Review

Carnitines and male infertility

Dr Ashok Agarwal is the Director of Research at the Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, and the Director of the Clinical Andrology Laboratory and Reproductive Tissue Bank. He holds these positions at The Cleveland Clinic Foundation, Ohio, USA, where he has been a full staff member in the Glickman Urological Institute, Departments of Obstetrics–Gynecology, Anatomic Pathology, and Immunology since 1993. Dr Agarwal has published extensively in different areas of Andrology. His current research interests include the role of oxidative stress, DNA integrity, and apoptosis in the pathophysiology of male and female reproduction, cryopreservation of spermatozoa in patients with cancer, epididymal physiology, and pathophysiology of sexual dysfunction.

Dr Ashok Agarwal

A Agarwal[1], Tamer M Said
Centre for Advanced Research in Human Reproduction, Infertility, and Sexual Function, Glickman Urological Institute and Department of Obstetrics and Gynecology, The Cleveland Clinic Foundation, 9500 Euclid Avenue, Desk A19.1, Cleveland, OH 44195, USA
[1]Correspondence: Tel: +1 216 4449485; Fax: +1 216 4456049; e-mail: agarwaa@ccf.org

Abstract

L-Carnitine (LC) and acetyl-L-carnitine (ALC) are highly concentrated in the epididymis and play a crucial role in sperm metabolism and maturation. They are related to sperm motility and have antioxidant properties. The objective of this review is to summarize the multiple roles played by LC and ALC in male reproduction, and to highlight their limitations as well as their benefits in the treatment of male infertility. A variety of studies support the conclusion that LC and/or ALC at total daily amounts of at least 3 g per day can significantly improve both sperm concentration and total sperm counts among men with astheno- or oligoasthenozoospermia. Although many clinical trials have demonstrated the beneficial effects of LC and ALC in selected cases of male infertility, the majority of these studies suffer from a lack of placebo-controlled, double blind design, making it difficult to reach a definite conclusion. Additional, well-designed studies are necessary to further validate the use of carnitines in the treatment of patients with male infertility, specifically in men with poor semen quality.

Keywords: *acetyl-L-carnitine, carnitine, male infertility, spermatozoa*

Introduction

Carnitines are highly polar compounds that are widely distributed in nature. Human requirements for carnitines are fulfilled through endogenous biosynthesis and diet (Bieber, 1988). Within the male genital tract, carnitines are concentrated in the epididymis and spermatozoa. While in ejaculated seminal fluid, most L-carnitine (LC) and acetyl-L-carnitine (ALC) are found in the seminal plasma; very little are found in the spermatozoon itself (Bohmer *et al*., 1978).

L-Carnitine and ALC play a key role in sperm metabolism by providing readily available energy for use by spermatozoa, which positively affects sperm motility, maturation and the spermatogenic process. This beneficial effect is mediated by the transport of long chain fatty acids across the inner membrane of the mitochondria for utilization in metabolism through β-oxidation (Matalliotakis *et al*., 2000). Carnitines also have a protective role against reactive oxygen species (ROS) by exerting antioxidant properties. These properties occur as a result of a repairing mechanism by which elevated intracellular toxic acetyl-coenzyme A (acetyl-CoA) is removed and/or fatty acids in membrane phospholipids are replaced (Vicari and Calogero, 2001). Based on these fundamental roles, numerous clinical trials have attempted to demonstrate a beneficial therapeutic effect of LC and/or ALC when administered to infertile men with various forms of sperm dysfunction. Moreover, several in-vitro studies have documented that carnitines enhance sperm motility when added *in vitro* and may also have a cryoprotectant effect.

This article provides an overview of carnitines, including their structure, origin, role in spermatogenesis and beneficial effects on sperm metabolism. In addition, it discusses how these compounds may potentially be used to diagnose male infertility. Finally, the article aims to clarify their limitations as well as their benefits in the treatment of male infertility.

Structure and sources of carnitine

L-Carnitine is a highly polar, water soluble, small quaternary amine. In contrast to other organisms, humans can biosynthesize LC *de novo*; however, the LC that is present in human tissues is mainly of exogenous origin (Engle and

Rebouche, 1984). Exogenous carnitine is dietary in origin; its sources include meat, poultry, fish and dairy products. It has long been assumed that, because humans have the ability to synthesize carnitine, this compound is not an essential component of the diet. However, when groups of strict vegetarians were studied, the results showed that average plasma concentrations of carnitine were significantly lower than those of the respective omnivorous controls. This may be attributed to the fact that strict vegetarians consume less than 0.1 µmol/kg per day of carnitine, whereas the average omnivorous diet provides a daily intake of 2–12 µmol/kg (Rebouche, 1988).

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. In general, the male genital tract contains several compartments that maintain the highest free carnitine concentrations in the body: epididymal tissue, seminal plasma and spermatozoa. In mammals, the origin of free carnitine in seminal plasma is mainly epididymal.

Numerous studies have investigated the presence of carnitine in the genital tract of animal models. A study in which luminal fluid was collected via micropuncture from the testis and epididymis of rats revealed that 1) carnitine was present in the testicular fluid in concentrations of <1 mmol/l and 2) concentrations increased as the epididymis was transversed and ultimately reached 53 mmol/l in the luminal fluid from the cauda epididymis. The high concentration was first found in the luminal fluid from the distal caput epididymis, at about the point where the spermatozoa become motile (Hinton *et al*., 1979). Similarly, the human epididymis possesses a concentrative mechanism for LC; its concentration is 10–50 times higher in the epididymis than in the plasma, following the same concentration patterns as in rats (Bohmer *et al*., 1978).

This high concentration seems to be, at least in rats, under androgen control, since treating young castrated rats with testosterone resulted in an increase in epididymal carnitine concentrations, and cryptorchidism in adult animals resulted in lower concentrations of carnitine (Brooks *et al*., 1974). Nevertheless, no correlation was found between the tissue concentration of testosterone in the testis and the concentrations of carnitine in the human epididymis. This discrepancy is probably due to the fact that concentrations of carnitine accumulate in the organ during a long period, whereas concentrations of testosterone change rapidly with gonadotrophin concentrations (Bohmer *et al*., 1978).

Carnitine transport

Free LC is taken up from blood plasma into the epididymal lumen. 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 (Yeung *et al*., 1980). Recently, a high affinity Na^+-driven, organic cation transporter, OCTN2, was shown to transport LC into the cells of the epididymal epithelium (Rodriguez *et al*., 2002).

Another carnitine transporter, termed carnitine transporter 2 (CT2), has been identified and characterized. Compared with OCTN2, which has broad substrate specificity, CT2 is unique because it is expressed exclusively in the human testis and displays substrate selectivity. The localization of CT2 was determined by immunohistochemistry; it was found in the luminal membrane of the human epididymis, which supports the hypothesis that CT2 facilitates the secretion of LC from the epididymal epithelium into the lumen (Enomoto *et al*., 2002).

Functions of carnitine

Carnitine primarily targets the matrix space within the mitochondria, which houses a system of enzymes responsible for fatty acid oxidation. LC essentially plays a key role in the mitochondrial β-oxidation of long chain free fatty acids (Jeulin and Lewin, 1996). By providing a shuttle system for free fatty acids and derivatives of acyl-CoA within the mitochondria, LC regulates the flux of acyl groups, and therefore energy balance, through the cellular membranes. 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 1**). The end result of these reactions is a modulation of mitochondrial concentrations of CoA implicated in various metabolic ways, such as the tricarboxylic acid cycle (Krebs cycle), the β-oxidation of organic acids and the oxidative degradation of amino acids (Bahl and Bresler, 1987).

Spermatozoal post-gonadal maturation occurs principally in the caput epididymis, where spermatozoa are bathed in plasma containing factors of testicular and epididymal origin. Spermatozoa first come in contact with significant amount of carnitine in the epididymal lumen at the location in which they develop the capacity for progressive motility. Thus, a relationship could be established between the potential initiation of progressive sperm motility (an end stage of sperm maturation) and the large increase in concentration of both free LC and ALC in the spermatozoa (Jeulin *et al*., 1987).

An ample concentration of carnitine has been detected in the rat testicle (half of the epididymal concentration), which suggests that it plays a role at the testicular level. In support, high concentrations of ALC transferase have been detected in primary spermatocytes and developing testicular tissue (Schanbacher *et al*., 1974). Carnitine may affect testicular sperm maturation indirectly via the stimulation of Sertoli cell glucose uptake. In general, Sertoli cells represent a very important site for the control of the spermatogenic process. The addition of LC to Sertoli cell cultures results in a considerable increase in pyruvate and lactate secretion, which are known to represent essential energy substrates for germ cell maturation (Palmero *et al*., 2000).

It has been postulated that the high carnitine concentration present in the epididymal fluid serves to maintain the spermatozoa in a quiescent stage (Rufo *et al*., 1984; Deana *et al*., 1989). The observation that high concentrations of carnitine inhibit the cellular efflux of enzymes and oxygen consumption and increase cellular viability strongly suggests that carnitine has a stabilizing effect on plasma membranes (Jenkins and Griffith, 1986). This hypothesis is supported by the finding that carnitine decreases the occurrence of
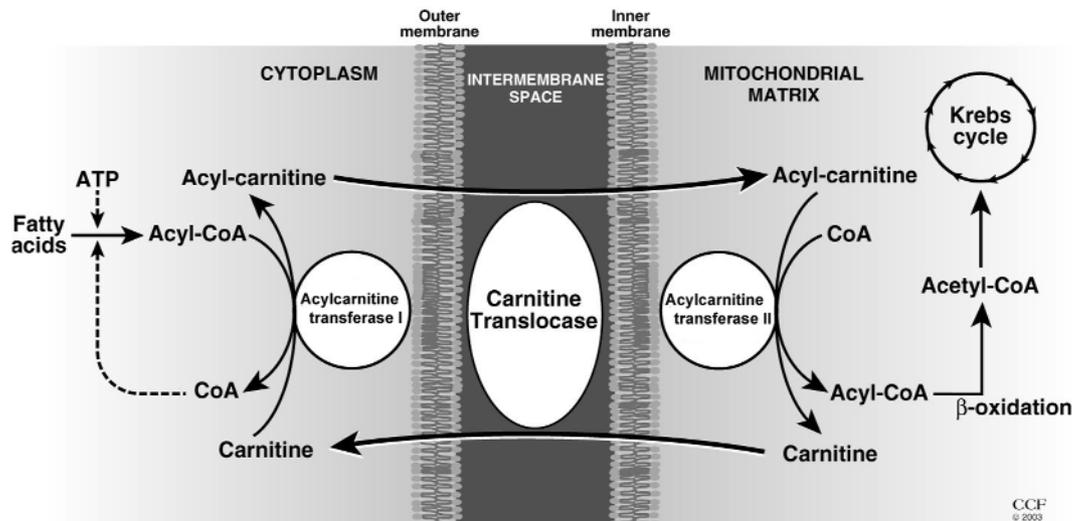
Figure 1. Transport of acyl groups and the role of carnitine in sperm mitochondrial metabolism.

acrosomal reaction in human spermatozoa (Deana *et al.*, 1988).

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 (Aitken *et al.*, 2003). In general, the antioxidant biochemical network may be depicted as a system operating on two different levels: a primary defence barrier that prevents oxidative injury by scavenging the initiating species, and a secondary defence mechanism that eventually repairs the damage that occurs after the oxidative attack (Arduini, 1992). As a result of oxidative stress, the fusogenicity of the sperm plasma membrane is lost due to peroxidative damage to unsaturated fatty acids (Aitken *et al.*, 2003). Carnitine may be responsible for removing excess intracellular toxic acetyl-CoA, which protects spermatozoa from oxidative damage (primary antioxidant defence) (Arduini, 1992; Vicari and Calogero, 2001).

On the other hand, ALC inhibits arachidonic acid incorporation into phospholipids. Arachidonic acid *per se* plays an important role in the formation of oxygen free radicals. In addition, it represents an important pool for accumulated lysophospholipids following radical attack to the cell membrane phospholipids. This role is critical as a repair mechanism following insult from ROS (secondary antioxidant defence) (Pignatelli *et al.*, 2003). Although the protective properties of carnitine against ROS have been documented (Schinetti *et al.*, 1989; Ochendorf, 1999), its actual effect on sperm quality remains controversial (Alvarez, 2003).

Carnitine as a diagnostic tool

Epididymal/testicular marker

Much research is being focused on objective biochemical markers for sperm maturity and function (Cayli *et al.*, 2003). The inclusion of indices of epididymal function in semen analysis has been recommended, since the epididymis is intimately involved in preparing spermatozoa for fertilization

(Wetterauer, 1986). Most important are reports that spermatozoa have the highest concentrations of the enzyme carnitine acetyltransferase and that carnitine itself is accumulated by spermatozoa during maturation in the epididymis, in addition to the fact that the epididymis has the highest content of carnitine (Marquis and Fritz, 1965; Frenkel *et al.*, 1974).

As most carnitine in human epididymal tissue is found in the caput, it reflects the regional secretory function of the epididymis (Bohmer *et al.*, 1978). Thus, it has been postulated that the ejaculate content of an epididymal marker such as LC would be significantly reduced in men with inflammation of the epididymis (Cooper *et al.*, 1990). In patients with epididymitis the carnitine concentration was half that found in males with normal epididymal function (Lewin *et al.*, 1976). Moreover, epididymal thickening, which may be *de novo* or a sequel of inflammation, was intimately associated with significant reduction of seminal fluid carnitine (Cooper *et al.*, 1988).

A potential important biomarker of testicular function is the active carnitine acetyltransferase, which is contained within the spermatozoa. The activity of this enzyme is 7-fold higher in the diplotene primary spermatocytes than in the spermatogonia, indicating that carnitine acetyltransferase may be useful as a marker enzyme of germ cell differentiation in the testis (Vernon *et al.*, 1971).

Carnitine and male infertility

The sperm quality and function improves with the intake of complementary LC and ALC (for review, see Comhaire and Mahmoud, 2003). In infertile human males, seminal plasma LC and ALC concentrations range anywhere from 200 to 1300 nmol/ml and from 60 to 280 nmol/ml respectively. A positive correlation has been reported between free LC and sperm count ($r = 0.617$; $P < 0.01$), sperm motility ($r = 0.614$; $P < 0.01$), and the number of motile spermatozoa/ml ($r = 0.646$; $P < 0.01$) (Menchini-Fabris *et al.*, 1984). Similar findings were

reported in a study consisting of 101 infertile men (Matalliotakis *et al*., 2000), in which, a strong positive relationship between semen LC content and sperm density ($r = 0.711$; $P < 0.0001$), sperm motility ($r = 0.579$; $P < 0.0001$), and sperm morphology ($r = 0.586$; $P < 0.001$) was detected. However, it is important to note that these studies lacked a double blind, controlled design. In addition, the patient inclusion criteria were not strictly defined, resulting in a mixing of various male factor aetiologies.

An association between the concentration of ALC and male fertility may be suggested, since ALC in infertile men with oligozoospermia demonstrated to be significantly lower than that of fertile controls (Kohengkul *et al*., 1977). A study in which infertile normozoospermic men were compared with a fertile control group found reduced concentrations of free carnitine (295 versus 521 μmol/l, $P < 0.001$) and total carnitine (513 versus 743, $P < 0.001$) in the infertile men. It may also be suggested that ALC have a potential role in diagnosing cases of unexplained infertility in men with normal semen parameters (Zopfgen *et al*., 2000).

Azoospermia

Another potential use for seminal LC would be in the diagnosis of the aetiology of azoospermia. The exact diagnosis may be based on the fact that carnitine in seminal plasma originates mainly from the epididymis and seminal vesicles. Men with obstructive azoospermia whose level of obstruction is high (post-epididymal), such as those with agenesis of the vas deferens, have extremely low concentrations of carnitine (Menchini-Fabris *et al*., 1984). On the other hand, men with obstruction below the level of the epididymis (intra-testicular or epididymo-testicular) have normal concentrations of carnitine in the seminal fluid (Saeed *et al*., 1994).

Asthenozoospermia

Findings suggest a relationship between carnitines and sperm motility. Spermatozoa are immotile when removed by micropuncture from a region in the caput epididymis in which they have not yet been exposed to appreciable concentrations of carnitine (Hinton *et al*., 1979). Furthermore, adding carnitine and ALC to human spermatozoa *in vitro* increases their motility (Tanphaichitr, 1977).

Although isolated values of LC and ALC may be higher in samples characterized by asthenozoospermia (Bartellini *et al*., 1987), the ALC/LC ratio and the per cent acetylation are always markedly reduced (Kohengkul *et al*., 1977; Golan *et al*., 1984; Bartellini *et al*., 1987). The significant difference in the ALC/LC ratio and the per cent acetylation found in samples with low degrees of motility could be explained by an impairment of the enzymatic system (carnitine transferase) that controls the reaction carnitine acetylcarnitine leading to defective spermatozoal motility.

Therapeutic effects of carnitine

The therapeutic roles of carnitines are based on multiple observations reported in the literature (Casillas, 1973; Lewin *et al*., 1976; Johansen and Bohmer, 1979; Golan *et al*., 1984; Bartellini *et al*., 1987; Bieber, 1988; Jeulin and Lewin, 1996;

Moore, 1998). Men with oligoasthenozoospermia have lower concentrations of LC (Lewin *et al*., 1976; Menchini-Fabris *et al*., 1984) and ALC (Kohengkul *et al*., 1977) than healthy and fertile subjects. A significant positive correlation was detected between LC concentrations and the number and motility of spermatozoa (Menchini-Fabris *et al*., 1984). When these observations are considered along with 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.

Non-human studies

In an attempt to characterize the protective action of ALC using an in-vivo test system, the recovery and maturation process of mouse spermatogenesis was investigated. Mice were exposed to irradiation to deplete the spermatogonia and then were given ALC at a rate of 100 mg/kg on alternate days for 4 weeks. The sperm population in the mice that received ALC demonstrated a quicker recovery throughout the maturation process than the spermatozoa in those that did not receive ALC (Amendola *et al*., 1989). Therefore, it appears that ALC could influence the early stages of spermatogenesis with consequent favourable effects on DNA repair and on proliferation of regenerating germ cells (Amendola *et al*., 1989). Similarly, shortening of the spermatogenesis recovery time following hyperthermic injury was reported (Amendola *et al*., 1991), which may be of clinical importance in humans as hyperthermia affects the reproductive capacity in cases of varicocoele, one of the most common aetiologies of male infertility (Comhaire *et al*., 1976).

Carnitines may also help protect from the hazardous effects of electric and magnetic fields (EMF), to which humans are frequently exposed. In one study, mice pretreated with LC before being subjected repeatedly to EMF had their sperm count and motility restored at a faster pace following the exposure compared with non-treated controls (Ramadan *et al*., 2002).

Administration of pivalic acid via drinking water decreases serum carnitine in rats by increasing urinary excretion of pivaloylcarnitine. Therefore, the acid was used in one study to decrease epididymal carnitine in rats and hamsters of proven fertility. Although the addition of pivalate (20 mmol/l) over a 5-week period lowered the carnitine content in rat epididymal fluid by 50–75%, neither the fertility nor the motility of spermatozoa was affected. Thus, carnitine depletion has not proved to be a successful mode of male contraception (Cooper and Yeung, 1999).

In-vitro studies

Enhancement of motility

Spermatozoa are able to retain 50% of their motility for up to 8 days if co-incubated with epididymal cell cultures (Moore, 1998). However, the ability of carnitines to induce a motility enhancer effect *in vitro* remains controversial. Exogenous acetylcarnitine and carnitine may be able to increase motility in human ejaculated semen, but carnitine itself fails to stimulate motility because its promoter effect is achieved only via its conversion to acetylcarnitine. This deduction is derived

from the observation that when acetate is added before or simultaneously with carnitine, the stimulatory effect is initiated. The exact mechanisms of this stimulation remain to be solved.

The observation that acetylcarnitine fails to stimulate sperm motility in washed spermatozoa and succeeds with raw semen samples suggests that it needs to be further metabolized and transported by or with factor (s) in the seminal plasma. In contrast with other compounds such as kallikrein, which act only on samples with low motility, carnitines potentiate the motility regardless of the sample's initial motility pattern (Tanphaichitr, 1977).

In-vitro fertilization

Techniques such as IVF and intracytoplasmic injection (ICSI) increase the chances of infertile couples to conceive. Many attempts have been made to investigate the relationship between seminal plasma constituents including carnitines and the fertilization potential of semen specimens in an IVF programme. In a recent study consisting of 24 males in an IVF programme, no significant differences were found in the concentrations of carnitine between those who achieved or did not achieve pregnancy (Lay *et al*., 2001). These findings are supported by another study, which was conducted using a larger group of patients ($n = 178$) and revealed no predictive value for seminal carnitine with regard to the fertilizing potential of the spermatozoa during IVF (Mieusset *et al*., 1989).

Cryopreservation

Cryo-induced damage is mainly manifested after thawing as loss of motility. The exact causes for this loss are unknown, but are probably multifactorial. It has been postulated that the decrease in sperm motility after cryopreservation may be associated with a disturbance in carnitine concentration in semen. Following cryo-thawing, acetylcarnitine content in spermatozoa was significantly reduced whereas no similar changes were found in the carnitine content in spermatozoa or ALC/LC ratio in seminal plasma (Grizard *et al*., 1992).

Many experiments have revealed that co-incubation of spermatozoa with epididymal cells promotes sperm motility and maintains its viability (Akhondi *et al*., 1997; Moore, 1998); this has rationalized the use of carnitine-rich epididymal cells as a cryoprotectant. The addition of epididymal cell medium improved post-thaw motility of spermatozoa when used during cryopreservation (Reyes-Moreno *et al*., 2000). However, there is a contradictory report that showed semen treatment with ALC not improving sperm motility or membrane damage after cryopreservation–thawing (Duru *et al*., 2000).

In-vivo studies

Many human clinical trials have found that LC and ALC therapy can optimize sperm motion parameters in men with astheno- or oligoasthenozoospermia (Costa *et al*., 1994; Vitali *et al*., 1995; Vicari and Calogero, 2001; Vicari *et al*., 2002; Lenzi *et al*., 2003). On the other hand, other studies failed to detect significant increases in sperm concentration following carnitine treatment (Moncada *et al*., 1992; Loumbakis *et al*., 1996). The relatively small doses and short duration of treatment employed may be the main reason why no substantial increases were detected.

The antioxidant properties of carnitines may be used as an effective tool against elevated concentrations of ROS in patients with recurrent or chronic abacterial genital tract/accessory gland inflammation. This therapeutic effect is best described in the work conducted by Vicari *et al*. (Vicari and Calogero, 2001; Vicari *et al*., 2002). Decrease in ROS concentrations coupled with an increase in sperm motility was observed in infertile male patients with abacterial prostatovesiculoepididymitis who received LC and ALC for durations of 3–4 months (Vicari and Calogero, 2001; Vicari *et al*., 2002). It was also noticed that the therapeutic effect of carnitine might be maximized in cases with leukocytospermia, if preceded by a 2-month course of non-steroidal anti-inflammatory drugs. Although significant, both studies over-looked the importance of a placebo-control group.

Most of the studies that have evaluated the therapeutic efficacy of carnitines lack a placebo-controlled, double-blind design (Moncada *et al*., 1992; Costa *et al*., 1994; Vitali *et al*., 1995; Loumbakis *et al*., 1996; Vicari and Calogero, 2001; Vicari *et al*., 2002). In the following section, some of the most relevant clinical studies on carnitine are highlighted. A summary of the main patient characteristics and efficacy results obtained from the recent trials is presented in **Table 1**.

ʟ-Carnitine (LC) human clinical trials

Vitali *et al*. evaluated the efficacy of oral LC 3 g/day for 3 months in 47 patients in an open prospective clinical trial (Vitali *et al*., 1995). Patients included in this study were young, and infertile with idiopathic asthenozoospermia of at least 2 years duration as the sole cause of infertility. At the end of the treatment period, 80% of patients had improved sperm motility levels nearly equal to that observed among a control group of 110 fertile donors, exceeding the WHO lower limit standard. The mean sperm counts increased 44.6% (from $88.0 \pm 8.9 \times 10^6$/ml to $159.0 \pm 5.8 \times 10^6$/ml), and the mean per cent motile spermatozoa increased 99.6% (from 26.8 to 53.5%). In addition, the mean percentage of spermatozoa with rapid linear motility increased 54.5% (from 20.7 ± 8.7 to $32.0 \pm 5.6\%$) after treatment. It is, however, important to note that although this study was characterized by precise patient selection, it lacked a placebo-control group.

Another multi-centre non-randomized, non-controlled clinical trial, which consisted of men with idiopathic astheno-zoospermia, evaluated the effect of long-term therapy of 3 g/day LC for 4 months on sperm motility (Costa *et al*., 1994). Basal semen parameters were determined from two pre-study semen specimens tested for homogeneity. If the samples were homogeneous, the two pretreatment values were averaged to determine the basal value (TO). When homogeneity was not found, the larger of the two values was used as the TO value. Semen analyses and computerized motility assessments were performed 2 months prior to treatment, at the start of treatment (TO), after 2 (T2) and 4 (T4) months of treatment and 2 (T6) months after the end of treatment. The total number of ejaculated sperm significantly increased at the 4th month ($P <$

Table 1. Summary of selected human clinical trials using carnitines. 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 findings 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 months 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, 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 b.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.

0.001). This was attributed to a parallel increase in both sperm concentration and seminal fluid volume. The mean velocity was significantly improved ($P < 0.001$) at all intervals (T2, T4 and T6), but the increases in linearity index and maximum amplitude were significant only at T4 and T6.

A recent study by Lenzi (Lenzi *et al*., 2003) consisted of 86 infertile patients in a placebo-controlled, double-blind design to test the efficacy of carnitine. Patients were subjected to a therapy of 2 g/day oral LC or an equal volume of seemingly identical placebo. The study design was 2 months of washout to minimize the effects of spontaneous variations in seminal characteristics, 2 months of therapy/placebo, 2 more months of washout to avoid incorrect attribution of the drug effects, 2 more months of placebo/therapy, and 2 months of follow-up.

Both groups (patients and controls) showed no differences in the pretreatment sperm parameters and no significant variation was seen after treatment in semen volume, sperm velocity analysed by CASA, seminal α-glucosidase, sperm lipid peroxidation potential or sperm morphology. The first analysis of differences between patients and controls in the percentage of total and forward sperm motility was not significant. However, after excluding five men who had the lowest motility values (10%) and showed spontaneous decrease in sperm motility during the first pretreatment washout period, the differences in total and forward motility were significant ($P = 0.04$ and $P = 0.05$ respectively). Sperm concentration ($P = 0.01$) and sperm linearity evaluated by CASA ($P = 0.03$) significantly increased during LC therapy after exclusion of these same five patients. The increase in the number of forward motile spermatozoa was more significant in those patients with an initial value of $<5 \times 10^6$ forward motile spermatozoa per ejaculate (55 patients; $P = 0.03$) and in those with $<2 \times 10^6$ forward motile spermatozoa/ml (71 patients; $P = 0.02$). It is of interest to note that in this study, which offers the best reliable design, there are no reports of the occurrence of spontaneous pregnancy as a result of the treatment (Comhaire and Mahmoud, 2003).

Acetyl-L-carnitine human clinical trials

The clinical efficacy of ALC on semen quality was evaluated in patients with idiopathic oligoasthenozoospermia in an open, non-placebo controlled trial (Moncada *et al*., 1992). Twenty patients who were diagnosed with idiopathic oligo-asthenozoospermia according to WHO criteria were enrolled in this clinical trial and treated with ALC 4 g/day for 60 days. Following treatment for 2 months, there was no apparent effect on sperm density, total motility or morphology. However, a 76% increase in the mean percentage of sperm with rapid progressive motility was observed ($21.7 \pm 3.24\%$ at baseline versus $38.2 \pm 4.71\%$ at 2 months). After withdrawal of ALC, semen parameters returned to baseline concentrations. This transient improvement is especially interesting as it occurred after only 2 months of treatment with ALC, even though constant exposure to carnitines is considered necessary for at least one full spermatogenic cycle (approximately 74 days) to show improvements in sperm quality.

Conclusion

Research has demonstrated the importance of LC and ALC to sperm metabolism and the benefits to sperm development and maturation. ALC and LC are highly concentrated in the epididymis. L-Carnitine optimizes sperm cell energy production by transporting long chain fatty acids into the mitochondria for utilization in metabolism through β-oxidation and consequent buffering of the acyl-CoA:CoA ratio. L-Carnitine also scavenges excess and potentially toxic free acyl groups and transports them out of the mitochondria. Acetyl-L-carnitine helps maintain cell membrane stability through its involvement in acetylation of membrane phospholipids. The role of ALC is not only confined to the epididymis, but is critical at the testicular level in spermatogenesis and is capable of shortening stem cell recovery following injury.

A positive correlation has been reported between total seminal carnitine concentration and the intra-spermatozoal ALC to LC ratios and sperm motility. Human clinical trials have demonstrated that LC and ALC supplementation may optimize sperm motion parameters among men with astheno- or oligoasthenozoospermia. However, most of these studies suffer from a lack of placebo-controlled, double-blind design, making it difficult to rely on their conclusions. In addition, regardless of the prospective nature of clinical trials of LC and/or ALC and the inclusion of placebo controls and double-blind design, there should be evidence of a real world clinical benefit. Even statistically significant improvements in semen characteristics do not always translate into clinical benefit; this can only be done by establishing a relevant clinical end point, ideally in-vivo fecundity (although IVF fertilization rate, embryo development post day 3, or reduction in early pregnancy loss can also be considered if appropriate), which must show statistically significant improvement after controlling for pertinent clinical factors such as female partners age. In conclusion, additional, well-designed studies are necessary to further validate the use of carnitines in the treatment of patients with male infertility, specifically those men with poor semen quality.

Acknowledgement

References

Aitken R, Baker M, Sawyer D 2003 Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. *Reproductive BioMedicine Online* **7**, 65–70.

Akhondi M, Chapple C, Moore H 1997 Prolonged survival of human spermatozoa when co-incubated with epididymal cell cultures. *Human Reproduction* **12**, 514–522.

Alvarez JG 2003 Nurture vs nature: how can we optimize sperm quality? *Journal of Andrology* **24**, 640–648.

Amendola R, Bartoleschi C, Cordelli E *et al*. 1989 Effects of L-acetylcarnitine 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 *et al*. 1991 Effects of L-acetylcarnitine (LAC) on post injury recovery of mouse spermatogenesis monitored by flow cytometry. 2. Recovery after hyperthermic treatment. *Andrologia* **23**, 135–140.

Arduini A 1992 Carnitine and its acyl esters as secondary antioxidants? *American Heart Journal* **123**, 1726–1727.

Bahl J, Bresler R 1987 The pharmacology of carnitine. *Annual Review of Pharmacology and Toxicology* **27**, 257–277.

Bartellini M, Canale D, Izzo P *et al*. 1987 L-Carnitine and acetylcarnitine in human sperm with normal and reduced motility. *Acta Europaea Fertilitatis* **18**, 29–31.

Bieber L 1988 Carnitine. *Annual Review of Biochemistry* **57**, 261–283.

Bohmer T, Hoel P, Purvis K *et al*. 1978 Carnitine levels in human accessory sex organs. *Archives of Andrology* **1**, 53–59.

Brooks D, Hamilton D, Mallek A 1974 Carnitine and glycerylphosphorylcholine in the reproductive tract of the male rat. *Journal of Reproduction and Fertility* **36**, 141–160.

Casillas E 1973 Accumulation of carnitine by bovine spermatozoa during maturation in epididymis. *Journal of Biological Chemistry* **248**, 8227–8232.

Cayli S, Jakab A, Ovari L *et al*. 2003 Biochemical markers of sperm function: male fertility and sperm selection for ICSI. *Reproductive BioMedicine Online* **7**, 462–468.

Comhaire F, Mahmoud A 2003 The role of food supplements in the treatment of the infertile man. *Reproductive BioMedicine Online* **7**, 385–391.

Comhaire F, Monteyne R, Kunnen M 1976 The value of scrotal thermography as compared with selective retrograde venography on the internal spermatic vein for the diagnosis of subclinical varicocele. *Fertility and Sterility* **27**, 694–698.

Cooper T, Yeung C 1999 Approaches to post-testicular contraception. *Asian Journal of Andrology* **1**, 29–36.

Cooper T, Yeung C, Nashan D *et al*. 1988 Epididymal markers in human infertility. *Journal of Andrology* **9**, 91–101.

Cooper T, 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. *International Journal of Andrology* **13**, 329–336.

Costa M, Canale D, Filicori M *et al*. 1994 L-Carnitine in idiopathic asthenozoospermia: a multicenter study. *Andrologia* **26**, 155–159.

Deana R, Indino M, Rigoni F *et al*. 1988 Effect of L-carnitine on motility and acrosome reaction of human spermatozoa. *Archives of Andrology* **21**, 147–153.

Deana R, Rigoni F, Francesconi M *et al*. 1989 Effect of L-carnitine and L-aminocarnitine on calcium transport, motility and enzyme release from ejaculated bovine spermatozoa. *Biology of Reproduction* **41**, 949–955.

Duru N, Morshedi M, Schuffner A *et al*. 2000 Semen treatment with progesterone and/or acetyl-L-carnitine does not improve sperm motility or membrane damage after cryopreservation-thawing. *Fertility and Sterility* **74**, 715–720.

Engle A, Rebouche C 1984 Carnitine metabolism and inborn errors. *Journal of Inherited Metabolic Disease* **7**, 38–43.

Enomoto A, Wempe M, Tsuchida H *et al*. 2002 Molecular identification of a novel carnitine transporter specific to human testis. Insights into the mechanism of carnitine recognition. *Journal of Biological Chemistry* **277**, 36262–36271.

Frenkel G, Peterson R, Davis J *et al*. 1974 Glycerylphosphorycholine and carnitine in normal human semen and in postvasectomy semen: differences in concentrations. *Fertility and Sterility* **25**, 84–87.

Golan R, Weissenberg R, Lewin L 1984 Carnitine and acetylcarnitine in motile and immotile spermatozoa. *International Journal of Andrology* **7**, 484–494.

Grizard G, Vignon N, Boucher D 1992 Changes in carnitine and acetylcarnitine in human semen during cryopreservation. *Human Reproduction* **7**, 1245–1248.

Hinton B, Snoswell A, Stechell B 1979 The concentration of carnitine in the luminal fluid of the testis and epididymis of the rat and some other mammals. *Journal of Reproduction and Fertility* **56**, 105–111.

Jenkins D, Griffith O 1986 Antiketogenic and hypoglycemic effects of aminocarnitine and acylaminocarnitine. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 290–294.

Jeulin C, Lewin L 1996 Role of free L-carnitine and acetyl-L-carnitine in post-gonadal maturation of mammalian spermatozoa. *Human Reproduction Update* **2**, 87–102.

Jeulin C, Soufir J, Marson J *et al*. 1987 The distribution of carnitine and acetylcarnitine in the epididymis and epididymal spermatozoa of the boar. *Journal of Reproduction and Fertility* **79**, 523–529.

Johansen L, Bohmer T 1979 Motility related to the presence of carnitine/acetyl-carnitine in human spermatozoa. *International Journal of Andrology* **1979**, 202–210.

Kohengkul S, Tanphaichitr V, Muangmun V *et al*. 1977 Levels of L-carnitine and L-acetylcarnitine in normal and infertile human semen: a lower level of L-acetylcarnitine in infertile semen. *Fertility and Sterility* **28**, 1333–1336.

Lay M, Richardson E, Boone W *et al*. 2001 Seminal plasma and IVF potential. *Journal of Assisted Reproduction and Genetics* **18**, 144–150.

Lenzi A, Lombardo F, Sgro P *et al*. 2003 Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial. *Fertility and Sterility* **2**, 292–300.

Lewin M, Beer R, Lunenfeld B 1976 Epididymis and seminal vesicle as sources of carnitine in human seminal fluid: the clinical significance of the carnitine concentration in human seminal fluid. *Fertility and Sterility* **27**, 9–13.

Loumbakis P, Anezinis P, Avengeliou A *et al*. 1996 Effect of L-carnitine in patients with asthenozoospermia. *European Urology* **30**, 255.

Marquis N, Fritz I 1965 Effects of testosterone on the distribution of carnitine, acetylcarnitine, and carnitine acetyltransferase in tissues of the reproductive system of the male rat. *Journal of Biological Chemistry* **240**, 2197–3001.

Matalliotakis I, Youmantaki Y, Evageliou A *et al*. 2000 L-Carnitine levels in the seminal plasma of fertile and infertile men: correlation with sperm quality. *International Journal of Fertility* **45**, 236–240.

Menchini-Fabris F, Canale D, Izzo P *et al*. 1984 Free L-carnitine in human semen: its variability in different andrologic pathologies. *Fertility and Sterility* **42**, 263–267.

Mieusset R, Parinaud J, Chap H *et al*. 1989 Absence of correlation between the levels of ATP and other seminal compounds in semen and the results of human in-vitro fertilization. *International Journal of Androlology* **12**, 346–353.

Moncada M, Vicari E, Cimino C *et al*. 1992 Effect of acetylcarnitine in oligoasthenospermic patients. *Acta Europaea Fertilitatis* **23**, 221–224.

Moore H 1998 Contribution of epididymal factors to sperm maturation and storage. *Andrologia* **30**, 233–239.

Ochendorf F 1999 Infections in the male genital tract and reactive oxygen species. *Human Reproduction Update* **5**, 399–420.

Palmero S, Bottazzi C, Costa M *et al*. 2000 Metabolic effects of L-carnitine on prepubertal rat Sertoli cells. *Hormone and Metabolic Research* **32**, 87–90.

Pignatelli P, Lenti L, Sanguigni V *et al*. 2003 Carnitine inhibits arachidonic acid turnover, platelet function, and oxidative stress. *American Journal of Physiology*. *Heart and Circulatory Physiology* **284**, H41–H48.

Ramadan L, Abd-Allah A, Aly H *et al*. 2002 Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice. *Pharmaceutical Research* **46**, 363–370.

Rebouche C 1988 Carnitine metabolism and human nutrition. *Journal of Applied Nutrition* **40**, 99–111.

Reyes-Moreno C, Gagnon A, Sullivan R *et al*. 2000 Addition of specific metabolites to bovine epididymal cell culture medium enhances survival and motility of cryopreserved sperm. *Journal of Andrology* **21**, 876–886.

Rodriguez C, Labus J, Hinton B 2002 Organic cation/carnitine transporter, OCTN2, is differentially expressed in the adult rat epididymis. *Biology of Reproduction* **67**, 314–319.

Rufo GJ, Schoff P, Lardy H 1984 Regulation of calcium content in bovine spermatozoa. *Journal of Biological Chemistry* **259**,

2547–2552.

Saeed S, Khan F, Khan D *et al*. 1994 Demonstration of the site of obstruction in azoospermia by biochemical markers. *Journal of the Pakistan Medical Association* **44**, 140–142.

Schanbacher B, Gomes W, Vandermark N 1974 Testicular carnitine acetyltransferase activity and serum testosterone levels in developmental stages of the rat and ram. *Journal of Animal Science* **39**, 889–892.

Schinetti M, Rossini D, Greco R *et al*. 1989 Protective action of acetylcarnitine on NADPH-induced lipid peroxidation of cardiac microsomes. *Drugs Under Experimental and Clinical Research* **13**, 509–515.

Tanphaichitr N 1977 In vitro stimulation of human sperm motility by acetylcarnitine. *International Journal of Fertility* **22**, 85–91.

Vernon R, Go V, Fritz I 1971 Studies on spermatogenesis in rats II. Evidence that carnitine acetyltransferase is a marker enzyme for the investigation of germ cell differentiation. *Canadian Journal of Biochemistry* **49**, 761–767.

Vicari E, Calogero A 2001 Effects of treatment with carnitines in infertile patients with prostato-vesiculo-epididymitis. *Human Reproduction* **16**, 2338–2342.

Vicari E, LaVignera S, Calogero A 2002 Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertility and Sterility* **6**, 1203–1208.

Vitali G, Parente R, Melotti C 1995 Carnitine supplementation in human idiopathic asthenospermia: clinical results. *Drugs Under Experimental and Clinical Research* **21**, 157–159.

Wetterauer U 1986 Recommended biochemical parameters for routine semen analysis. *Urological Research* **14**, 241–246.

Yeung C, Cooper T, Waites G 1980 Carnitine transport into the perfused epididymis of the rat: regional differences, stereospecificity, stimulation by choline, and the effect of other luminal factors. *Biology of Reproduction* **23**, 294–304.

Zopfgen A, Priem F, Sudhoff F *et al*. 2000 Relationship between semen quality and the seminal plasma components carnitine, alpha-glucosidase, fructose, citrate and garnulocyte elastase in infertile men compared with a normal population. *Human Reproduction* **15**, 840–845.