Carnitine and Male Fertility

National Center for Complementary and Alternative Medicine
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Carnitine: The Science Behind a Conditionally Essential Nutrient
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Presentation Overview

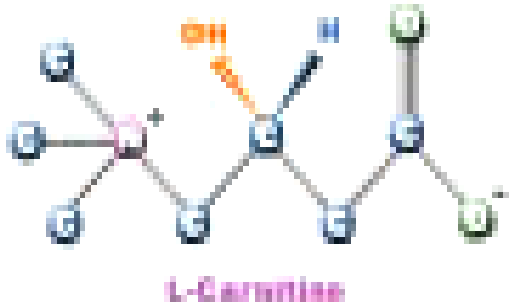
- **Carnitine physiology**
- **Preclinical studies of carnitine's effects in the testis**
- **Anti-apoptotic effects of carnitine**
- **Clinical studies of use of carnitine in male infertility**

Biological Research - It's All "Natural"…!

"People can be induced to swallow anything, provided it is sufficiently seasoned with praise."

Jean Moliere

Carnitine-metabolic functions



- Trimethylated aminoacid-ester
- Synthesized in liver, brain, and kidney from dietary amino acids- methylation of lysine
- Most derived from diet: red meat, fish and dairy products

- Facilitates LCFA transfer into the mitochondria and their oxidation (beta-oxidation and Krebs' cycle)
- Buffers the mitochondrial acylCoA/CoA ratio – important for carbohydrate metabolism (PDH inhibitor)
- Regulates of acetylCoA/malonylCoA ratio- important for appetite control, insulin release from pancreas and liver neoglycogenesis
- Carnitine system-enzymes involved in peroxisomal FAO

Pharmacokinetics

- Absorbed in the intestine by a combination of active transport and passive dilution. (Li B, et al. The effect of enteral carnitine administration in humans. Am J Clin Nutr 1992;55:838-845)
- Mucosal absorption is saturated at about a 2 g dose. (Harper P, et al Eur J Clin Pharmacol 1988;35:555-562)
- Max blood concentrations are reached approximately 3.5 hrs after an oral dose, with a half-life of about 15 hrs.
- Stored in skeletal muscles, myocardium, epididymis, liver and adrenal glands
- Eliminated by kidneys (Bach AC, Schirardin H, Sihr MO, Storck D. Free and total carnitine in human serum after oral ingestion of L-carnitine. Diabete Metab 1983;9:121-124)
- Bioavailability varied 54-87% (Rebouche CJ, et al. J Nutr 1991;121:539-546)

Testicular Carnitine Transport

- Free carnitine is taken up from the blood plasma, actively transported into the epididymal plasma, under the regulation of androgen

- Carnitine is then accumulated in the spermatozoa by passive dilution

Benefits of carnitine in sperm

- High concentrations of carnitine within the epididymis may be beneficial.

- Epididymal sperm use fatty acid oxidation as the main source of energy metabolism, carnitine is crucial to transport fatty acids into mitochondria matrix within spermatozoa for energy production.

- Carnitine contributes directly to sperm motility and sperm maturation.

Benefits of carnitine

- Energy metabolism: Transportation of fatty acid into mitochondria for oxidation
- Cellular metabolism: Anti-apoptosis

Anti-apoptotic effects

- Carnitine has been used in Alzheimer disease, congestive heart failure, chronic fatigue syndrome, end-stage renal failure, peripheral vascular disease, supplementation during exercise

- Carnitine reduces apoptotic cell death in growth factor-deprived murine C2.8 hepatocytes, lymphocytes, teratocarcinoma cell lines, neuronal cells, cardiac myocytes after doxorubicin, skeletal muscle.

Anti-apoptotic effects

- Carnitine exerts its anti-apoptotic effects in diverse tissues
  - Does carnitine exert anti-apoptotic effects in the testis ?
  - Which step(s) of apoptosis does carnitine influence ?

In vivo study in testes (1)

- Effects of L-acetylcarnitine (L-ACAR) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry.
  - 1. Recovery after X-irradiation.

Amendola R et al. *Andrologia. 1989 Nov.-Dec; (6):568-75.*

Carnitine in irradiated testes (1)

- First report to characterize the in vivo actions of carnitine on the testes
- The testes of mice were irradiated with a single dose of 10 Gy.
- The treatment group was given intraperitoneal

  L- ACAR (100mg/kg body weight) on alternate days for 4 weeks, starting from the day of irradiation.
- The effects on spermatogenesis were assessed at 28, 35, 40, 45, 50, 55, 60 days after irradiation.
- The effects were examined by flow cytometric analysis of cellular DNA content.

  - Amendola R et al. Andrologia. 1989 Nov.-Dec; (6):568-75.

Carnitine in irradiated testes (1)

- In the treatment group:
- The fraction of tetraploid cells was greater at days 28 ($P<0.05$) and 45 ($P<0.02$).
- The round spermatid fraction was greater at 45 days ($p< 0.1$) and the elongated spermatid fraction was higher at 50 days ($P<0.1$).
- The recovery period throughout the maturation process was shortened.
- Conclusion: L-ACAR enhanced the recovery of spermatogonial cells after X ray damage
  - **N.B. Despite the favorable outcome in the carnitine group, however, no significant difference was detected in the fraction of round and elongated spermatids in the control vs L-ACAR groups. It is speculative that the beneficial effect of L-ACAR would be seen throughout the maturation process of spermatogenesis.**

Amendola R et al. Andrologia. 1989 Nov.-Dec; (6):568-75.

In vivo study in testes (2)

- Effects of L-acetylcarnitine (L-ACAR) on the post-injury recovery of mouse spermatogenesis monitored by flow cytometry.

  2. Recovery after hyperthermic treatment.

Amendola R et al, Andrologia. 1991 Mar-Apr; 23(2): 135-40.

Carnitine in heated testis (2)

- Heat was applied by local immersion of mice in a water bath maintained at 42C for 1 hour.
- The same dose of intra-peritoneal L- ACAR was administered in treatment group as in prior expts, starting from the day of heat treatment.
- The effects of spermatogenesis were studied at 8,14, 21, 28, 35, 40, 45 and 60 days after heat treatment.
- The testes were excised and weighed. Their cellular DNA contents were examined by the flow cytometry.

Amendola R et al, Andrologia. 1991 Mar-Apr; 23(2): 135-40

Carnitine in heated testis (2)

- In control group, the number of primary spermatocytes was markedly reduced with complete absence of haploid cells one week after heat treatment.
- In treatment group: the haploid cell fraction was higher at day 45 in the treatment vs control group ($P < 0.01$).
- Histological examination of tissue sections indicated that the reorganization of the seminiferous epithelium was faster.
- Weight of the testis was higher ($p < 0.05$) at the above time points.
- Conclusion: There was more rapid recovery of spermatogenesis after heat treatment with L-ACAR administration.

Amendola R et al, Andrologia. 1991 Mar-Apr; 23(2): 135-40

In vivo study in testis (3)

- Testicular toxicity effects of magnetic field exposure and prophylactic role of coenzyme Q10 and L-carnitine in mice.

Ramadan LA et al. Pharmacol Res. 2002 Oct; 46(4): 363-70

Carnitine in testes after magnetic field (3)

- Testes of mice were damaged by high magnetic field exposure.
- The animals were injected either with carnitine intraperitoneally (200mg/kg) or coenzyme Q10 orally (200 mg/ kg).
- Parameters like total sperm count, motility, daily sperm production, testicular LDH- X activity as well as histopathological examinations were assessed.
- There was a significant decrease in above-mentioned parameters in the control group.
- Pre-treatment with carnitine or coenzyme Q10 1 hr before exposure to magnetic field caused a significant recovery of mice testicular damage.

Ramadan LA et al. Pharmacol Res. 2002 Oct; 46(4): 363-70

Administration of L-ACAR can speed recovery of spermatogenesis in mice after various insults

- **Possible mechanisms:**
  1) Provision of an extra source of acetyl groups for CoA, enhancing the cellular energy metabolism with consequent favorable outcome on DNA repair, on Sertoli cells and regenerating germ cells.
- **2 Anti-apoptotic effects in the testis**

Possible mechanisms of carnitine's anti-apoptotic effects

- Anti-oxidant
- Inhibition of extrinsic mitochondrial-independent Fas-triggered apoptotic signals (e.g. caspases), and of ceramide generation
- Inhibition of intrinsic mitochondrial-dependent pathways
- Others

Possible mechanisms of carnitine's anti-apoptotic effects

- Increased reactive oxygen species (ROS) have been detected in patients with idiopathic and post-inflammatory oligoasthenospermia.
- Carnitine as an anti-oxidant
  - reduces ROS
  - increases sperm forward motility and viability in infertile patients with prostato-vesculo-epididymitis

Vicari E, Calogero AE. Effects of treatment with carnitine in infertile patients with prostato-vesiculo-epididymitis. Hum Reprod 2001 Nov; 16 (11): 2338-42.

Possible mechanisms of carnitine's anti-apoptotic effects

- ?? Antioxidant effect
- Antioxidant: ascorbate and alpha-tocopherol
- Result: production of ROS by sperm was reduced by supplementation in vitro with ascorbate and alpha-tocopherol.
- Supplementation of preparation media with ascorbate and alpha -tocopherol, either singly or in combination, was not beneficial to sperm motility.

Donnelly ET, et al, Antioxidant supplementation in vitro does not improve human sperm motility. Fertil Steril. 1999 Sep;72(3):484-95

Possible mechanisms of carnitine's anti-apoptotic effects

- **Inhibit extrinsic Fas-mediated apoptosis**
- Jurkat cells were induced to undergo apoptosis with Fas ligation in the presence or absence of carnitine.
- Carnitine protected Jurkat cells against Fas-mediated apoptosis in a dose dependent fashion
- Carnitine exerts an inhibitory effect on the activity of recombinant capases 3, 7 and 8.
- Concentration of endogenous carnitine reduced during apoptosis.
- => endogenous carnitine might play a regulatory role in apoptosis.

Martha C et al. Regulation of the activity of caspases by L-carnitine and palmitoylcarnitine. FEBS Letters 478 (2000) 19-25.

Possible mechanisms of carnitine's anti-apoptotic effects

- **Inhibit intrinsic mitochondrial-dependent pathway**
- In a rat model of heart failure, an increase in apoptosis of the skeletal muscle was noted.
- Pro-apoptotic agents, capase 3 and 9, serum TNF-alpha and its second messenger sphingosine were elevated.
- Rats were treated with carnitine (50mg/kg) orally for 28 days.
- In the treatment group, there were fewer TUNEL- positive nuclei and DNA break strands (ELISA ladder), which was associated with a lower expression of capases 3 and 9 and with increased expression of Bcl-2. The levels of TNF-alpha and sphingosine were also increased.
  - **N.B.: Although carnitine prevents apoptosis of skeletal muscle, whether this effect is secondary to improvement of heart function or is of genuine protective role of skeletal muscle is open to question.**

G. Vescovo et al, Am J Physiol Cell Physiol 283: C802-C810, 2002.

Possible mechanisms of carnitine's anti-apoptotic effects

Other:

- **Increase cardiolipin, enhance pyruvate transport into mitochondria and facilitates oxidation (in heart mitochondria of aging rats).**
- **Remove acyl CoA, a potentially toxic intermediate.**
- **Activate the GH/IGF-I axis.**

Use of Carnitine in Male Infertility

- In one study of 101 men, a positive correlation was found between carnitine content in semen and sperm motility, number and morphology ($p < 0.01$)

Matalliotakis I, et al, Int Fertil Women Med 2000: 45(3): 236-40

Use of Carnitine in Male Infertility

- 100 patients with idiopathic oligoasthenozoospermia all received 3g/day of oral L-CAR x 4 months
- Sperm motility determined before, during, after study
- % of motile spermatozoa increased: 26.9 ± 1.1→ 37.7 ± 1.1% (P<0.01)
- Sperm with rapid linear progression: 10.8→ 18% (P<0.01)
- Total no. of spermatozoa per ejaculate: 142 → 163 x 10^6 (P<0.01)
- **N.B. uncontrolled study**

Costa M et al. Italian Study Group on Carnitine and Male infertility Andrologia 1994 May-Jun; 26(3): 155-9.

Use of Carnitine in Male Infertility

- Placebo-controlled, double-blind, cross over trial
- 86 infertile men with: sperm conc 10-20 x 10^6; total motility 10-30%; forward motility < 15%; atypical forms< 70%; Velocity: 10-30u/s; Linearity <4
- L-CAR- 2g/d orally or placebo x 2 months → 2 months of washout→ 2 months placebo/L-CAR therapy.
- Significant increase in semen quality- sperm conc, total and forward sperm motility, especially in groups with lower baseline levels.
- **N.B. L-CAR therapy may improve semen quality in selected cases of male infertility, but effects on fertility not examined.**

A. Lenzi, et al, Reproductive endocrinology 7: 292-300, 2003

Limitations of carnitine clinical studies

- Bioavailability of carnitine is small and varied from 5 to 15%.
- Intake of food related to carnitine contents was not standardized
- Dosing of carnitine may have been further complicated by the variable carnitine content in the formulation with poor dissolution properties
- Unclear whether exogenously administered carnitine can reach the epididymis or inside spermatogonia
- Action of substrates may be limited by the rate-limiting step of carnitine uptake and its metabolism

Carnitine and Male Fertility
Conclusions

- Carnitine administration improves sperm quality and/or quantity in testes of mice exposed to physical insults, and in clinical trials conducted in men with idiopathic oligoasthenospermia.
- The benefits are partly related to improvement in motility of epididymal sperm, possibly due to increased mitochondrial fatty acid oxidation.
- The anti-apoptotic properties of carnitine in the testis are likely to contribute to these beneficial effects, but require further study.

Carnitine and Male Fertility
Future Research Questions

- Basic studies:
  - Explore the protective mechanism(s) of carnitine's effects in the testis
  - Enhance understanding of the pathophysiology of germ cell apoptosis
  - Develop strategies to prevent germ cell death
  - Identify specific therapy for some forms of male infertility
- Clinical studies:
  - Who is the optimal candidate for carnitine therapy?
  - What dose, route, duration, and formulation of carnitine therapy is best?
  - Does carnitine truly improve sperm quality and quantity, and does that result in increased fertility with normal offspring?

Acknowledgments

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