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L- carnitine in idiopathic asthenozoospermia: a multicenter study

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Summary. The aim of the study described here was to evaluate any possible effect of L-carnitine on spermatozoal motility in a group of patients with unexplained asthenozoospermia in four different infertility centres. One hundred patients received 3 g d^{-1} of oral L-carnitine for 4 months. Sperm parameters were studied before, during and after this treatment. Motility was also studied by means of a computer-assisted sperm analysis.

The results of the study indicate that L-carnitine is able to increase spermatozoal motility, both in a quantitative and in a qualitative manner (per cent motile spermatozoa increased from $26.9 \pm 1.1\%$ to $37.7 \pm 1.1\%$ [$P<0.001$]; per cent spermatozoa with rapid linear progression increased from $10.8 \pm 0.6\%$ to $18.0 + 0.9\%$ [$P<0.001$]; mean velocity increased from 28.4 ± 0.6 μm s^{-1} to 32.5 ± 0.8 μm s^{-1} [$P<0.001$]; linearity index increased from 3.7 ± 0.1 to 4.1 ± 0.1 [$P<0.001$], especially in the subgroup of patients with poor rapid linear progression of spermatozoa (per cent of motile spermatozoa increased from $19.3 \pm 1.9\%$ to $40.9 \pm 1.4\%$ [$P<0.001$], and per cent of spermatozoa with rapid linear progression increased from $3.1 \pm 0.4\%$ to $20.3 \pm 1.6\%$ [$P<0.001$]) An increase in spermatozoal output was also observed (total number of ejaculated spermatozoa increased from 142.4 ± 10.3 10^6 to $163.3 \pm 11.0 \times 10^6$ [$P<0.001$]). The authors conclude that oral administration of L-carnitine may improve sperm quality at least in patients with idiopathic asthenozoospermia.

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Introduction

Carnitine is a zwitterionic compound (betaine of γ-trimethylamino-β-hydroxy buthirryc acid). Its role is to carry long-chain fatty acids from the cytosolic compartment to the mitochondrial matrix, where they undergo β-oxydation. Car-nitine is produced by hepatocytes and secreted into the bloodstream. It is taken up by the epididymal cells and then released in the epididymal lumen by active epithelial pumps (Brooks *et al.*, 1980; Yeung *et al.*, 1980). Then carnitine is partially esterified as acetylcarnitine, and partially taken up by the spermatozoa during their passage through the epididymis and esterified within the cells (Casillas, 1973). Both extracellular and intracellular acetylcarnitine play an important role in sperm energy metabolism providing the primary fuel for sperm motility (Milkowski *et al.*, 1976; Bruns & Casillas, 1989; Bruns & Casillas, 1990).

The increase of L-carnitine and L-acetylcarnitine content in the spermatozoa during the epididymal passage is contemporaneous with acquisition of progressive motility (Jeulin *et al.*, 1988).

Previous studies have shown that seminal-free L-carnitine content correlates with sperm count and motility (Menchini Fabris *et al.*, 1984, Bornman *et al.*, 1989); a reduction of L-acetylcarnitine/L-carnitine ratio has been found in patients with defective sperm motility (Bartelloni *et al.*, 1987); moreover, when lactate and pyruvate were supplied to washed spermatozoa, the ratio increased only in motile spermatozoa but not in samples from asthenozoospermic males, thus indicating differences in substrate utilization in motile and immotile cells (Golan *et al.*, 1986).

Given the putative *in vivo* role played by carnit-

ine in activating sperm motility, the authors evaluated the possibility that orally administered L-carnitine could affect sperm motility and other semen parameters both in a quantitative and in a qualitative manner.

Subjects and methods

Patient population

The multi-centre study described here was conducted according to an open design, in four different infertility centres (Genova, Rome, Pisa and Bologna). The study included 100 patients (20–40 years of age) with infertility of at least 2 years duration, concomitant with unexplained asthenozoospermia, according to the following seminal laboratory parameters: (1) concentration of equal or more than 10^7 sperm ml^{-1}; (2) motility of 20–40% at 2 h; (3) rapid linear progression <20%; (4) abnormal morphology <50%; (5) white blood-cell count (WBC) in seminal plasma of $<10^6$ ml^{-1}; (6) mean velocity ranging between 20 and 40 $\mu m\ s^{-1}$ (CASA, Cellsoft Cryo Research, NY, USA); and (7) linearity index ranging between 2 and 4 (CASA).

None of the patients had antisperm antibodies nor systemic illness, endocrine disease, genital-tract infection (including chlamydia), mono-bilateral testicular hypotrophy, varicocele, confirmed by doppler flowmetry and/or ultrasound scanning, nor had any a history of chryptorchidism, orchitis, or post-pubertal mumps.

Protocol

L-carnitine ($3\ g\ d^{-1}$) was administered orally in three doses after meals. L-carnitine was supplied in 10-ml phials each containing 1 g. Semen analysis and computerized motility assessment were performed 2 months before the beginning of treatment (T − 2), at the start of treatment (T 0), after 2 months (T + 2) and 4 months (T + 4) of treatment and finally 2 months after the end of treatment (T + 6).

Laboratory procedures

The following parameters were evaluated in each semen analysis: (A) spermatozoa concentration; (B) absolute number of ejaculated spermatozoa; (C) per cent of motile spermatozoa at 2 h; (C1) per cent of spermatozoa with rapid linear progression at 2 h; (D) per cent of spermatozoa showing abnormal morphology; (A × C1) concentration of spermatozoa with rapid linear progression at 2 h; and (B × C1) absolute number of ejaculated spermatozoa with rapid linear progression.

Semen examination was performed according to World Health Organization (WHO) criteria (WHO 1987).

CASA analysis was performed to evaluate: (E) mean velocity; (F) linearity index; (G) maximal amplitude of sperm lateral head displacement; (H) mean amplitude of sperm lateral head displacement; and (I) beat-cross frequency.

Sperm from all patients was also tested for the presence of antisperm antibodies (specific anti IgA, IgG, IgM immunobeads).

Patients' data from the four centres were considered together for statistical evaluation. For each variable the homogeneity of the two basal values was tested with a Student's *t*-test for paired data.

When the basal values were homogenous (variables A, B, C, D, E, G, H, I), the average of the two values (T-basal) was used to evaluate response to treatment. When the basal values were not homogenous (variables C1, A × C1, B × C1, F), the highest value was used as basal value (T basal) to evaluate response to treatment, in order to choose the lowest interval between the basal values and the values during or after treatment. Throughout the study (T + 2, T + 4, T + 6) the values of each variable were compared to the T-basal values by the means of the Student's *t*-test for paired data.

Further evaluation was carried out dividing the patients into four groups, according to the per cent of rapid-linear progressing spermatozoa at T-basal value (0–5%, 6–10%, 11–15%, 16–20%). The variables mentioned above were evaluated throughout the study.

Differences were considered statistically significant when $P<0.001$, for a two-tailed *t*-test.

Results

The results of semen analysis are summarized in Table 1. The spermatozoa concentration showed only a slight increase after 4 months of treatment. However, the total number of ejaculated spermatozoa was significantly increased throughout the study, probably in relation to the parallel increase of both spermatozoa production and seminal-fluid volume.

The percentage of motile spermatozoa and the rapid linear progressing fraction increased gradually throughout the study. Consequently, there was an increase in concentration and total number of rapidly progressing spermatozoa in the ejaculate.

The percentage of spermatozoa showing abnor-

Table 1. Semen analysis (WHO, 1987); all patients

	Variable (mean ± SEM)	T0	T+2	T+4	T+6
(A)	Concentration (10^6 ml^{-1})	49.4 ± 3.7	52.0 ± 3.7	53.2* ± 3.4	51.9 ± 3.5
(B)	Number of ejaculated spermatozoa (10^6)	142.4 ± 10.3	156.6* ± 11.6	163.3* ± 11.0	161.1* ± 11.6
(C)	Motile spermatozoa (%)	26.9 ± 1.1	31.7* ± 1.2	36.4* ± 0.9	37.7* ± 1.1
(C1)	Spermatozoa with rapid linear progression (%)	10.8 ± 0.6	13.9* ± 0.7	17.4* ± 0.8	18.0* ± 0.9
(A × C1)	Concentration of spermatozoa with rapid linear progression (10^6 ml^{-1})	5.4 ± 0.5	7.3* ± 0.7	9.4* ± 0.8	9.5* ± 0.8
(B × C1)	Number of spermatozoa with rapid linear progression in the ejaculated (10^6 ml^{-1})	15.8 ± 1.5	22.6* ± 2.2	29.0* ± 2.5	29.8* ± 2.6
(D)	Spermatozoa with abnormal morphology (%)	45.9 ± 0.8	45.3 ± 1.0	43.9 ± 0.9	42.9* ± 0.8

* $P \leq 0.001$ vs. T0.

mal morphology decreased only 2 months after discontinuation of the treatment.

The variables of computerized analysis of sperm motion are summarized in Table 2. The mean velocity was increased significantly throughout the study, while the linearity index showed increased values only after 4 months of treatment and at the follow-up control (T + 6). The beat-cross frequency was unchanged. The maximum amplitude of lateral head displacement was increased at 4 months and at the follow up control, while the average amplitude increased only at follow up.

Evaluation of the data obtained after separation into the four groups, according to the basal percentage of rapid-linear progression showed large differences between the groups. In fact the group with the greatest improvement was the one with 0–5% of rapid-linear progressing spermatozoa. This group had significant increases in both the percentage of rapid-linear motility and in mean velocity throughout the study (Table 3). The amplitude of the improvement was lower, but still significant, in the group with rapid-linear motility 5–10%, but was not significant in the other two groups (11–15 and 16–20%).

Figure 1 shows clearly that the lower the basal rapid-linear motility the wider the improvement during treatment.

Discussion

The reduction sperm motility observed in seminal samples from infertile patients is still attributed to 'unknown causes' in most cases. When a uniform impaired-motion pattern is present in all the spermatozoa a genetic defect has been demonstrated (Eliasson *et al.*, 1977; Feneux *et al.*, 1985; Chemes *et al.*, 1987). Alternatively, a metabolic alteration can be postulated. Since fatty-acid oxidation, involving the carnitine-dependent systems, seems to be the major energy-supplying process (Bruns

Table 2. Computerized analysis of sperm motion (Cellsoft CASA); all patients

	Variable (mean ± SEM)	T0	T2	T4	T6
(G)	Mean velocity (μ s^{-1})	28.4 ± 0.6	30.9* ± 0.7	32.5* ± 0.8	31.9* ± 0.7
(H)	Linearity index	3.7 ± 0.1	3.9 ± 0.1	4.1* ± 0.1	4.1* ± 0.1
(I)	Maximal amplitude (μ)	2.5 ± 0.1	2.6 ± 0.1	2.7* ± 0.1	2.7* ± 0.1
(L)	Mean amplitude (μ)	2.0 ± 0.1	2.1 ± 0.1	2.2 ± 0.1	2.2* ± 0.1
(M)	Beat cross frequency (Hz)	11.3 ± 0.1	11.3 ± 0.1	11.6 ± 0.1	11.3 ± 0.1

* $P \leq 0.001$ vs. T0.

Table 3. Semen analysis: patients with percent rapid linear progression <5% at 2 h (25 patients)

Variable (mean ± SEM)	T0	T2	T4	T6
Motile spermatozoa (%)	19.3 ± 1.9	34.2* ± 2.6	40.9* ± 1.4	32.6* ± 2.4
Spermatozoa with rapid linear progression (%)	3.1 ± 0.4	15.9* ± 1.3	20.3* ± 1.6	20.0* ± 2.2
Velocity (μ s^{-1})	26.9 ± 1.1	30.9* ± 1.7	31.7* ± 1.7	30.5* ± 1.4

* $P \leq 0.001$ vs. T0.

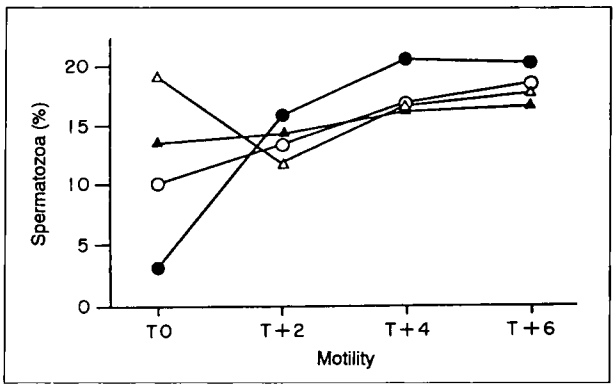

Figure 1. Computerized analysis of sperm motility. Percentage of spermatozoa with rapid linear progression; patients divided in four classes according to T0 motility. ●, 0–5% (25 patients); ○, 6–10% (27 patients); ▲, 11–15% (26 patients); △, 16–20% (22 patients).

& Casillas, 1989), a defective function of one of these systems may lead to a reduction of energy-dependent sperm functions, such as motility. Thus, an alteration of the carnitine/acetylcarnitine ratio has been shown in asthenozoospermic samples (Bartelloni *et al.*, 1987).

The authors[1] data, collected with both the standard semen examination and the computerized study of sperm motion, showed a significant improvement in spermatozoal motility after administration of oral carnitine. Analysis of motility variables showed a progressive increase, with a maximum of 30%, in the percentage of motile forms when compared to the basal value. Considered separately, the percentage of spermatozoa with rapid linear motility, is improved by 80%, with a net doubling of the total number of rapid progressing spermatozoa in the ejaculate. Further data from analysis of the four groups divided according to basal linear progressive motility, show that the greatest increase of this variable was recorded in the group with severe asthenozoospermia, with an increase from a mean value of $3.1 \pm 0.4\%$ to $20.3 \pm 1.6\%$ ($P < 0{,}001$). Thus, reaching values close to normal ones.

Although in these patients the assessment of carnitine and its esters both within sperm cells and in the ejaculate was not carried out, one could speculate that in this subgroup of infertile patients with defective motility there was impairment either in epididymal function (with subsequent reduction of available carnitine, as shown by Ben-Ali *et al.* (1991), or in the ability of sperm to capture and utilize carnitine (Bartelloni *et al.*, 1987). A reduction of seminal carnitine content in patients with impaired motility was shown also by Bornman *et al.* (1989). Thus, the administration of carnitine would provide additive substrate for sperm energy metabolism and motility.

With regard to computerized analysis of sperm motion the authors observed that the variable affected most by the treatment was velocity. Linearity and amplitude of lateral-head displacement increased later, while beat-cross frequency was unchanged. These modifications are of potential clinical relevance, considering that the spermatozoal ability to fertilize *in vitro* is correlated strongly to sperm motion parameters (Jeulin *et al.*, 1986; Liu *et al.*, 1991).

To the authors' knowledge no effect of carnitine has been described at the level of the seminiferous tubule or on accessory sex glands to date. Thus, the authors were surprised to find an increase in the number of ejaculated spermatozoa during treatment, while concentration did not change. Caution must be exercised in assessing the relevance of these data due to the well-known variability in sperm count and to the limited amplitude of the increase. However, the authors' data still reach statistical significance, although clearly, such a small increase in sperm count is not necessarily clinically relevant to the patient's fertility. The authors think that these findings deserve further study to test for any possible effect of carnitine on spermatogenesis.

In conclusion the authors' results show the clear effect of oral administration of L-carnitine on spermatozoal motility in a group of patients with idiopathic asthenozoospermia. They believe that this treatment could be useful, given the limited treatment available for this 'mysterious' but common cause of infertility.

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