ANDROLOGY

ISSN: 2047-2919

ANDROLOGY

REVIEW ARTICLE

Correspondence:
Sandro La Vignera, Policlinico 'G. Rodolico', Via Santa Sofia 78, 95123 Catania, Italy.
E-mail sandrolavignera@unict.it

Keywords:
carnitine, male infertility, sperm parameters

Received: 5-Feb-2016
Revised: 26-Feb-2016
Accepted: 1-Mar-2016

doi: 10.1111/andr.12191

The role of carnitine in male infertility

[1]L. Mongioi, [1]A. E. Calogero, [1]E. Vicari, [1]R. A. Condorelli, [2]G. I. Russo, [2]S. Privitera, [2]G. Morgia and [1]S. La Vignera

[1]Department of Clinical and Experimental Medicine, and [2]Department of Urology, University of Catania, Catania, Italy

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-

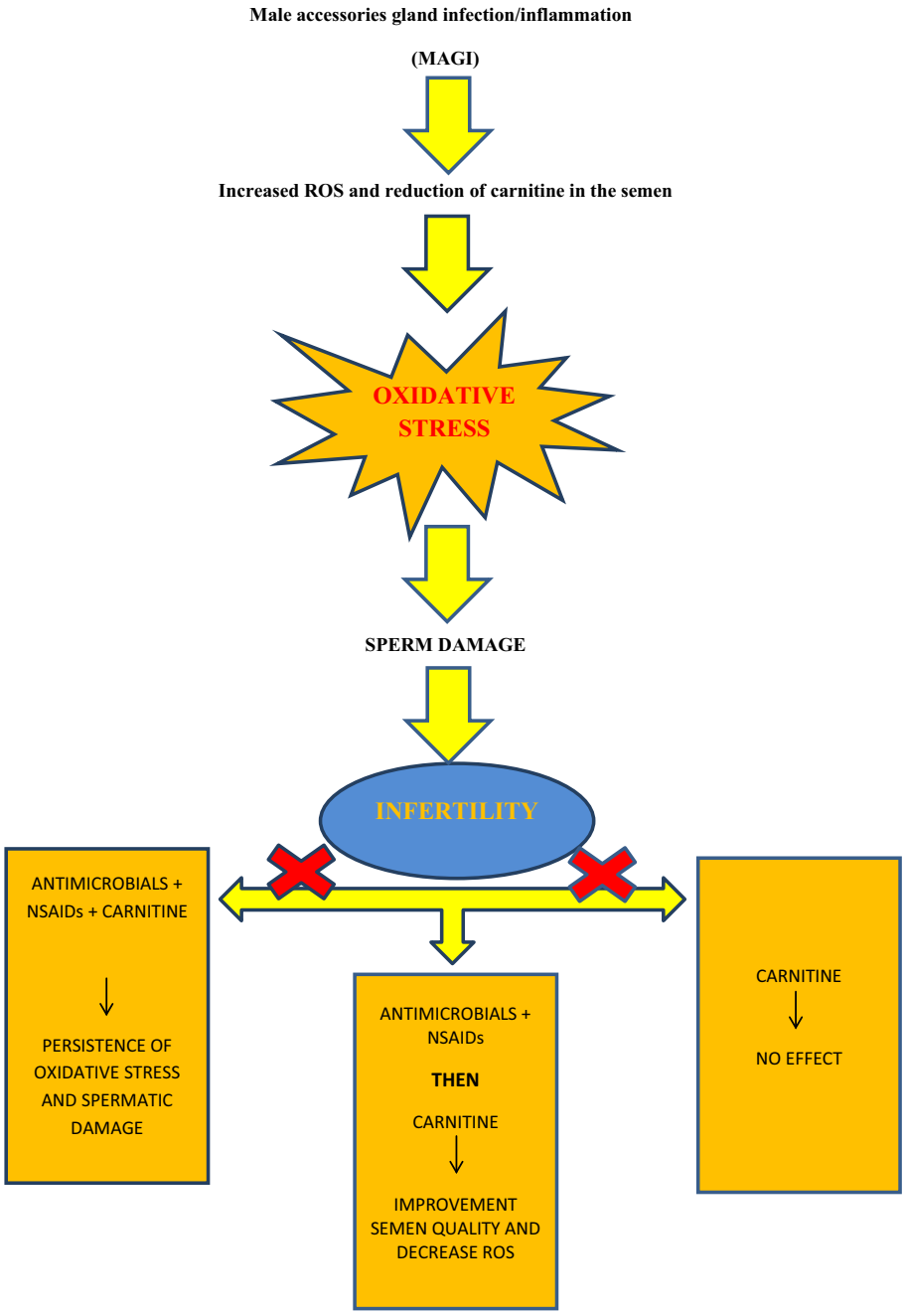

Figure 1 Role of reduction of carnitine in the semen and consequent inferility.

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.

ACKNOWLEDGMENT

Each author declares no conflict of interest.

REFERENCES

Abad C, Amengual MJ, Gosálvez J, Coward K, Hannaoui N, Benet J, García-Peiró A & Prats J. (2013) Effects of oral antioxidant treatment upon the dynamics of human sperm DNA fragmentation and subpopulations of sperm with highly degraded DNA. *Andrologia* 45, 211–216.

Agarwal A & Said TM. (2004) Carnitines and male infertility. *Reprod Biomed Online* 8, 376–384.

Amendola R, Bartoleschi C, Cordelli E, Mauro F, Uccelli R & Spanò M. (1989) Effects of L-acetylcarnitine (LAC) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry. 1. Recovery after X-irradiation. *Andrologia* 21, 568–575.

Amendola R, Cordelli E, Mauro F & Spanò M. (1991) Effects of L-acetylcarnitine (LAC) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry. 2. Recovery after hyperthermic treatment. *Andrologia* 23, 135–140.

Balercia G, Regoli F, Armeni T, Koverech A, Mantero F & Boscaro M. (2005) Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. *Fertil Steril* 84, 662–671.

Banihani S, Sharma R, Bayachou M, Sabanegh E & Agarwal A. (2012) Human sperm DNA oxidation, motility and viability in the presence of L-carnitine during in vitro incubation and centrifugation. *Andrologia* 44(Suppl 1), 505–512.

Banihani S, Agarwal A, Sharma R & Bayachou M. (2014) Cryoprotective effect of l-carnitine on motility, vitality and DNA oxidation of human spermatozoa. *Andrologia* 46, 637–641.

Banihani SA, Bayachou M & Alzoubi K. (2015) L-carnitine is a calcium chelator: a reason for its useful and toxic effects in biological systems. *J Basic Clin Physiol Pharmacol* 26, 141–145.

Bornman MS, du Toit D, Otto B, Müller II, Hurter P & du Plessis DJ. (1989) Seminal carnitine, epididymal function and spermatozoal motility. *S Afr Med J* 75, 20–21.

Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G & Vitali G. (2004) Cinnoxicam and L-carnitine/acetil-L-carnitine treatment for idiopathic and varicocele-associated oligoasthenospermia. *J Androl* 25, 761–772.

Cooper TG, Weidner W & Nieschlag E. (1990) The influence of inflammation of the human male genital tract on secretion of the seminal markers alpha-glucosidase, glycerophosphocholine, carnitine, fructose and citric acid. *Int J Androl* 13, 329–336.

Costa M, Canale D, Filicori M, D'lddio S & Lenzi A. (1994) L-carnitine in idiopathic asthenozoospermia: a multicenter study. Italian Study Group on Carnitine and Male Infertility. *Andrologia* 26, 155–159.

De Rosa M, Boggia B, Amalfi B, Zarrilli S, Vita A, Colao A & Lombardi G. (2005) Correlation between seminal carnitine and functional spermatozoal characteristics in men with semen dysfunction of various origins. *Drugs R D* 6, 1–9.

Enomoto A, Wempe MF, Tsuchida H, Shin HJ, Cha SH, Anzai N, Goto A, Sakamoto A, Niwa T, Kanai Y, Anders MW & Endou H. (2002) Molecular identification of a novel carnitine transporter specific to human testis. Insights into the mechanism of carnitine recognition. *J Biol Chem* 277, 36262–36271.

Garolla A, Maiorino M, Roverato A, Roveri A, Ursini F & Foresta C. (2005) Oral carnitine supplementation increases sperm motility in asthenozoospermic men with normal sperm phospholipid hydroperoxide glutathione peroxidase levels. *Fertil Steril* 83, 355–361.

Gulcin I. (2006) Antioxidant and antiradical activities of L-carnitine. *Life Sci* 78, 803–811.

Jeulin C & Lewin LM. (1996) Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. *Hum Reprod Update* 2, 87–102.

Kanter M, Topcu-Tarladacalisir Y & Parlar S. (2010) Antiapoptotic effect of L-carnitine on testicular irradiation in rats. *J Mol Histol* 41, 121–128.

Kobayashi D, Tamai I, Sai Y, Yoshida K, Wakayama T, Kido Y, Nezu J, Iseki S & Tsuji A. (2007) Transport of carnitine and acetylcarnitine by carnitine/organic cation transporter (OCTN) 2 and OCTN3 into epididymal spermatozoa. *Reproduction* 134, 651–658.

Lenzi A, Lombardo F, Gandini L & Dondero F. (1992) Metabolism and action of L-carnitine: its possible role in sperm tail function. *Arch Ital Urol Nefrol Androl* 64, 187–196.

Lenzi A, Lombardo F, Sgrò P, Salacone P, Caponecchia L, Dondero F & Gandini L. (2003) Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. *Fertil Steril* 79, 292–300.

Lenzi A, Sgrò P, Salacone P, Paoli D, Gilio B, Lombardo F, Santulli M, Agarwal A & Gandini L. (2004) A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia. *Fertil Steril* 81, 1578–1584.

Mathur A, Zhong D, Sabharwal AK, Smith KJ & Bajaj SP. (1997) Interaction of factor IXa with factor VIIIa. Effects of protease domain Ca^{2+} binding site, proteolysis in the autolysis loop, phospholipid, and factor X. *J Biol Chem* 272, 23418–23426.

Moncada ML, Vicari E, Cimino C, Calogero AE, Mongioì A & D'Agata R. (1992) Effect of acetylcarnitine treatment in oligoasthenospermic patients. *Acta Eur Fertil* 23, 221–224.

Mutomba MC, Yuan H, Konyavko M, Adachi S, Yokoyama CB, Esser V, McGarry JD, Babior BM & Gottlieb RA. (2000) Regulation of the activity of caspases by L-carnitine and palmitoylcarnitine. *FEBS Lett* 478, 19–25.

Ng CM, Blackman MR, Wang C & Swerdloff RS. (2004) The role of carnitine in the male reproductive system. *Ann N Y Acad Sci* 1033, 177–188.

Okada FK, Stumpp T & Miraglia SM. (2009) Carnitine reduces testicular damage in rats treated with etoposide in the prepubertal phase. *Cell Tissue Res* 337, 269–280.

Vicari E & Calogero AE. (2001) Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. *Hum Reprod* 16, 2338–2342.

Vicari E, Rubino C, De Palma A, Longo G, Lauretta M, Consoli S & Arancio A. (2001) Antioxidant therapeutic efficiency after the use of carnitine in infertile patients with bacterial or non bacterial prostato-vesiculo-epididymitis. *Arch Ital Urol Androl* 73, 15–25.

Vicari E, La Vignera S & Calogero AE. (2002) Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertil Steril* 78, 1203–1208.

Vicari E, La Vignera S, Condorelli R & Calogero AE. (2010) Endothelial antioxidant administration ameliorates the erectile response to PDE5 regardless of the extension of the atherosclerotic process. *J Sex Med* 7, 1247–1253.

Vitali G, Parente R & Melotti C. (1995) Carnitine supplementation in human idiopathic asthenospermia: clinical results. *Drugs Exp Clin Res* 21, 157–159.

Weiss HJ & Lages B. (1997) Platelet prothrombinase activity and intracellular calcium responses in patients with storage pool deficiency, glycoprotein IIb-IIIa deficiency, or impaired platelet coagulant activity – a comparison with Scott syndrome. *Blood* 89, 1599–1611.

Yuan YV, Carrington MF & Walsh NA. (2005) Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation in vitro. *Food Chem Toxicol* 43, 1073–1081.