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REVIEW ARTICLE

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The role of carnitine in male infertility

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SUMMARY

This review explores the role of carnitine in male infertility. The structure of this review is organized into short paragraphs that address the following aspects: antiapoptotic effect of L-carnitine on germ cells, effects of L-carnitine on conventional sperm parameters, in vitro effects of L-carnitine on sperm function, and the role of L-carnitine on erectile function.

INTRODUCTION

From almost a century, the beneficial effect that carnitine exerts on the human organism, especially in its forms L-carnitine and acetyl-L-carnitine, is now known.

The L-carnitine (that man is able to synthesize, but which is mainly of exogenous origin) is a quaternary amine highly polar and water soluble in nature. It acts as an essential co-factor for the transport of long-chain fatty acids within the mitochondrial matrix in order to facilitate the oxidative processes and to enhance cellular energy production (Agarwal & Said, 2004; Ng *et al.*, 2004). The acetyl-L-carnitine, instead, is formed in a reversible manner from the enzyme acetyl-L-carnitine transferase which modulates the intracellular and mitochondrial concentrations of CoA and acetyl-CoA (Lenzi *et al.*, 1992).

It is believed that the contribution necessary with the daily supply of L-carnitine is about 8–11 mg. Approximately 98% of the body's L-carnitine is stored in the skeletal muscles and heart, liver contains between 1 and 6%, while in extracellular fluids we find a concentration ranging between 0 and 6% (Lenzi *et al.*, 1992).

CARNITINE AND MALE REPRODUCTIVE SYSTEM

An interesting aspect is the high concentration of carnitine that is found in the male reproductive tract, especially in the epididymis, suggesting its crucial role in energy metabolism and in the maturation of spermatozoa (Lenzi *et al.*, 1992; Vicari *et al.*, 2001).

The L-carnitine located in the epididymis is derived from plasma and it is actively transported across the epithelial cells into the epididymal plasma (Ng *et al.*, 2004). Based on several research, it seems that this process of active transport may be mediated by specific carnitine/organic cation transporters (OCTNs) located in the testis, especially in the luminal epithelium of the seminiferous tubules and Sertoli cells, and expressed in humans, rats, and mice (Enomoto *et al.*, 2002). The first member of OCTNs, OCTN1 (solute carrier 22A4), transports cationic xenobiotics, such as tetraethylammonium, and has a low activity for carnitine transport. OCTN2 (SLC22A5) is a Na^+-dependent, high-affinity (K_m = 4–25 μM) carnitine transporter. Human carnitine transporter CT2 (SLC22A16) and mouse carnitine transporter OCTN3 (SLC22A21) transport carnitine with high affinity (K_m = 20 and 3 μM, respectively) in a sodium-independent manner (Kobayashi *et al.*, 2007). Finally, the L-carnitine would be accumulated inside the spermatozoa by passive diffusion (Jeulin & Lewin, 1996).

Based on several research, it seems that this process of active transport is mediated by a specific carnitine carrier (CT2), located in the testis, especially in the luminal epithelium of the seminiferous tubules and in the Sertoli cells (Enomoto *et al.*, 2002). Finally, the L-carnitine would be accumulated inside the spermatozoa through passive diffusion (Jeulin & Lewin, 1996). Because the sperm in the epididymis are able to use fatty acids and phospholipids as energetic source, probably L-carnitine also here acts as a co-factor for the mitochondrial transport and the subsequent oxidation of fatty acids. Furthermore, high concentrations of this molecule, seem to suppress the metabolic activity of the ejaculated spermatozoa (whose metabolism is mainly glucose), but not those of the epididymis whose main energy source is represented by fatty acids (Lenzi *et al.*, 1992).

ANTIAPOPTOTIC EFFECT OF L-carnitine on germ cells

Another finding of particular interest is the effect of the antiapoptotic L-carnitine, which seems to be explained by the inhibition of programmed cell death mediated by the FAS-FAS ligand and the caspase 3, 7, and 8 (Mutomba *et al.*, 2000).

In the male reproductive system, the apoptosis process can occur spontaneously or be induced by several factors, such as heat or androgen deprivation. To confirm this, Amendola and colleagues evaluated the effects of treatment with L-carnitine on spermatogenesis in mice irradiated with a single dose of 10 Gy on the testicles. Mice of the treatment group were treated with intraperitoneal administration of 100 mg/kg of acetyl-L-carnitine on alternate days for 4 weeks: the effects on spermatogenesis were evaluated after 1, 28, 35, 40, 45, 50, 55, and 60 days from irradiation. The authors concluded that acetyl-L-carnitine improves the possibility of identifying spermatogonia after radiation damage (Amendola *et al.*, 1989).

The same authors, furthermore, have conducted a study to assess the protective effect of L-carnitine on the spermatogenesis after heat-induced damage with similar results (Amendola *et al.*, 1991). Kanter also suggested a reduction in germ cells apoptosis and a significant reduction in the incidence of sperm morphological abnormalities in rats subjected to testicular irradiation and simultaneous administration of L-carnitine (Kanter *et al.*, 2010). In addition, this powerful antioxidant seems to cause a cytoprotective effect in rats treated with etoposide, a chemotherapeutic agent that acts by blocking the catalytic function of topoisomerase II, thereby causing cell death (Okada *et al.*, 2009). Human studies appear to confirm this antiapoptotic action, in addition to the antioxidant, at the level of the reproductive male system, with a consequent improvement in sperm parameters (Abad *et al.*, 2013).

According to the above, it is not difficult to understand what is the rational use of L-carnitine and of its ester, acetyl-L-carnitine, in the treatment of men with reduced fertility (Ng *et al.*, 2004).

EFFECTS OF L-carnitine on conventional sperm parameters (Table 1)

In 1992, Moncada and colleagues enrolled 20 couples with a history of infertility lasting from 16 to 24 months; the male partners of these couples had a diagnosis of idiopathic oligoasthenozoospermia and had an average age of 30 $\pm$ 3 years. All patients were treated with acetyl-L-carnitine 4 g/day for 60 days; the sperm parameters were then evaluated at the end of the treatment and after 4 months from the end of the same. At the end of treatment with acetyl-L-carnitine, 60% of the patients showed a statistically significant increase in the progressive sperm motility that appeared to be drug related; 4 months after the end of therapy, indeed, the values of progressive motility were returning similar to pretreatment value (Moncada *et al.*, 1992).

Similarly, a study of 100 patients with idiopathic asthenozoospermia, treated with L-carnitine administered orally at a dose of 3 g/day for 4 months, showed an improvement in progressive and total sperm motility and an increase in sperm concentration, confirming, then, a real advantage on the quality and quantity of semen of treated subjects (Costa *et al.*, 1994). As further proof, in 1995, the efficacy of treatment with L-carnitine in 47 infertile patients from at least 2 years, with idiopathic asthenozoospermia as the only known cause of infertility after exclusion of patients with a history of cryptorchidism, postinfectious testicular atrophy or trauma, severe varicocoele, obstruction, urogenital tract inflammation or infection, endocrine hypothalamic–pituitary–gonadal axis disorders, and with antisperm antibodies evidence was evaluated. For each patient enrolled in the study, it was administered orally 3 g/day of L-carnitine (divided into three doses with meals) for 3 months. At the end of the treatment, 80% of patients had a significant improvement in sperm motility, with values nearly equal to those found in a control group consisting of 110 fertile donors (Vitali *et al.*, 1995).

As the L-carnitine concentrations are particularly high in the epididymis, it is now known that its concentration in the ejaculate may be a marker of epididymal function. Because the major cause of reduced male fertility is represented by urogenital tract inflammation, including epididymitis, some studies have shown a reduction in the concentration of L-carnitine in the seminal fluid in patients with epididymitis (Bornman *et al.*, 1989; Cooper *et al.*, 1990). The inflammatory state could determine fertility reduction through the over production of reactive oxygen species (ROS) from leukocyte and/or spermatozoa with a consequent increase in oxidative stress.

Because the prostato-vesiculo-epididymitis (PVE), including all possible urogenital infections, are the diagnostic category with a higher level of oxidative stress (that often persistent even after antibiotic therapy), Vicari and colleagues have evaluated the antioxidant properties of L-carnitine in the treatment of patients with PVE. For this purpose, they were examined two groups of infertile men: group A, consisting of 55 patients with abacterial PVE (average age 34 years old); group B, formed by 35 subjects with bacterial PVE (average age 35 years old). They were excluded from the study patients with primary testicular disease, testicular atrophy, endocrine disorders, obstruction of spermatozoa, those who practiced drug therapies in the 3 months prior to the study, patients with oligozoospermia (<5 million/mL), and/or severe teratozoospermia (>87% of atypical forms) and monomorphic teratozoospermia. The two groups were then randomly divided into different subgroups based on the treatment received: A1 and B1 subsets received, respectively, for 3 months an antibiotic and/or nonsteroidal anti-inflammatory drugs (NSAIDs) (14 days monthly for 3 months), followed by L-carnitine (1 g twice daily) together with acetyl-L-carnitine (0.5 g twice a day), and finally no drug for 3 months. A2 and B2 subgroups received, for a 3 month period, in the meantime the combined antibiotic and/or anti-inflammatory regimen ($\times$14 days monthly) and L-carnitine (1 g $\times$ 2/day) + acetyl-L-carnitine (0.5 g $\times$ 2/day) followed by 3 months without any therapy. A3 and B3 subsets received for a 3-month period treatment with L-carnitine 1 g twice a day and acetyl-L-carnitine 0.5 g twice a day, followed by 3 months without any treatment. Each patient was subjected to an examination of the seminal fluid, microbiological analysis, and in 60 of the 90 patients ROS production was also investigated before, during, and after treatment. The results obtained showed a significant reduction of ROS in seminal fluid and an improvement in the progressive motility and vitality of spermatozoa, in particular in the subgroups A1 and B1. These results suggest that the best antimicrobial and antioxidant response is obtained administering first antibiotic therapy and/or anti-inflammatory regimen, and second by the treatment with

Table 1 Clinical studies on the effects of treatment with carnitine in infertile patients

Authors	Patients	Treatment	Dosage	Result
Moncada *et al.* (1992)	20 patients with idiopathic OAT	Acetyl-L-carnitine	4 g/day × 60 days	Improvement in sperm motility
Costa *et al.* (1994)	100 patients with idiopathic asthenozoospermia	L-carnitine	3 g/day × 4 months	Sperm concentration increased Improvement in progressive and total motility
Vitali *et al.*, (1995)	47 patients with history of infertility for at least 2 years	L-carnitine	3 g/day × 3 months	Significant improvement in sperm motility
Vicari *et al.* (2001)	55 patients with abacterial PVE (group A); 35 patients with bacterial PVE (group B)	Groups A1 and B1: antimicrobials + NSAIDs and L-carnitine + acetyl-L-carnitine; Groups A2 and B2: antimicrobials + NSAIDs + L-carnitine + acetyl-L-carnitine; Groups A3 and B3: L-carnitine + acetyl-L-carnitine	Groups A1 and B1: antimicrobials + NSAIDs 14 days monthly for 3 months and L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day for 3 months; Groups A2 and B2: antimicrobials + NSAIDs 14 days monthly + L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day for 3 months; Groups A3 and B3: L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day for 3 months	Groups A1 and B1: significant decrease in the ROS production, increase in some semen parameters (sperm motility and viability) Groups A2 and B2: no significant improvement in sperm parameters, ROS persist in semen; Groups A3 and B3: treatment ineffective
Vicari & Calogero (2001)	34 patients with normal seminal WBC $<1 \times 10^6$/mL (group A) and 20 patients with elevated seminal WBC $> 1 \times 10^6$/mL (group B), infertile patients with ROS overproduction and PVE after antimicrobials treatment	L-carnitine + acetyl-L-carnitine	2 g/day + 1 g/day × 3 months	Group A: improvement in sperm vitality and sperm motility, increased pregnancy rate (significantly higher than group B) Group B: only improvement in sperm vitality
Vicari *et al.* (2002)	94 patients with abacterial PVE and leukocytospermia	Group A: L-carnitine + acetyl-L-carnitine Group B ($n = 16$): nimesulide Group C ($n = 26$) nimesulide and L-carnitine + acetyl-L-carnitine Group D ($n = 26$) nimesulide + L-carnitine + acetyl-L-carnitine	Group A: L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day × 4 months Group B: nimesulide × 4 months Group C: nimesulide × 2 months and after L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day × 2 months Group D: nimesulide + L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day × 4 months	Significant increase in sperm vitality and motility and ROS reduction in seminal fluid only in subjects of group C (sequential treatment NSAIDs and carnitine)
Lenzi *et al.* (2003)	Randomized placebo-controlled study of 100 patients with infertility history >2 years	L-carnitine or placebo	2 g/day or placebo according to the scheme: 2 months washout, 2 months therapy/placebo, 2 months more washout and finally a further 2 months therapy/placebo	Improvement in sperm parameters in the group treated with carnitine
Lenzi *et al.* (2004)	Randomized placebo-controlled study of 60 infertile patients with OAT	L-carnitine + acetyl-L-carnitine or placebo	2 g/day + 1 g/day or placebo, according to the scheme: 2 months washout, 6 months therapy/placebo, 2 months follow-up	Total and progressive motility improvement in patients treated with carnitine
Cavallini *et al.* (2004)	123 patients with OAT and subclinical varicocoeles	Group 1: placebo; Group 2: L-carnitine + acetyl-L-carnitine; Group 3: L-carnitine/ acetyl-L-carnitine and cinnoxicam	Group 1: placebo × 6 months; Group 2: L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day × 6 months; Group 3: L-carnitine/ acetyl-L-carnitine (same dose of group 2) and cinnoxicam (30 mg every 4 days) × 6 months	Group 1: no significant change in sperm parameters; pregnancy rate 1.7%; Group 2: improvement in the spermatic patterns in patients with varicocoele of I, II, and III degree; pregnancy rate in group 1 was 21.8%; Group 3: improvement in sperm parameters, pregnancy rate 38%

(continued)

Table 1 (continued)

Authors	Patients	Treatment	Dosage	Result
Garolla *et al.* (2005)	30 normozoospermic controls 30 asthenozoospermic patients divided into two groups based on the seminal concentrations PHGPx (normal or increased)	Placebo and after L-carnitine	Placebo × 3 months, after L-carnitine 2 g/day × 3 months	Sperm motility improved after treatment with L-carnitine in asthenozoospermic patients with baseline normal PHGPx
Balercia *et al.* (2005)	60 infertile men with asthenozoospermia	L-carnitine Acetyl-L-carnitine L-carnitine + acetyl-L-carnitine Placebo	3 g/day × 6 months 3 g/day × 6 months 3 g/day + 3 g/day × 6 months 6 months	Acetyl-L-carnitine leads to an improvement in sperm motility and reduction of ROS in semen; great improvement when the acetyl-L-carnitine was administered in combination with L-carnitine
Balercia *et al.* (2005)	170 infertile men divided into two groups depending on whether the total sperm motility was greater than or less than the stated range by the WHO (50%)	Not azoospermic patients with motility <50% treated with L-carnitine + acetyl-L-carnitine	1 g/day + 1 g/day × 6 months	Improvement in sperm parameters of I and II level

L-carnitine; co-administration of antimicrobial agents and antioxidants is less effective, while treatment with only L-carnitine has no effect (Vicari *et al.*, 2001).

On the basis of these data, which show the persistence of oxidative stress despite the antibiotic therapy, 54 asymptomatic infertile patients with overproduction of ROS and ultrasonographic evidence of PVE, already receiving antimicrobial therapy, were treated with acetyl-L-carnitine. The patients excluded from the study were the patients with microbial reinfection, azoospermia, severe oligozoospermia, teratozoospermia, and/or necrozoospermia, high FSH levels, primary testicular disease, smoking cigarette, alcohol consumption, occupational exposure to chemical, kidney or liver disease, myopathies, or consumption of drugs in the 3 months preceding the study. According to the concentration of seminal leukocytes, patients were divided into two groups: group A, with normal numbers of leukocytes in the ejaculate; group B, with leukocytes higher than the norm (>1 million/mL). The seminal parameters, the production of ROS, and the rate of spontaneous pregnancy were assessed before, during, and after treatment with L-carnitine. The results obtained showed an increase in the motility and viability of spermatozoa in subjects of group A. Treatment with L-carnitine in group B patients, however, only led to an improvement in sperm vitality. Finally, the rate of spontaneous pregnancy in patients of group A was significantly higher than patients in group B (Fig. 1) (Vicari & Calogero, 2001).

To evaluate whether the association of antioxidants and anti-inflammatory compounds may be beneficial in treatment of patients with abacterial PVE and elevated seminal leukocyte concentrations, the same working group has conducted a prospective randomized study on 98 patients with the clinical characteristics just before mentioned. The cohort was divided into four groups: group A (n = 30) received oral carnitine (1 g every 12 h) and acetyl-L-carnitine (500 mg every 12 h) for 4 months; group B (n = 16) received non-steroidal anti-inflammatory drugs (nimesulide) for 4 months; group C (n = 26) received non-steroidal anti-inflammatory compounds for 2 months followed by carnitine for 2 months (carnitine 1 g every 12 h and acetyl-L-carnitine 500 mg every 12 h); group D (n = 26) received non-steroidal anti-inflammatory compounds and carnitine for 4 months. The sperm parameters, the ROS production in seminal fluid, and the spontaneous pregnancy rate were assessed before, during, and after treatment with a 3-months washout period. The collected data have stressed that the patients in group C had the highest reduction in production of ROS associated with increased sperm motility and viability, namely in those patients who have received the first treatment with NSAIDs and at a later time the one with L-carnitine (Vicari *et al.*, 2002).

With the aim to evaluate the effects of therapy with L-carnitine on the male infertility, it has been realized a randomized double-blind placebo control on 100 patients, aged between 20 and 40 years, with infertility lasting longer than 2 years (Lenzi *et al.*, 2003). They were excluded from the study patients with endocrine diseases, present or previous cryptorchidism, genital infections or genital tract obstructions, varicocoele, and testicular hypotrophy. The seminological inclusion criteria were normal rheological characteristics, sperm concentration between 10 and 20 million/mL, total motility 10–30%, progressive motility <15%, atypical forms <70%, semen leukocytes <1 million/mL. The study design involved the administration of L-carnitine (2 g/day) or placebo according to the scheme: 2 month of washout, 2 months of therapy/placebo, other 2 months washout, and finally a further 2 months of therapy/placebo. During each control sperm parameters seminal concentrations of L-carnitine and those of seminal α-glycosidase concentration (neutral, SDS inhibitable form used as a marker of epididymal function) and the lipid peroxidation potential of the sperm membrane were evaluated. The results have revealed an improvement in some of the variables analyzed both after treatment with L-carnitine than after placebo, especially in the first period of administration, stressing the importance of the psychological aspect on the infertility etiopathogenesis. However, L-carnitine therapy was effective in increasing semen quality, especially in groups with lower baseline levels compared with those in the group that received the placebo, in both cases, however, there have been no changes in the concentration of seminal α-glycosidase and in the lipid peroxidation of membrane (Lenzi *et al.*, 2003).

In 2004, a double-blind randomized placebo-controlled trial to evaluate the efficacy of combination therapy with L-carnitine and acetyl-L-carnitine in infertile men with oligoasthenoteratozoospermia was conducted. The study enrolled 60 subjects between the ages of 20 and 40 years: 30 patients were treated with placebo and 30 with L-carnitine (2 g/day) plus acetyl-L-

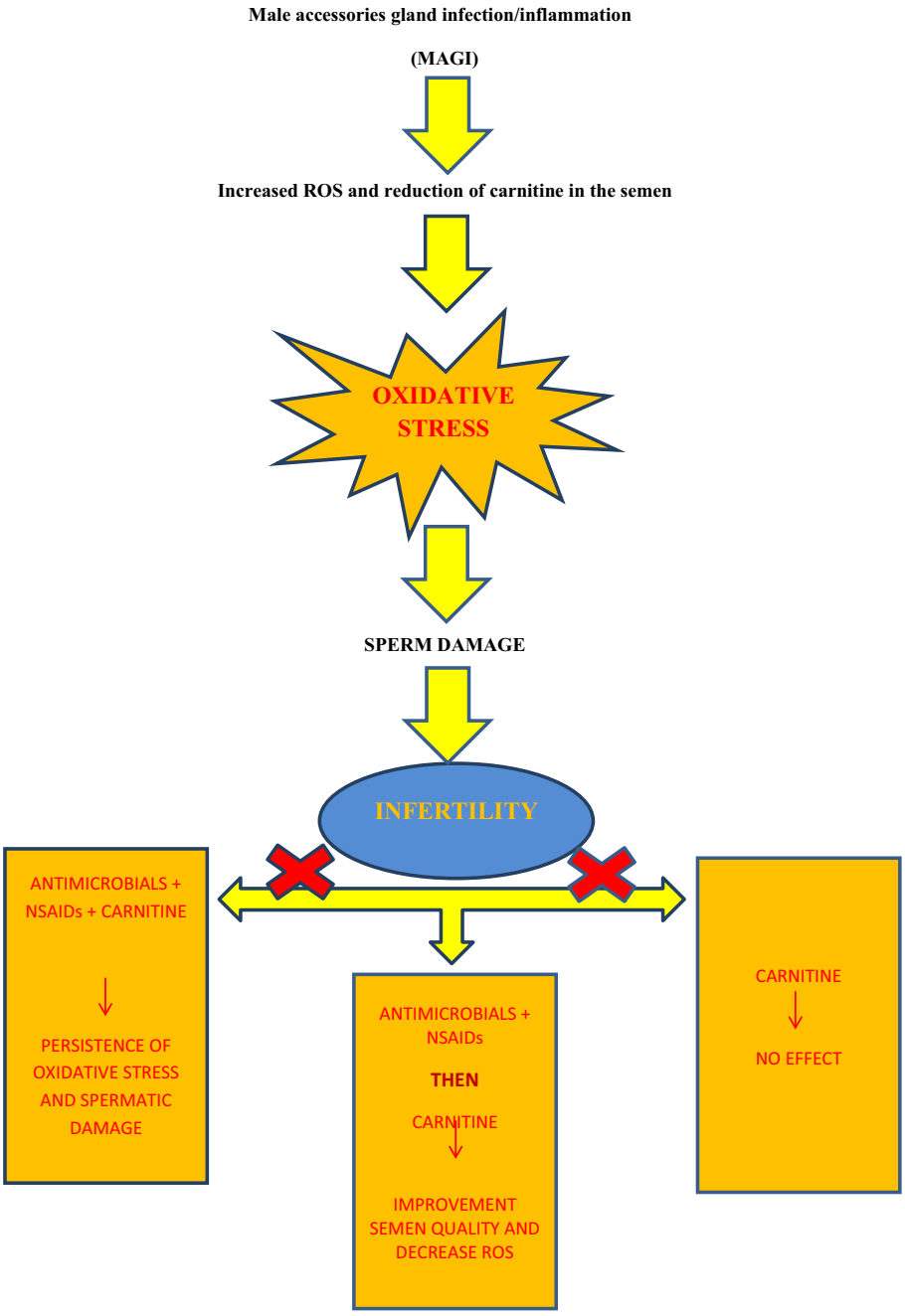

Figure 1 Role of reduction of carnitine in the semen and conseguent inferility.

carnitine (500 mg every 12 h), according to the following scheme: 2 months washout, 6 months of therapy/placebo, and another 2-month follow-up; the criteria for inclusion and exclusion were similar to those of the study described previously (Lenzi *et al.*, 2003). The results showed that combined treatment with L-carnitine and acetyl-L-carnitine compared to controls was effective in increasing sperm motility, especially in groups with lower baseline levels. In addition, during the intake of carnitine, four pairs have reached spontaneous pregnancy. Therefore, the authors concluded that combined treatment with L-carnitine and acetyl-L-carnitine is effective in improving semen quality in infertile patients (Lenzi *et al.*, 2004).

In the same year, Cavallini and colleagues conducted a study to evaluate the effect of therapy with carnitine and cinnoxicam (drug belonging to the family of NSAIDs) on sperm parameters in patients with idiopathic oligoasthenoteratozoospermia or associated with 'subclinical' varicocoeles. The 123 patients enrolled in the study were randomly divided into three groups: group 1, which received placebo; group 2 treated with L-carnitine (2 g/day) + acetyl-L-carnitine (1 g/day); group 3 treated with L-carnitine/acetyl-L-carnitine (same dosages of the group 2) and cinnoxicam (30 mg every 4 days). Drugs were administered for 6 months. The sperm concentration, motility, and morphology were assessed before, during, and after treatment. At the end of the study, the first group showed no significant change in sperm parameters; the second group had significantly increased sperm parameters after 3 and 6 months of therapy, but only in patients with grades I, II, and III varicocoele. Finally, all patients in group 3 had a significant improvement in sperm parameters, except for those suffering from grade V varicocoele. After treatment

discontinuation semen parameters return to baseline. The pregnancy rate in group 1 was 1.7%, in group 2 was 21.8%, and in group 3 was 38%. The authors concluded, therefore, that the co-administration of L-carnitine/acetyl-L-carnitine and cinnoxicam may be a feasible therapeutic strategy in patients with low-grade varicocoele and idiopathic oligoasthenoteratozoospermia (Cavallini *et al.*, 2004).

To clarify the role of carnitine supplementation in patients with idiopathic asthenozoospermia, Garolla and collaborators enrolled 30 controls normozoospermic and 30 idiopathic asthenozoospermia patients, the latter were divided in two groups according to the seminal concentrations (normal or reduced) of phospholipid hydroperoxide glutathione peroxidase (PHGPx). The therapeutic scheme adopted was the administration of placebo for 3 months, followed by L-carnitine 2 g/day for another 3 months. Semen samples and the assessment of levels of PHGPx were collected at baseline, after placebo, after carnitine administration, and again after 3 months with no drugs. After treatment with L-carnitine, asthenozoospermic subjects with normal PHGPx seminal concentrations had a mean sperm motility improvement, highlighting the important role played by this enzyme in male fertility (Garolla *et al.*, 2005).

In the study of Balercia and collaborators, 60 patients affected by idiopathic asthenozoospermia were divided into different treatment groups: L-carnitine 3 g/day, acetyl-L-carnitine 3 g/day, a combination of the two drugs or placebo for 6 months (Balercia *et al.*, 2005). The therapy with acetyl-L-carnitine alone led to an improvement in sperm motility after 3 months, but the latter was significantly higher when the acetyl-L-carnitine was administered in combination with L-carnitine. This therapy improves, moreover, the total oxyradical scavenging capacity of the seminal fluid in the same population (Balercia *et al.*, 2005). Similarly, in the same year, De Rosa and coworkers enrolled 170 infertile men; patients were divided into two groups depending on whether the total sperm motility was higher or lower than the range set by the WHO (50%). Patients with total motility <50% were further divided into two groups: group 1A without azoospermia; group 1B with primary or secondary azoospermia. The group 1A has been treated with L-carnitine 1 g/day and acetyl-L-carnitine 500 mg twice daily for 6 months; at the end of treatment there was an improvement in sperm parameters of conventional and non-conventional sperm parameters (De Rosa *et al.*, 2005).

IN VITRO EFFECTS OF L-carnitine on sperm function

Recent studies have shown that the addition of L-carnitine in spermatozoa intended for incubation, and subsequent centrifugation improves their vitality and motility (Banihani *et al.*, 2012). Starting from this observation, it is therefore been postulated that the addition of L-carnitine in semen samples intended for cryopreservation and for medically assisted procreation would improve the semen quality (Banihani *et al.*, 2014). In a recent paper, in particular, semen samples obtained from 22 infertile patients were analyzed and subjected to addition of L-carnitine before being cryopreserved; samples from the controls, instead, were cryopreserved without any supplementation. Twenty-four hours after cryopreservation, the thawed samples were analyzed for motility, viability, and spermatozoa DNA oxidation. Although the presence of some methodological limitation such as the lack of placebo control showed that the addition of L-carnitine improved significantly motility and viability compared to controls, while no statistically significant difference was found in the levels of DNA oxidation between samples and controls (Banihani *et al.*, 2014).

THE ROLE OF L-carnitine on erectile function

In 2010, Vicari and colleagues evaluated the effect of treatment with Ezerex (Sigma-Tau, Industrie farmaceutiche riunite s.p.a., Rome, Italy) (a nutraceutical containing arginine, vitamin B3, and propionyl-L-carnitine) on the erectile response to sildenafil in patients with arterial ED already treated with phosphodiesterase type 5 in order to increase the amount of nitric oxide (NO) bioavailable. Propionyl-L-carnitine is an ester of L-carnitine that is required for the transport of fatty acids into the mitochondria, within the cell it splits into L-carnitine and propionyl-CoA, an intermediate product of the Krebs cycle which is thereby stimulated. Fifty-three patients with arterial ED, hypertension, and diabetes mellitus were randomly treated, for 8 weeks, with Ezerex (1 dose/day) and then, after a wash out of 8 weeks, every day with sildenafil 100 mg and a Ezerex. Patients were divided into the following groups: group A, patients with ED isolated; group B, patients with erectile dysfunction plus atheromatous plaques and/or increased intima-media thickness of carotid arteries; group C, patients with ED plus arterial anomalies of the lower limbs; group D, subjects with ED and carotid disease and lower limbs arterial disease. The study had showed that co-administration of sildenafil and Ezerex significantly improved erectile response compared to isolated treatment with sildenafil in all groups of patients. These data suggest that the combination therapy with phosphodiesterase inhibitor 5 and Ezerex is effective in increasing the bioavailable NO and reduce ROS, which in turn inactivates NO (Vicari *et al.*, 2010). However, despite the encouraging data, available data do not demonstrate that propionyl-L-carnitine was able to increase the bioavailable NO and reduce ROS.

L-carnitine and toxicity

Although many studies provided evidences about the clinical benefits of L-carnitine, there are also some data on its toxicity. Beside the antioxidant activity, it should be considered that compounds with chemical structures containing two or more of the following functional groups: –COOH, –OH, –SH, –S–, C = O, –O–, and amino groups are known to exhibit metal chelating activity (Yuan *et al.*, 2005; Gulcin, 2006). To this regard in fact, L-carnitine with –COOH and –OH groups may act as a metal chelator. A previous study from Banihani *et al.* (2012) showed that a dosage of 0.5 mg/mL of L-carnitine significantly increased the motility of human spermatozoa (5×10^6 cell/mL) after in vitro incubation and centrifugation. However, high L-carnitine concentration (50 mg/mL) was toxic to sperm and significantly decreases sperm motility. As concerning the metal chelator activity of L-carnitine, it has been shown that L-carnitine can effectively compete for the chelation of calcium ions. In fact, the detrimental effect of the high dosage of L-carnitine may be mainly due to its ability to bind Ca^{2+}, a vital ion needed for sperm motion (Banihani *et al.*, 2015). In fact, L-carnitine exhibited 13.8 and 40.1% chelation of calcium ions at 0.075 and 0.75 mM, respectively (Banihani *et al.*, 2015).

It should be in fact considered that in the human body, a number of enzymes require Ca^{2+} as a co-factor for optimal

activity. Some examples of those enzymes are the ones involved in the blood clotting cascade such as prothrombinase and tenase (Mathur *et al.*, 1997; Weiss & Lages, 1997). Accordingly, decreased Ca^{2+} concentrations may reduce the activity of these enzymes and affect their activity. LC supplementation may, thus, decelerate the blood clotting by lowering the level of unbound calcium.

Beside clinical effect of L-carnitine, physicians should be aware about the detrimental effect of high dosage of L-carnitine and that improvement in sperm parameters should not be achieved by the increasing dosage.

CONCLUSION

According to what has been said, it is clear that the use of L-carnitine and its esters, acetyl-L-carnitine and propionyl-L-carnitine, is effective in determining an improvement in sperm parameters and in particular of the total motility and progressive motility, reduces the levels of ROS in seminal fluid, and would be able to improve the quality of the semen, also in case of cryopreservation. The administration of these molecules in the treatment of male infertility (alone or in combination) is, therefore, a rational and effective therapeutic strategy. However, clinical benefits should not be achieved at high dose, since the evidence of calcium chelator activity of L-carnitine that may determine cell damage and decrease in serum calcium.

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左卡尼汀在男性不育中的作用

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摘要：

此综述研究左卡尼汀在男性不育中的作用。将此文组织成短的段落来阐述以下几个方面的内容：左卡尼汀抗生殖细胞凋亡作用，左卡尼汀对常规精子参数的作用，体外试验中左卡尼汀对精子功能的作用以及左卡尼汀对勃起功能的作用。

关键词：左卡尼汀　　男性不育　　精子参数

引言

从近一个世纪以来，卡尼汀，尤其是左卡尼汀和乙酰左卡尼汀，对人体组织的有益作用已被广泛认识。

左卡尼汀是一种极性、水溶性的季铵化合物，人体可以自身合成，但主要来源于外源性摄取。它作为一种必不可少的辅酶因子转运长链脂肪酸进入线粒体基质内，从而促进氧化代谢过程，增强细胞能量产生 (Agarwal & Said, 2004; Ng et al., 2004)。而左卡尼汀在乙酰卡尼汀转移酶的作用下以可逆的方式形成乙酰左卡尼汀，以此调节细胞和线粒体内 CoA 和乙酰 CoA 的浓度(Lenzi et al., 1992)。

公认的每天必需提供大约 8-11mg 的左卡尼汀以满足机体需要。大约机体 98%的左卡尼汀存储于骨骼肌和心脏，肝脏含 1-6%，而我们发现细胞外液中左卡尼汀的浓度范围为 0-6%之间 (Lenzi et al.,1992)。

左卡尼汀与男性生殖系统

有意思的是，人们发现男性生殖道，尤其是附睾中，左卡尼汀浓度异常高，暗示左卡尼汀在精子细胞能量代谢和精子成熟中起关键作用。 (Lenzi et al., 1992; Vicari et al.,2001).

位于附睾的左卡尼汀是来自血浆，它通过主动运输穿过上皮细胞进入附睾细胞质(Ng et al., 2004)。从几项研究来看，这一主动运输过程可能是由位于睾丸，尤其是曲细精管腔上皮和支持细胞上的特定的卡尼汀/有机阳离子转运体（OCTNs）介导的，其在人类、大鼠和小鼠中表达 (Enomoto et al., 2002)。OCTNs 家族中的第一个成员， OCTN1 （溶质载体家族

22A4）转运阳离子外源化学物，如四乙铵。OCTN2(SLC22A5)是一种 Na+依赖的高亲和力卡尼汀转运子。人类卡尼汀转运子 CT2 (SLC22A16) 和小鼠卡尼汀 OCTN3 (SLC22A21) 以高亲和力(Km 分别为 20 和 30M) 不依赖于钠离子的方式转运卡尼汀 (Kobayashi et al., 2007)。最终，左卡尼汀通过被动扩散聚集在精子内(Jeulin & Lewin, 1996)。

从几项研究来看，这一主动运输过程可能是由位于睾丸，尤其是曲细精管腔上皮和支持细胞上的特定的卡尼汀/有机阳离子转运体（CT2）介导的(Enomoto et al., 2002)。最终，左卡尼汀通过被动扩散聚集在精子内(Jeulin & Lewin, 1996)。由于附睾中的精子能使用脂肪酸和磷脂作为能量来源，很可能左卡尼汀在此也是作为线粒体转运的辅酶因子，随后进行脂肪酸氧化。而且，卡尼汀分子的高浓度似乎抑制射出精子的代谢活性（其代谢底物主要是葡萄糖），但不会抑制附睾中那些主要以脂肪酸为代谢底物的精子的代谢活性。 (Lenzi et al., 1992)。

左卡尼汀抗生殖细胞凋亡作用

另一个特别有趣的发现是左卡尼汀的抗凋亡作用，这似乎可以用通过 FAS-FAS 配体和 caspase3,7 和 8 介导的对细胞程序性死亡的抑制来解释 (Mutomba et al., 2000)。在男性生殖系统，凋亡过程能自发发生或由其他一些因素，如热火雄激素阻断等引起。为了证实这一点，Amendola 及其团队评价了左卡尼汀治疗对接受单一剂量 10 Gy 照射睾丸小鼠精子生成的影响。治疗组小鼠用 100 mg/kg 腹腔注射乙酰左卡尼汀，隔天一次，连续 4 周：照射后第 1，28, 35, 40, 45, 50, 55 和 60 天评价对精子生成的作用。作者得出结论：乙酰左卡尼汀改善辐射损伤后识别精原细胞的可能性(Amendola et al., 1989)。

同样的作者进而又完成了一项研究评价左卡尼汀对热损伤后精子生成的作用，得到了类似的结果(Amendola et al.,1991).。Kanter 也认为接受睾丸照射并同时给予左卡尼汀补充的大鼠生殖细胞凋亡减少，精子形态异常的发生率也明显降低 (Kanter et al.,2010)。而且，左卡尼汀强大的抗氧化作用似乎也在用依托泊苷（一种化疗药物，通过阻断拓扑异构酶 II 的催化作用而引起细胞死亡）处理过的大鼠中起到一种细胞保护作用(Okada et al., 2009)。人体研究也证实了左卡尼汀的这种抗凋亡作用，除了抗氧化作用，在男性生殖系统水平，还具有改善精子参数的作用(Abad et al., 2013)。

根据以上所述，不难理解左卡尼汀及其酯化物，乙酰左卡尼汀在治疗男性生育力下降中的合理用药机制(Ng et al., 2004).。

左卡尼汀对常规精子参数的作用（表 1）

表 1 关于卡尼汀治疗对男性不育患者作用的临床研究

作者	患者	治疗	剂量	结果
Moncada et al. (1992)	20 例特发性少弱精子症患者	乙酰左卡尼汀	4g/天×60 天	精子活动力提高
Costa et al. (1994)	100 名特发性少弱精子症患者	左卡尼汀	3g/天×4 个月	精子浓度增加；总精子活动力、前向运动精子活动力改善
Vitali et al., (1995)	47 名 2 年以上不育史的患者	左卡尼汀	3g/天×3 个月	精子活动力明显改善
Vicari et al. (2001)	55 名非细菌性前列 PVE*患者（A 组） 35 名细菌性 PVE 患者（B 组）	A1 和 B1 亚组：抗生素+NSAIDs 和左卡尼汀+乙酰左卡尼汀； A2 和 B2 亚组：抗生素+NSAIDs+左卡尼汀+乙酰左卡尼汀； A3 和 B3 亚组：左卡尼汀+乙酰左卡尼汀；	A1 和 B1 亚组：抗生素+NSAIDs 每个月 14 天，持续 3 个月，左卡尼汀（2g/天）+乙酰左卡尼汀（1 g/天），持续 3 个月； A2 和 B2 亚组：抗生素+NSAIDs 每个月 14 天+左卡尼汀（2g/天）+乙酰左卡尼汀（1 g/天），持续 3 个月； A3 和 B3 亚组：左卡尼汀（2g/天）+乙酰左卡尼汀（1 g/天），持续 3 个月；	A1 和 B1 亚组：ROS 生成明显减少，某些精子参数（精子活动力和活力）增加； A2 和 B2 亚组：精子参数没有明显改善，ROS 仍然存在于精液中； A3 和 B3 亚组：治疗无效
Vicari & Calogero (2001)	34 名患者具有正常的精液 WBC<1×10^6/ml；20 名患者具有升高的精液 WBC>1×10^6/ml;抗生素治疗后仍有 ROS 过量产生和 PVE 的不育患者	左卡尼汀+乙酰左卡尼汀	左卡尼汀（2g/天）+乙酰左卡尼汀（1 g/天），治疗 3 个月	A 组：患者精子的活动力和活力改善，妊娠率增加（明显高于 B 组）； B 组：仅精子活力改善
Vicari et al. (2002)	94 例非细菌性 PVE 和白细胞精子症患者	A 组（n=30）：左卡尼汀+乙酰左卡尼汀；B 组（n=16）：尼美舒利；C 组（n=26）：尼美舒利，然后左卡尼汀+乙酰左卡尼汀；D 组（n=26）：尼美舒利+左卡尼汀+乙酰左卡尼汀	A 组：左卡尼汀（2g/天）+乙酰左卡尼汀（1 g/天）×4 个月；B 组（n=16）：尼美舒利×4 个月；C 组（n=26）：尼美舒利×2 个月，然后左卡尼汀+乙酰左卡尼汀×2 个月；D 组（n=26）：尼美舒利+左卡	仅在 C 组(NSAIDs 和卡尼汀的序贯治疗组）精子活力和活动力明显增加，精液中 ROS 的生成明显减少

			尼汀（2g/天）+乙酰左卡尼汀（1 g/天）×4 个月	
Lenzi et al. (2003)	100 名不育史持续两年以上的患者的随机安慰剂对照研究	左卡尼汀或安慰剂	按照以下方案补充左卡尼汀（2g/天）或安慰剂：2 个月的洗脱期、2 个月服用治疗药物/安慰剂、再经历 2 个月洗脱期、最终 2 个月的服用治疗药物/安慰剂	左卡尼汀治疗组精子参数改善
Lenzi et al. (2004)	60 名少弱畸精症患者的随机安慰剂对照研究	左卡尼汀+ 乙酰左卡尼汀或安慰剂	按照以下治疗周期用左卡尼汀（2g/天）+ 乙酰左卡尼汀（500mg/12h）或安慰剂治疗： 2 个月洗脱期，6 个月治疗药物/安慰剂补充，2 个月随访	卡尼汀治疗组患者总精子活动力和前向精子活动力改善
Cavallini et al. (2004)	123 名特发性少弱畸精子症或伴有“亚临床”精索静脉曲张的患者	组 1：安慰剂； 组 2：左卡尼汀+乙酰左卡尼汀；组 3：左卡尼汀/乙酰左卡尼汀和辛诺昔康	组 1:安慰剂治疗×6 个月； 组 2：:左卡尼汀(2 g/天) + 乙酰左卡尼汀（1 g/天）×6 个月；组 3：左卡尼汀/乙酰左卡尼汀（剂量同组 2)和辛诺昔康(30mg/4 天）×6 个月	组 1：精子参数没有明显改变，妊娠率为 1.7%； 组 2：仅在 1-3 级精索静脉曲张的患者中精子参数有改善，妊娠率为 21.8%； 组 3：精子参数改善，妊娠率为 38%；
Garolla et al. (2005)	30 名正常精子对照组和 30 名特发性弱精子症患者，后者被按照磷脂过氧化氢谷胱甘肽过氧化物酶(PHGPx)的精液浓度（正常或降低)分成两组	安慰剂治疗，然后左卡尼汀治疗	安慰剂治疗×3 个月，随后用左卡尼汀 2 g/天×3 个月	左卡尼汀治疗后，PHGPx 精液浓度正常的弱精子症患者精子活动力改善
Balercia et al. (2005)	60 名特发性弱精子症患者	左卡尼汀； 乙酰左卡尼汀； 左卡尼汀+乙酰左卡尼汀； 安慰剂	3g/天×6 个月； 3g/天×6 个月； 3g/天+3g/天×6 个月； 6 个月	单用乙酰左卡尼汀治疗精子活动力提高，ROS 生成减少，但当乙酰左卡尼汀与左卡尼汀联合治疗时，以上指标的改善作用增强
De Rosa et al. (2005)	170 名男性不育患者根据总精子活动力是高于还是低于 WHO 设置的标准范围(50%)分成两组	没有无精子症，总精子活动力<50%的患者用左卡尼汀+乙酰左卡尼汀治疗	1g/天+1g/天×6 个月	常规和非常规精子参数均有明显改善

* PVE，prostato-vesiculo-epididymitis，腺水疱附睾炎

在 1992 年，Moncada 及其团队招募了 20 对有不育史的夫妇，随访时间为 16-24 个月，夫妇中男方诊断为特发性少弱精子症，平均年龄 30±3 岁。所有这些患者用乙酰左卡尼汀 4g/天，治疗 60 天，治疗结束时和治疗后 4 个月评价精子参数。乙酰左卡尼汀治疗结束时，60%的患者前向运动精子活力呈现与药物相关的明显增加；治疗结束后 4 个月，前向运动精子活力的值又回复到治疗前的水平 (Moncada et al., 1992)。

同样，一项 100 名特发性少弱精子症患者的研究，用口服左卡尼汀 3g/天持续治疗 4 个月，显示总精子活动力、前向运动精子活动力改善，精子浓度增加，从而证实治疗组患者在精子质量和数量上确有优势 (Costa et al., 1994)。为进一步证明，在 1995 年，有研究用抗精子抗体证据评价了左卡尼汀对 2 年以上不育史的 47 名伴特发性弱精子症的男性不育的治疗效果。排除隐睾史、感染后睾丸萎缩、创伤、严重精索静脉曲张、阻塞、 泌尿生殖道炎症火感染、内分泌下丘脑-垂体-性腺轴紊乱后，特发性弱精子症是唯一已知的不育因素在本研究中，每一位入组患者口服补充左卡尼汀 3g/天（分三次随餐服用），连服 3 个月。治疗后，80%的患者精子活力明显改善，精子活力值几乎等同于由 110 名正常生育志愿者组成的对照组 (Vitali et al.,1995)。

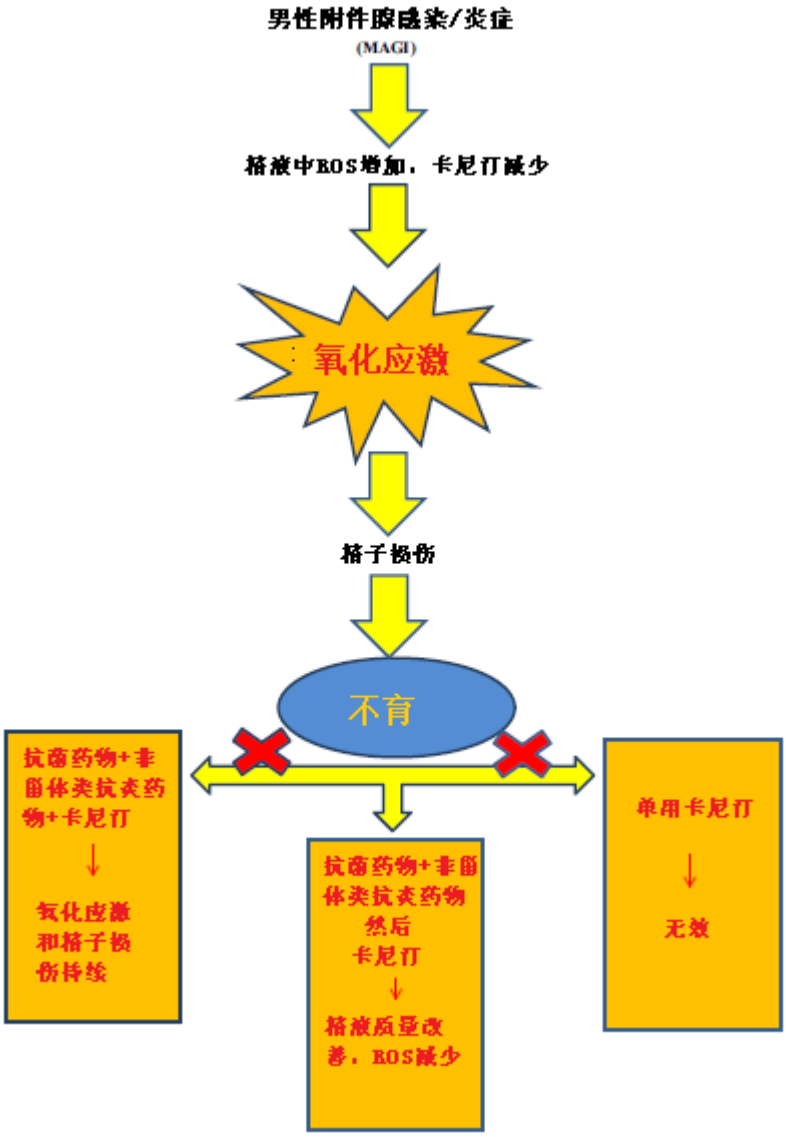
由于附睾中左卡尼汀浓度特别地高,现在已知射出的精液中左卡尼汀的浓度可能是附睾功能的一个标志物。因为降低男性生育力的主要原因是泌尿生殖道炎症，包括附睾炎，一些研究显示附睾炎患者精液中左卡尼汀浓度降低 (Bornman et al., 1989; Cooper et al., 1990)。炎症状态能通过从白细胞和/或精子细胞过度产生 ROS（活性氧簇），增加氧化应激来降低生育力。由于前列腺水疱附睾炎（PVE），包括所有可能的泌尿生殖道感染，是用更高水平的氧化应激（常常即使在抗生素治疗后仍然持续）进行诊断分类。Vicari 及其团队评价了左卡尼汀治疗 PVE 患者的抗氧化作用。为此目的，他们研究了两组男性不育患者：A 组由 55 名非细菌性 PVE 组成（平均年龄 34 岁）；B 组由 35 名细菌性 PVE 组成（平均年龄 35 岁）。以下患者从研究中排除：伴有原发性睾丸疾病，研究前 3 个月内接受过药物治疗，少精子症患者（(<5 百万/mL），和/或严重的畸形精子症(>87% 非正常形态）和单形性畸形精子症。然后将两组患者按照接受的治疗随机分成不同的亚组： A1 和 B1 亚组分别接受 3 个月的抗生素和/或非甾体类抗炎药(NSAIDs)（每月 14 天，持续 3 个月），随后继以左卡尼汀（1g,每天两次）和乙酰左卡尼汀（0.5g,每天两次），最后停止药物治疗 3 个月。A2 和 B2 亚组同时接受为期 3 个月的联合抗生素和/或抗炎药物（×每月 14 天）和左卡尼汀（1g×每天两次）和乙酰左卡尼汀（0.5g×每天两次）治疗，随后 3 个月不接受任何治疗。A3 和 B3 亚组接受 3 个月的左卡尼汀（1g, 每天两次）和乙酰左卡尼汀（0.5g, 每天两次）治疗，随

后 3 个月不进行任何治疗。每位患者接受精液检查、微生物分析，90 名患者中 60 名患者检测治疗前、中、后的 ROS 生成情况。得到的结果显示精液中 ROS 生成明显减少、精子的运动能力和活力明显改善，尤其是在 A1 和 B1 亚组。这些结果提示先进行抗微生物治疗和/或抗炎治疗，然后继以左卡尼汀治疗的方案得到了最佳的抗菌和抗氧化效果；抗微生物治疗和抗氧化治疗同时联用效果次之，而仅用左卡尼汀治疗没有效果(Vicari et al., 2001)。

这些结果显示尽管进行了抗微生物治疗，氧化应激状态仍持续。用乙酰左卡尼汀治疗已经接受抗菌药物治疗的 54 名无临床症状伴 ROS 产生过量和有 PVE 超声证据的男性不育患者。有以下情形的患者从研究中排除：微生物再次感染、无精子症、严重少精子症、畸形精子症，和/或死精症、高 FSH 水平，原发性睾丸疾病，吸烟，饮酒，暴露于化学物质的职业、肾脏或肝脏疾病、肌病、或在研究前 3 个月内使用药物。根据精液中白细胞浓度，将患者分为分为两组：A 组，射出的精液中白细胞数正常；B 组，射出的精液中白细胞数高于正常值（> 100 万/毫升）。评价左卡尼汀治疗前、中、后的精液参数，ROS 的生成情况，和自然妊娠率。得到的结果显示 A 组患者精子的活动力和活力增加。而 B 组患者用左卡尼汀治疗后仅观察到精子活力改善。最终，A 组患者的自然妊娠率明显高于 B 组患者(图 1)(Vicari & Calogero, 2001)。

为了评价是否抗氧化剂和抗炎化合物的联合可以有效治疗非细菌性 PVE 患者，提升精液中白细胞的浓度，同一个工作小组在 98 名患者（临床特征如前所述）中开展了一项前瞻性随机研究。这一队列被分成四组：A 组（n=30）接受口服左卡尼汀（1g/12h）和乙酰左卡尼汀（500mg/12h），持续治疗 4 个月；B 组（n=16）接受非甾体抗炎药（尼美舒利）治疗 4 个月；C 组（n=26）先接受非甾体抗炎药治疗 2 个月，然后用卡尼汀治疗（左卡尼汀 1g/12h 和乙酰左卡尼汀 500mg/12h）2 个月；D 组（n=26）接受非甾体抗炎药和卡尼汀治疗 4 个月。经历 3 个月的洗脱期后，评价患者治疗前、中、后精子参数、精液中 ROS 生成、自然妊娠率。收集的数据强烈提示 C 组患者，即在那些首先接受 NSAIDs 药物治疗，之后进行左卡尼汀治疗的患者中 ROS 生成的减少以及精子运动力和活力增加最明显 (Vicari et al., 2002)。为评价左卡尼汀对男性不育的治疗效果，已经完成了一项在年龄 20-40 岁之间，不育史至少持续两年以上的 100 名患者中的随机双盲安慰剂对照研究 (Lenzi et al., 2003)。以下患者从研究中排除：内分泌疾病、现患或有隐睾症史、生殖道感染或生殖道梗阻、精索静脉曲张以及睾丸萎缩。纳入患者标准是流变特性正常、精子浓度为 1000-2000 万/ml, 精子活动力为 10-30%，前向运动精子率<15%，非典型形态精子率 <70%，精液白细胞<1 百万/mL。研究设计包括按照以下方案补充左卡尼汀（2g/天）或安慰剂：2 个月的洗脱期、2 个月服用治疗

图 1 卡尼汀减少在精液和不育中的作用



药物/安慰剂、再经历 2 个月洗脱期、最终 2 个月的服用治疗药物/安慰剂。在每一过程中，评价精子参数、精液左卡尼汀浓度、精浆α -葡萄糖苷酶（中性，其 SDS 可抑制形式常用作附睾功能的标志物）的浓度以及精子细胞膜脂质过氧化电位。结果显示左卡尼汀组于安慰剂相比，治疗后一些变量分析上有明显改善，尤其在治疗的第一阶段，强调心理方面在不孕不育病因中的重要性。然而，左卡尼汀治疗能有效提高精液质量，尤其是更低基线值的患者与补充安慰剂相比，左卡尼汀效果更佳明显。但在两组中，精浆α -葡萄糖苷酶浓度和精子细胞膜脂质过氧化电位方面没有变化 (Lenzi et al., 2003)。

2004 年，一项双盲随机安慰剂对照研究评价了左卡尼汀和乙酰左卡尼汀联合治疗少弱畸精症患者的疗效。这项研究纳入了 60 名 20-49 岁之间的患者：30 名患者用安慰剂治疗，

30 名患者用左卡尼汀（2g/天）+ 乙酰左卡尼汀（500mg/12h）治疗，治疗周期如下：2 个月洗脱期，6 个月治疗药物/安慰剂补充，2 个月随访；纳入和排除标准与先前描述的研究相似(Lenzi et al., 2003)。研究结果表明，与安慰剂组相比，左卡尼汀和乙酰左卡尼汀的联合治疗能有效增强精子活动力，尤其是在基线值更低的组效果更明显。而且，在补充卡尼汀治疗期间，有 4 对成功的完成自然妊娠。因此，作者得出结论左卡尼汀和乙酰左卡尼汀的联合治疗能有效改善不育患者的精液质量 (Lenzi et al., 2004)。在同一年，Cavallini 及其团队开展了一项研究来评价卡尼汀和辛诺昔康（属于非甾体类抗炎药家族中的药物）对特发性少弱畸精子症或伴有“亚临床”精索静脉曲张的患者精子参数的作用效果。将 123 名纳入该研究的患者随机分成三组：组 1，接受安慰剂治疗；组 2，接受左卡尼汀(2 g/天) + 乙酰左卡尼汀（1 g/天)；组 3 用左卡尼汀/乙酰左卡尼汀（剂量同组 2）和辛诺昔康（30mg/4 天)，药物治疗持续 6 个月。评价治疗前、中、后患者的精子浓度、精子活动力和形态。研究结束时，第一组显示在精子参数方面没有明显变化；第二组治疗 3 个月和治疗 6 个月后精子参数有明显改善，单仅限于在 1-3 级精索静脉曲张的患者。最终，组 3 中除了那些患有 4 级精索静脉曲张的患者，其余所有患者精子参数均有明显改善。治疗中止后又回到基线水平。组 1、组 2 和组 3 的怀孕率分别为 1.7%， 21.8%和 38%。因此，作者得出的结论是左卡尼汀/乙酰左卡尼汀和辛诺昔康联用对低级别精索静脉曲张和特发性少弱畸精症患者而言是一种可行的治疗策略(Cavallini et al., 2004)。

为了阐明卡尼汀补充对特发性弱精子症患者的作用，Garolla 及其团队纳入 30 名正常精子对照组和 30 名特发性弱精子症患者，后者被按照磷脂过氧化氢谷胱甘肽过氧化物酶(PHGPx)的精液浓度（正常或降低）分成两组。采用的治疗方案为安慰剂治疗 3 个月，随后用左卡尼汀 2 g/天再持续治疗 3 个月。收集治疗前，安慰剂治疗后、卡尼汀治疗后的精液样本和 PHGPx 水平评估，停药 3 个月后再次收集。左卡尼汀治疗后，PHGPx 精液浓度正常的弱精子症患者平均精子活动力得到改善，突出了这种酶在男性不育中所起的重要作用(Garolla et al., 2005)。

在Balercia及其团队的研究中,60名患有特发性弱精子症的患者被分成不同的治疗组：左卡尼汀 3 g/天，乙酰左卡尼汀 3g/天，以上两种药物的组合治疗或安慰剂治疗，治疗均持续 6 个月。仅用乙酰左卡尼汀治疗组 3 个月后精子活动力改善，但当乙酰左卡尼汀与左卡尼汀联合治疗时，这一指标的改善明显更高。而且，在同一群人中这种治疗提高精液中总氧自由基清除能力(Balercia et al., 2005)。类似地，在同一年， De Rosa 及其团队招募了 170 名男性不育患者；患者根据总精子活动力是高于还是低于 WHO 设置的标准范围(50%)。

总精子活动力<50%的患者又进一步分成两组： 1A 组没有无精子症；1B 组伴有原发性或继发性的无精子症。 1A 组一直用左卡尼汀 1 g/天和乙酰左卡尼汀 500 mg，每天两次，持续治疗 6 个月，在治疗结束时，常规和非常规精子参数均有明显改善(De Rosa et al., 2005)。

体外条件下左卡尼汀对精子功能的作用

最近的研究显示，在用于孵育的精子中添加左卡尼汀，然后离心，能改善精子的活力和活动力(Banihani et al., 2012)。因此，从这个观察开始，一直被推定在用于冷冻保存和医学辅助生育的精液中添加左卡尼汀将改善精子质量 (Banihani et al., 2014)。尤其是最近的一篇论文，将来自 22 名不育患者的精液样本进行分析，并在冷冻保存前进行添加左卡尼汀的处理；相反，来自对照组的精液样本冷冻保存前未进行任何添加。冷冻保存 24 小时后，对解冻的样本分析精液的活动力、活力和精子 DNA 氧化情况。虽然存在一些方法学上的限制，如缺乏安慰剂对照，但结果显示与对照组相比，添加左卡尼汀明显改善精子的运动力和活力，而样本和对照组之间在 DNA 氧化水平上没有发现有统计学意义的差异(Banihani et al., 2014)。

左卡尼汀对勃起功能的作用

2010 年， Vicari 及其团队评价了勃艾精（Sigma-Tau 制药集团，意大利罗马）（一种含精氨酸、维生素 B3 和丙酰左卡尼汀产品）对已经接受磷酸二酯酶 5 型抑制剂治疗以增加可生物利用的 NO 含量的动脉性 ED 患者对西地那非勃起反应的作用。丙酰左卡尼汀是一种左卡尼汀（转运脂肪酸进入线粒体所必需的物质）的酯化物，它在细胞内裂解成左卡尼汀和丙酰 CoA（一种三羧酸循环的中间产物，可刺激三羧酸循环）。53 名动脉性 ED、高血压和糖尿病患者被随机治疗，持续 8 周，然后用勃艾精（1 次/天），经过 8 周的洗脱期后，每天补充西地那非 100mg 和勃艾精。患者被分成以下 4 组：A 组，仅患 ED 的患者；B 组，勃起功能障碍伴动脉粥样斑块的患者；C 组，勃起功能障碍伴下肢动脉病变的患者；D 组，勃起功能障碍同时伴有颈动脉疾病和下肢动脉疾病的患者。研究表明，在以上各组的患者中，与单用西地那非相比，西地那非和勃艾精的联合治疗可以明显改善患者的勃起反应。这些结果表明，磷酸二酯酶抑制剂与勃艾精联合治疗能有效增强有生物效应的 NO 含量，减少 ROS（反过来又灭活 NO 的物质）生成 (Vicari et al., 2010)。然而，尽管这些结果是令人鼓舞的，但现有数据并不支持丙酰左卡尼汀单用能增强 NO 生物活性，减少 ROS 生成。

左卡尼汀和毒性

虽然许多研究提供了关于左卡尼汀临床获益的证据，也有一些研究结果是关于它的毒性的。除了抗氧化活性以外，还应该考虑含有以下两个或两个以上功能基团： －COOH, －OH, －SH, －S－, C＝O, －O－, 氨基，化学结构的化合物被认为表现出金属螯合活性 (Yuan et al., 2005; Gulcin, 2006)。关于这一点，事实上，左卡尼汀拥有－COOH 和 －OH 基团，可以作为金属螯合剂。先前一项来自 Banihani(2012) 等人的研究显示剂量为 0.5 mg/mL 的左卡尼汀明显增加经过体外培养和离心的人类精子细胞的活动力（5×10^6 个细胞/mL）。然而，高浓度的左卡尼汀(50 mg/mL) 对精子是有毒性的，明显减少精子活动力。关于左卡尼汀的金属螯合活性，已被证明左卡尼汀能有效竞争钙离子螯合作用。事实上，高剂量左卡尼汀的这个不良效应可能主要取决于它的钙离子结合能力，而钙离子是精子运动所需的一种离子(Banihani et al., 2015)。事实上，在钙离子浓度分别为 0.075 mM 和 0.75 mM 时，左卡尼汀分别螯合 13.8%和 40.1%的钙离子 (Banihani et al., 2015)。事实上应该考虑到，在人体中，许多酶需要钙离子作为辅酶以达到最佳活性。这些酶中的例子包括参与凝血级联反应的酶，如凝血因子和 tenase 凝血酶(Mathur et al., 1997; Weiss & Lages, 1997)。相应地，减少钙离子浓度可以降低这些酶的活性，影响它们的生理功能。这样，补充左卡尼汀可以通过降低非结合钙离子的水平而减缓血液凝固。

除了左卡尼汀的临床效果外，临床医生也应该意识到高剂量左卡尼汀的不利影响，不应该为了改善精子参数而盲目加大左卡尼汀剂量。

结论

根据以上所述，显然使用左卡尼汀及其酰化物，乙酰左卡尼汀和丙酰左卡尼汀，具有确切的改善精子参数，尤其是总精子活动力和前向运动力，减少精液中 ROS 水平的疗效，也可以用作精子冷冻保存的保护剂。因此，服用左卡尼汀及其酯化物治疗男性不育（单用或联用）是一种合理、有效的治疗策略。然而，不应该以过高剂量达到临床获益，因为有左卡尼汀具有钙离子螯合活性的证据，它可能产生细胞损伤，减少血清中钙离子含量。

声明

每一位作者声明不对任何方造成利益冲突

参考文献（略）