The effect of L-carnitine administered to pregnant mice on reproductive performance of female offsprings

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تأثير مادة أل-كارنيتين على الكفاءة التناسلية لذرية الأناث في الفئران

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المستخلص

اكتشف ال-كارنيتين في بداية القرن الماضي .في السنوات الثلاثين الاخيرة زاد الاهتمام في ايض ووظيفة ال-كارنيتين . يلعب ال-كارنيتين دورا رئيسيا في ايض الحوامض الدهنية بواسطة تسهيل نقل السلاسل الحرة الطويلة للحامض الدهني الى بيوت المايتوكوندريا لجعلها متوفرة لاكسدة بيتا,الذي يكون غالبا ممر فعال ايضيا لانتاج الطاقة .

الغرض:انجزت تلك الدراسة لتحديد تاثير اعطاء ال-كارنيتين للاناث الحوامل على بعض الصفات التكاثرية لذرية الاناث.

طريقة العمل: استخدمت ستون انثى فار بعمر ستة اسابيع قسمت الى مجموعتين متساويتين تتضمن مجموعة السيطرة (جرعت الامهات بالماء المقطر اثناء فترة الحمل)ومجموعة المعالجة (جرعت الامهات 1ملغم/كغم من ال-كارنيتين اثناء فترة الحمل). اخذت نماذج من الدم من الاجيال الاناث لتحديد الهرمونات(الهرمون المحفز للحويصلة والهرمون اللوتيني والهرمون المثير للدورة النزوية)كماوزنت الاعضاء التكاثرية للاجيال الاناث ، تم تحضير المقاطع النسيجية للرحم والمبيض لقياس فطر المبيض والغدد الرحمية بالاضافة الى قياس بطانة الرحم .

النتائج :اظهرت نتائج هذه الدراسة زيادة معنوية عالية بمستوى (الهرمون المحفز للحويصلة والهرمون اللوتيني والهرمون المثير للدورة النزوية) ووزن الاعضاء التكاثرية لمجموعة الاختبارمقارنة بمجموعة السيطرة كذلك لوحضت زيادة معنوية في سمك طبقة بطانة الرحم بالاضافة الى قطر المبيض , الغدد الرحمية لكلا مجموعتي الاختبارمقارنة بمجموعة السيطرة.

الاستنتاج:ان التجريع بالجرعة المنخفضة لمادة ال-كارنيتين1ملغم/كغم تمتلك تاثير ايجابي على الكفاءة التكاثرية للاجيال الاناث .توصي الدراسات لبحث تاثير ال-كارنيتين على تكوين البيوض للاجيال الاناث .

المفتاح: ال-كارنيتين,فار,انثى,حمل,اجيال

Abstract

Carnitine was detected at the beginning of the previous. In the last 30 years, interest in the metabolism and functions of carnitine has steadily increased. Carnitine plays an essential role in fatty acid metabolism by facilitating the transport of long-chain free fatty acids into the mitochondrial matrix, making them available for β-oxidation, which is the most efficient metabolic pathway for energy production.

The present study was performed to determine the effects of L-carnitine administered to pregnant mice on some parameters of reproductive performance for their female offspring.

Sixty healthy female mice (age: 6 weeks) were used in this study, and divided into two equal groups including control group (mothers administered distilled water during gestation period)

and treated group (mothers administered 1 mg/Kg L-carnitine during gestation period). Blood samples were taken from their female offspring for assessment of serum hormones (FSH, LH and E_2). Also, weight of reproductive system for female offspring was recorded, and then histological sections were prepared for the uterus and ovary to measure the diameters uterine gland and ovary, in addition to measure endometrial thickness.

Significant elevation ($P<0.01$) was observed in the levels of serum gonadotropins (FSH and LH) and E_2 for the treated group as compared to the control group. Weight of female reproductive system for the treated group was highly significant increased ($P<0.01$) as compared to the control group. Also, significant increment ($P<0.05$) was noticed in the thickness of endometrium for the treated as compared to the control group. Similarly, diameters of ovary and uterine glands for treated group were increased significantly ($P<0.05$) when compared to the control group.

Conclusion: Administration low doses of L-carnitine to pregnant mice have positive effects on reproductive performance of their female offspring. Further studies are recommended to investigate effects of L-carnitine to pregnant mice on oogenesis for their female offspring.

Key words: Carnitine, Mice, Female, Gestation, Offspring.

Introduction

Carnitine is a small (chemical structure $C_7H_{15}NO_3$; M.W. 161.2), water soluble, quaternary nitrogen-containing compound that is present in both L- and D- forms, while, L-carnitine being the biologically active form (1). Carnitine is synthesized from the amino acids L-methionine and L-lysine. Lysine provides the carbon backbone of carnitine (2) and methionine provides the 4-*N*-methyl groups (3). Carnitine is present as free carnitine and as acylcarnitines, which are products of reactions catalyzed by carnitine acyltransferases that utilize acyl-CoA. Free carnitine is the major carnitine pool representative (4, 5).

Although discovered in 1905, the crucial role of L-carnitine in metabolism was not elucidated until 1955, and its deficiency was not described until 1972. L-carnitine is naturally occurring in all mammalian species and is found in almost all cells. The human pool of L-carnitine is around 20 g with 98% of this within the cardiac and skeletal muscle pool, 1.4% in the liver and kidney, and 0.6% in extracellular fluid (6). However, carnitine biosynthesis accounts for one third to one half of the total carnitine sources when omnivorous diet is consumed (7). The most significant source of L-carnitine in human nutrition is meat, although humans can synthesize L-carnitine from dietary amino acids (8). If carnitine food intake is reduced, the biosynthesis of carnitine can account for more than 90% of the body requirements (9).

L-carnitine is present in most parts of the body, mainly in the skeletal muscle and heart (10). Experiments of carnitine uptake by different tissues, either using cells or organelles, perfused tissues or directly *in vivo* (11). Oral supplementation of L-carnitine in individual dosages greater than 2 g appears to offer no advantage, since the mucosal absorption of carnitine appears to be saturated at about a 2-g dose (12). Maximum blood concentration is reached approximately 3.5 hours after an oral dose and slowly decreases, with a half-life of about 15 hours. Elimination of carnitine occurs primarily through the kidneys (13). Following oral administration of 2-6 g carnitine in healthy humans, maximum blood concentrations are reached approximately 3.5 hours following an oral dose, with a half-life of about hours (12, 14). Therefore, the present study was performed to determine the effects of L-carnitine

administered to pregnant mice on some parameters of reproductive performance for their female offspring.

Materials and methods

Animals

Sixty healthy female mice (age: 6 weeks) were used in this study, and divided into two equal groups including control group (mothers administered distilled water during gestation period) and treated group (mothers administered 1 mg/Kg L-carnitine during gestation period). Females were supplied from Animal House of High Institute for Infertility Diagnosis and ARTs at Al-Nahrain University.

Preparation of L-carnitine dose

Dose of L-carnitine was prepared by dissolving one crushed tablet (1000 mg tablet; Harbin Yeekong Herb Inc.; Australia) in 50 mL of distilled water. Throughout pregnancy period, each pregnant mouse was orally administered 0.05 mL from either distilled water or L-carnitine solution for control and treated groups; respectively.

Measurements

Blood samples were taken from their female offspring under a light anesthesia by heart puncture using 2 mL syringe attached to 21-gauge needle and put in 1.5 mL tube and left for 10 minutes. Then, serum of hormonal assay (FSH, LH and E_2) was collected using centrifugation for 2500 RPM for 8 minutes and preserved at -20 C^{o} till the time of the analysis using radioimmunoassay (RIA) technique.Weight of reproductive system for female offspring were recorded after cleaning from adipose tissues, and then histological sections were prepared for the uterus and ovary to measure the diameters uterine gland and ovary in addition to measure endometrial thickness (15).

Statistical analysis

All values were presented as mean and standard error of mean (Mean ± S.E.M) using Statistical Analysis Package for Social Sciences (SPSS, version 14). To compare among means of three groups, multiple analysis of variance (MANOVA) test and student t-test were used. ***P*** value $\leq$ 0.05 was considered statistically significant (16).

Results

The results of serum reproductive hormones for female offspring where mothers administered 1 mg/Kg L-carnitine during gestation period were presented in the table (1). As compared to the control group, significant elevation ($P<0.01$) was observed in the levels of serum gonadotropins (FSH and LH) for the treated group. Similarly, the level of serum E_2 for the treated group was elevated significantly ($P<0.05$) when compared to the control group (Table 1).

In the present work, weight of female reproductive system for the treated group was highly significant increased ($P<0.01$) as compared to the control group (Table 2). From the same table, significant increment ($P<0.05$) was noticed in the thickness of endometrium for the

treated as compared to the control group. Similarly, diameters of ovary and uterine glands for treated group were increased significantly (P<0.05) when compared to the control group (Table 2).

Table (1): Effect of L-carnitine administered to pregnant mice during gestation period on levels of serum reproductive hormones for their female offspring

Study groups	Serum reproductive hormones		
	FSH (mIU/mL)	LH (mIU/mL)	E_2 (Pg/mL)
Control group (C)	3.34 + 0.09 *	1.32 + 0.012 *	6.41 + 0.01 *
Treated group (T)	5.31 + 0.042	2.32 + 0.020	8.22 + 0.021

* Significant difference (P<0.01) between control and treated groups.
#: Number of female offspring groups (Control =**(30);** Treated = **(30)**.

Table (2): Effect of L-carnitine administered to pregnant mice during gestation period on some parameters of reproductive system for their female offspring

Study groups	Parameters			
	Weight of reproductive organs (g)	Diameter of ovary (mm)	Endometrium thickness (□)	Diameter of uterine glands (□)
Control group (C)	4.03+0.034 *	6.41+0.013 *	1.32+0.012 *	3.21+0.05 *
Treated group (T)	6.72+0.031	8.24+0.021	2.33+0.021	5.5+0.07

* Significant difference (P<0.05) between control and treated groups.
#: Number of female offspring groups (Control = (30); Treated = (30).

Discussion

In the present study, administration of L-carnitine (LC) to female mice increases levels of serum FSH and LH of their female offspring as compared to the control group. An explanation for this hormonal changes may be under several direct and/or indirect factors related to the pituitary gland or hypothalamus either alone or both. Once LC oxidized they enhance the mitochondrial production of adenosine triphosphate (ATP). Enhancing ATP production improves the metabolic efficiency in the tissues involved (6). Also, carnitine is involved in the metabolism of ketones for energy (17) and the conversion of branched-chain amino acids – Valine, Leucine, and Isoleucine – into energy (18).

Moreover, 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 (19). The simultaneous reduction of acetyl-CoA levels in the cytosol further contributes to activate the glycolytic pathway (20). L-carnitine mediated changes in lipid metabolism (21). Carnitine has also an antioxidant capacity and improves oxidative stress (22). Furthermore, sex hormones may also influence carnitine distribution. In rats, females have been reported to have a significantly higher liver/plasma carnitine concentration ratio than male rats, and the high carnitine uptake and concentration in the epididymis depend on an androgen-induced transport mechanism (23).

As compared to the control group in the present study, an increase in the weight of female reproductive organs for the group treated with LC may be related to several biochemical and metabolic factors which cooperate to build and repair tissues of female reproductive organs. In fact, LC essentially plays a key role in the mitochondrial β-oxidation of long chain free fatty acids (24). By providing a shuttle system for free fatty acids and derivatives of acetyl-CoA within the mitochondria, LC regulates the flux of acetyl groups, and therefore energy balance, through the cellular membrane. During their passage through the cellular membranes, acetyl groups are temporarily transferred to LC, producing LAC (25). Furthermore, carnitine improves repair mechanisms for oxidative stress-induced damage to membrane phospholipids (26), and also maintains general antioxidant status (27). It protects cells from reactive oxygen species (22) by acting as a free radical scavenger (28). Carnitine plays essential roles in energy production, oxidative stress and glucose metabolism (29).

On the basis of the present results, LC administration causes a significant increase in the thickness of endometrium as compared to the control group. Therefore, LC may be enhancing the endocrinological function of ovary which controls the growth of endometrium. It is well known that the ovarian function under the control of gonadotropins (30). As previously mentioned, an elevation of gonadotropins for the LC treated offspring group increases endometrial thickness indirectly through endocrinological action of ovaries. In addition to indirect action of LC, LC may be has direct effect supports endometrial growth. Several *in vivo* studies have appeared on the uptake and exchange of carnitine in different tissues. Because most tissues have a carnitine concentration that is >lO-fold higher than that of blood plasma, an active uptake of carnitine must take place; this uptake, however, must occur with widely varying rates (31). L-carnitine supplementation of hen diets improved embryo yolk lipid mobilization. The mobilized yolk lipid may be used to produce energy or be incorporated into body tissues (32). Previous researches have shown the addition of L-carnitine to maternal gestation diets increased body weight gain (33), plasma IGF-II (34) of gestation mothers and increased total number of new born and born alive (35).An increment of endometrial thickness improve many biological factors and biochemical pathways which collectively support implantation of blastocyst and embryonic development (36).

Carnitine plays a key role in glucose metabolism and in fuel-sensing, because it behaves as a shuttle for acetyl groups from inside to outside the mitochondrial membrane (37). The intrauterine milieu is a complex mixture of substances originating from serum and endometrium that support blastocyst growth and development (36, 38). For mouse embryos, lysine is used for the synthesis of protein, but whether lysine is oxidized or converted to carnitine, a substance needed for transport of long-chain fatty acids from the cytosol into

mitochondria for oxidation (4). The carnitine system is important in determining body composition, because it plays a critical role in insulin regulation of fat and glucose metabolic rate in skeletal muscle (39). The mechanism of carnitine action includes an increase in glucose metabolism via stimulating glucose disposal and oxidation (26). Also, carnitine may improve insulin sensitivity in insulin resistance (40). In contrast, carnitine deficiency may also be provoked in infants given artificial carnitine-free diets, presumably because of a slow endogenous rate of carnitine biosynthesis in the neonatal period. Newborn infants normally receive substantial amounts of carnitine from their mothers' milk (41).

From the results of the present study, it was concluded that the administration low doses of L-carnitine to pregnant mice have positive effects on reproductive performance of their female offspring. Further studies are recommended to investigate effects of L-carnitine to pregnant mice on oogenesis for their female offspring.

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