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Contents

Editorial

S-etodolac gel in the treatment of osteoarthritis
N.J. Karne

Role of vitamin D in osteoarthritis
M.S. Shah

Challenges in the management of iron deficiency anemia
V. Ramanan

Daptomycin – First of a new class of antimicrobial agents
R. Upadhyay

Reteplase: Convenient fixed bolus dose thrombolytic for rapid reperfusion 26
R.S. Shinde

L-carnitine – A potential therapeutic agent for male infertility 34
A.R. Kale

Review Article

L-carnitine – A potential therapeutic agent for male infertility

A.R. Kale[1]

[1] Asha Kiran Hospital, Pune

Correspondence: Dr. Ashish R. Kale, Consultant gynecologist, Asha Kiran Hospital, Near Narayan Peth Police station, Kelkar Road, Pune - 411030; Email:drashishkale1978@yahoo.com

Abstract

Carnitines, including L-carnitine (LC) and L-acetyl-carnitine (ALC), are widely used in various diseases including male infertility. LC is a bioactive form of carnitine. Within the male genital tract, carnitines are concentrated in the epididymis and spermatozoa. Oxidative stress in the male germ line leads to the induction of damage in the spermatozoa and loss of integrity in the nucleus and mitochondria. LC essentially plays a key role in the mitochondrial β-oxidation of long chain free fatty acids. There is a positive correlation between LC levels in the seminal plasma and the number of spermatozoa, the percentage of motile spermatozoa, and the percentage of normal forms. The administration of LC and/or ALC may be effective in improving pregnancy rate and sperm kinetic features in patients affected by male infertility. The well-established role LC have in sperm energy production, maturation and antioxidant properties; creates a rationale for the treatment with LC and/or ALC in many cases of male infertility.

Keywords: L-carnitine, male infertility, motile spermatozoa

Introduction

Carnitine (3-hydroxy-4-N-trymethylaminobutyric acid) was first isolated from bovine muscle by Russian scientists in 1905 and only the L-isomer (L-carnitine, LC) was found bioactive. Its role in metabolism was only elucidated in 1955 and primary LC deficiency was defined in 1972.[1] Although LC could be biosynthesized *de novo* by human body, LC present in human tissues is mainly of exogenous origin from meat, poultry and fish. Thus, L-carnitine is a conditionally-essential component of diet and meat is the richest source for it. Average plasma concentration of carnitine was found to be significantly lower in strict vegetarians than those of the respective omnivorous controls.[2] L-carnitine is approved by USFDA for the treatment of carnitine nutritional deficiency induced by hemodialysis in chronic renal failure patients. However, carnitines, including LC and L-acetyl-carnitine (LAC), are widely used as health supplements by athletes and in various diseases including cardiomyopathy, congestive heart failure, peripheral vascular disease and male infertility.[1]

Male infertility is a significant problem affecting 7.5% of the male population. Approximately 60% of these cases are idiopathic and related to sperm dysfunctions such as oligo-astheno-teratozoospermia (OAT). No "etiological" treatment has been found; therefore "empirical" drugs have been used for OAT. Idiopathic OAT males were regarded as impervious to any therapy until a few years ago.[3]

LC is suggested to play a key role in sperm metabolism by positively affecting sperm motility, maturation and the spermatogenic process. This effect is mediated by transport of free fatty acid across the inner mitochondrial membrane required for beta-oxidation. Several studies have reported high concentration of LC in epididymal fluid and spermatozoa. The concentration of LC is reported as being positively correlated to the number and motility of spermatozoa.[2,4] LC has a protective role against reactive oxygen species (ROS) by exerting antioxidant properties. It binds to free coenzyme-A and removes toxic concentrations of acetyl-coenzyme A (acetyl-CoA) from cells. Numerous clinical trials have attempted to demonstrate a beneficial therapeutic effect of LC and/or ALC in various forms of

sperm dysfunction.[5] This article elucidates the role of LC in the treatment of various forms of male infertility and summarizes clinical studies on the same.

L-carnitine

Biochemistry

L-carnitine is a cofactor required for transformation of free long-chain fatty acids into acylcarnitines which are transported into the mitochondrial matrix for beta oxidation. Mitochondrial fatty acid oxidation is an essential source of energy production especially in heart and skeletal muscles.[1] Structure of L-carnitine and acetyl –L-carnitine is depicted in Figure 1.

Steps in synthesis

Carnitine synthesis begins with methylation of the amino acid L-lysine by S-adenosylmethionine (SAM) [Figure 2]. L-lysine undergoes three consecutive methylation reactions with SAM acting as the methyl donor. Trimethyl lysine thus formed is converted to hydroxytrimethyl lysine in presence of alphaketoglutarate, oxygen, ascorbic acid and iron. This compound in the next step that requires pyridoxal 5'-phosphate (vitamin B_6) results in the formation of trimethylaminobutyraldehyde.

Trimethylaminobutyraldehyde after NADH-dependent reduction and hydroxylation using alpha-ketoglutarate, oxygen, ascorbic acid and iron Trimethylaminobutyrate (or gamma-butyrobetaine) is then formed in a reaction requiring NADH finally transfoms into carnitine.[1]

Pharmacokinetics

L-carnitine is absorbed in the intestine by a combination of active transport and passive diffusion. As mucosal absorption of carnitine is saturated at about a 2 g dose, there is no significant advantage of supplementing an oral dose of L-carnitine in amounts greater than 2 g. Maximum blood concentrations are reached approximately 3.5 hours following an oral dose, with a half-life of about 15 hours. Elimination of carnitine occurs primarily through the kidneys. Bioavailability of L-carnitine is reported to be variable ranging from 16% to 87% in various studies.[1]

Distribution of carnitine in the genital tract

L-Carnitine is secreted from mammalian epithelium into epididymal plasma and ultimately into spermatozoa, where it accumulates as free and acetylated L-carnitine. Epididymal tissue, seminal plasma and spermatozoa have the highest free concentrations of carnitine in the body. Carnitine is secreted into the seminal fluid by epididymis.

Studies in rats have revealed that carnitine is present in testicular fluid and concentrations increase in epididymis towards from distal caput epididymis point from where sperms become motile. Similarly, in human epididymis, concentration of LC is 10–50 times higher than in the plasma. The mechanism of LC transport in the epididymis has been previously suggested to involve an active transport system consisting of both a basolateral as well as apical transporter. Recently, a high affinity Na^+- driven, organic cation transporter (OCTN2), was shown to transport LC into the cells of the epididymal epithelium.

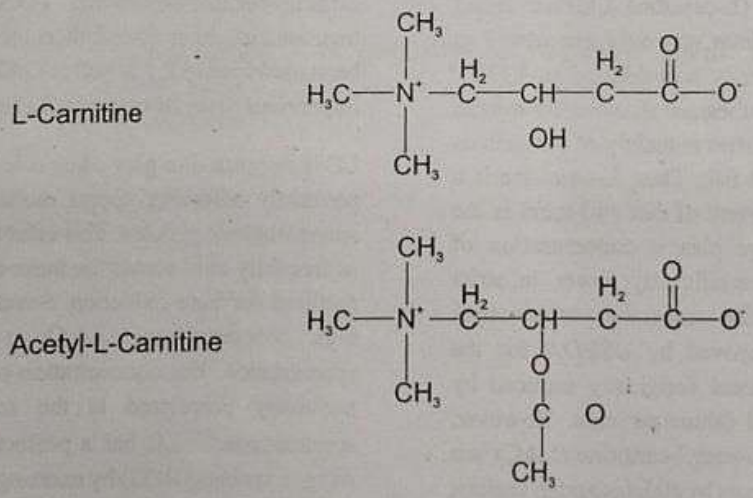

Figure 1. Chemical structure of L-carnitine and Acetyl-L-carnitine[1]

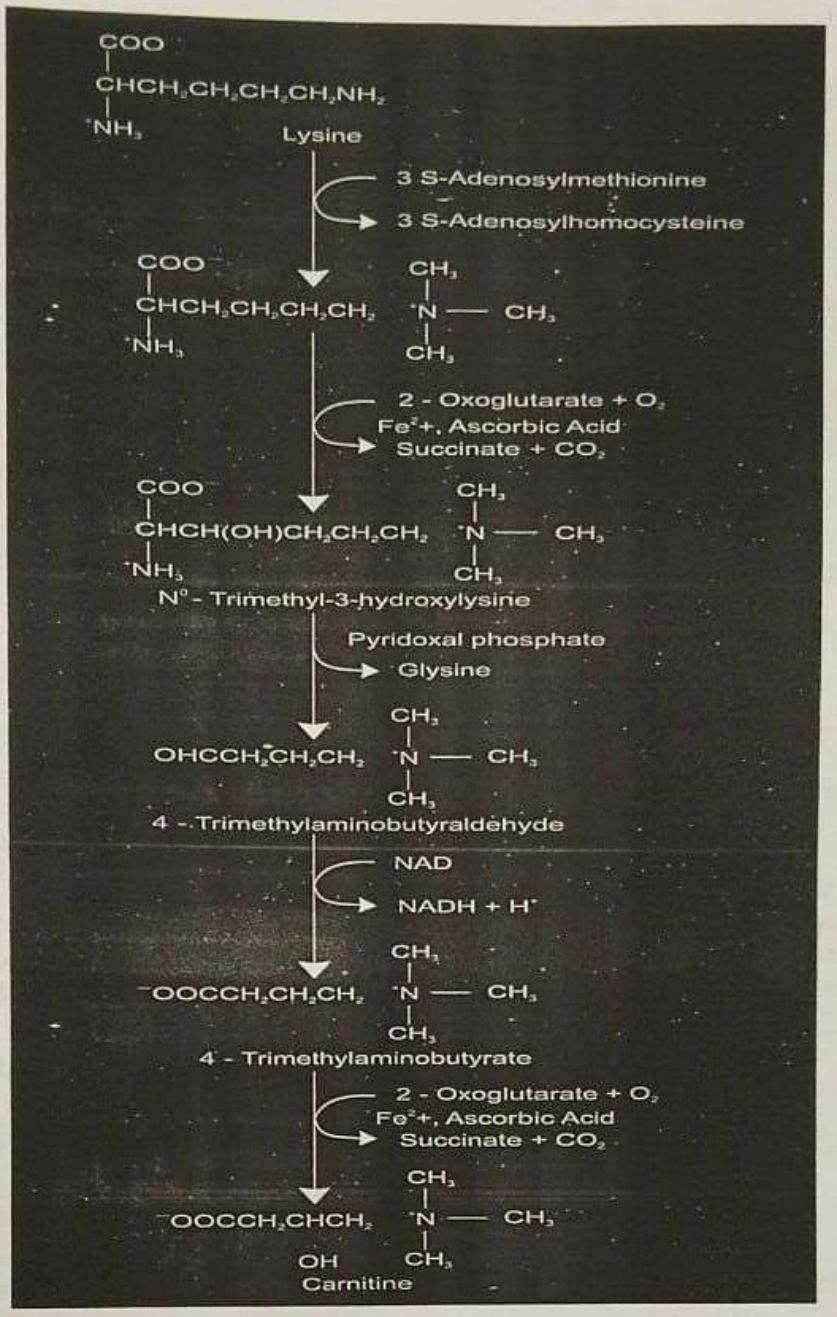

Figure 2. Synthesis of carnitine[1]

The high concentration of LC in epididymis in rats was found to be under the control of androgens, thus animals with cryptorchidism showing lower concentrations of carnitine. Nevertheless, no correlation has been found between the tissue concentration of testosterone in the testis and the concentrations of carnitine in the human epididymis. This is probably because carnitine accumulates in testis during a long period and testosterone concentrations change rapidly with gonadotrophin concentrations.[5]

Mechanism of action

LC provides a shuttle system for free fatty acids and derivatives of acyl-CoA within the mitochondria. During their passage through the cell membrane, acyl groups are temporarily transferred to LC, producing ALC. In a similar way, carnitine facilitates the transport of acetyl groups via ALC [Figure 3]. The end result of these reactions is a modulation of mitochondrial concentrations of CoA implicated in various metabolic ways, such as the

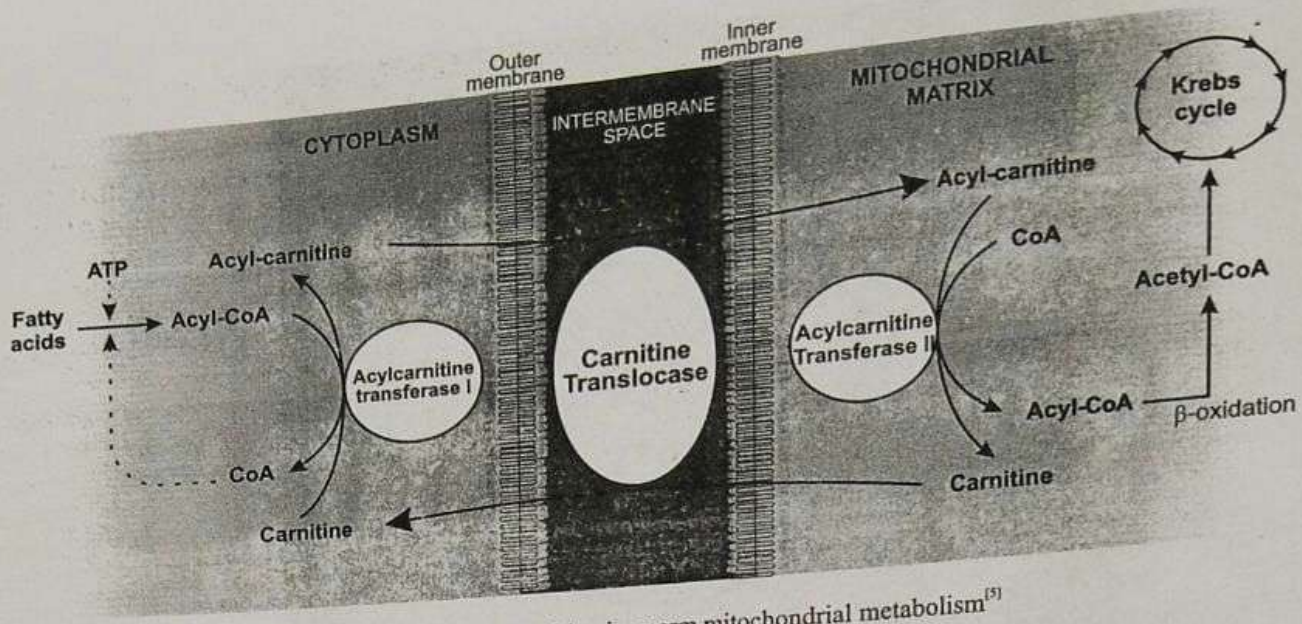

Figure 3. Transport of acyl groups and the role of carnitine in sperm mitochondrial metabolism[5]

tricarboxylic acid cycle (Krebs cycle), the β- oxidation of organic acids and the oxidative degradation of amino acids.[5] In rat tissue, ample concentration of carnitine has been detected in the rat testicle and high concentrations of ALC transferase have been detected in primary spermatocytes. Carnitine may affect testicular sperm maturation indirectly via the stimulation of Sertoli cell glucose uptake. The addition of LC to Sertoli cell cultures results in a considerable increase in pyruvate and lactate secretion, which are known essential energy substrates for germ cell maturation. Carnitine may be responsible for removing excess intracellular toxic acetyl-CoA, which protects spermatozoa from oxidative damage Oxidative stress in the male germ line can lead to damage to spermatozoa. Oxidative stress can cause peroxidative damage to the membrane unsaturated fatty acids and reduce fusogenicity of sperm plasma membrane.[5]

Dosage

As a general guideline, the average therapeutic dose is 1000

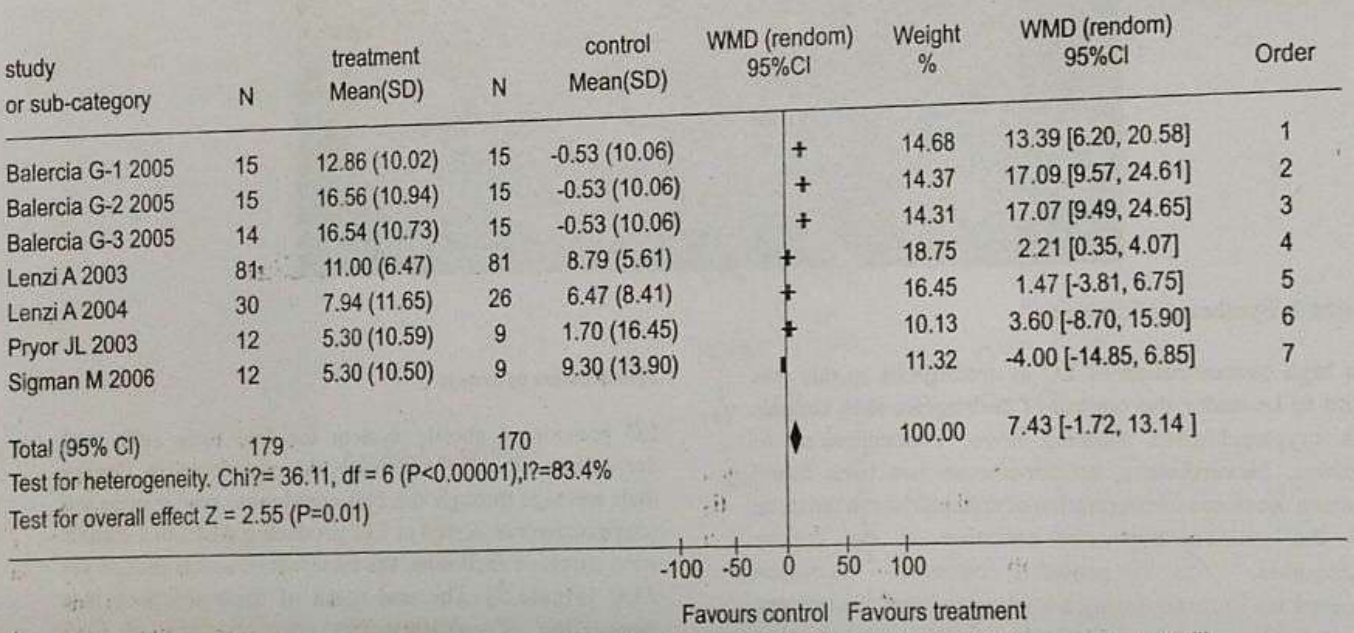

Figure 4. Comparison of effects of carnitine therapy with placebo (control) on percentage of total sperm motility[2]

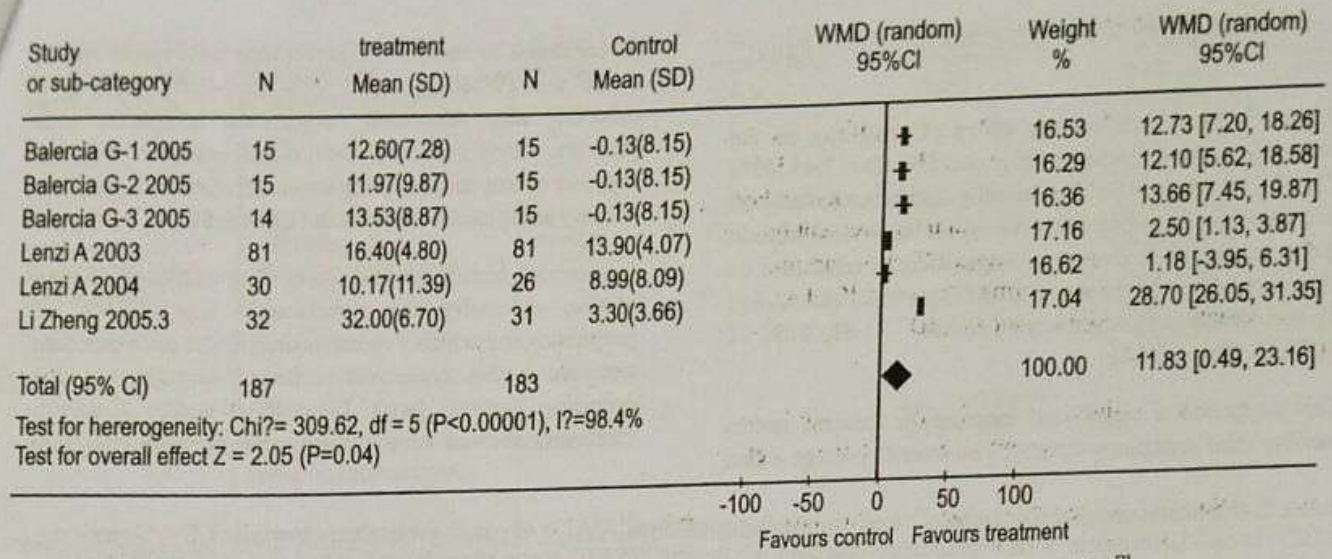

Figure 5. Comparison of effects of carnitine therapy with placebo on percentage of forward sperm motility[2]

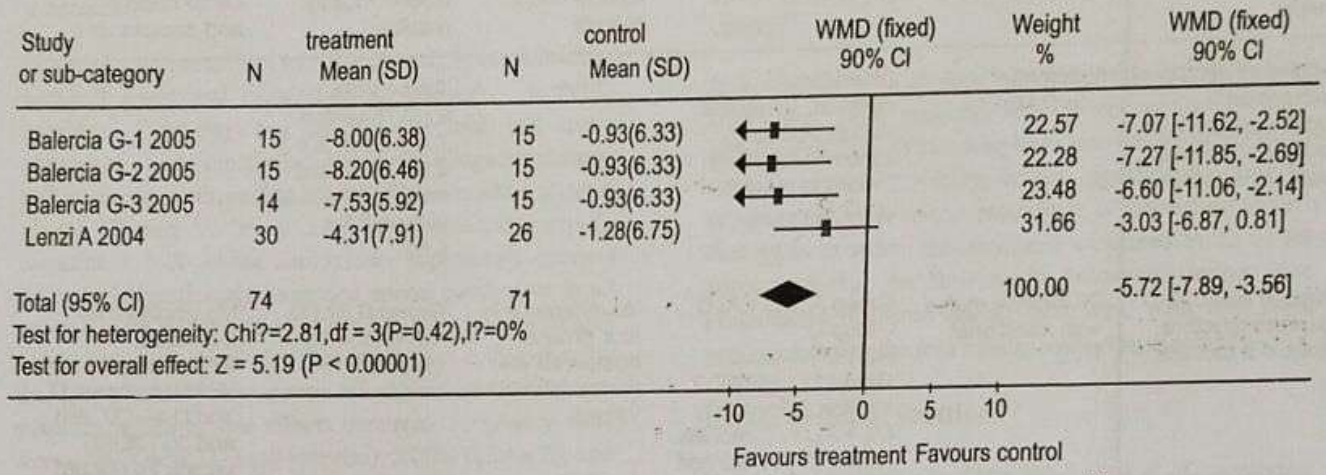

Figure 6. Comparison of effects of carnitine therapy with placebo on percentage of atypical sperm forms[2]

mg given two to three times daily for a total of 2000-3000 mg. No advantage appears to exist in giving an oral dose greater than 2000 mg at one time, since absorption studies indicate saturation at this dose.[1]

Adverse reactions

A variety of mild gastrointestinal symptoms have been reported, including transient nausea and vomiting, abdominal cramps, and diarrhea. A change in body odor has also been observed in a few individuals. Typically, reducing the dose will result in improvements in these adverse reactions. No reports of L-carnitine toxicity from overdosage exist. In mice, the LD_{50} is 19.2 g/kg. Studies indicate no mutagenicity; however, experiments to determine the long-term carcinogenicity have not been conducted.[1]

Clinical studies in male infertility

A review [the meta-analysis of 7 randomized clinical trials compared LC and /or L-acetyl-carnitine (LAC) therapy to placebo treatment] by Zhou *et al.* indicated that there was marked significant effect of carnitines on pregnancy rate was [OR = 4.10, 95% CI (2.08, 8.08); $p < 0.0001$). This supported the hypothesis that pregnancy rate could be significantly improved after administration of carnitines in infertile men.

Meta-analysis of 3 trials showed that the overall average effect of carnitines on sperm concentration was not statistically significant as compared to placebo [Weighted

mean difference (WMD) = 5.69, 95% CI (-4.47, 15.84); p = 0.27].

However, the overall average effect of carnitines on the percentage of total sperm motility was [WMD = 7.43, 95% CI (1.72, 13.14);p=0.01], statistically significant to indicate that carnitines could be effective on the increment of total sperm motility. The overall average effect of carnitines on the percentage of forward sperm motility (WHO class A and B) was statistically significant [WMD = 11.83, 95% CI (0.49,23.16);p=0.04].

This suggested a significant increase in forward sperm motility after carnitines therapy. The overall average effect of carnitines to reduce the percentage of atypical sperm forms was [WMD = -5.72, 95% CI (-7.89, -3.56; p < 0.00001] also statistically significant compared with placebo, which supported their effectiveness to decrease atypical sperm forms. Comparison of effects of carnitine therapy with placebo is shown in Figure 4, 5 & 6.

In summary, based on the results of meta-analysis presented above, especially the significantly improvement in pregnancy rate which was considered as the main outcome measure in this systematic review, it is supported that carnitine therapy (with L-carnitine and/or L-acetyl-carnitine) showed some considerable positive effects in

Table 2. Summary of selected human clinical trials using carnitines[5] OAT = oligoasthenoteratozoospermia; LC = L-carnitine; ALC = acetyl-L-carnitine; PVE = prostaticovesiculoepididymitis; NSAID = non-steroidal anti-inflammatory drug; ROS = reactive oxygen species.

Author and design	*Study population*	*Treatment*	*Sperm count results*	*Sperm motility results*	*Other finding and comments*
Lenzi *et al.* (2003): randomized placebo-controlled double-blind cross-over trial, 8 months	86 infertile with OAT	2 months washout, 2 months placebo/LC (2 g), 2 month washout, 2 months placebo / LC (2 g)		Total motile: 9± 6.75 versus 7.4 ± 5.58[bc] 5.4 ± 4.82[bc]. Forward motile: 7.2 ± 6.39 versus 5.8 ± 6.02[bc]	
Vicari *et al.*(2002): open prospective study, 6-9 months	98 infertile males with abacterial PVE	Group A: LC + ALC Group B: NSAID - 4 months Group C: . NSAID 2 months followed by LC + ALC - 2 months. Group D: NSAID and LC + ALC concomitantly - 4 months. LC: 1 g/12 h. ALC: 500 mg/12 h.	No difference in four groups compared with pretreatment values	Group C 32 (18, 40) versus 14 (10,19)[ab]	Morphology: no significant difference. Viability: groups C and D 44 (32, 60) and 38 (28,50) versus 24 (19,38) and 24 (18,39)[ab] leukocyte count: groups C and D 0.7 (0.4,1.0) and 1 (0.6, 1.1) versus 1.7 (1.1, 2.0) and 1.7 (1.1,2.1)[ab]. ROS production: group C 14.7 versus 51.7 [ab]
Vicari and Calogero (2001): open prospective study, 3 months	54 infertile males with abacterial PVE Group A: (*n* = 34) no leukocytospermia. Group B: (*n* = 20) leukocytospermia	LC (1 g) + ALC (500 mg)/12 h for 3 months	No difference compared with pretreatment	Forward motility 28 (22,35) versus 14 (10,20)[ab]	Viability: groups A and B 42 (32,56) and 33 (28,56) and 33 (28,46) versus 29.5 (25,32) and 27.5 (25,40)[ab] ROS production group A 48.8 (26.2,66.8) versus 61.1 (30.2,79.5)[ab]

Vitali et al. (1995): open prospective study 3 months	47 infertile males with idiopathic asthenozoospermia	LC (1 g t.i.d., 3 months)	159 ± 5.8 versus 88.9 ± 8.9[bc]	53.5 ± 7.7 versus 26.8 ± 10.5[bc]	3/47 patients had no change and 7/47 had worsening of semen parameters
Costa *et al.* (1994): open prospective study, multicentre trial, 6 months	100 infertile males with idiopathic asthenozoospermia	LC (1 g t.i.d., 4 months)	49.4 ± 3.7 to 53.2 ± 3.4[bc]	26.9 ± 1.1 to 36.4 ± 0.9[bc]	Abnormal morphology significantly decreased 45.9 ± 0.8 to 42.9 ± 0.8[bc]
Moncada *et al.* (1992): 2 months	20 infertile males with idiopathic asthenozoospermia	ALC (2 g t.i.d., 2 months)		21.7 ± 3.24 to 38.2 ± 4.71[bc]	

[a]Results expressed as median (10th percentile, 90th percentile). [b]Compared with pretreatment values in the same group considered significant ($P < 0.05$). [c]Results expressed as mean ± standard deviation.

improving sperm quality compared with placebo treatment.[2]

In a study conducted by Cavallini G *et al.*, it was found that group 2 (used oral L-carnitine (2 gm/day) + acetyl-L-carnitine (1 gm/day) had significantly increased sperm patterns at 3 and 6 months into therapy in idiopathic patients and in patients with grades I, II, and III varicocele, but not in grades IV and V. Group 3 (used L-carnitine/acetyl-L-carnitine + 1 X 30-mg cinnoxicam suppository every 4 days) had significantly increased sperm parameters in all patients, with the exception of grade V varicocele. Group 3 sperm patterns proved significantly higher during therapy than group 2. All sperm patterns fell to baseline after therapy suspension. Minor side effects occurred. Pregnancy rates were 1.7% (group 1- used placebo), 21.8% (group 2), and 38.0% (group 3) ($p< 0.01$). Thus, L-carnitine/acetyl-L-carnitine + cinnoxicam suppositories proved a reliable treatment for low-grade varicoceles and idiopathic OATs.[3]

In one multi-center trial by Costa *et al.*, 100 patients received 3 g/day of oral L-carnitine for four months. Sperm parameters were assessed before, during, and after the study. Motility was determined by computer-assisted sperm analysis. The results clearly demonstrate carnitine has a positive effect on sperm motility. The percentage of motile spermatozoa increased from 26.9 ± 1.1 to 37.7 ± 1.1 %. The percent of sperm with rapid linear progression increased from a baseline of 10.8% to 18%. Not only did carnitine significantly affect sperm motility, but the total number of spermatozoa per ejaculate also increased.[6]

In an open prospective clinical trial by Vitali *et al.*, similar results were reported with 3 g carnitine given daily for three months. Thirty seven of the 47 participants had increases in sperm motility, rapid linear progression, and total number of sperm.[4]

In a related study, 20 men with idiopathic asthenospermia (defective sperm motility) were given acetylcarnitine, 4 g/day for 60 days. While acetylcarnitine did not affect sperm density or total motility, it did significantly increase progressive linear sperm motility. It is interesting to note that gains in sperm motility were sustained in 12 of the subjects during the four-month follow-up period. Five pregnancies occurred during treatment, with two more occurring during the four months following the trial.[7]

Observational studies

In a study conducted by Menchini-Fabris *et al.*, involving 124 infertile patients, a direct correlation between semen carnitine content and sperm motility ($P<0.01$), was found. The results also showed a positive correlation between free L-carnitine and both sperm count and the number of motile sperm per milliliter ($p<0.01$).[8]

Similar findings were reported in a study consisting of 101 infertile men in whom the group with normal spermiogram had a mean value for L-carnitine of 478.4 while the abnormal one comes to 100.58. This difference was statistically significant ($p<0.0001$). There was a statistically significant, positive correlation between L-carnitine and the number of spermatozoa, the percentage of motile spermatozoa, and the percentage of normal forms ($p < 0.0001$).[9]

However, it is important to note that above mentioned

studies lacked a double blind, controlled design. Summary of selected human clinical trials using carnitines is shown in Table 2.

Conclusion

Human clinical trials have demonstrated that L-carnitine (LC) and Acetyl-L-carnitine (ALC) supplementations improve sperm motility parameters among men with astheno- or oligoasthenozoospermia. Due to the well-established role LC and ALC have in sperm energy production, maturation and antioxidant properties, it creates a rationale for treatment with LC and/or ALC in many cases of male infertility.

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