Significance was set at $\boldsymbol{P} \leq 0.05$ (18).

Results and Discussion

Significant increment ($P<0.05$) in the weight of reproductive organs for both treated groups was assessed as compared to the control group. For the same parameter, non significant differences ($P>0.05$) were noticed between both treated groups. Litter sizes for both treated groups were increased significantly ($P<0.05$) as compared to the control group. However, non significant ($P>0.05$) differences were assessed for the litter size between both treated groups. Also, non significant ($P>0.05$) differences were observed in the female sex ratio among the control and both treated groups (Table 1).

Figure (1) shows the changes in the endometrial thickness for the control and both treated groups. Significant increment ($P<0.05$) in the thickness of endometrium was observed for both treated groups as compared to the control group. However, non significant ($P>0.05$) differences were appeared between both treated groups.

The results showed that the gonadotropins (FSH and LH) and estradiol (E_2) for both treated groups (T1 and T2) were elevated significantly ($P<0.05$) as compared to the control group. However, non significant differences ($P>0.05$) were reported for levels of all serum reproductive hormones between both treated groups (Table 2).

Table 1: Litter size, percentage of female sex and weight of reproductive organs for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=35 pregnant mice/group; Mean± S.E.)

Groups	Parameters		
	Litter size	Percentage of female sex	Weight of reproductive organs (g)
Control group	6.72 ± 0.11 *	0.76 ± 0.09	4.23 ± 0.041 *
Low dose group (T1)	8.64 ± 0.12	0.73 ± 0.09	6.61 ± 0.024
High dose group (T2)	8.81 ± 0.08	0.70 ± 0.05	6.82 ± 0.031

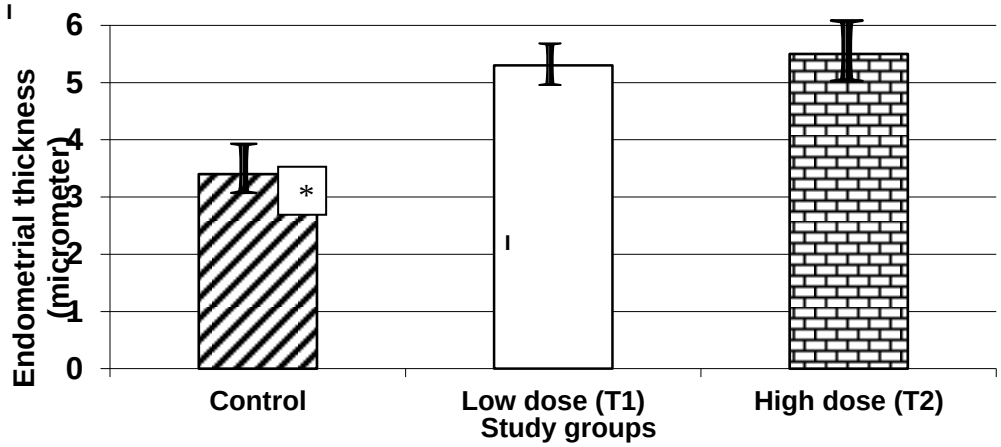

Figure 1: Endometrial thickness for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=10 pregnant mice/group; Data are Mean± S.E.)

Table 2: Levels of serum FSH, LH and E_2 for pregnant mice# administered two doses of L-carnitine throughout pregnancy (No.=10 pregnant mice/group; Data are Mean± S.E.)

Groups	Reproductive hormones		
	FSH (mIU/mL)	LH (mIU/mL)	E_2 (Pg/mL)
Control group	3.41 ± 0.020 *	1.30 ± 0.031 *	6.81 ± 0.012 *
Low dose group (T1)	5.10 ± 0.027	2.32 ± 0.022	8.42 ± 0.018
High dose group (T2)	5.22 ± 0.020	2.41 ± 0.026	8.34 ± 0.020

In the present study, a significant increment ($P<0.05$) in the weight of reproductive system and endometrial thickness was assessed for both L-carnitine treated groups as compared to the control group. The recently study, the intrauterine milieu is a complex mixture of substances originating from serum and endometrium that support blastocyst growth and development (19). Therefore, use of LC in patients with anorexia nervosa has been shown to accelerate body weight gain, normalize gastrointestinal function, and improve physical performance. Although LC biosynthesis increases during embryonic development, its levels are still much lower than those measured in adults (12). Thus, if carnitine food intake is reduced, the biosynthesis of carnitine can account for more than 90% of the body requirements (20).

Table 1 and Figure 1, changes in the weight of reproductive organs and endometrium thickness may be as a result of changes in the number of implantation sites and metabolism in several body organs and systems. There is experimental evidence that

LC stimulates the activity of the pyruvate dehydrogenase (PDH) complex by decreasing the intramitochondrial acetyl-CoA/CoA ratio through the trapping of acetyl groups (21). The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway (22). In general, L-carnitine transports long-chain fatty acids into the mitochondria where they are oxidized (metabolized). Once oxidized enhance the mitochondrial production of adenosine triphosphate (ATP). Enhancing ATP production, improves the metabolic efficiency in the tissues involved (23). In hearts containing raised concentrations of carnitine, there was a significant increase in glucose oxidation (24), subsequently, leads to increase ATP production and tissue formation.

Results show significant differences ($P<0.05$) were reported in the litter size between the control group and both treated groups. Previous researches had shown the addition of LC to maternal gestation diets increased body weight gain (25), plasma insulin like growth factor-II (26) of gestation mothers and increased total number of new born and born alive (27). Although LC is supplied exogenously as a component of the diet and can also be synthesized endogenously, evidence suggests both primary and secondary deficiencies do occur. On the other hand, carnitine deficiency can be acquired or a result of inborn errors of metabolism (16). Carnitine degradation in mammals is restricted to the non-absorbed carnitine in the intestinal tract, whereas absorbed or intravenously administered carnitine and endogenous carnitine are mostly eliminated in urine (13), and also excreted in milk (14).

Although much is known concerning the utilization and/or metabolism of specific nutrients, such as glucose and amino acids, by embryos before hatching from the zona pellucida (28 and 29). More recently, the impact of select nutrients on development of hatched blastocysts is limited, and this is especially true for species in which hatched blastocysts must undergo extensive elongation before implantation (29). Furthermore, a decrease in the production of free radicals, less tissue damage and reduced muscle soreness after exercise and a better utilization of fat as energy source during recovery (30). Carnitine had also an antioxidant capacity and decreases oxidative stress (31).

In conclution that administration of low concentration of L-carnitine to pregnant mothers had beneficial effects on pregnancy and offspring outcomes.

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