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(*Director: prof. Francesco Trimarchi)*

PROGRAMMA DI ENDOCRINOLOGIA MOLECOLARE CLINICA
(*Chief: prof. Salvatore Benvenga)*

EFFECT OF CARNITINE ON THYROID HORMONE ACTION

Salvatore Benvenga, MD

INTERNATIONAL WORKSHOP ON
CARNITINE: THE SCIENCE BEHIND AN ESSENTIAL NUTRIENT
Bethesda, Md, USA
March 25 & 26, 2004

LITERATURE ON THE THYROID HORMONE ANTAGONIST PROPERTY OF **CARNITINE** (I)

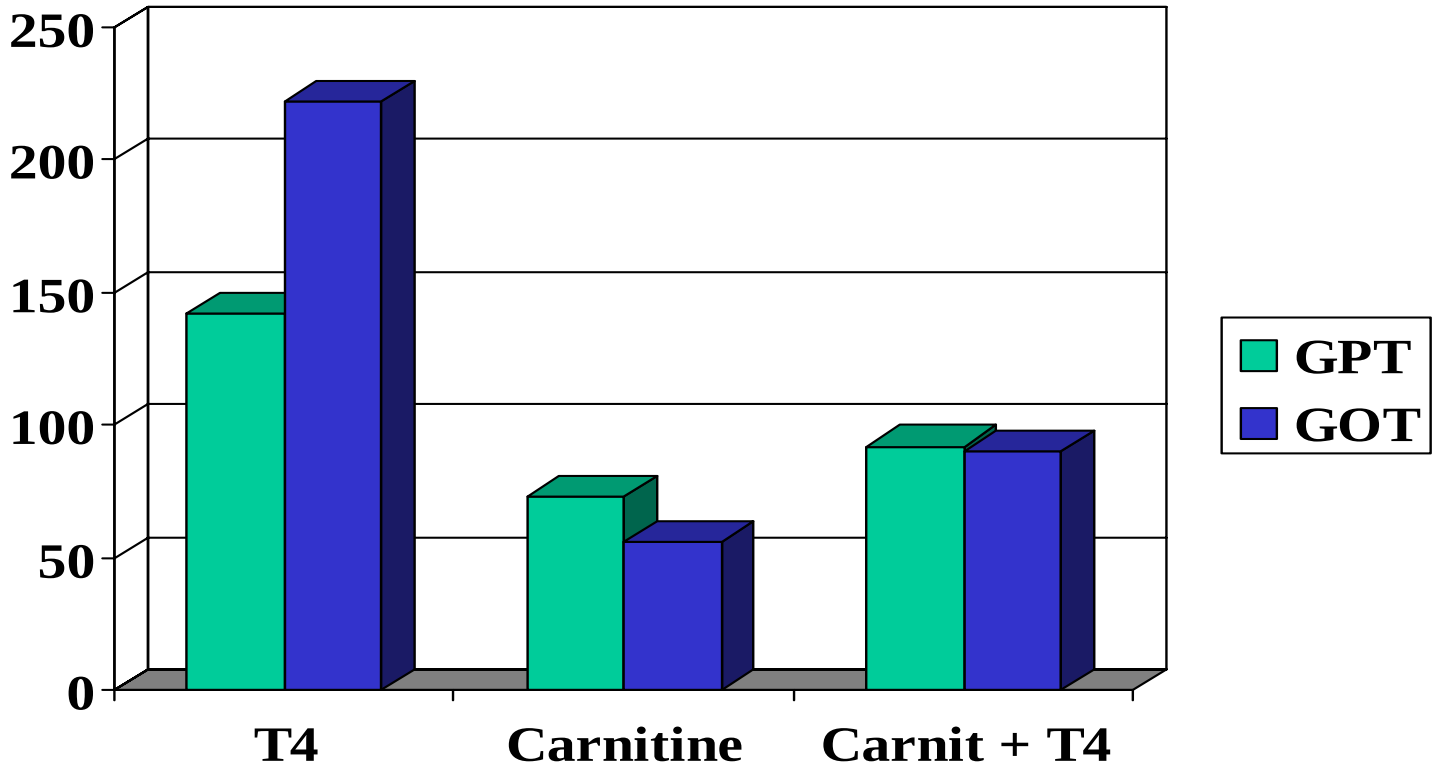
- **Rotzsch W, Strack E. Umstaz Und Wirkung des Carnitins im Tierkorper.** ***Int Abstr Biol Sci 1958; 11: 80***
- **Strack E, Rotzsch W. Der einfluss des carnitins auf die Stoffwechselgrosse in Organismus. In:** ***Proceedings of the 7th Colloquium on Protides of the biological fluids. Bruges, Belgium. Amsterdam: Elsevier;1959, p. 263***
- **Strack E, Wortz G, Rotzsch W. Wirkungen von Karnitin bei Uberfunction der Schildrusse.** ***Endokrinologie 1959; 38: 218-225.***
- **Strack E, Blosche H, Bemme H, Rotzsch W. Anwendung von L-carnitin bei Schildrussenuberfunktion.**
  Dtsch Z Verdau Stoffwechselkr 1962; 21: 253-259
- **Strack E, Han Y-Z, Aurich H, Rotzsch W. Die aktivitat von Serumtransaminasen nach Carnitingabe.**
  Hoppe-Sey Z Physiol Chemie 1963; 331: 33-40.
- **Hellthaler G, Wenzel KW, Rotzsch W. Aminotransferasen unter thyroxin und Karnitin.** ***Acta Biol German 1967; 19: 641-652.***

LITERATURE ON THE THYROID HORMONE ANTAGONIST PROPERTY OF **CARNITINE** (II)

- **Gilgore SG, DeFelice SL. Evaluation of carnitine – an antagonist of thyroid hormone action. *J New Drugs 1966; 6: 349-350***
- **DeFelice Sl, Gilgore SG. The antagonistic effect of carnitine in hyperthyroidism. Preliminary report. *J New Drugs 1966; 6: 351-353*.**
- **Benvenga S, Lakshmanan M, Trimarchi F. Carnitine is a naturally occurring inhibitor of thyroid hormone nuclear uptake. *Thyroid 2000; 12: 1043-1050*.**
- **Benvenga S, Ruggeri RM, Russo A, Lapa D, Campennì A, Trimarchi F. Usefulness of L-carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab 2001; 86: 3579-3594*.**
- **Benvenga S, Lapa D, Cannavò S, Trimarchi F. Successive thyroid storms treated with L-carnitine and low doses of methimazole. *Am J Med 2003; 115: 417-418.***

EFFECT OF L-T4 OR CARNITINE INJECTION ON TRANSAMINASE ACTIVITY OF RAT LIVER HOMOGENATES

[G. Hellthaler et al. Aminotranferasen unter thyroxin und carnitin. Acta Biol Med German 19: 641-652, 1967]

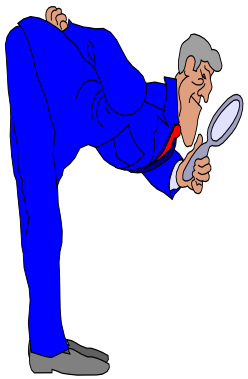


Strack E et al. Wirkungen von Carnitin bei Uberfunktion der Schildruse [EFFECTS OF CARNITINE IN CASES OF THYROID HYPERFUNCTION] .

Endocrinologie 38: 218-225, 1959.

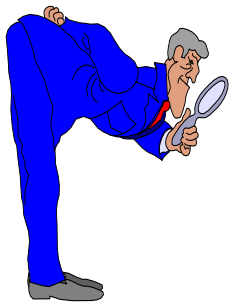
- **"… We have 3 patients available with hyperactive thyroids who were treated with carnitine … Synthetic D,L-carnitine as well as D and L-carnitine was administered to the patients orally… (0.25 to 1.25 g per patient per day were deliberately administered at first)… One g is completely harmless, as we have seen by taking in this quantity ourselves".**

Strack E et al. Wirkungen von Carnitin bei Uberfunktion der Schildruse [EFFECTS OF CARNITINE IN CASES OF THYROID HYPERFUNCTION] . ***Endocrinologie 38: 218-225, 1959.***



- **Patient 1 (53-yr-old woman).** She noted increasing nervousness, insomnia, weight loss, sweating, tachycardia. She was so weak that became bedridden. On admission, there was atrial fibrillation (170 b/min), exophthalmus, ↑ basal metabolic rate [BMR] (+ 82%) and PBI (16 μg/dl).
- Starting at the day 9, we administered **1 g/d D,L-carnitine**. After 10 days, BMR unchanged but "one week later" it fell to +59%. Dose **increased to 1.25 g/d**. Five weeks after starting **D,L-carnitine**, BMR +50%. We **switched to L-carnitine**, which occurs naturally. **(continued …)**

Strack E et al. Wirkungen von Carnitin bei Uberfunktion der Schildruse [EFFECTS OF CARNITINE IN CASES OF THYROID HYPERFUNCTION] . ***Endocrinologie 38: 218-225, 1959***



- **(continued Patient 1)** . "After only 10 days during which a total of 9 g **L-carnitine** had been administered, BMR fell more rapidly; it was now + 8%. Particularly noteworthy was the improvement in general well-being… Atrial fibrillation disappeared and heart rate was 80-90 b/min. **To prove that improvement was due to L-carnitine**, this was withdrawn in the 7 week from admission. BMR rose to + 39%.
- **L-carnitine** was given again, and BMR fell to +18, 10 days later. Exophthalmus unchanged.

Strack E et al. Wirkungen von Carnitin bei Uberfunktion der Schildrusse [EFFECTS OF CARNITINE IN CASES OF THYROID HYPERFUNCTION] . ***Endocrinologie 38: 218-225, 1959***

- **Patient 2** (38-yr-old woman) “with typical Basedow symptoms and BMR + 45%. BMR fell to +3% on day 10, after **1 g D,L-carnitine every other day**. After a further 10 days with half the dose, BMR rose to +19%. The nervous system were substantially alleviated”.
- **Patient 3** (69-yr-old woman) “with typical hyperthyroid symptoms. ↑BMR (+ 54%) and PBI (15μg/dl). BMR fell to +21 % on day 5, after **1 g D,L-carnitine every other day**.

The antagonistic effect of carnitine in hyperthyroidism. Preliminary report *[DeFelice SL, Gilgore SG. J New Drugs 6: 351-53, 1966]*

- "**Three subjects** [*age ?, gender ?]* with overt hyperth. [*cause of hyperthyroidism ?]* were studied". **DL-carnitine** (1 g t.i.d. for 5 weeks) was the sole mode of therapy. After one week of wash-out, placebo was given from week 6 through 13. "The subjects were seen weekly and were clinically evaluated".
- PBI, BMR, radioactive iodine uptake measured periodically. In 2 patients, urinary cathecolamines measured (week 5 and 7).

The antagonistic effect of **carnitine** in hyperthyroidism. Preliminary report *[DeFelice SL, Gilgore SG. J New Drugs 6: 351-53, 1966]*

- **Results**.- "**By the end of 2nd week of the carnitine treatment**, the tremors, tachycardia, emotional instability, nervousness intolerance, and fatigues along with other manifestations had virtually disappeared. However, the laboratory data did not correlate with the clinical changes. [….].
  Within 2 weeks [of the **placebo** treatment] subjects A and B were again overtly manifesting their previous clinical hyperthyroid pattern ".

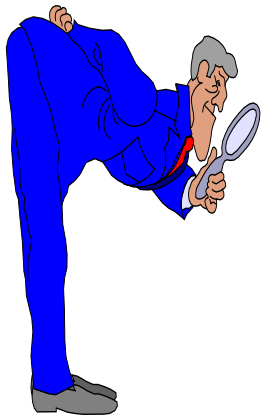
LABORATORY DATA IN 3 HYPERTHYROID PATIENTS TREATED WITH 3 g/d **CARNITINE (weeks 1-5)** OR PLACEBO *[DeFelice SL, Gilgore SG. J New Drugs 6: 351-53, 1966]*

	0	1	2	4	5	7	9	13
PBI (μg/dl)	**12.9 (11.2-14.6)**	**9.7 (9.6 ; 9.8)**	**9.4 (7.7; 11.2)**	**11.2 (8.1 - 14.0)**	**12.7 (11.6 - 13.8)**	**11 (11.4 - 13)**	**7.7**	**9.3**
% ^{131}I thyroid uptake	**59 (49- 72)**			**60 (54- 68)**				**61**
BMR	**33 (27-41)**			**27.6 (26-29)**		**43 (40;47)**	**31**	**40**
Pulse	**109 (96-120)**	**92 (80-104)**	**86**	**84**	**91 (82-100)**	**112 (112; 112)**	**96**	**108**
Weight (Kg)	**66 (62-73)**	**68 (62-74)**	**66 (62-74)**	**62 (62; 62)**	**68 (62-74)**	**67 (61; 72)**	**63**	**64**

The antagonistic effect of **carnitine** in hyperthyroidism. Preliminary report *[DeFelice SL, Gilgore SG. J New Drugs 6: 351-53, 1966]*

- **Conclusions**. - “The observation that the patients became clinically euthyroid without any consistent changes in the thyroid function studies supports the notion that the antithyroid effect of **carnitine** is one of peripheral antagonism of thyroid hormone, rather a direct inhibition of thyroid gland function “.

Gilgore SC, DeFelice SL. Evaluation of carnitine - An antagonist of thyroid hormone. Clinical pharmacology report.
J New Drugs 6:349-350, 1966



- 18 hypercholesterolemic male volunteers divided into 3 groups based on 4-week oral administration of : a) **Carnitine** alone; b) T3 alone; c) **Carnitine** + T3.
- **Parameters evaluated**.- "Serum cholesterol, triglycerides and NEFA measured twice weekly after 2 baseline evaluations. Serum tyrosine, basal metabolic rate, pulse rate and body weight measured periodically".
- Data (*only mean*) reported in different ways (*see next slide*), with no statistical analysis.

EFFECT OF A 4-WEEK ADMINISTRATION OF **CARNITINE (2 g/day),** T3 (0.1 mg/day) OR CARNIT. + T3 IN HYPERCHOLESTEROLEMIC MALES.

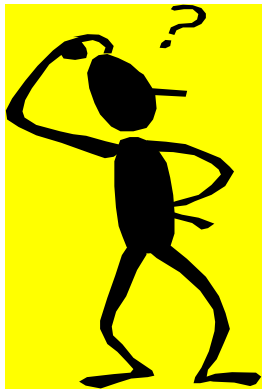
[Gilgore SC, DeFelice SL. Evaluation of carnitine - An antagonist of thyroid hormone. Clinical pharmacology report. *J New Drugs 6:349-350, 1966*]

Drug	Pulse	BMR	Weight	Cholest. ***Triglyc.***	NEFA
Carnit. (n= 6)	**83/min**	**Not given**	**- 1.4 Kg**	**- 3 %** **- *22%***	**- 10 %**
0.1 mg T3 (n=6)	**98/min**	**+ 15**	**- 4.8 Kg**	**- 38%** **- *32%***	**+ 20%**
Carnit + T3 (n=6)	**90/min**	**+ 6**	**- 4.7 Kg**	**- 22%** **- *26%***	**- 2 %**

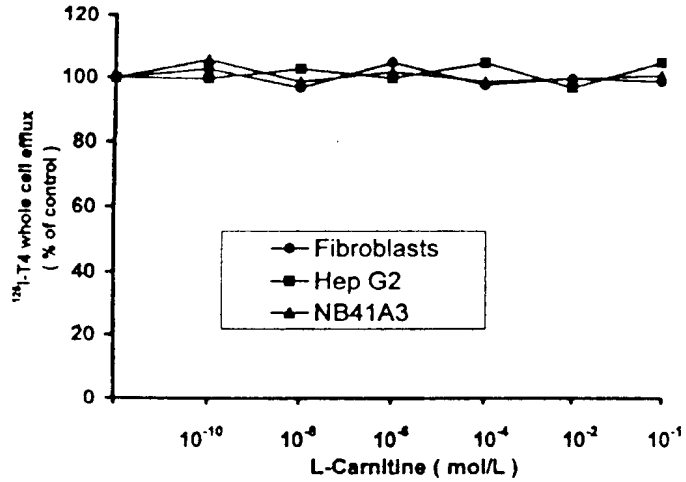
Gilgore SC, DeFelice SL. Evaluation of carnitine - An antagonist of thyroid hormone. Clinical pharmacology report. *J New Drugs 6:349-350, 1966*

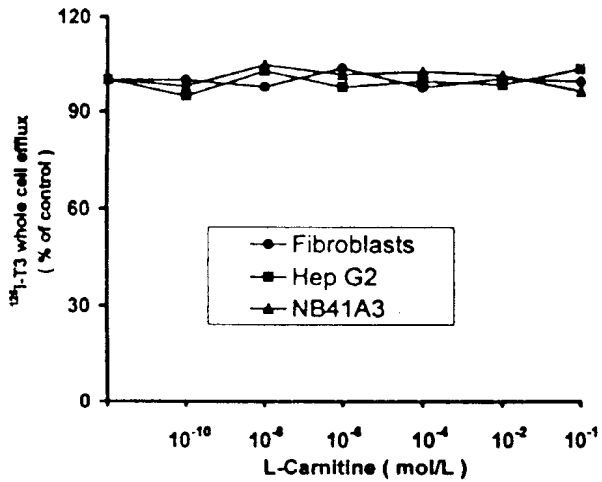
- **Conclusions**. - "**Carnitine** significantly inhibited T3-induced elevations of NEFA and lowering of cholest. **Carnitine** also inhibited to some extent the expected T3 effects on serum tyrosine, BMR and pulse rate. … The notion that the antithyroid action of **Carnitine** is a peripheral one is strongly supported ".

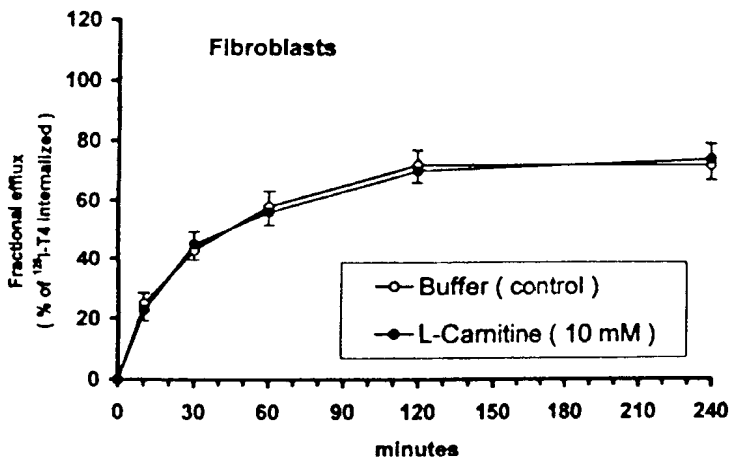
HYPOTHESIS.

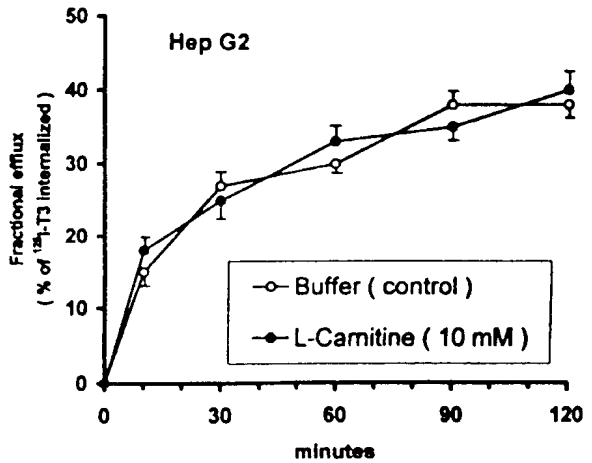


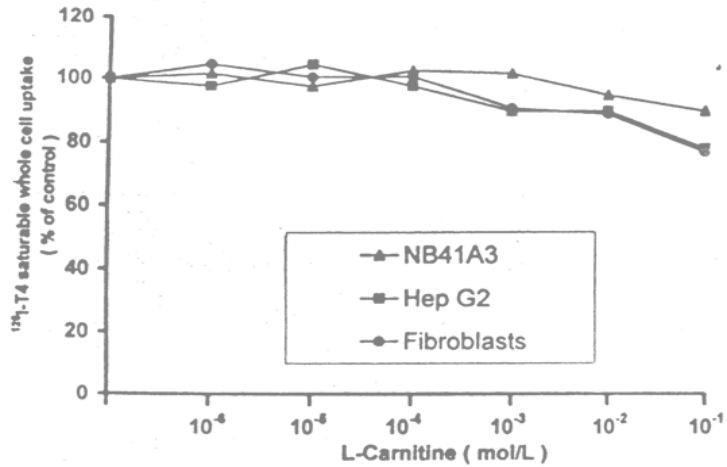
- If **carnitine** is a peripheral antagonist of thyroid hormone (TH) action, it should cause one or more of the following:
  1) ↑ the efflux (exit) of TH from cells;
  2) ↓ the entry of TH into cells at the level of the plasma membrane and/or nuclear membrane ;
  3) ↓ interaction of TH with nuclear receptors.

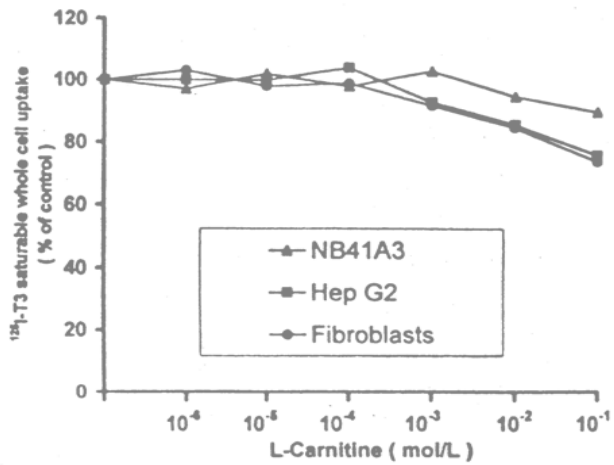

120
100
80
60
40
20
0
^{125}I-T4 whole cell efflux
(% of control)
Fibroblasts
Hep G2
NB41A3
10^{-10}
10^{-8}
10^{-6}
10^{-4}
10^{-2}
10^{-1}
L-Carnitine (mol/L)

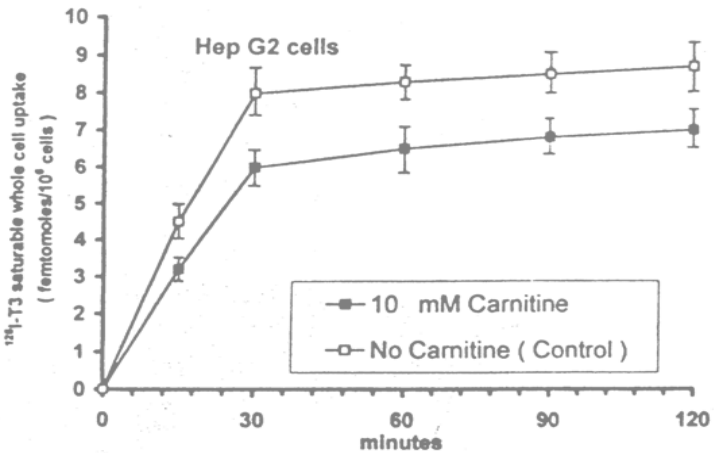

120
90
60
30
0
^{125}I-T3 whole cell efflux
(% of control)
Fibroblasts
Hep G2
NB41A3
10^{-10}
10^{-8}
10^{-6}
10^{-4}
10^{-2}
10^{-1}
L-Carnitine (mol/L)

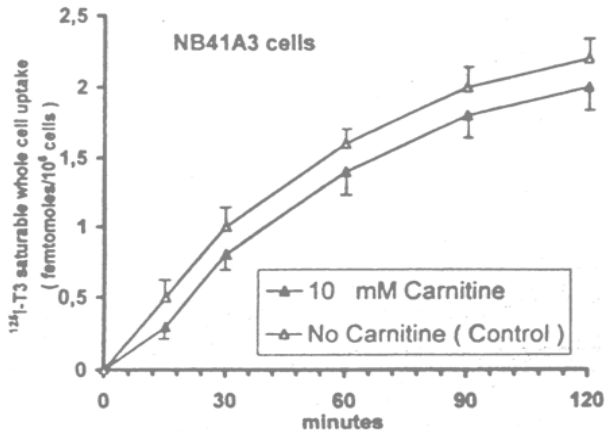

Fibroblasts
120
100
80
60
40
20
0
Fractional efflux
(% of ^{125}I-T4 internalized)
Buffer (control)
L-Carnitine (10 mM)
0
30
60
90
120
150
180
210
240
minutes

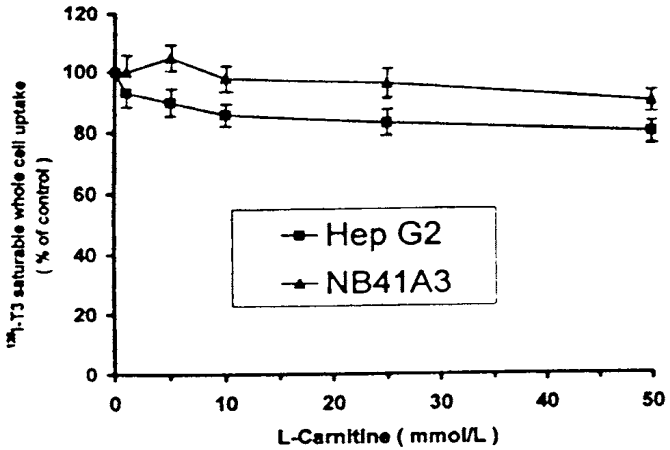

Hep G2
50
40
30
20
10
0
Fractional efflux
(% of ^{125}I-T3 internalized)
Buffer (control)
L-Carnitine (10 mM)
0
30
60
90
120
minutes

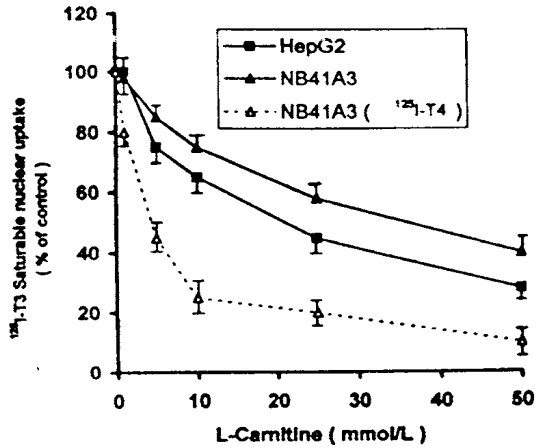

120
100
80
60
40
20
0
^{125}I-T4 saturable whole cell uptake
(% of control)
NB41A3
Hep G2
Fibroblasts
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
L-Carnitine (mol/L)

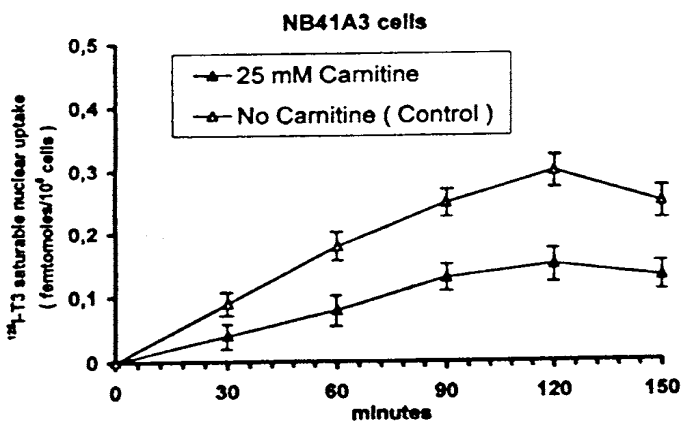

120
100
80
60
40
20
0
^{125}I-T3 saturable whole cell uptake
(% of control)
NB41A3
Hep G2
Fibroblasts
10^{-6}
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
L-Carnitine (mol/L)

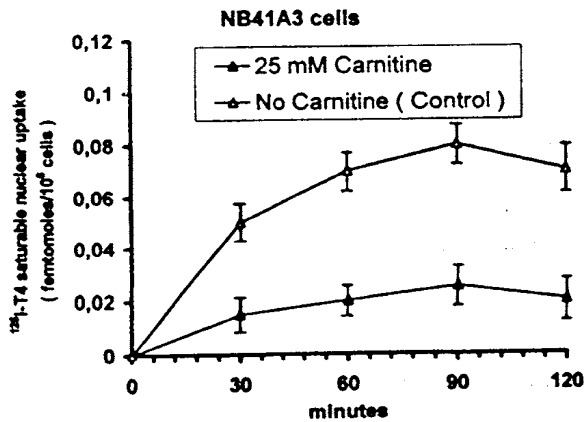

Hep G2 cells
10
9
8
7
6
5
4
3
2
1
0
^{125}I-T3 saturable whole cell uptake
(femtomoles/10^6 cells)
10 mM Carnitine
No Carnitine (Control)
0
30
60
90
120
minutes

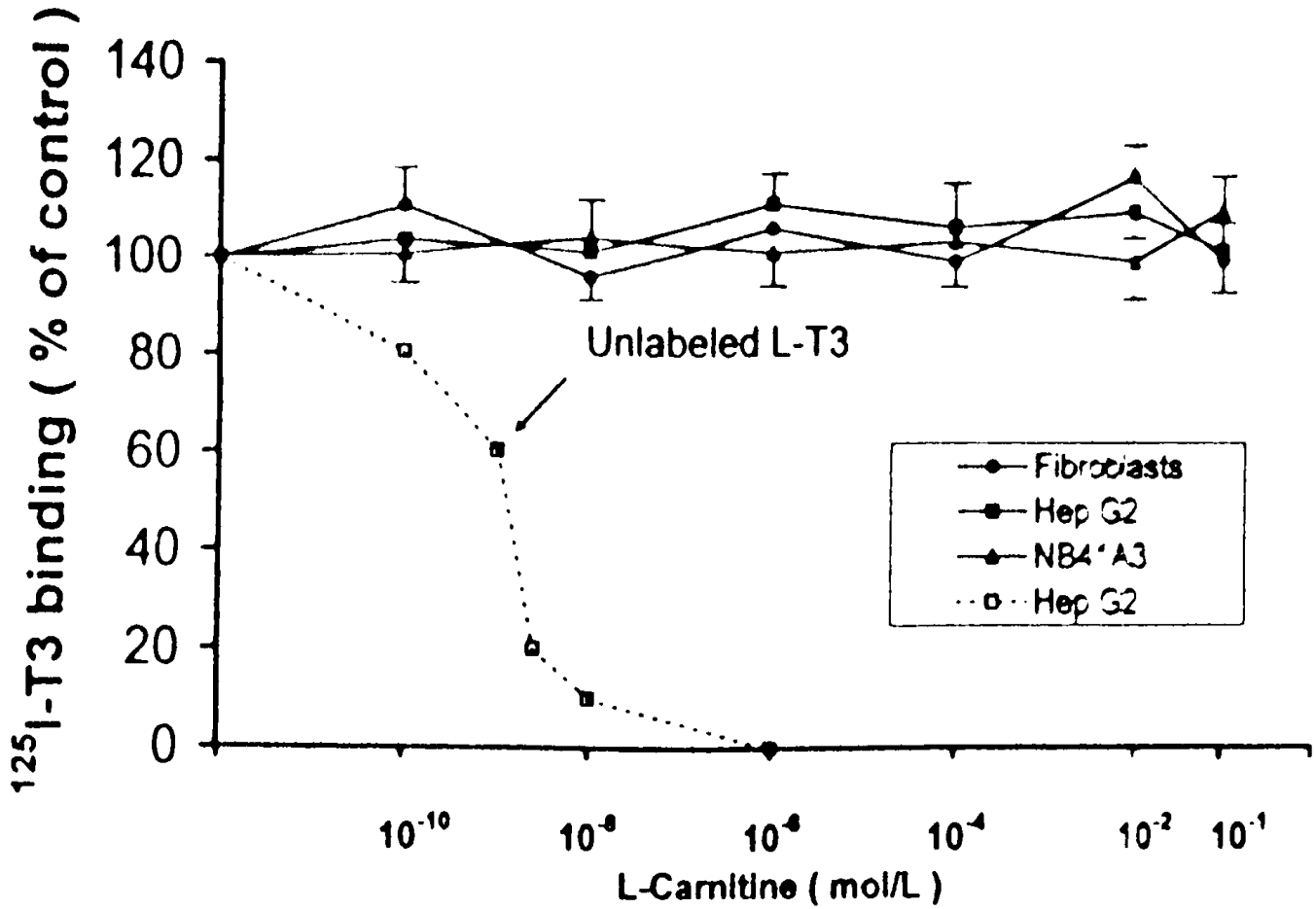

NB41A3 cells
2,5
2
1,5
1
0,5
0
^{125}I-T3 saturable whole cell uptake
(femtomoles/10^6 cells)
10 mM Carnitine
No Carnitine (Control)
0
30
60
90
120
minutes


120
100
80
60
40
20
0
0
10
20
30
40
50
L-Carnitine (mmol/L)
Hep G2
NB41A3


120
100
80
60
40
20
0
0
10
20
30
40
50
L-Carnitine (mmol/L)
HepG2
NB41A3
NB41A3 (125I-T4)


NB41A3 cells
0,5
0,4
0,3
0,2
0,1
0
0
30
60
90
120
150
minutes
25 mM Carnitine
No Carnitine (Control)


NB41A3 cells
0,12
0,1
0,08
0,06
0,04
0,02
0
0
30
60
90
120
minutes
25 mM Carnitine
No Carnitine (Control)


^{125}I-T3 binding (% of control)
140
120
100
80
60
40
20
0
Unlabeled L-T3
Fibroblasts
Hep G2
NB4 A3
Hep G2
10^{-10}
10^{-8}
10^{-6}
10^{-4}
10^{-2}
10^{-1}
L-Carnitine (mol/L)

DRUGS OTHER THAN **CARNITINE** (and antithyroid drugs) THAT HAVE BEEN USED OR COULD BE USED TO COUNTERACT HYPERTHYROIDISM

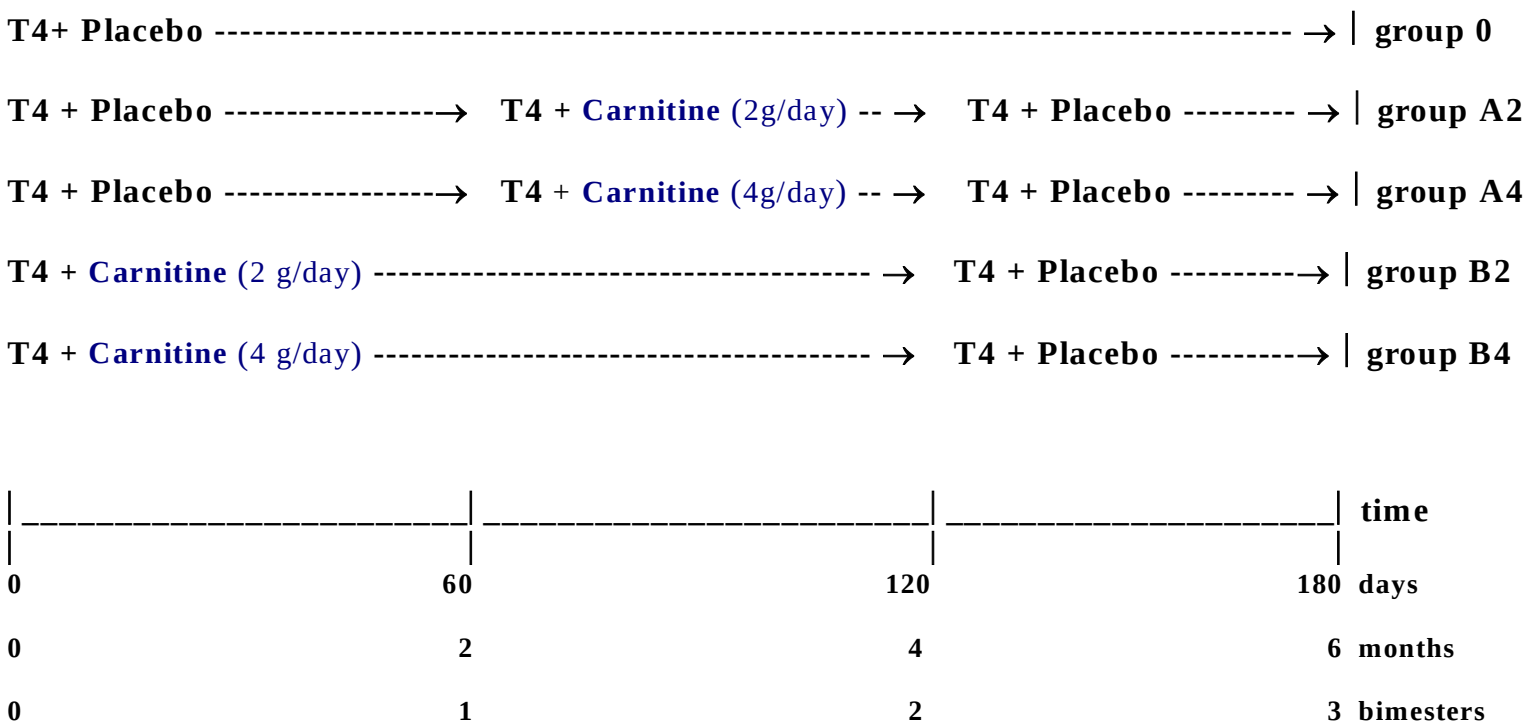
- Beta-blockers
- Benzodiazepines
- Bile sequestrants
- Corticosteroids
- Nonsteroidal antiinflammatory drugs
- Diphenylhydantoin
- Amiodarone
- Others

COST OF 2 grams/d oral **L-carnitine** as compared to other drugs used to counteract hyperthyroidism.

Drug	Cost per day (US dollars)
β-blockers	0.1 (60 mg propranolol)
Benzodiazepines	0.42-0.60 (3 mg bromazepam or 2mg lorazepam)
Carnitine	**1.30**
Bile sequestrants	5.0 (8 g cholestyramine or 20 g colestipol)

S. Benvenga et al. Usefulness of L-Carnitine, a naturally occurring peripheral antagonist of thyroid hormone action, in iatrogenic hyperthyroidism: a randomized, double-blind, placebo-controlled clinical trial. ***J Clin Endocrinol Metab 86:3579-94, 2001*****.**

STUDY DESIGN



SYMPTOMS EVALUATED IN THE 50 PATIENTS OF TRIAL

[S. Benvenga et al. J Clin Endocrinol Metab 86: 3579-94, 2001]

Symptoms	Evaluation *	Change in hyperthyroidism
Asthenia	Subjective 5-point scale (score 1 to 5)	Increase
Dyspnea	Subjective 5-point scale (score 1 to 5)	Increase
Palpitations	Subjective 5-point scale (score 1 to 5)	Increase
Nervousness	Subjective 5-point scale (score 1 to 5)	Increase
Insomnia	Subjective 5-point scale (score 1 to 5)	Increase
Tremors	5-point objective scale (score 1 to 5)	Increase
Knee reflexes	5-point objective scale (score 1 to 5)	Increase
Heart rate	Beats per min, by cardiac auscultation	Increase
Body weight	Kilograms (Kg), using the same scale	Decrease

* Patients were required to score their subjective symptoms on this 5-point scale: 1= absent; 2= occasional, but disturbing when present; 3= frequent, disturbing; 4= more frequent, more disturbing; 5= constant, intolerable. Hand tremors were evaluated on this 5-point scale: 1= absent; 2= very fine; 3= fine; 4= mildly gross; 5= overtly gross, shaking of the hands. Knee reflexes were evaluated on this 5-point scale: 1= normal; 2= barely brisk; 3= moderately brisk; 4= markedly brisk; 5= extremely exaggerated, polyphasic.

Biochemical parameters of thyroid hormone action evaluated in the 50 patients of trial.
[S. Benvenga et al. JCEM 86: 3579-94, 2001]

Parameters *	Target tissue	Change in hyperthyroidism
ALT	Liver	Increase
AST	Liver	Increase
GGT	Liver	Increase
SHBG	Liver	Increase
Ferritin	Liver	Increase
Total cholesterol	Liver	Decrease
CPK	Skeletal muscle	Decrease
Osteocalcin	Bone (osteoblasts)	Increase
Urinary OH-proline	Bone (osteoclasts)	Increase

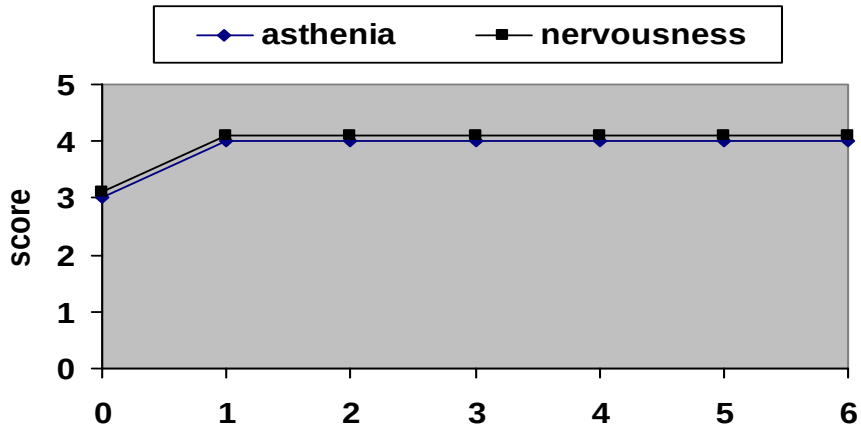
*** Abbreviations are: ALT = alanine aminotransferate; AST = aspartate aminotransferase, GGT= γ–glutamyltransferase; SHBG = sex hormone binding globulin ; CPK = creatine phosphokinase ; OH-proline = hydroxyproline.**

CURATIVE EFFECT OF CARNITINE. Mean ± SE percent changes of the nine clinical parameters * [*S. Benvenga et al. JCEM 86: 3579-94,2001].*

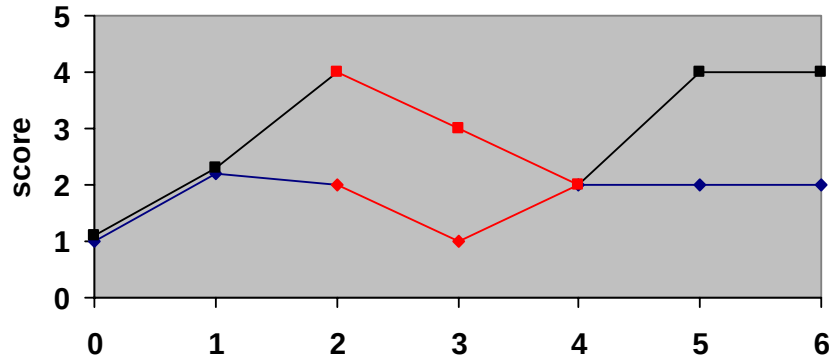
Symptoms	Group 0 (n = 10)	Group A2 (n= 10)	Group A4 (n= 10)
Asthenia	9 ± 3	- 29 ± 5	- 43 ± 2
Dyspnea	10 ± 6	-15 ± 6	-15 ± 4
Palpitations	12 ±6	- 35 ± 6	- 39 ± 3
Nervousness	18 ± 10	- 33 ± 8	- 31 ± 5
Insomnia	18 ± 11	- 29 ± 5	- 21 ± 5
Tremors	18 ± 10	- 22 ± 6	- 25 ± 5
Knee reflexes	21 ± 5	- 20 ± 5	- 17 ± 6
Heart rate	2 ± 3	- 7 ± 1	- 7 ± 1
Body weight	- 0.8 ± 0.1	- 0.5 ± 0.1	- 0.3 ± 0.2

*** Percent change refers to the second bimester score, beats per min or Kg body weight over the corresponding value of the first bimester times 100.** All comparisons between the three groups were analyzed by ANOVA, and yielded P values < 0.001, except dyspnea, palpitations, insomnia (P< 0.01) and body weight (P> 0.05). Concerning difference in carnitine dose (A2 *vs* A4), only asthenia improved significantly better (P < 0.05) at the higher dose.

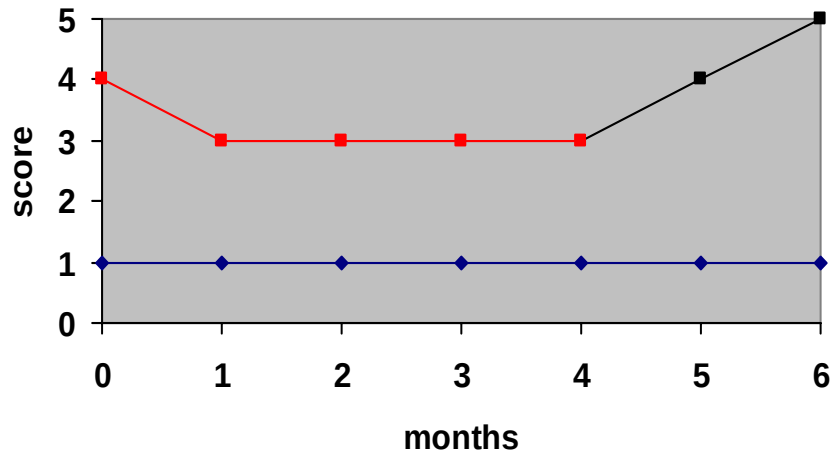
- Group 0



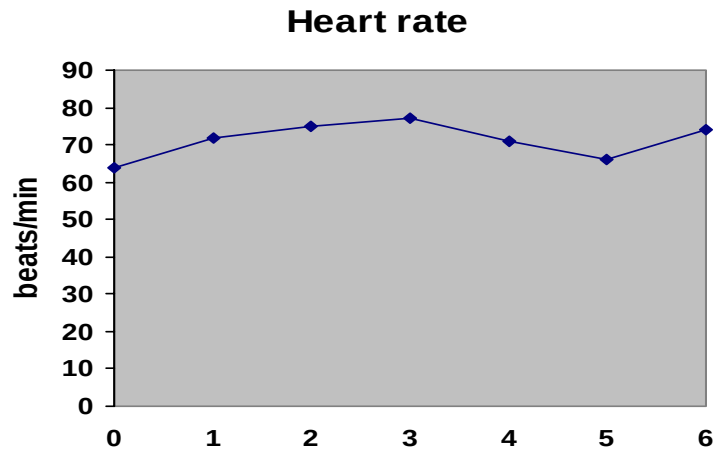
- Group A2



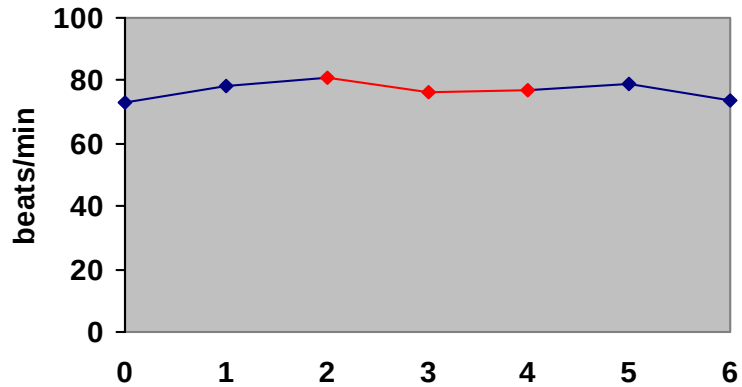
- Group B2



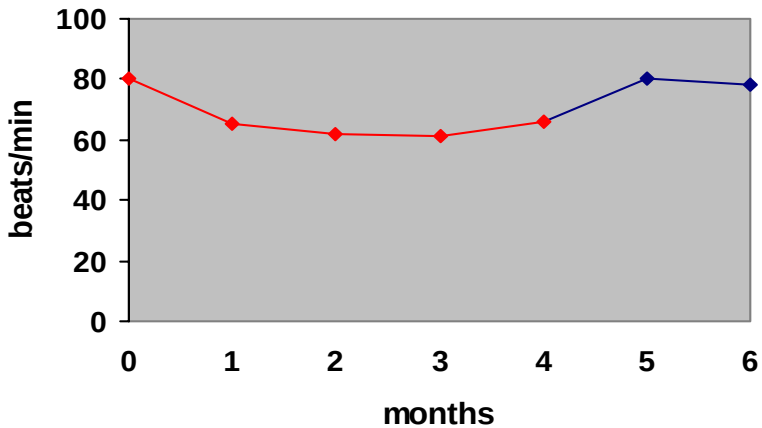
Group 0



Group A2



Group B2

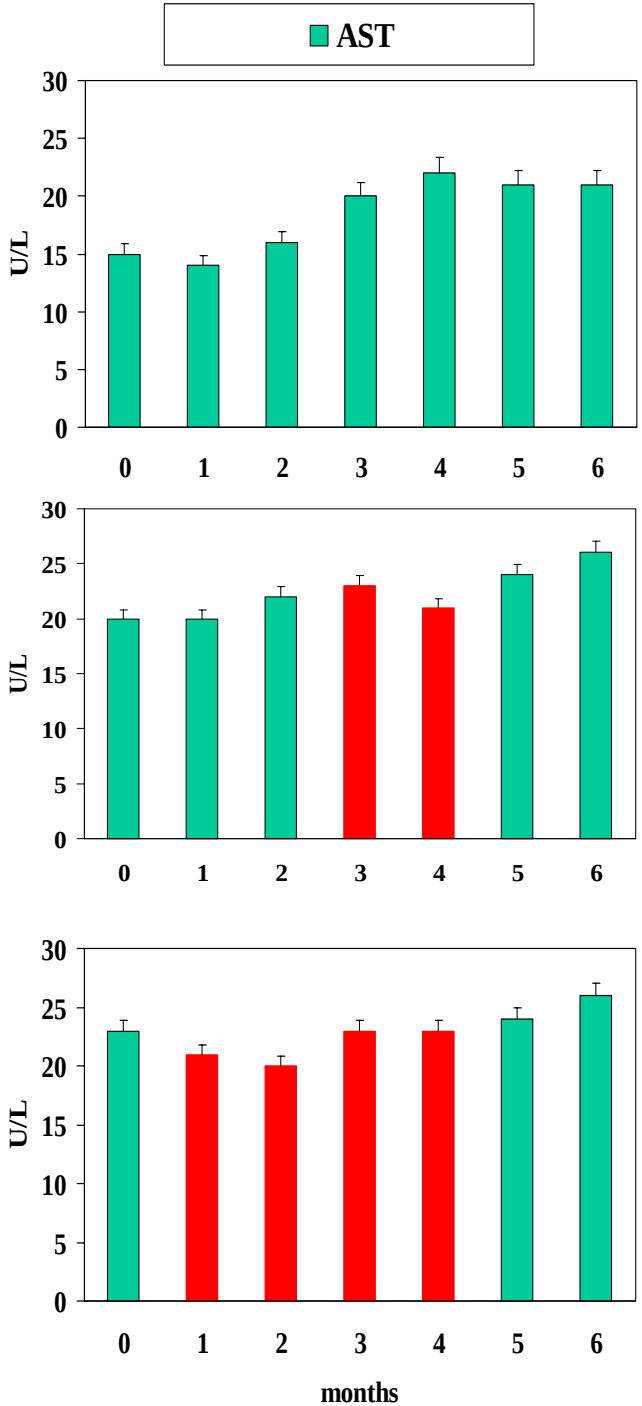


PREVENTIVE EFFECT OF CARNITINE. Mean ± SE percent changes of the nine clinical parameters * [*S. Benvenga et al. JCEM 86: 3579-94,2001].*

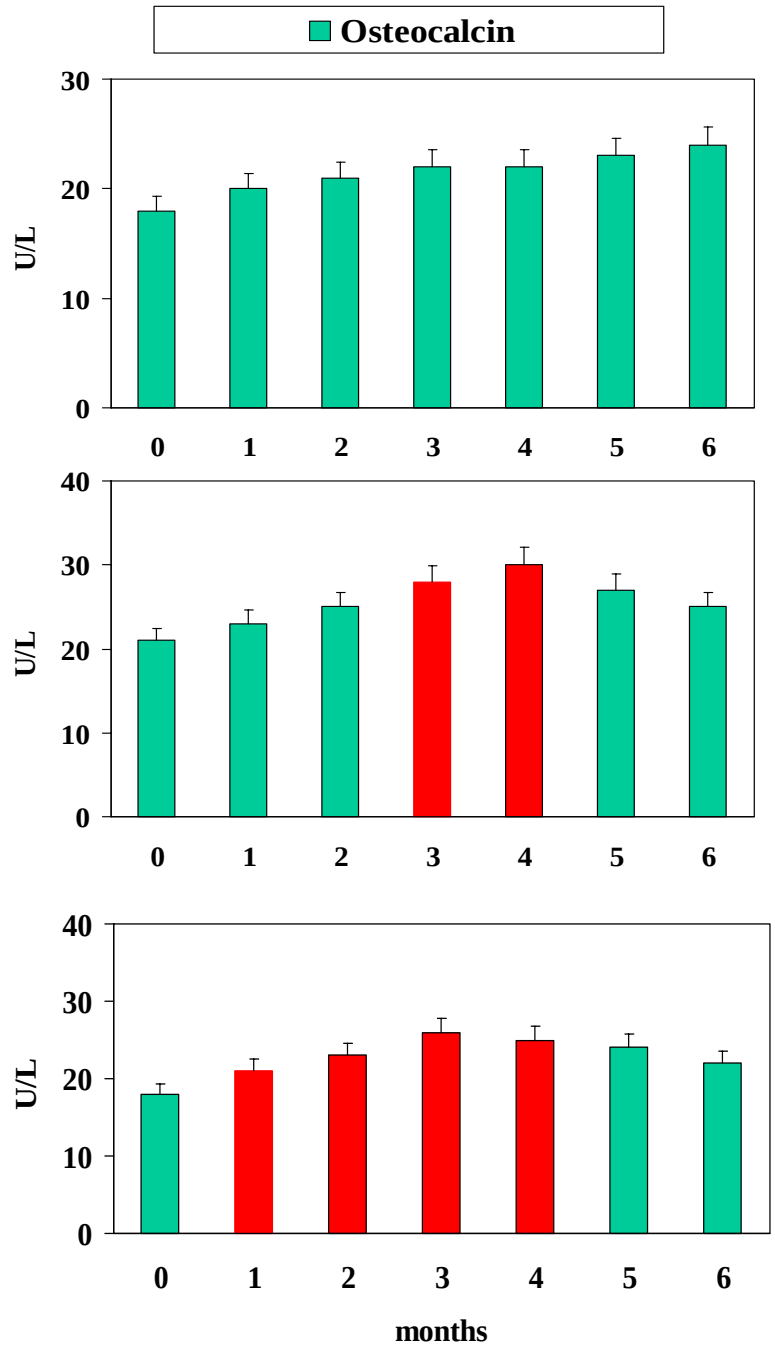
Symptoms	Group 0 (n= 10)	Group B2 (n= 10)	Group B4 (n= 10)
Asthenia	50 ± 13	- 22 ± 6	- 32 ± 4
Dyspnea	25 ± 10	- 10 ± 5	- 11 ± 5
Palpitations	52 ± 13	- 18 ± 6	- 23 ± 6
Nervousness	62 ± 11	- 26 ± 6	- 24 ± 7
Insomnia	37 ± 12	- 12 ± 5	- 23 ± 6
Tremors	40 ± 12	7 ± 8	12 ± 8
Knee reflexes	37 ± 9	- 1 ± 5	6 ± 9
Heart rate	10 ± 1	- 3 ± 1	- 4 ± 1
Body weight	- 0.8 ± 0.2	- 0.6 ± 0.1	- 0.5 ± 0.1

*** percent change refers to the pooled first two bimester value over the corresponding baseline (day 0) value. All comparisons between the three groups were analyzed by ANOVA, and yielded P values < 0.001, except dyspnea, reflexes (P< 0.01), tremors (P < 0.05) and body weight (P> 0.05). There was no statistical difference (P > 0.05) in carnitine dose (B2 *vs* B4) for any symptom**

- Group 0
- Group A2
- Group B2



- Group 0
- Group A2
- Group B2



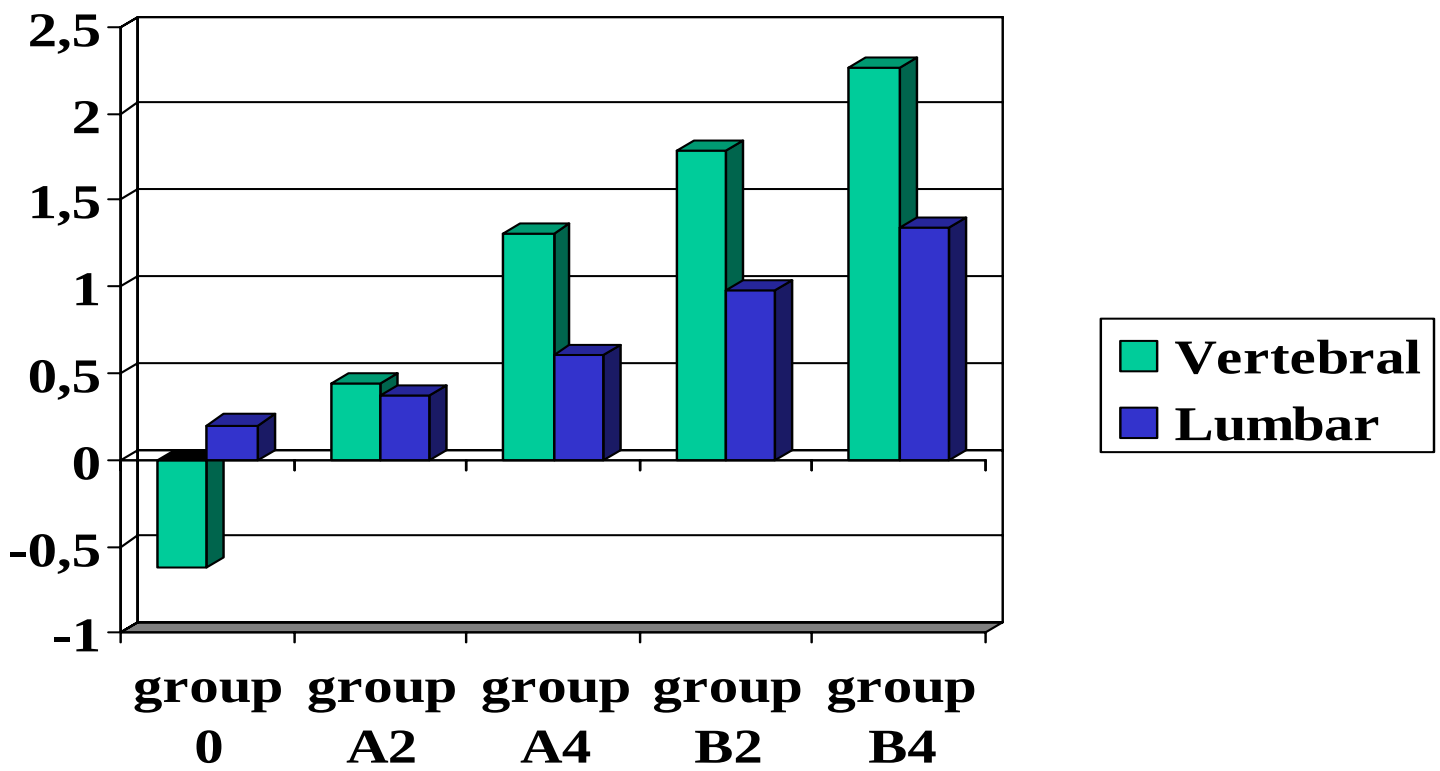
OSTEOCALCIN LEVELS (ng/ml; m ± SE) IN 3 to 7 MALE RATS TREATED FOR 6 or 12 WEEK WITH PLACEBO OR L-CARNITINE *[Abdenabi A et al. National Association of Geriatrics, Washington, D.C., November 1992 (poster)]*

	Dose (mg/kg/d)	6 weeks	12 weeks
Control	0	124 ± 8 ng/ml	96 ± 2 ng/ml
L-Carnitine	50	151 ± 40 ng/ml *(P< 0.05)*	153 ± 2 ng/ml *(P < 0.01)*
L-Carnitine	100	199 ± 7 ng/ml *(P < 0.01)*	115 ± 8 ng/ml *(P < 0.05)*

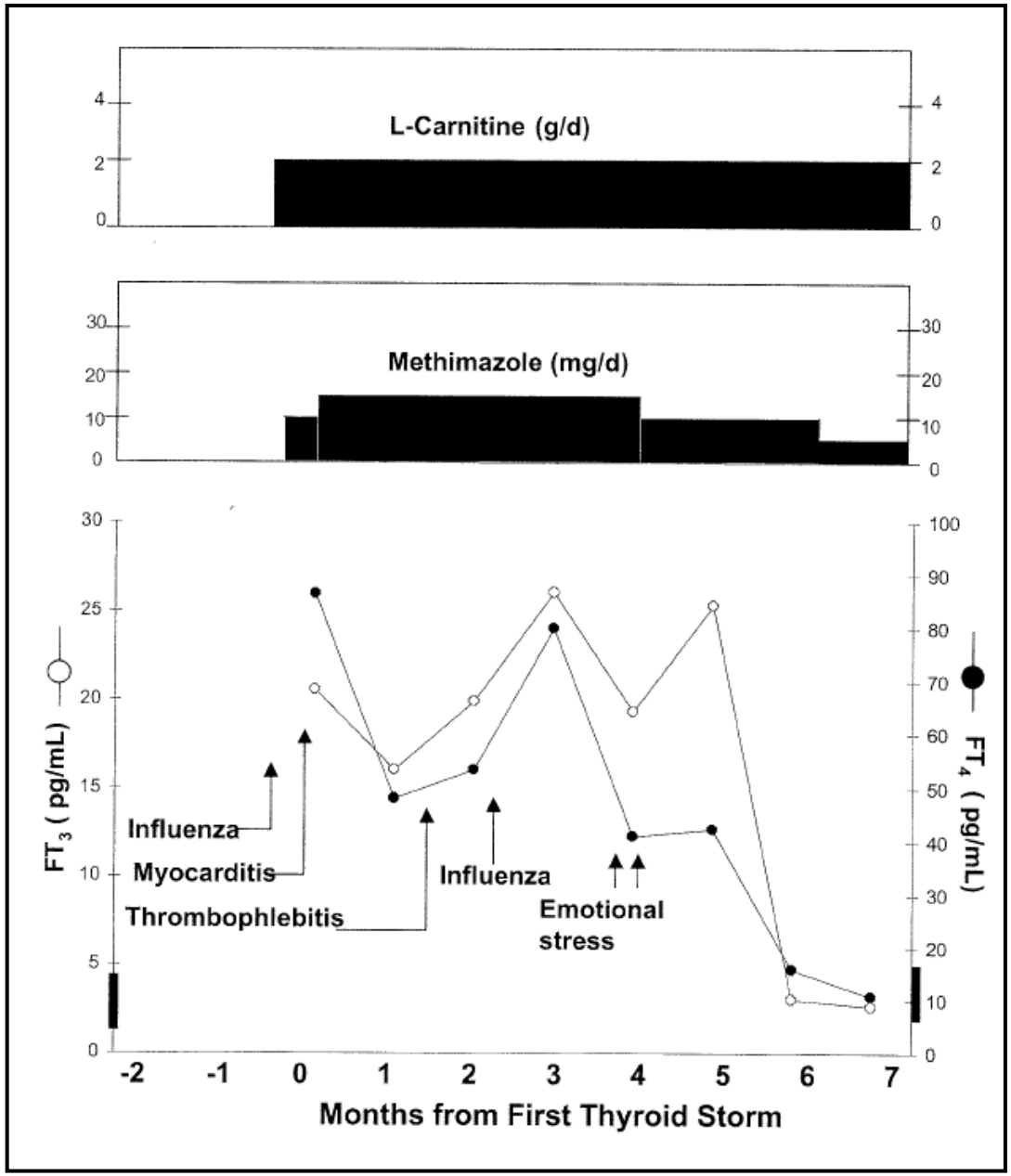
STIMULATION OF ALKALINE PHOSPHATASE ACTIVITY IN OSTEOBLAST-LIKE CELLS BY L-CARNITINE *[Chiu KM et al. J. Bone Miner Res 9 (suppl 1), S-354, 1994]*

- **"An osteoblast enriched population was obtained from young pig and adult Rhesus monkey femoral bone marrow stromal cells. … ALP activity , a marker of bone formation, was measured …**
  ALP activity from pig cell culture with 10^{-1} M L-carnitine were 2-fold higher than control *($p < 0.005$)*. ALP activity from monkey culture with 10^{-1} M L-carnitine were 1.6-fold above control *($p < 0.01$)*".

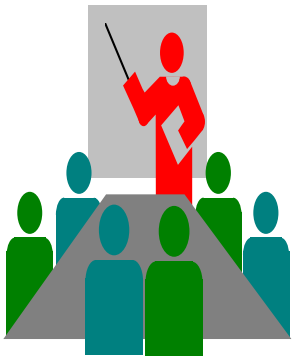
% CHANGES IN BONE MINERAL DENSITY (BMD) AT THE END OF CLINICAL TRIAL WITH RESPECT TO BASELINE. *[S Benvenga et al. JCEM 86: 3579-94, 2001]*



Successive thyroid storms treated with L-Carnitine and low doses of Methimazole (Benvenga S. et al., *Am J Med 115:417-418)*

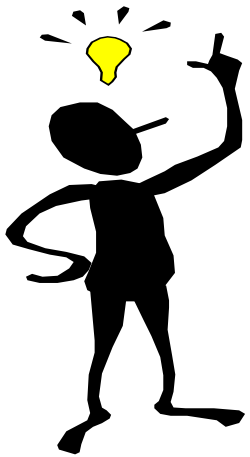


CONCLUSIONS (Highlights)



- Peripheral antagonism of **Carnitine** on thyroid hormone (TH) action is supported by:
- lack of “central” antagonism (TSH)
- inhibition of TH (both T3 and T4) entry into cell nuclei (quantitative cell difference exists).
- human studies on iatrogenic hyperthyroidism and naturally occurring thyroid storms. Antagonism is seen on both clinical and biochemical parameters of TH action.
- However, some peripheral targets are insensitive to carnitine inhibition, while others are potentiated by **carnitine**.

FUTURE RESEARCH DIRECTIONS



- To exploit the diverse effect of **carnitine** on peripheral targets of thyroid hormone for therapeutically useful purposes.
  For instance, a) prevention of osteoporosis in post-menopausal women who depend on life-long TSH-suppressive L-T4 therapy for the management of thyroid cancer.
  b) prophylaxis and/or ancillary therapy of serious hyperthyroidism in patients (especially the elderly ones) in whom amiodarone is the sole effective anti-arrhytmic drug.

ACKNOWLEDGEMENTS

All this work would have not been possible without the support from the friends at Sigma-Tau:

Tony Amato
Menotti Calvani
Luigi Mosconi
Carlo Trevisani