Original Article

Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review

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The aim of this systematic review was to quantify the efficacy of L-carnitine (LC) and/or L-acetyl-carnitine (LAC) in nutrition treatment for male infertility according to present clinical evidence. Biomedical databases were searched to collect related clinical trials and nine relevant randomized controlled trials (RCTs) were included. The quality of the RCTs was assessed based on their performance in randomization, blinding, and allocation concealment. The meta-analysis compared LC and /or LAC therapy to placebo treatment found significant improvement in pregnancy rate (OR = 4.10, 95% CI (2.08, 8.08), $p < 0.0001$), total sperm motility（WMD = 7.43, 95% CI (1.72, 13.14), $p = 0.04$, forward sperm motility (WMD = 11.83, 95% CI (0.49, 23.16), $p = 0.04$) and atypical sperm cell (WMD = -5.72, 95% CI (-7.89, -3.56), $p < 0.00001$). However, no significant difference was found in the sperm concentration (WMD = 5.69, 95% CI (-4.47, 15.84), $p = 0.27$) and semen volume (WMD = 0.28, 95% CI (-0.02, 0.58), $p = 0.07$). In conclusion, the administration of LC and/or LAC may be effective in improving pregnancy rate and sperm kinetic features in patients affected by male infertility. However, the exact efficacy of carnitines on male infertility needs to be confirmed by further investigations.

Key Words: food aid, expert system, disaster relief, monitoring, evaluation Male infertility, carnitine, pregnancy rate, sperm motility, systematic review

Introduction

Carnitines are are widely distributed in nature and their potential health benefits have been popularized. Free carnitine (3-hydroxy-4-N-trymethylaminobutyric acid) was first isolated from bovine muscle by Russian scientists in 1905 and only the L-isomer (L-carnitine, LC) was found bioactive.[1] In 1955, Fritz found that LC could accelerate lipid metabolism and then identified its pivotal role in mitochondrial β-oxidation of long-chain fatty acids for cellular energy production.[1, 2] Moreover, carnitine protects cell membrane and DNA against damage induced by free oxygen radicals. It also prevents protein oxidation and lactate oxidative damage.[3]

In fact, LC could be biosynthesized *de novo* by human body. However, LC present in human tissues is mainly of exogenous origin from meat, poultry and fish in dietary.[4] It has long been assumed that carnitine is not an essential component of diet as humans have the ability to synthesize this compound. However, when groups of strict vegetarians were studied, the results showed that their average plasma concentration of carnitine was significantly lower than those of the respective omnivorous controls, which may be attributed to the much less carnitine that strict vegetarians consumed per day.[5] In 1973, Engel reported the first case of carnitine deficiency and treated it with carnitine supplementation.[4] In 1985, carnitine was identified as an essential nutrient of multifunction for the body by the International Nutritional Conference held in Chicago.

Carnitines for medication use are mainly approved to treat carnitine nutritional deficiency induced by hemodialysis in chronic renal failure patients by Food and Drug administration (FDA). However, considering their safety and multifunction, carnitines, including LC and L-acetyl-carnitine (LAC), are widely used in various diseases including male infertility.

Male infertility is a significant problem affecting 7.5% of the male population.[6] Approximately 60% of these cases are idiopathic and related to sperm dysfunctions such as oligo-astheno-teratozoospermia (OAT). By providing readily available energy for use by spermatozoa thus positively affecting sperm motility, maturation and the spermatogenic process,[7, 8] a key role in sperm metabolism is strongly suggested by the high levels of LC found in epididymal fluid due to an active secretary mechanism,[9] and there is also evidence that the initiation of sperm motility is related to an increase of LC in the epididymal lumen and LAC in sperm cells.[10-12] Based on these fundamental roles, numerous clinical trials have attempted to demonstrate a beneficial therapeutic effect of LC and/or LAC when administered to infertile men with various forms of sperm dysfunction.

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However, there has been no in-depth systematic overview of efficacy of carnitines in infertile treatment yet. This systematic review of available randomized controlled trials (RCTs) was conducted to evaluate the effectiveness of carnitines in male infertility, trying to give clinical evidence with meta-analysis and provide guidance for rational drug use.

Materials and methods

Inclusion and exclusion criteria

Study type:

All related RCTs were included, whether to reserve blinding or not.

Subjects:

According to World Health Organization (WHO) criteria,[13] male patients, aged 18-65 years old with infertility >1year, having regular sexual intercourse with a gynecologically normal partner who has no apparent factors of female factor infertility were chosen.

Treatments:

The study group was submitted to one of the following therapeutic approaches: (A) LC alone, (B) LAC alone, (C) combined LC and LAC, (D) combined carnitines and some other drugs (see Table 1). At least one control group treated with proper placebo or some other drugs was established.

Treatment effect measures:

Pregnancy rate was taken as primary outcome measure. The second outcome measure was semen analysis, including sperm concentration ($n\times10^6$/mL), total and forward sperm motility (percentage at one hour after ejaculation) and sperm morphology (percentage of atypical forms), according to WHO standard procedures.[13]

Search strategy

A computer-aided systematic search of MEDLINE (1950-2006), EMBASE (1966-2006), Cochrane Central Register of Controlled Trials (*The Cochrane Library* Issue 2, 2006), The Chinese Biomedical Database (CBM) (1978-2006) and China National Knowledge Internet (CNKI) (1994-2006) was conducted in March 2006, applying combinations of the following search terms: "carnitine", "acetylcarnitine", "levocarnitine", "L-carnitine", "L-acetyl- carnitine", "infertility", "fertility", "semen", "sperm", "sperm motility", "asthenzoospermia", "oligozoospermia", "oligoasthenospermia". Only studies published in English or Chinese language were selected.

Quality assessment and data collection

According to the Cochrane Reviewers' Handbook, a qualified reviewer assessed each potentially eligible study to see whether it met the inclusion criteria. The Jadad Quality Scale was used for methodological quality assessment of each report and a total score was computed by summing up the scores of all criteria (range 0-5).[14] Low quality was defined by a 0-2 score and high quality by 3 or higher.[15] Data collection should include study characteristics such as methodology, cases, characteristics of participants (e.g. age, sex and ethnic population etc), detailed experimental and control interventions, main outcomes and variations in the parameters of treatment effect. The original investigators were contacted for the missing information that we needed and unclear data were not used before their reply.

Data statistics and analysis

Cochrane Review writing software- RevMan 4.2.8 was used for the combination of results from two or more separate studies. Statistical heterogeneity should be identified and measured by using Chi-square test before this combination ($p = 0.05$). When heterogeneity is identified among a group of trials ($p < 0.05$), random effect models should be applied and heterogeneity should be incorporated by the analysis of its causes. Otherwise ($p > 0.05$) fixed effect models were employed and confidence intervals (CIs) of pooled effect were calculated. Odds ratio (OR) was calculated for dichotomous outcomes while weighted mean difference (WMD) for continuous outcomes, expressing with 95% CI. Statistical significance was set at $p < 0.05$. Regarding some important factors such as the difference in the studies' quality, therapy course, diverse interventions (preparations, dosage), degree of disease and complications, sensitivity analysis was suggested by excluding some trials to assess the stability and reliability of the results. A subgroup analysis was performed with more than 2 trials to answer specific questions about particular patient groups or types of interventions.

Results

Literature search results

The first selection was based on titles, keywords and abstracts. No meta-analysis on carnitines' role for male infertility medication was done before. 92 studies (83 in English and 9 in Chinese) were found initially. However, only 9 RCTs (7 in English from MEDLINE and 2 in Chinese from CNKI) met the selection criteria and were included in the review,[16-24] among which 5 were of high quality (all in English) [16, 17, 18, 20, 22] and only 1 was multi-centre.[24] The characteristics and Jadad score of each study are presented in Table 1 and Table 2, showing that the difference among the 9 trials is quite significant. The total number of participants included is 862 with the largest sample size of 325 [17] and the smallest of 21.[21, 22]

Heterogeneity analyses- the comparison of efficacy among different treatments with carnitines

As widely applied treatments in clinical practice, drugs of carnitines mainly include L-carnitine (LC) and L-acetyl-carnitine (LAC). Though they both belong to carnitines, there is still clinical heterogeneity between the two. Considering for that, we tried to find if there was statistical difference in the efficacy on main sperm parameters and pregnancy rate among the treatments with LC, LAC and combined LC+LAC. The RCT of Balercia G. 2005 had three experimental groups treated with LC, LAC, combined LC+LAC respectively and one control group treated with placebo, which was appropriate for our analysis as below.[16]

Treatment with LC alone versus LAC alone

To allow a comparison of effect between LC and LAC, variations in sperm concentration, total motility, forward motility, atypical forms and pregnancy rate were ex

Table 1. General characteristics of included 9 RCTs

Studies reference	infertile Type	Cases (T/C)	Intervention	Duration (month or week)	Ages (years)	Course (years)
Balercia G. 2005 [16]	Idiopathic OAT	59 (15/15/14/15)	LC3g/d LAC3g/d LC2g/d+LAC1g/d Placebo	1m wash-out+6m intervention+3m follow-up	20-40	≥2
Cavallini G. 2004 [17]	Idiopathic and varicocele–ass-ociated OAT	325[1] (101/106/118)	LC2g/d+LAC1g/d LC2g/d+LAC1g/d +Cinnoxicam[2]30mg/4d Placebo	6m intervention+3m follow-up	27-40	≥1
Lenzi A. 2003 [18]	Selected OAT	81[3]	LC(2g/d) Placebo	2m wash-out+2m therapy/placebo+2m wash-out +2m placebo/therapy+2m wash-out	20-40	≥2
Lenzi A. 2004 [19]	OAT	56 (30/26)	LC2g/d+LAC1g/d Placebo	2m wash-out+ 6m intervention+2m follow-up	20-40	≥2
Vicari E. 2002 [20]	PVE[4] and seminal WBC↑	98 (30/16/26/26)	LC1g/12h+Nicetile500mg/12h NSAID[5] NSAID[5](2m)+LC1g/12h(2m) NSAID[5]+LC1g/12h(4m)	4m intervention+3m follow-up	22-42	2.6-13
Pryor JL 2003 [21]	Idiopathic OAT	21 (12/9)	345mgLC＋1180mgLAC/d placebo	24w intervention	-	-
Sigman M. 2006 [22]	Idiopathic OAT	21 (12/9)	2000mgLC+1000mgLAC/d placebo	24w intervention	18-65	≥0.5
Li Zheng 2005.3 [23]	No define	63 (32/31)	LC1g, Bid or Tid VE100mg+VC100mg, Tid	3m intervention	23-40	1-10
Li Zheng 2005.10 [24]	Idiopathic OAT	138 (85/53)	LC1g+LAC0.5g, Bid VE100mg+VC100mg, Tid	2m wash-out 3m intervention	23-46	≥1

Note: 1-195 idiopathic OAT and 130 aricocele-associated OAT; 2-as a suppository of a non-steroidal anti-inflammatory drug (NSAID); 3-81 cases in a crossover trial; 4-Prostato-vesiculo-epididymitis; 5-NSAID therapy consist of nimesulide100mg+serratiopeptidase 5mg /12h intermittently administered for 14 days per month

Table 2. Quality assessment of included 9 RCTs

Studies	Randomization		Allocation Concealment	Inclusion criteria	Comparability	Blinding			Quitting or side effects	Reason of quitting	Compliance	Jadad score
	Yes/No	Method description				Patients	Doctor	Measurement				
Balercia G. 2005 [16]	Yes	-	-	Yes	Yes	Yes	Yes	-	Yes	-	Yes	3
Cavallini G. 2004 [17]	Yes		-	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	4
Lenzi A. 2003 [18]	Yes	-	-	Yes	Yes	Yes	Yes	-	Yes	Yes	Yes	3
Lenzi A. 2004 [19]	Yes	-	-	Yes	Yes	Yes	Yes	-	Yes	-	Yes	2
Vicari E. 2002 [20]	Yes	Yes	-	Yes	Yes	-	-	Yes	-	-	-	3
Pryor JL 2003 [21]	Yes	-	-	Yes	Yes	Yes	Yes	-	-	-	-	2
Sigman M. 2006 [22]	Yes	-	-	Yes	Yes	Yes	Yes	Yes	Yes	-	Yes	3
Li Zheng 2005.3 [23]	Yes	-	-	Yes	-	-	-	-	Yes	-	Yes	1
Li Zheng 2005.10 [24]	Yes	-	-	Yes	-	-	-	-	Yes	-	Yes	1

Table 3. *t*-test of semen parameter variation - LC (15 patients) vs. LAC (15 patients)

Parameter	*conc* ($n\times10^6$/mL)	*Mot tot* (%)	*Mot frw* (%)	*Atyp* (%)
T values	0.36	0.93	0.19	0.08
p values	0.72	0.36	0.85	0.94
Statistically significance （α=0.05）	No	No	No	No

Note: Conc=sperm concentration; Mot tot=percentage of total sperm motility; Mot frw=percentage of forward sperm motility; Atyp=percentage of atypical forms.

Table 4. *t*-test of semen parameter variation - LC+LAC (14 patients) vs. LC (15 patients)

Parameter	*conc* ($n\times10^6$/mL)	*Mot tot* (%)	*Mot frw* (%)	*Atyp* (%)
T values	0.23	0.92	0.30	0.20
p values	0.82	0.37	0.77	0.844
Statistically significance （α=0.05）	No	No	No	No

Note: Conc=sperm concentration; Mot tot=percentage of total sperm motility; Mot frw=percentage of forward sperm motility; Atyp=percentage of atypical forms.

Table 5. t-test of semen parameter variation - LC+LAC (14 patients) vs. LAC (15 patients

Parameter	*conc* ($n\times10^6$/mL)	*Mot tot* (%)	*Mot frw* (%)	*Atyp* (%)
T values	0.19	0.005	0.43	0.28
p values	0.85	0.996	0.67	0.78
Statistically significance （α=0.05）	No	No	No	No

Note: Conc=sperm concentration; Mot tot=percentage of total sperm motility; Mot frw=percentage of forward sperm motility; Atyp=percentage of atypical forms.

tracted from the study of Balercia G. 2005. Student's *t*-test for independent samples was used to evaluate whether the overall semen parameter variations were significantly different between the two treatments as presented in Table 3. No statistical significance was found between the two drugs in the efficacy on sperm quality. Pregnancy rates of the two groups were both 2/15 without difference.

Treatment with combined LC and LAC versus LC alone: Similar to the analysis described above, the comparison of combined LC and LAC with LC alone in

Balercia G. 2005 was performed to evaluate their effect on semen parameters including sperm concentration, total motility, forward motility, atypical forms by using the *t*-test. The results were presented in Table 4, showing no significant difference between the two treatments for male infertility. Although an increase of pregnancy rate was observed after treatments with combined LC+LAC (5/14) versus LC alone (2/15), there was no significant difference ($p = 0.17$).

Treatment of combined LC and LAC versus LAC alone: Similar to the analysis described above, the comparison of combined LC and LAC with LAC alone in Balercia G. 2005 was performed to evaluate the variations in semen parameters including sperm concentration, total motility, forward motility, atypical forms, using the *t*-test. The results also showed no significant difference between the two treatments for male infertility (see **Table 5)**. Although an increase of pregnancy rate was observed after using combined LC+LAC (5/14) versus LC alone (2/15), there was still no significant difference ($p = 0.17$).

Summary:

No significant difference in efficacy among the three interventions was observed, according to the results presented above. It supported the feasibility to pool all groups treated with LC and/or LAC as experimental groups of carnitines therapy into the meta-analysis of overall effect evaluation. However, larger RCTs are recommended for further confirmation of this conclusion considering the small sample size in this trial.

Pregnancy rate

Pregnancy rates of female partners, as the most important outcome measure linked to the effect of carnitines on male infertility, were reported by almost all studies except for one trial.[21] Another one was excluded as a crossover trial (Lenzi A. 2003), which was quite different from the non-crossover trials.[18]

General analysis

The data from 7 trials were taken into meta-analysis (see Figure 1) which found a marked significant difference in overall effect of carnitines on pregnancy rate [OR = 4.10, 95% CI (2.08, 8.08), $p < 0.0001$], using the fixed effect model (heterogeneity test $p = 0.15$). [16, 17, 19, 20, 22, 23, 24]

Sensitivity analysis:

In the 7 RCTs mentioned above,[16, 17, 19, 20, 22, 23, 24] 1 took NSAID as combined intervention in both the two groups,[20] and another had a pregnancy occurred in the treatment arm after in vitro fertilization.[22] To avoid potential effect to the results, the two RCTs were excluded and meta-analysis of the other 5 RCTs were carried out.[16, 17, 19, 23, 24] Similarly, the pooled effect showed statistically significance in spontaneous pregnancy rates between carnitines (LC and/or LAC) and placebo [OR = 5.05, 95% CI (2.38, 10.72), $p < 0.0001$].

Subgroup analysis:

Dividing different carnitines treatments into different subgroups, the pooled effect of 4 trials in combination of LC and LAC subgroup was statistically significant [OR = 6.56, 95% CI (2.88, 14.97), $p < 0.0001$].[16, 17, 19, 24] However, comparing LC alone to placebo treatment, no significant difference was found in the pooled result of only 2 trials [OR = 1.31, 95% CI (0.30, 5.80), $p = 0.72$], which recommended more well-designed RCTs considering for the few participants included in this analysis (47 in LC group vs. 46 in control).[16, 23]

Sperm concentration

6 studies reported the variations in sperm concentration after the interventions of carnitines or placebo. Among them, 1 was a randomized crossover trial (Lenzi A. 2003) [18] and 2 presented their results with quantile,[17, 20] which were unable to combine with others. At last, 3 studies

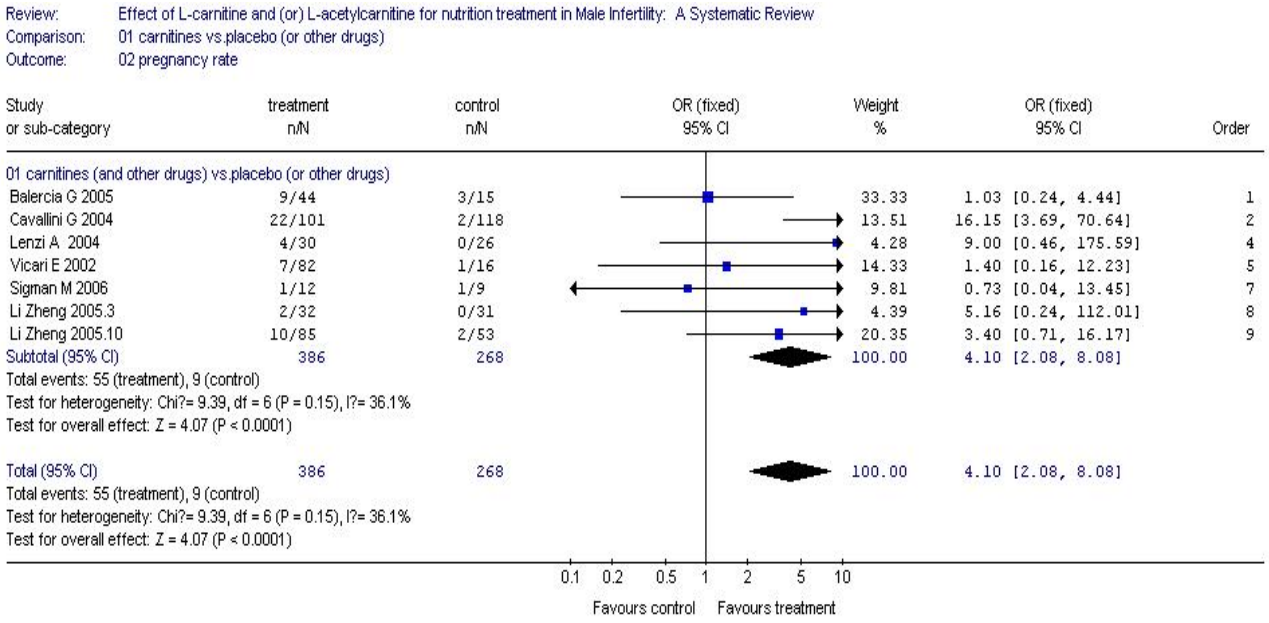

Figure 1. Comparison of effects of carnitine therapy with placebo (or NSAIDs) on pregnancy rate

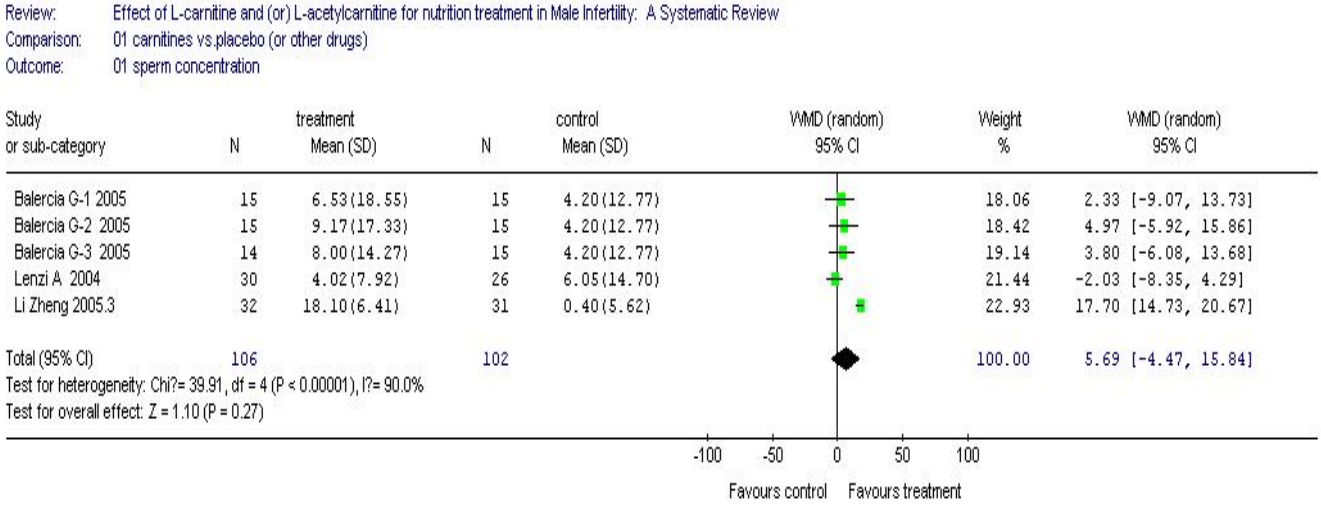

Figure 2. Comparison of effects of carnitine therapy with placebo on sperm concentration

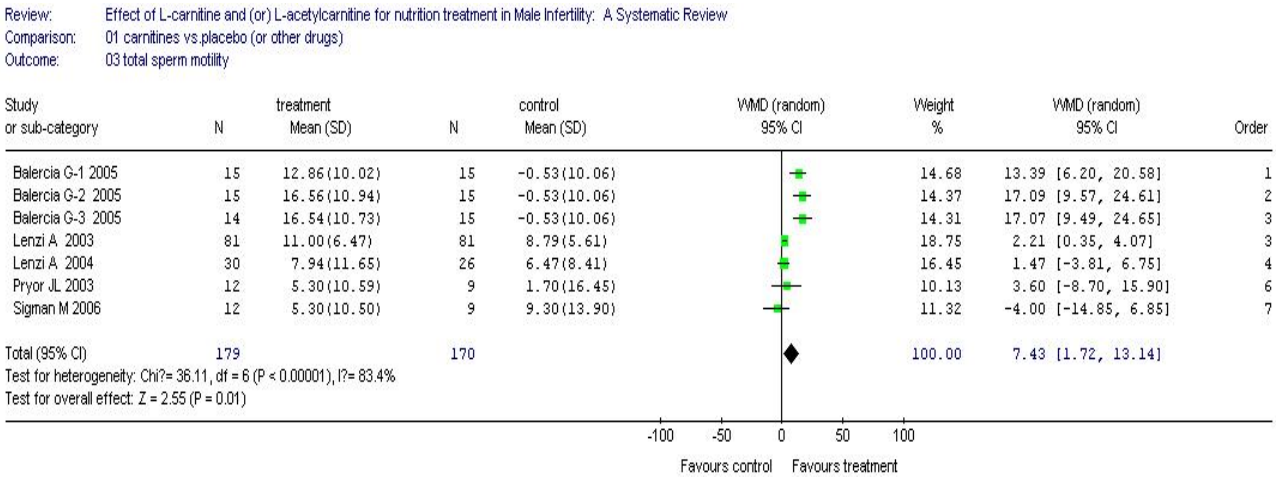

Figure 3. Comparison of effects of carnitine therapy with placebo on percentage of total sperm motility

were subjected to meta-analysis with results of mean ± S.D.[16, 19, 23] Random effect model was employed because statistically significant in sperm concentration [WMD = 5.69, 95% CI (-4.47, 15.84), $p = 0.27$] (see Fig 2). However, one (Balercia G. 2005) of the 3 studies had different design compared with the other two by setting three

of the marked difference among the 3 trials (heterogeneity) test $p < 0.00001$) and the pooled effect was not

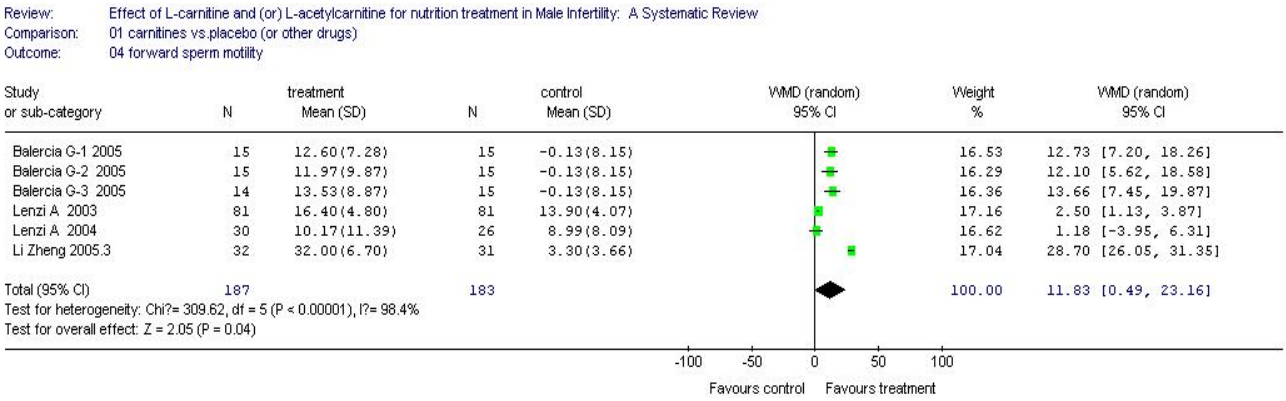

Figure 4. Comparison of effects of carnitine therapy with placebo on percentage of forward sperm motility

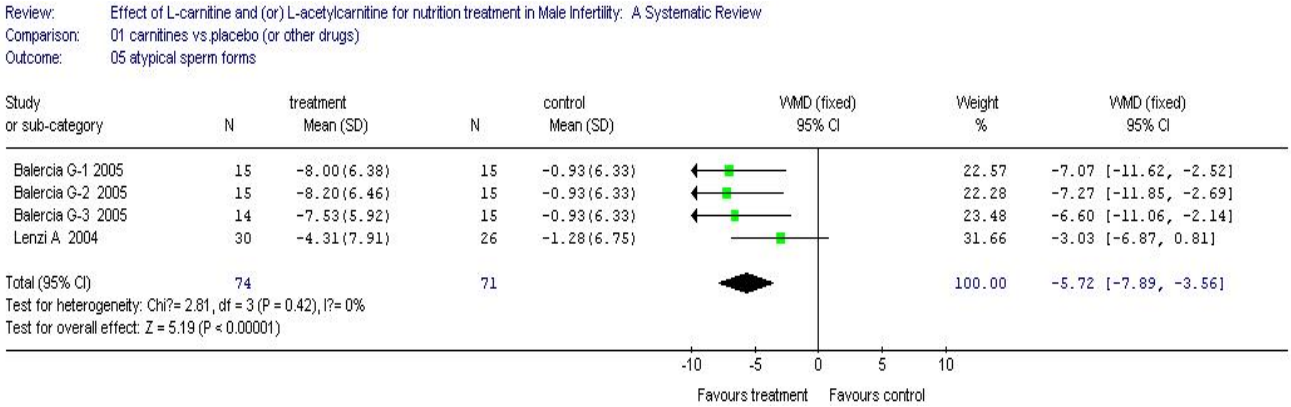

Figure 5. Comparison of effects of carnitine therapy with placebo on percentage of atypical sperm forms

experimental groups and a control group, and each experimental group (treated with LC, LAC and combined LC+LAC respectively) was compared to the control group independently.[16] Therefore, the data of each experimental group were considered as an independent study when imported into RevMan 4.2. To rule out any potential influence, either two groups of data from this study were excluded and the rest data were analyzed again. The results showed no significant difference among the three analyses or compared with the original one [WMD_1 = 6.35, 95% CI (-8.54, 21.24), p = 0.40; WMD_2=7.15, 95% CI (-7.28, 21.58), p = 0.33; WMD_3= 6.77, 95% CI (-7.55, 21.09), p = 0.35]*.

**Note*:

WMD_1 —— excluding LAC vs. placebo and LC/LAC vs. placebo

WMD_2 —— excluding LC vs. placebo and LC/LAC vs. placebo

WMD_3 —— excluding LC vs. placebo and LAC vs. placebo

Total sperm motility

5 studies reported the total sperm motility after treating with carnitines or placebo.[16, 18, 19, 21, 22] Regarding the wide heterogeneity among these trials (heterogeneity test $p < 0.00001$), we applied random effect model in pooled analysis, which showed a significant difference in overall effect [WMD = 7.43, 95% CI (1.72, 13.14), p = 0.01] as shown in Figure 3. The data from Balercia G. 2005 were processed as mentioned above.[16] After excluding the results of Balercia G. 2005 divergent from the other 4 studies, the pooled effect was still statistically significant [WMD = 2.00, 95% CI (0.28, 3.72), p = 0.02]. This sensitivity analysis indicated the fair confidence of the result.

Forward sperm motility (including WHO class A and B motile sperm)

The changes in forward sperm motility were measured in 5 trials,[16, 18, 19, 20, 23] among which the result of Vicari E. 2002 was presented in quartiles and thus unable to be subjected into meta-analysis.[20] The pooled analysis of the other 4 trials showed a significant effect of carnitines in increasing forward sperm motility [WMD = 11.83, 95% CI (0.49, 23.16), p = 0.04] (see Fig 4).[16, 18, 19, 23] The data from Balercia G. 2005 were processed as described above.[16] Otherwise, when the low quality study (Li Zheng 2005.3) was excluded,[23] the difference between two groups was still statistically significant [WMD = 8.03, 95% CI (2.54, 13.52), p = 0.004]. A similar result [WMD = 13.78, 95% CI (2.43, 25.12), p = 0.02] was observed when excluding the study of Lenzi A. 2003 with the heaviest weight.[18] According the above sensitivity analyses, it could be concluded that this outcome was quite consistent and confident.

Atypical sperm forms

4 studies reported the variations in the percentage of atypical sperm forms,[16, 17, 19, 20] among which the results of Cavallini G. 2004 and Vicari E. 2002 were presented in quantiles that were unable to combine with other trials.[17, 20] The pooled analysis of the other 2 trials found a statistical significance in overall effect of carnitines in reducing the atypical sperm forms [WMD = -5.72, 95% CI (-7.89, -3.56), $p < 0.00001$] as shown in Figure 5.[16, 19]

The data from Balercia G. 2005 were processed as described above.[16] However, the conclusion needs to be further confirmed by large RCTs with more participants regarding the small sample size included in this analysis (74 in experimental group versus 41 in control).

Discussion

OAT is a relevant issue in male infertility management. The efficiency of sperm motility, required for fertilization capacity, might decrease in the presence of different factors, eventually leading to infertility. A failure in producing metabolic energy is one of the most reasonable causes of OAT. Spermatozoa are cells sentenced to death, and it seems reasonable that reduced sperm motility represents the initial hallmark of depressed mitochondrial function, eventually leading to sperm death.

A number of drugs have been proposed as being possible causes of male factor infertility associated with OAT of unknown origin. In consequence, both general practitioners and specialists (andrologists, endocrinologists, urologists, gynecologists) around the world frequently employ, for the purpose of improving sperm quality, drugs (e.g., progesterone, zinc sulfate, Vitamin C, Vitamin E, Vitamin B_{12} and many others) of dubious efficacy based on anecdotal indications and without consideration for good medical practice. However, several controlled studies have supported a potential positive effect of therapy with LC and its acyl derivatives LAC for male infertility.[16-24] As we know, free LC is much more concentrated at the epididymal level than in blood. In the epididymis, free LC is transported from blood plasma into the epididymal fluid and spermatozoa and accumulates as both free and acetylated L-carnitine. Carnitines may be also responsible for removing excess intracellular toxic acetyl-CoA, which protects spermatozoa from oxidative damage.[25] Although some evidence suggests a key role of carnitine for sperm motility, its real effective role still remains an interesting open question.

In order to in-depth evaluate the efficacy of carnitines for male infertility, we selected the pregnancy rate, sperm concentration, percentage of total sperm motility, forward sperm motility and atypical forms as main treatment effect measures in this systematic review, according to WHO standard procedures.[13] Further analyses and explanations were performed as below to answer specific questions about this review.

Analyses of efficacy of carnitines in male infertility

The overall average effect of carnitines on pregnancy rate was 4.10 (2.08, 8.08) ($p < 0.0001$), showing a large statistical significance compared with placebo, which supported that pregnancy rate, as the primary end point in this review, could be significantly improved after administration of carnitines in infertile men.

The overall average effect of carnitines on sperm concentration was 5.69 (-4.47, 15.84) ($p = 0.27$), indicating that there is no difference between carnitines and placebo. No conclusion of carnitines to increase sperm concentration could be drawn.

The overall average effect of carnitines on the percentage of total sperm motility was 7.43 (1.72, 13.14) ($p = 0.01$), statistically significant to indicate that carnitines could be effective on the increment of total sperm motility.

The overall average effect of carnitines on the percentage of forward sperm motility (WHO class A and B) was 11.83 (0.49, 23.16) ($p = 0.04$). The statistical difer-ence in favor of carnitines suggested a significant increase in forward sperm motility after carnitines therapy.

The overall average effect of carnitines to reduce the percentage of atypical sperm forms was -5.72 (-7.89, -3.56) ($p < 0.00001$), showing a statistical significance compared with placebo, which supported their effectiveness to decrease atypical sperm forms.

However, considering the wide heterogeneity among the trials included in this review, the evidence was not sufficient enough and more certain conclusions should be drawn from more well-designed RCTs.

About the comparison of efficacy among different treatments with carnitines

LC essentially plays a key role in the mitochondrial β-oxidation of long chain free fatty acids.[26] By providing a shuttle system for free fatty acids and derivatives of acetyl-CoA within the mitochondria, LC regulates the flux of acetyl groups, and therefore energy balance, through the cellular membrane. During their passage through the cellular membranes, acetyl groups are temporarily transferred to LC, producing LAC. Similarly, carnitine facilitates the transport of acetyl group via LAC.[27] It could be concluded that LAC is a bioactive production from LC and they both participate in the energy metabolism, which positively affects sperm motility, maturation and the spermatogenic process.

According to the heterogeneity analyses presented above, no significant difference in efficacy among the three interventions (LC, LAC, LC+LAC) was observed, which supported the feasibility to pool all groups treated with LC and/or LAC as experimental groups of carnitines therapy into the meta-analysis of overall effect evaluation. However, further studies should concentrate on the difference between their sperm-fertilizing abilities for confirmation of this conclusion, considering the small sample size in this trial.

Limitations of this review

This review has a few important limitations. Although we had tried to consult the authors to collect additional information either on methodology or about non-published outcomes in their studies, we still can't get all the information we were interested in. Supplementary information on methodological assessment might have resulted in (slightly) higher method scores. The number of studies that provided statistical data needed to perform quantitative analyses limited the actual performed analyses, as we had to eliminate several studies from the meta-analyses of some effect measures due to some data unavailable, or lack of standard deviations (S.D.s) and means. Because of the few studies present available, the patient inclusion criteria for this review were not very strictly defined despite various forms of sperm dysfunction. That would result in a mixing of various male factor etiologies and a large heterogeneity among the few included studies, which indicated that further investigations should be

conducted with selected specific cases. In addition to these, a publication bias caused by unpublished negative results or publication languages could not be excluded, which might attenuate the validity of the conclusions. Because of the limitations existing, the results of this systematic review should be considered deliberately when applying.

Conclusions

In summary, based on the results of meta-analysis presented above, especially the significantly improvement in pregnancy rate which was considered as the main outcome measure in this systematic review, it is supported that carnitine therapy (with L-carnitine and/or L-acetyl-carnitine) showed some considerable positive effects in improving sperm quality compared with placebo treatment, which merit further researches with well–designed large RCTs regarding the limitations of this review mentioned above. Also needed are biological studies of the effect of carnitines on the metabolism of the male gamete, using molecular and cellular studies on single intracellular functions or organelles.

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