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Correlation between Seminal Carnitine and Functional Spermatozoal Characteristics in Men with Semen Dysfunction of Various Origins

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Abstract

Background and objective: L-carnitine is an essential molecule involved in mitochondrial metabolism, controlling the transport of acetyl and acyl groups across the mitochondrial inner membrane. Carnitine and acetylated carnitine (L-acetylcarnitine) are found in high concentrations in the epididymis, where they also act as antioxidants, protecting spermatozoa against damage caused by reactive oxygen species. In this open study we investigated the correlation between seminal carnitine levels and spermatozoal function, and the effect of combined L-carnitine + L-acetylcarnitine therapy, in infertile men.
Patients and methods: 170 infertile men were enrolled in this study. Patients were divided into those with a total sperm motility below the normal WHO range (<50% motility, group 1 [n = 102]) and those with total sperm motility within the normal range (≥50% motility, group 2 [n = 68]). Patients in group 1 were further divided into two groups: those with primary or secondary azoospermia (1B [n = 36]), and those without (1A [n = 66]). Patients in group 1A received L-carnitine 1 g/day and L-acetylcarnitine 500mg twice daily for 6 months. Seminal carnitine levels were measured and correlated with sperm count and motility, eosin test, hypo-osmotic swelling test, acridine orange test for sperm nuclear DNA integrity and sperm kinetics evaluated by computer-assisted sperm analysis in all patients.
Results: There was a significant correlation between seminal carnitine concentration and sperm concentration, total sperm count, sperm total motility, rapid forward progression, live sperm count, membrane function, nuclear DNA inte-

grity, capacity for cervical mucus penetration, linearity of spermatic movement, and amplitude of lateral sperm head movement (all $p < 0.0001$) in the entire study population. In group 1A, there was a significant increase in total motility, live sperm count, membrane integrity and linearity of spermatic movement after 3 and 6 months of L-carnitine/L-acetylcarnitine treatment, and in capacity for cervical mucus penetration after 6 months of treatment, compared with baseline.

Conclusion: Seminal carnitine concentration may be an appropriate marker of sperm and epididymal function. L-carnitine/L-acetylcarnitine treatment may be an effective therapy to improve mainly functional seminal parameters.

L-carnitine (levocarnitine), first isolated from beef muscle in 1905, is an essential molecule involved in mitochondrial metabolism. Free carnitine is obtained from the diet, or produced in the liver and kidney, and only the L-isomer is biologically active in mammals. L-carnitine controls the transport of acetyl and acyl groups across the mitochondrial inner membrane, and its concentration was found to be regulated and stable in humans.[1] The total concentration of carnitine within a cell consists of both free L-carnitine and acylcarnitine esters formed by reaction with fatty acids, catalysed by the enzyme carnitine acyltransferase.[2-5]

Carnitine and acetylated carnitine are found in high concentrations in the epididymis,[6] and epididymal spermatozoa are extraordinarily rich in carnitine acetyltransferase. The main site of carnitine accumulation is the cauda epididymis, but a high concentration can also be detected in luminal fluid collected by micropuncture from the caput. In the epididymis, the epithelium takes up free L-carnitine from the blood plasma and transports it into the epididymal fluid, where it is concentrated 100-fold. L-carnitine is subsequently taken up by the spermatozoa, where it is accumulated as both free and acetylated (L-acetylcarnitine, levacecarnine) forms.[7]

Free L-carnitine plays a pivotal role in mitochondrial β-oxidation of long-chain fatty acids,[8] which must be activated by binding to coenzyme A (CoA) to form acyl-CoA. Acyl-CoA molecules are not able to passively cross the internal mitochondrial membrane and must be transported by a specific enzymatic shuttle system, the constituents of which include free carnitine.[9] Carnitine also acts as an antioxidant, protecting spermatozoa against damage caused by reactive oxygen species (ROS).

With the exception of subjects with sperm total motility below the normal range established by the World Health Organization (WHO), it is now well recognised that there is a high correlation between seminal carnitine levels and several functional spermatozoal parameters,[10] indicating that carnitine is important for sperm fecundity. In oligozoospermia, asthenozoospermia and necrospermia, the L-acetylcarnitine concentration in seminal plasma is below that of normal controls.[7] In particular, the spermatozoal L-carnitine to L-acetylcarnitine ratio is reduced in asthenospermia.[11] It has been demonstrated that azoospermic patients have a low semen concentration of free carnitine,[12,13] and there is a strong correlation between seminal carnitine levels and the anatomical site of occlusion in patients with obstructive azoospermia.[14]

We investigated the association between seminal carnitine levels and sperm function, and the effect of combined L-carnitine and L-acetylcarnitine treatment, in infertile men.

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Table I. Seminal parameters in 170 infertile males with various pathologies divided in groups

Parameter	Total group			Group 1			Group 1A			Group 1B			Group 2		
	mean	±SEM	range	mean	±SEM	range	mean	±SEM	range	mean	±SEM	range	mean	±SEM	range
Sperm concentration ($\times 10^6$/mL)	23.64	1.78	0–70	11.87	1.61	0–60	18.35	2.09	0.5–60	0	0	0	41.29	2.51	1–70
Sperm total count ($\times 10^6$)	73.58	6.24	0–300	34.99	5.35	0–240	54.08	7.26	2–240	0	0	0	131.50	9.86	1.5–300
Sperm total motility (%)	30.06	1.94	0–80	11.67	1.34	0–40	18.03	1.58	0–40	0	0	0	57.60	0.84	50–80
Rapid forward progression (%)	13.76	1.23	0–60	2.55	0.46	0–20	3.93	0.64	0–20	0	0	0	30.59	1.44	0–60
Eosin test (%)	43.39	2.28	0–90	24.51	2.24	0–65	37.88	2.05	10–65	0	0	0	71.71	1.21	40–90
Hypo-osmotic swelling test (%)	38.48	2.19	0–75	18.43	1.79	0–60	28.48	1.81	5–60	0	0	0	6.96	0.63	55–75
Cervical mucus penetration capacity (mm)	16.24	1.12	0–36	5.43	0.75	0–25	8.39	0.97	0–25	0	0	0	32.44	0.28	27–36
Amplitude of lateral movement of the head (μm)	1.22	0.09	0–4.8	0.66	0.94	0–4	1.01	0.13	0–4	0	0	0	2.06	0.09	1.5–4.8
Linearity of spermatic movement	38.45	2.06	0–75	21.85	2.13	0–56.2	33.77	2.77	0–56	0	0	0	63.34	1.06	18.9–75
Acridine orange test (%)	42.31	2.31	0–85	21.86	1.96	0–65	3.79	1.75	2.65	0	0	0	72.97	1.23	20–85
Carnitine (μmol/L)	266	25.58	2.66–1779	191	22.79	2.66–987.7	227.70	32.84	5–987	123.70	19.37	2.66–421.4	379.60	51.31	15.05–1779

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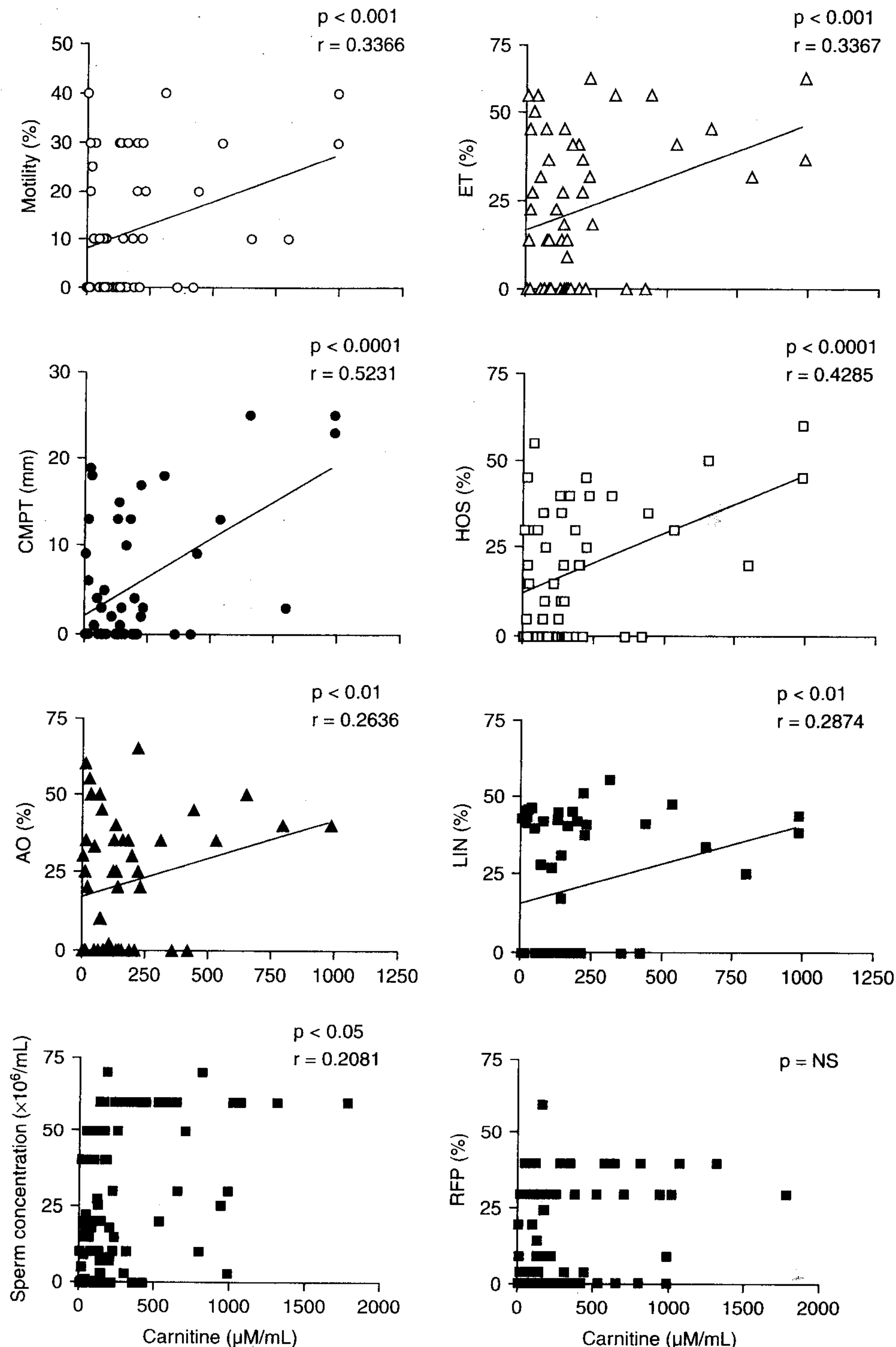

Fig. 1. Correlation analysis between seminal carnitine levels and sperm parameters in patients with low sperm motility (group 1A). **AO** = acridine orange test; **CMPT** = cervical mucus penetration test; **ET** = eosin test; **HOS** = hypo-osmotic swelling test; **LIN** = linearity of spermatozoal movement; **RFP** = rapid forward progression.

Patients and Methods

A total of 170 (duration 1–2 years) infertile patients (aged between 23 and 38 years) without any general illness, presenting with various pathologies that affect fertility without any female factor, including previous cryptorchidism, testicular atrophy, varicocoele, surgically treated varicocoele, idiopathic asthenozoospermia and asymptomatic bacterio-

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Table II. Linear regression analysis between carnitine and seminal parameters in 134 male infertile patients (total study group except for males with azoospermia)

Parameter	Mean	±SEM	Range	p-Value	r-Value
Sperm concentration (×10^6/mL)	29.99	1.91	0.5–70	<0.0001	0.41
Sperm total count (×10^6)	93.34	6.99	1.5–300	<0.01	0.23
Sperm total motility (%)	38.13	1.93	0–80	<0.001	0.30
Rapid forward progression (%)	17.46	1.4	0–60	<0.01	0.25
Eosin test (%)	55.04	1.88	10–90	<0.001	0.31
Hypo-osmotic swelling test (%)	48.82	1.99	5–75	<0.001	0.33
Cervical mucus penetration capacity (mm)	20.6	1.16	0–36	<0.001	0.29
Acridine orange test (%)	53.67	2.0	2–85	<0.01	0.28
Amplitude of lateral movement of the head (μm)	1.55	0.09	0–4.8	<0.01	0.23
Linearity of spermatic movement	48.78	1.75	0–75	<0.01	0.25

spermia, were enrolled in this study between February 2000 and June 2003. Patients provided written informed consent to be enrolled in the study.

The study group was divided into two groups on the basis of total sperm motility, as defined by the WHO. Group 1 included 102 patients with sperm total motility below the normal range (<50%), while the remaining 68 patients, with total sperm motility within the normal range (≥50%), constituted group 2 (table I). None of the patients had had any treatment affecting spermatogenesis for almost 3 months before study inclusion, including antibiotic treatment for bacteriospermia (washout period).

Within group 1, 66 patients did not have azoospermia and made up group 1A; the remaining 36 patients with primary or secondary azoospermia made up group 1B. Patients in group 1A received L-carnitine 1 g/day and L-acetylcarnitine 500mg twice daily for 6 months. Both drugs were delivered orally and commercially obtained.

All semen analyses were performed according to the 1999 WHO laboratory manual guidelines. The eosin test was used to evaluate live spermatozoa percentage (normal range ≥75%), the hypo-osmotic swelling test for sperm membrane integrity (normal range ≥60%), the bovine cervical mucus penetration test for the sperm kinetic index (normal range ≥30mm), and the acridine orange test for sperm nuclear DNA integrity (normal range ≥70%). Sperm kinetics were evaluated by computer-assisted sperm analysis (CASA) using the Cell Track/S® apparatus (Motion Analysis Corporation, Santa Rosa, CA, USA). This technique was used to determine sperm curvilinear velocity (normal range ≥46 μm/sec), sperm linear velocity (normal range ≥25 μm/sec), the linearity of sperm motion (curvilinear velocity divided by linear velocity; normal range ≥58%), and the amplitude of lateral sperm head movement (normal range ≥1.5μm).

The quantitative evaluation of sperm carnitine levels was performed by spectrophotometry, after enzymatic reaction, according to the method suggested by Marquis and Fritz.[9]

Semen analysis was carried out and carnitine levels evaluated in all patients at baseline, and at 3 and 6 months during L-carnitine + L-acetylcarnitine therapy in patients in group 1A.

Statistical Analysis

A Pearson correlation analysis was performed to investigate the relationship between seminal carnitine concentration and all seminal parameters in group 1A in the entire study population as well as in group 1. Seminal carnitine levels in groups 1 and 2 were compared by t-test to evaluate the differences between the two groups.

In patients treated with L-carnitine + L-acetylcarnitine, seminal parameters after 3 and 6 months

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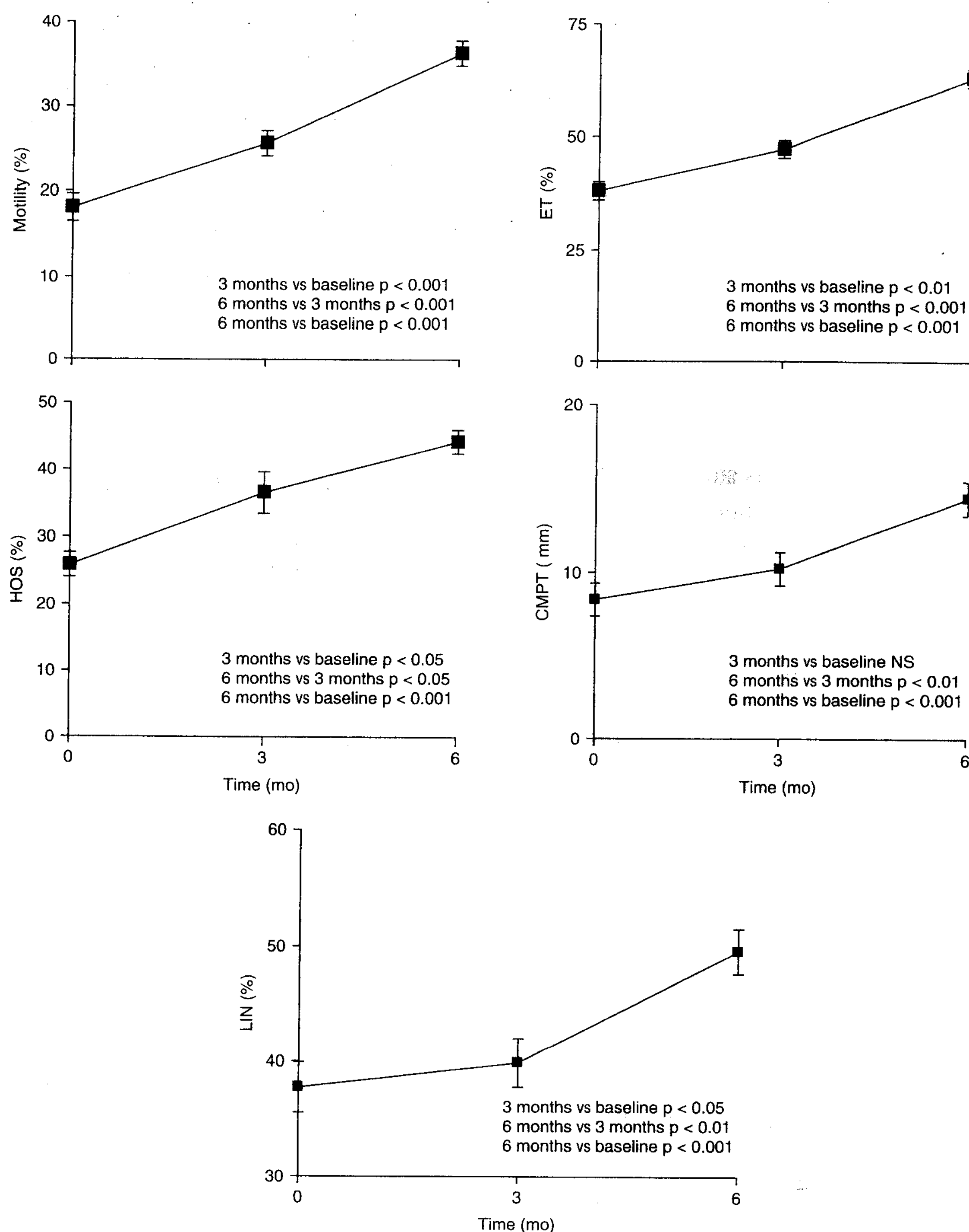

Fig. 2. Seminal parameters at baseline and after 3 and 6 months of treatment in patients with low sperm motility and without azoospermia (group 1A). **CMPT** = cervical mucus penetration test; **ET** = eosin test; **HOS** = hypo-osmotic swelling test; **LIN** = linearity of spermatozoal movement; **NS** = not significant.

of therapy were compared with baseline using a Neumann and Keuls test. p-Values <0.05 were considered significant.

Results

Table I shows the linear regression analysis between seminal parameters and carnitine concentration in the entire study population. There was a

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significant correlation between seminal carnitine concentration and sperm concentration, total sperm count, sperm total motility, rapid forward progression, live sperm count, membrane function, nuclear DNA integrity, capacity for cervical mucus penetration, linearity of spermatic movement, and amplitude of lateral sperm head movement (all $p < 0.0001$).

Seminal carnitine levels were significantly lower in group 1 than in group 2 ($p < 0.001$). In group 1A, out of 66 patients with sperm total motility <50%, there was a significant positive correlation between seminal carnitine concentration and sperm total motility ($p < 0.05$), live sperm count ($p < 0.05$), membrane function ($p < 0.001$), and capacity for cervical mucus penetration ($p < 0.0001$). The relationship between these seminal parameters and carnitine concentration is shown in figure 1.

In the patients with total sperm motility below the normal range (group 1A), there was a significant increase in total motility, live sperm count, membrane integrity and linearity of spermatic movement after 3 and 6 months of L-carnitine + L-acetylcarnitine treatment, and in capacity for cervical mucus penetration after 6 months of treatment, compared with baseline. Table II and figure 2 summarise results from the Neumann-Keuls test carried out in this group.

Discussion

The hypothesis that, in epididymal spermatozoa, carnitine facilitates fatty acid transport and lipid oxidation enabling the storage of acetyl groups and thereby regulating the level of acetyl–CoA,[15] is in concordance with several observations: the presence of significant amounts of free fatty acids in the epididymal plasma,[16] the stimulatory effect of carnitine on the incorporation of exogenous palmitic acid into sperm phospholipids and 1,2-diglicerides,[17] the ability of spermatozoa to oxidise their own endogenous phospholipids, thereby providing metabolic energy,[18,19] and the presence in the spermatozoa of an appreciable reserve of intracellularly-bound acetate after exhaustive washing.[20]

Acetylated carnitine is probably the main source of intracellularly bound acetate carried by the epididymal spermatozoa into the ejaculated semen. In human semen, where the concentration of total carnitine as well as acetylated carnitine is much higher in spermatozoa than in seminal plasma, the intracellular level of acetylated carnitine is positively correlated with sperm motility.[21] It is highly probable that carnitine and acetylated carnitine are metabolised by spermatozoa in the same way as elsewhere in the body, especially the liver.[22] Intracellular free L-carnitine accumulated by spermatozoa might participate in a buffering role, trapping excess mitochondrial acetyl-CoA as L-acetylcarnitine. This system would protect the activity of pyruvate dehydrogenase, a key enzyme for mitochondrial respiration, which is inhibited by excess acetyl-CoA. Exchanges of free L-carnitine and L-acetylcarnitine between the mitochondrion and the cytoplasm (via a translocase-like enzyme) constitute a carrier system that allows cytoplasmic storage of acetyl groups.

In our study we showed that seminal carnitine concentrations are significantly ($p < 0.0001$) but weakly correlated with a variety of seminal parameters, regardless of the underlying pathology causing their infertility. Notably, the correlation between carnitine levels and a number of seminal parameters was also significant among the 66 patients with sperm total motility below the normal range (group 1A).

Treatment with L-carnitine in combination with L-acetylcarnitine resulted in significant improvements in several seminal parameters, indicating that carnitine has a beneficial metabolic effect on spermatozoa function, specifically post-testicular sperm maturation, confirming other previous studies.[23] Sperm count and sperm morphology, including nu-

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clear DNA integrity, were unaffected by L-carnitine + L-acetylcarnitine therapy. As these parameters are a product of intratesticular development,[24-27] this suggests that L-carnitine + L-acetylcarnitine treatment has no effect in this compartment. In spite of the lack of previous double-blind, placebo-controlled studies,[28] Lenzi et al.[29] have demonstrated in a double-blind study on carnitine and L-carnitine treatment of asthenozoospermia that it is effective in increasing sperm motility, especially in groups with lower baseline levels.

These data demonstrate that in a heterogeneous population of infertile men, seminal carnitine concentration plays an important role in spermatogenesis and may be a useful marker of testicular and epididymal function.[30,31]

Conclusion

In conclusion, our data suggest that seminal carnitine concentration is an appropriate marker of sperm and epididymal function. L-carnitine + L-acetylcarnitine treatment may be an effective therapy to improve mainly functional seminal parameters, acting in the post-testicular compartment. Further studies are warranted to confirm the efficacy of L-carnitine + L-acetylcarnitine treatment and to investigate the exact site of action of this combination therapy.

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