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 \( \beta \)-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 \textit{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.

**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).

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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 \textit{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 epididymal plasma and ultimately into spermatozoa, where it 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 acyl 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

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). Carnitine may affect testicular 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).

### 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).

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).

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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 \((295 \text{ versus } 521 \mu\text{mol/l}, P < 0.001)\) and total carnitine \((513 \text{ versus } 743, P < 0.001)\) 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 obstruction of the vas deferens, have extremely low concentrations of carnitine (Menchini-Fabris et al., 1984). Furthermore, 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 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 varicocele, 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 \((20 \text{ mmol/l})\) 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 acetyl carnitine 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 intracytoplasmatic 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 (Mieuisset 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 (Akhoundi 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; Lenni 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 prostatovesiculooepididymitis 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-inflammatorv drugs. Although significant, both studies overlooked 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.

**L-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 ± 9.9 x 10⁶/ml to 159.0 ± 5.8 x 10⁶/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 ± 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 asthenozoospermia, 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.

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<tr>
<th>Author and study design</th>
<th>Study population</th>
<th>Treatment</th>
<th>Sperm count and motility results</th>
<th>Other findings and comments</th>
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<td><strong>Lenzi et al. (2003):</strong> randomized placebo-controlled double-blind cross-over trial, 8 months</td>
<td>86 infertile with OAT</td>
<td>2 months placebo/LC (2 g), 2 months placebo/LC (2 g)</td>
<td>Total motile: 9 ± 6.75 versus 7.4 ± 5.58&lt;sup&gt;bc&lt;/sup&gt;, Forward motile: 7.2 ± 6.39 versus 5.8 ± 6.02&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td><strong>Vicari et al. (2002); open prospective study, 6–9 months</strong></td>
<td>98 infertile males with abacterial PVE</td>
<td>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</td>
<td>No difference in four groups compared with pretreatment values</td>
<td>Group C 32 (18, 40) versus 14 (10, 19)&lt;sup&gt;ab&lt;/sup&gt; Morphology: no significant difference. Viability: groups C and D 44 (32, 60) and 38 (28, 50) versus 24 (19, 38) and 24 (18, 39)&lt;sup&gt;ab&lt;/sup&gt;. Leukocyte count: groups C and D 0.7 (0.4, 1.3) and 1 (0.6, 1.1) versus 1.7 (1.1, 2.0) and 1.7 (1.1, 2.1)&lt;sup&gt;ab&lt;/sup&gt;. ROS production: group C 14.7 versus 51.7&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td><strong>Vicari and Calogero (2001): open prospective study, 3 months</strong></td>
<td>54 infertile males with abacterial PVE Group A: (&lt;i&gt;n&lt;/i&gt; = 34) no leukocytospermia, Group B: (&lt;i&gt;n&lt;/i&gt; = 20) leukocytospermia</td>
<td>LC (1 g) + ALC (500 mg)/12 h for 3 months</td>
<td>No difference compared with pretreatment values</td>
<td>Forward motility 28 (22, 35) versus 14 (10, 20)&lt;sup&gt;ab&lt;/sup&gt; Viability: groups A and B 42 (32, 56) and 33 (28, 46) versus 29.5 (25, 32) and 27.5 (25, 40)&lt;sup&gt;ab&lt;/sup&gt; ROS production group A 48.8 (26.2, 66.8) versus 61.1 (30.2, 79.5)&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td><strong>Vitali et al. (1995): open prospective study, 3 months</strong></td>
<td>47 infertile males with idiopathic asthenozoospermia</td>
<td>LC (1 g t.i.d., 3 months)</td>
<td>159 ± 5.8 versus 88.9 ± 8.9&lt;sup&gt;bc&lt;/sup&gt; versus 53.5 ± 7.7 ± 10.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3/47 patients had no change and 7/47 had worsening of semen parameters</td>
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<td><strong>Costa et al. (1994): open prospective study multicentre trial, 6 months</strong></td>
<td>100 infertile males with idiopathic asthenozoospermia</td>
<td>LC (1 g t.i.d., 4 months)</td>
<td>49.4 ± 3.7 to 53.2 ± 3.4&lt;sup&gt;bc&lt;/sup&gt; to 26.9 ± 1.1 to 36.4 ± 0.9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>Abnormal morphology significantly decreased 45.9 ± 0.8 to 42.9 ± 0.8&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td><strong>Moncada et al. (1992): 2 months</strong></td>
<td>20 infertile males with idiopathic asthenozoospermia</td>
<td>ALC (2 g b.i.d., 2 months)</td>
<td>21.7 ± 3.24 to 38.2 ± 4.71&lt;sup&gt;bc&lt;/sup&gt;</td>
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<sup>a</sup>Results expressed as median (10th percentile, 90th percentile).

<sup>b</sup>Compared with pretreatment values in the same group considered significant (<i>P</i> < 0.05).

<sup>c</sup>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 same five patients. The increase in linearity index and maximum amplitude were significant only at T4 and T6.

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.

### 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

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