The Role of Carnitine in the Male Reproductive System

CHIU MING NG,[a] MARC R. BLACKMAN,[b] CHRISTINA WANG,[a] AND RONALD S. SWERDLOFF[a]

[a]*Department of Medicine, Harbor-UCLA Medical Center and Research and Education Institute, Torrance, California 90509, USA*

[b]*Endocrine Section, Laboratory of Clinical Investigation, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, Maryland 20892, USA*

ABSTRACT: **Carnitine is highly concentrated in the epididymis and spermatozoa, where it may serve as an intramitochondrial vehicle for the acyl group, which in the form of acyl CoA acts as a substrate for the oxidation process producing energy for sperm respiration and motility. To date, studies in rodents and humans suggest that sperm count, motility, and maturation are related to epididymal free carnitine concentrations. Moreover, supplementation with carnitine improves sperm quality and/or quantity in testes of mice exposed to physical insults, such as heat and X-irradiation, and in men with idiopathic oligoasthenospermia. These benefits may be due to increased mitochondrial fatty acid oxidation resulting in improvement in motility of epididymal sperm. The antiapoptotic effect(s) of carnitine in the testes may also contribute, but this remains speculative and requires further investigation. Research to uncover the many characteristics and mechanisms of action of carnitine in somatic and germ cells may provide insights into the pathophysiology of germ cell apoptosis, the prevention of germ cell death, and possibly specific therapy of some forms of infertility. Further well-controlled, carefully designed, larger-scale studies are necessary and desirable before widespread clinical use as an infertility therapy can be contemplated.**

KEYWORDS: **apoptosis; spermatogenesis; testis; epididymis; infertility**

INTRODUCTION

Carnitine has been recognized to be physiologically important for nearly a century; yet, its fundamental roles in health and disease remain to be understood. It is generally accepted that carnitine's main function is to serve as an important intracellular cofactor for the transport of activated long-chain fatty acids into the mitochondrial matrix, thereby facilitating oxidation and enhancing energy production (FIG. 1). Because carnitine is highly concentrated in the epididymis, its possible role

Address for correspondence: Marc R. Blackman, M.D., Endocrine Section, Laboratory of Clinical Investigation, National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD 20892.
blackmam@mail.nih.gov

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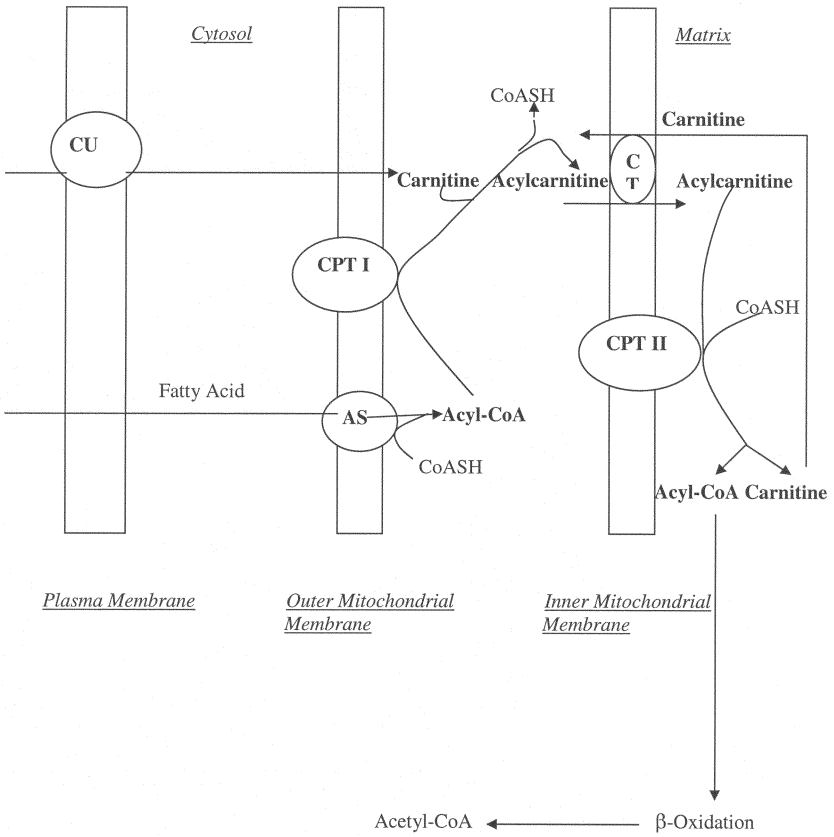

FIGURE 1. Carnitine cycle. Intracellular concentration of carnitine is maintained via the uptake of carnitine by its transporter, located in the plasma membrane. Long-chain fatty acid and coenzyme A (CoASH) are activated by acyl CoA synthase (AS) to form acyl CoA. Carnitine palmitoyltransferase I (CPT I) is located in the outer mitochondrial membrane, converting carnitine and acyl CoA to form acylcarnitine. Acylcarnitine is then transported over the inner mitochondrial membrane by the specific carrier, carnitine/acylcarnitine translocase (CT). On the matrix side, acylcarnitine is reesterified to form acyl CoA under the action of CPT II. The released acyl CoA undergoes β-oxidation to generate energy. The released carnitine can leave mitochondria by CT to enter another cycle.

in the acquisition of functional capacity and motility of spermatozoa is of particular interest to reproductive biologists. This high concentration of carnitine within the epididymis and spermatozoa serves as an intramitochondrial vehicle for the acyl group, which in the form of acyl CoA acts as a substrate for the oxidation process producing energy for sperm respiration and motility. This critical role of carnitine as the energy substrate for spermatozoa has led to its application as a treatment to increase sperm motility in patients with asthenozoospermia.[1]

Carnitine may have other therapeutic properties, as discussed elsewhere in this volume. For example, it has been proposed as an agent capable of protecting the heart against ischemia/perfusion injury leading to myocyte death.[2] This presumptive antiapoptotic effect of carnitine has also been observed in different cells, including neurons,[3] myocytes,[4] teratoma cells,[5] hepatocytes,[6] and lymphocytes.[7] Although the exact mechanisms of the antiapoptotic property remain uncertain, carnitine has been widely used as an alternative therapy in patients with Alzheimer's disease, chronic fatigue syndrome, end-stage renal failure, and peripheral vascular disease,[8] and as a supplement to enhance exercise tolerance.[9,10] Carnitine has been studied as a treatment for erectile dysfunction in elderly men. In a recently published placebo-controlled study, carnitine was found to improve nocturnal penile erections and the International Index of Erectile Function score.[11] Additional benefits have also been reported for selected cardiovascular indices (e.g., peak systolic velocity, end diastolic velocity), as well as fatigue, melancholia, and depression scores.

The antiapoptotic effects of carnitine are manifested in many tissues, suggesting that carnitine may also exert inhibitory effects on apoptosis of germ cells within the testis. This concept is supported, but not proved, by reports that the quality and quantity of spermatozoa are improved after carnitine supplementation in patients with asthenozoospermia.[12] Data derived from animal models also suggest the beneficial role of carnitine to protect against various stressors to germ cells. In this review, we summarize current knowledge of the possible antiapoptotic effects of carnitine in testes and other tissues, focusing on the mechanism in different animal models and cell systems. We also evaluate the literature on the effects of carnitine on human male infertility.

CARNITINE

Carnitine (L-3-hydroxy-4-*N*,*N*,*N*-trimethylaminobutyrate) is a trimethylated, conditionally essential amino acid. Meat and milk are the most significant dietary sources of exogenous carnitine for humans. Carnitine can also be synthesized endogenously from hepatic methylation of dietary amino acids such as lysine. Carnitine plays an important role in transferring long-chain fatty acids across the mitochondrial membranes, facilitating oxidation within mitochondria and subsequent energy production (FIG. 1). Carnitine is concentrated in high energy demanding tissues such as skeletal and cardiac muscles and in a specialized reproductive tract organ, the epididymis. In the epididymis, free carnitine is taken up from the circulating plasma and actively transported through the epithelial cells into the epididymal plasma, using a specific carrier under the regulation of androgens. Recent studies suggest that this process is modulated by a novel carnitine transporter in the testis, located in the luminal epithelium of the seminiferous tubules, and in the Sertoli cells.[13] Carnitine is then accumulated in the spermatozoa by passive diffusion.[14] The concentration of L-carnitine in epididymal plasma and spermatozoa varies from 2 to 100 mM, which is nearly 2000-fold greater than circulating levels (10–50 μM). Because epididymal spermatozoa use fatty acid oxidation as the main source of energy metabolism, carnitine's role in transporting fatty acids into the sperm mitochondrial matrix for energy production is crucial. The recent findings that L-carnitine and L-acetyl-carnitine, particularly in combination, elicit a dose-dependent decrease in Sertoli

cell amino acid incorporation, and both enhance mRNA expression of the glucose transporter (Glut-1) and reduce that for IGF binding protein-4 (IGFBP-4), suggest that Sertoli cells play a role in carnitine's effects on male fertility.[15] Not surprisingly, it has been suggested that carnitine contributes directly to sperm motility and sperm maturation.[16]

MECHANISMS OF CARNITINE ACTION

In addition to its contribution to cellular energy metabolism, carnitine can protect a variety of cells from apoptosis through several mechanisms. Addition of carnitine reduces apoptotic cell death in hepatocyte growth factor–deprived murine C2.8 hepatocytes[6] and lymphocytes.[7] Moreover, acetyl-L-carnitine has been reported to inhibit apoptosis triggered by serum deprivation in a teratocarcinoma cell line.[5] Carnitine possesses antioxidant properties. Increased reactive oxygen species have been found in patients with idiopathic and postinflammatory oligoasthenozoospermia. Carnitine has been shown to reduce reactive oxygen species and increase sperm forward motility and viability in infertile patients with prostato-vesiculo-epididymitis.[17] This beneficial antioxidant property has also been shown in tissues such as peripheral blood lymphocytes from patients during acute HIV syndrome[18] and heart muscles subjected to ischemia.[19] Despite the potential role of carnitine as an antioxidant, its actual protective significance is open to argument.[20] Given that various medications that purport to be free radical scavengers may not improve sperm motility,[21] other mechanisms for carnitine's action on spermatozoa may come into play. These alternative protective mechanisms have been investigated in *in vitro* studies of different tissues.

CARNITINE AS AN ANTIAPOPTOTIC AGENT

Data derived from many *in vitro* studies suggest that apoptosis of mammalian cells can be activated through the extrinsic Fas-FAS Ligand (Fas-FAS-L)–mediated pathway (FIG. 2). The latter pathway is the common effector of stress-induced apoptosis. It has been suggested that carnitine can act by interfering with the transduction of Fas-triggered apoptotic signals and inhibition of ceramide generation (FIG. 2). Mutomba *et al.*[22] attempted to define a role for carnitine in the protection of Fas-FAS-L–mediated apoptosis. They found that carnitine protected Jurkat cells against Fas-mediated apoptosis and exerted inhibitory effects on the activity of recombinant caspases 3, 7, and 8 (the "executioners" of cell apoptosis). They also found that the concentration of endogenous carnitine was reduced during apoptosis. These *in vitro* findings suggested that endogenous carnitine might play a regulatory role in the apoptotic process. Results from a number of *in vitro* studies suggest that apoptosis is associated with increased ceramide generation.[23] Carnitine has been found to reduce formation of ceramide through the inhibition of sphingomyelin degradation. For instance, Andrieu-Abadie *et al.*[20] showed that carnitine prevented doxorubicin-induced apoptosis of cardiac myocytes through downregulation of ceramide.

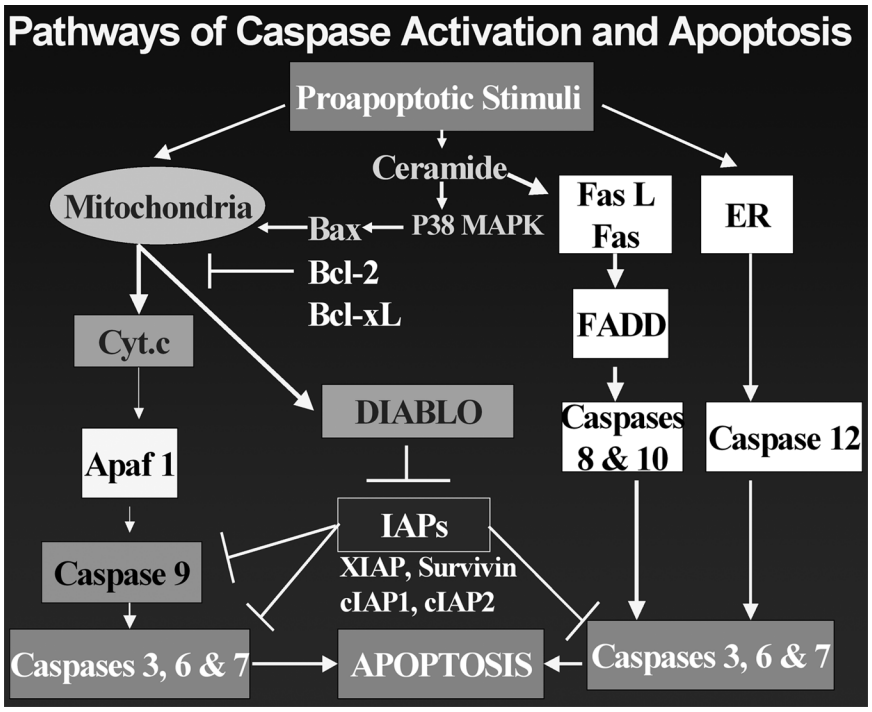

FIGURE 2. Pathways of caspase activation and apoptosis.

Apart from the Fas-FAS-L–mediated extrinsic pathway, the intrinsic mitochondrial-dependent pathway (FIG. 2) is considered to be a crucial step of programmed cell death. This intrinsic mechanism involves the release of cytochrome c from mitochondria into the cytosol, leading to activation of the initiator caspase 9 and subsequent activation of executioner caspases 3, 6, and 7. Mitochondrial Bcl-2 protein has been implicated to suppress the mitochondrial-dependent pathway for apoptosis. Vescovo *et al.*[24] demonstrated that carnitine intervenes in the intrinsic pathway protecting the cells from apoptosis. In a rat model of heart failure, an increase in apoptosis of the skeletal muscle was noted. Proapoptotic agents such as caspases 3 and 9, serum TNF, and its second messenger sphingosine were elevated. The rats were treated with carnitine (50 mg/kg) orally for 28 days. Cells from the treatment group exhibited a substantially lower number of TUNEL-positive nuclei and DNA break strands. These cells also demonstrated decreased expression of caspases 3 and 9 and increased expression of Bcl-2. The levels of TNF and sphingosine were also decreased. In the latter study, carnitine prevented apoptosis of skeletal muscle, although it was uncertain whether this effect was secondary to improvement of heart function or due to a direct protective role of carnitine on skeletal muscle.

Carnitine exerts other complex effects that may protect tissue. Carnitine can protect cells from apoptosis by removing acyl CoA, a potentially toxic intermediate.

Carnitine also interacts with cardiolipin, modifying membrane permeability and protecting the functions of the mitochondria. This mechanism has been proposed to explain the protective effects of carnitine against doxorubicin-induced cardiotoxicity, ammonium acetate, and zidovudine-induced mitochondrial ultrastructural and functional alterations.[4] Carnitine has also been reported to activate the growth hormone–insulin-like growth factor-I axis;[25] the implications of this action are beyond the scope of this review.

In general, carnitine appears to provide antiapoptotic protection, as shown in different *in vivo* and *in vitro* studies. However, various authors have proposed several alternative mechanisms. Controversial results in the literature might have arisen because outcome parameters varied, several animal models were used under diverse conditions, and carnitine might exert its effects at multiple levels. Nevertheless, knowledge about the protective mechanism of carnitine in different tissues may provide insight in its effect on the male reproductive system. In contrast to the clear-cut antiapoptotic effects of carnitine on cardiac muscle, it remains to be determined whether carnitine's beneficial effects on testis function include protection against germ cell apoptosis.

IN VIVO STUDIES OF CARNITINE ON MOUSE TESTES

In the male reproductive system, germ cell apoptosis can occur spontaneously or be induced by various insults, such as heat treatment and androgen deprivation.[26,27] Most recently, the apoptotic pathway for heat-induced programmed cell death in the testis has been reported. Hikim *et al*.[28] reported that the intrinsic mechanism was the key apoptotic pathway, involving the release of cytochrome c from mitochondria into the cytosol, resulting in activation of the initiator caspase 9 and subsequent activation of executioner caspases 3, 6, and 7 (FIG. 2). The latter caspases were responsible for cleavage of DNA resulting in apoptosis. On the other hand, the Fas-FAS-L–mediated pathway has also been shown convincingly to be the extrinsic drive to events of programmed cell death resulting from other testicular insults.[29]

Whether carnitine exerts any protective effects against apoptosis in testicular germ cells needs to be better defined. There are a large number of studies supporting the antiapoptotic effects of carnitine in various tissues, but only a few on the testis (TABLE 1). The strongest evidence of the beneficial effects of carnitine on germ cells was reported by Amendola *et al*.,[30] who studied the effects of carnitine on the post-injury recovery of mouse spermatogenesis. In this study characterizing the action of carnitine on the testes *in vivo*, the testes of each mouse were irradiated with a single dose of 10 Gy. Mice in the treatment group were treated with intraperitoneal administration of L-acetylcarnitine (100 mg/kg body weight) on alternate days for 4 weeks, starting from the day of irradiation. The effects of spermatogenesis were assessed at 28, 35, 40, 45, 50, 55, and 60 days after irradiation. The effects were examined by flow cytometric analysis of cellular DNA content. In the treatment group, the fraction of tetraploid cells was higher at days 28 ($P < 0.05$) and 45 ($P < 0.02$). The round spermatid fraction in the treatment group was higher at 45 days ($P < 0.1$), and the elongated spermatid fraction was higher at 50 days ($P < 0.1$). The recovery period throughout the maturation process was shorter in the treatment group. The investigators concluded that L-acetylcarnitine enhanced the recovery of mouse spermatogonia

TABLE 1. Animal studies showing effects of carnitine in the testes

Authors (year)	Species	Insults to testis	Route of Administration	Dosage of carnitine	Results
Amendola *et al.* (1989)[30]	Mice	X-ray irradiation (10 Gy)	Intraperitoneal	100 mg/kg on alternate days × 4 weeks	↑ tetraploid cells at days 28 & 45
Amendola *et al.* (1991)[31]	Mice	Local heat (42°C × 1 h)	Intraperitoneal	100 mg/kg on alternate days × 4 weeks	↑ haploid cells at day 45; improve histological changes
Ramadan *et al.* (2002)[32]	Mice	Magnetic field (20 mT)	Intraperitoneal	200 mg/kg, 3 times per week × 2 weeks	↑ sperm count, motility, daily sperm production, and testicular LDH-X activity; improve histological changes
Palmero *et al.* (2000)[33]	Rats	Nil	*In vitro* supplement	10–100 mM	↑ fatty acid breakdown; ↑ glycolysis of Sertoli cells

after X-ray damage. Despite the more favorable outcome in the carnitine group, however, no statistically significant difference was detected in the fractions of round and elongated spermatids between the control group and the carnitine-treated group. The beneficial effects of carnitine might be expected to occur more concordantly throughout the maturation process of spermatogenesis.

The same authors reported similar protective effects of carnitine on the *in vivo* recovery process of spermatogenesis of mice after heat treatment.[31] Heat was applied by immersion of mouse testes in a water bath maintained at 42°C for 1 h. The same dose of intraperitoneal carnitine as above was administered to the treatment group, starting from the day of heat treatment. The effects on spermatogenesis were studied at 8, 14, 21, 28, 35, 40, 45, and 60 days after heat treatment. The cellular DNA contents of testes were examined by flow cytometry. Testis weight in the treatment group was significantly higher than that in the control group ($P < 0.05$) at all time points, except day 60. In the control group, the number of primary spermatocytes was markedly reduced, with complete absence of haploid cells at 1 week after heat treatment, whereas the haploid cell fraction was significantly higher at day 45 in the treatment group ($P < 0.01$). Of note was that the treated animals showed no statistically significant increase of haploid cell fraction at other time points. It was not certain whether the difference shown only in a single time point was adequate to confirm the efficacy of carnitine. Histological examination of tissue sections indicated that the reorganization and recovery of the seminiferous epithelium were faster in the treatment group. The authors suggested that a more rapid recovery of spermatogenesis after heat treatment occurred with carnitine administration.

Ramadan *et al.*[32] also demonstrated the protective effect of carnitine against physical insult to mouse testes following damage by high magnetic field exposure.

The magnetic field (20 mT) was delivered either in fractions (30 min per day, 3 times per week for 2 weeks) or in one session for 3 h. The control group was injected with normal saline intraperitoneally. The treatment group was administered either carnitine intraperitoneally (200 mg/kg) or coenzyme Q10 orally (200 mg/kg) at 1 h before exposure to the magnetic field. One day after the magnetic field exposure, animals were sacrificed; the testes were then dissected and analyzed. Parameters, including total sperm count, motility, daily sperm production, and testicular LDH-X activity, as well as histopathological examinations were assessed. There was a significant decrease in all the above-mentioned parameters in the control group. Pretreatment with carnitine or coenzyme Q10 caused a significant improvement in all sperm parameters and histopathological changes.

In summary, the possible efficacy of carnitine in maintaining spermatogenesis in mice in the face of physical insult was demonstrated by the above studies. However, the exact pathways of the protective mechanism were not addressed in these models. Benefits, if any, appeared to be related to the antiapoptotic effect(s) of carnitine. It is also possible that exogenous carnitine can provide an extra source of acetyl groups for the CoA, enhancing cellular energy metabolism with consequent favorable outcomes on DNA repair, and regeneration of germ cells and Sertoli cell function.[33]

CLINICAL STUDIES OF CARNITINE ON MALE INFERTILITY

Based on the knowledge of the action of carnitine on cellular energy metabolism and on its antiapoptotic effect(s), the possibility that carnitine could enhance sperm motility and maturation has been clinically evaluated. Previous studies have shown that seminal fluid free carnitine content is directly related to sperm count and motility,[16,34] further suggesting that carnitine may be used in the treatment of male infertility.

There are few, if any, prospective, placebo-controlled, randomized studies of the use of carnitine in human male infertility (TABLE 2). Performance of randomized, placebo-controlled clinical trials of treatment of male infertility is fraught with procedural and interpretive difficulties because the causes of infertility can be multifactorial; there are variations in confounding factors such as the partnering female's fertility, the frequency of sexual intercourse, and varying comorbid conditions; semen analyses are technician-dependent and not adequately standardized; semen quality and quantity may vary within the same individual at different time points without intervention; and the selection criteria for patients are often arbitrary. Many of the studies have design problems,[9] which included, but are not limited to, the following: the bioavailability of carnitine is small and can vary from 5% to 15%;[35,36] intake of food related to carnitine content is not standardized; dosing of carnitine can be complicated by variable carnitine content in the formulation with poor dissolution properties; exogenous supplementation of carnitine substrate may not reach the epididymis or the spermatozoa; and action on substrates may be limited by the rate-limiting step of carnitine uptake and its metabolism.

In the study by Costa *et al.*,[12] 100 patients with idiopathic asthenozoospermia received carnitine orally at 3 g/day for 4 months. Sperm motility was determined before, during, and after study. After carnitine treatment, the percent of motile spermatozoa increased from $26.9 \pm 1.1\%$ to $36.4 \pm 0.9\%$ ($P < 0.01$), with concomi-

TABLE 2. Clinical studies of carnitine in male infertility

Authors (year)	Types of study	Subjects (*n*)	Carnitine dosage (per day)	Duration (months)	Results
Costa *et al.* (1994)[12]	Prospective uncontrolled	Idiopathic astheno-zoospermia ($n = 100$)	3 g	4	↑ % of motile sperm; ↑ rapid linear progression; ↑ total number of spermatozoa
Vitali *et al.* (1995)[37]	Prospective uncontrolled	Idiopathic astheno-zoospermia ($n = 47$)	3 g	3	↑ % of mobile spermatozoa; ↑ rapid linear progression; ↑ total number of spermatozoa
Vicari *et al.* (2001)[17]	Prospective nonrandomized	Infertility with abacterial prostato-vesiculo-epididymitis ($n = 54$)	1 g of L-carnitine + 1 g of acetyl-carnitine	3	↓ reactive oxygen species; ↑ sperm forward motility and viability; ↑ spontaneous pregnancy rate
Lenzi *et al.* (2003)[1]	Prospective randomized double-blind crossover	Idiopathic oligo-astheno-teratozoo-spermia ($n = 100$)	2 g	2	↑ sperm concentration; ↑ total and forward motility

tant increase in mean velocity and linearity index. Similarly, sperm with rapid linear progression increased from $10.8 \pm 0.6\%$ to $17.4 \pm 0.8\%$ ($P < 0.01$). The total number of spermatozoa per ejaculate also increased from $142.4 \pm 10.3 \times 10^6$ to $163.3 \pm 11.0 \times 10^6$ ($P < 0.01$), albeit the sperm concentration was only slightly increased. The authors concluded that oral administration of carnitine improved sperm quality in patients with idiopathic asthenozoospermia. Although these increases may have been statistically significant, the clinical relevance of the findings is not known. Other investigators also reported a similar effectiveness of carnitine in improving sperm motility.[37] These latter studies were not placebo-controlled or randomized, and neither patients nor investigators were blinded to the treatment. Some of the improvement of sperm quality might have been due to bias of the interpretation of semen analysis, secondary to spontaneous improvement, or the phenomenon of "regression towards the mean", as is frequently encountered in clinical trials for male infertility. Furthermore, the concentrations of carnitine in the seminal fluid and spermatozoa were not measured and the concentration of carnitine within the epididymis was not known. Thus, while interesting, these findings require further validation.

Lenzi *et al.*[1] reported a prospective, randomized, double-blind, crossover trial, comparing the usefulness of carnitine versus placebo in male infertility. One hundred male patients with a history of infertility for more than 2 years were enrolled. The inclusion criteria included sperm concentration of $10–20 \times 10^6$/mL, total motility of 10–30%, forward motility of <15%, atypical forms of >70%, velocity of 10–30 microns/s, and linearity of <4. Subjects underwent a washout period for 2 months and then were randomized to treatment with oral carnitine (2 g/day) or placebo for another 2 months. This was followed by a 2-month washout period and a crossover study where those who initially received carnitine were allocated to placebo for

2 months and those who initially received placebo were assigned to carnitine. All participants were followed up for 2 more months. Carnitine treatment significantly increased sperm concentration and total and forward sperm motility, especially in groups with lower baseline levels. The authors concluded that carnitine therapy might improve semen quality in selected cases of male infertility. The investigators also showed that the seminal carnitine concentrations after carnitine therapy were not significantly increased, probably because the initial seminal concentrations were too high to allow the detection of any statistically significant increase after treatment.

Although this study was not designed to examine pregnancy rates as an end point, 8 spontaneous pregnancies occurred in the female partners during the period of carnitine therapy, suggesting the possible beneficial effect of carnitine on fertility. However, each treatment period, placebo or carnitine, was for 2 months with a 2-month washout in between. A washout period lasting for 2 months might not be long enough to eliminate the carryover effect of carnitine. In addition, the treatment period of 2 months might be too short, thus making it impossible to interpret the pregnancy data. Furthermore, one of the inclusion criteria was that the sperm concentration should range from 10 to 20×10^6/mL. Because patients with sperm counts in this range are subfertile with a substantial background rate of fertility, the efficacy of carnitine therapy might not be applicable to patients with more severe oligozoospermia. Thus, any beneficial effect of carnitine in fertility should be confirmed by a properly controlled, adequately powered, longer duration, parallel-designed intervention study.

In summary, from our review of the available literature, carnitine appears to elicit antiapoptotic actions in the testes, as shown in several *in vivo* and *in vitro* studies. Carnitine also appears to improve sperm quality in human clinical trials. However, many questions remain regarding the dose, route, and duration, and the selection of suitable candidates for clinical trials of carnitine therapy. The exact mechanisms for its beneficial effects, if any, on spermatogenesis are not clear. As the action of carnitine is at an intracellular level, the underlying protective mechanism in spermatogenesis may be more easily explored in *in vitro* studies. However, the translation of *in vitro* effects to the clinically relevant outcomes remains to be elucidated. The positive effect of carnitine on semen quality requires additional well-designed placebo-controlled clinical trials to determine its clinical significance and mechanisms of action.

CONCLUSIONS

Carnitine has widespread actions and has been proposed, and is used, as a health supplement for many medical conditions including those associated with male reproductive dysfunction. Carnitine has been widely used in the treatment of male infertility. Reported benefits may be related to improvement of epididymal function and sperm motility consequent to increased mitochondrial fatty acid oxidation. The antiapoptotic effect(s) of carnitine in the testes may also contribute, but this remains speculative and requires further investigation. Research to uncover the many characteristics and mechanisms of action of carnitine in somatic and germ cells may provide insights into the pathophysiology of germ cell apoptosis, the prevention of germ cell death, and possibly specific therapy of some forms of infertility. Further well-

controlled, carefully designed, larger-scale studies are necessary and desirable before widespread clinical use as an infertility therapy can be contemplated.

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