



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2434962 A1 2002/06/27

(21) **2 434 962**

(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2001/12/04
(87) Date publication PCT/PCT Publication Date: 2002/06/27
(85) Entrée phase nationale/National Entry: 2003/07/14
(86) N° demande PCT/PCT Application No.: IT 2001/000614
(87) N° publication PCT/PCT Publication No.: 2002/049639
(30) Priorité/Priority: 2000/12/21 (RM2000A000688) IT

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 31/22
(71) Demandeur/Applicant:
SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE
S.P.A, IT
(72) Inventeurs/Inventors:
KOVERECH, ALEARDO, IT;
ZALLONE, ALBERTA, IT
(74) Agent: FETHERSTONHAUGH & CO.

(54) Titre : UTILISATION DE L'ISOVALERYLE L-CARNITINE POUR LA PREPARATION D'UN MEDICAMENT DESTINE
A LA PREVENTION ET AU TRAITEMENT DE L'OSTEOPOROSE
(54) Title: USE OF ISOVALERYL L-CARNITINE FOR THE PREPARATION OF A MEDICAMENT FOR THE
PREVENTION AND CURE OF OSTEOPOROSIS

(57) **Abrégé/Abstract:**

The use of isovaleryl L-carnitine for the preparation of a medicament for the prevention and cure of osteoporosis and for the prevention and cure of fractures occurring during osteoporosis post-menopause or during the ageing, is described.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
27 June 2002 (27.06.2002)

PCT

(10) International Publication Number
WO 02/49639 A1

(51) International Patent Classification⁷: **A61K 31/22**

(21) International Application Number: PCT/IT01/00614

(22) International Filing Date: 4 December 2001 (04.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
RM2000A000688
21 December 2000 (21.12.2000) IT

(71) Applicant (for all designated States except US):
SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE S.P.A. [IT/IT]; Viale Shakespeare, 47, I-00144 Rome (IT).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KOVERECH, Aleardo** [IT/IT]; c/o Sigma-Tau Industrie Farmaceutiche Riunite S.p., a., Via Pontina, km 30,400, I-00040 Pomezia (IT). **ZALLONE, Alberta** [IT/IT]; Viale Venezia, 15, I-70123 Bari (IT).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,

GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for all designations
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for the following designation US
- of inventorship (Rule 4.17(iv)) for US only

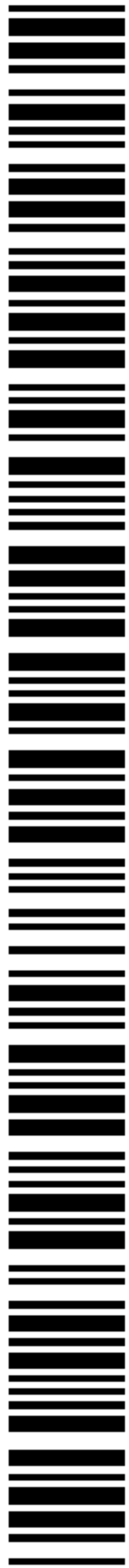
Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: USE OF ISOVALERYL L-CARNITINE FOR THE PREPARATION OF A MEDICAMENT FOR THE PREVENTION AND CURE OF OSTEOPOROSIS

(57) Abstract: The use of isovaleryl L-carnitine for the preparation of a medicament for the prevention and cure of osteoporosis and for the prevention and cure of fractures occurring during osteoporosis post-menopause or during the ageing, is described.



WO 02/49639 A1

**Use of isovaleryl L-carnitine for the preparation of a
medicament for the prevention and cure of ^{FRACTURES} osteoporosis.**

The present invention relates to the use of isovaleryl L-carnitine for the preparation of a medicament for the prevention and
5 cure of osteoporosis, ~~for the prevention and cure of fractures,~~
~~occurring during post-menopause osteoporosis or during ageing.~~

Aged people or people which are in post-menopause are subjected to fractures due to the fragility of the bone caused by osteoporosis.

10 The hospitalisation period of patients with bone fractures, which may last some day or some months, depends from the type of fracture, the age, and from the general conditions of the patients.

The convalescence period depends on the gravity of the pathology. At the end of this period patients come back to the
15 hospital for the radiographic controls of the bony callus formation.

Unfortunately not all patients present the same degree of recovery, in fact some of them present a minor, some time a complete lack of bony callus formation. Because of these events patients are obliged to remain inactive for a further period of time,
20 with a loss of working day and an increases of the hospital expenses.

To date exist few specific therapeutical compounds which act directly on the bone cells, the osteoblasts, favouring in a specific way their propagation with formation of bony callus and wound healing.

In the orthopaedic section of the hospital, as adjuvant therapy
25 of the fractures are used calcitonine or alendronates, drugs specific

WO 02/49639

PCT/IT01/00614

2

for the prevention of the post-menopausal osteoporosis, eventually in combination with vitamins, particularly with vitamin D and mineral salts, which act limiting the osteoclastic activity.

Therefor, the discovery of new drugs which exert their action
5 directly on the osteoblasts and are capable both to support their multiplication and the bony callus formation, and to decrease the recovering period, would have a beneficial effect on the patients.

Considering the relevant number of patients involved, it is evident both the socio-economics benefit obtainable, reducing the
10 hospital expenses, increasing the working day during the year, and particularly improving the quality life of the patients.

Is therefore the scope of the present invention to furnish a drug, which overcoming the drawbacks and the unsatisfactory efficacy of the therapeutical compounds used to date, is capable to
15 ~~limits the osteoclastic activity accelerating the formation of bony~~ matrix (stimulating the osteoblastic activity) and is an useful agent for the prevention and treatment of FRACTURES ~~osteoporosis~~.

Compounds useful for the cure of fractures ~~and osteoporosis~~ are already known.

20 WO 98/46233 describes a pharmaceutical compositions comprising an alkanoyl L-carnitine in combination with the dehydroepiandrosterone (DHEA) or the dehydroepiandrosterone sulphate (DHEA-S) capable to stimulate the multiplication and growth of the osteoblast.

WO 99/66913 describes compositions useful for the treatment of the osteoporosis and menopausal syndromes which comprise propionyl L-carnitine in combination with 4'-5,7-trihydroxyflavone.

The patent FR 2765804 describes compositions useful for the treatment of osteoporosis containing calcium pidolate, calcium carbonate, and carnitine.

WO 98/01128 describes the use of acetyl L-carnitine, isovaleryl L-carnitine and propionyl L-carnitine for increasing the IGF-1 levels for the treatment and prevention of cytological disorders and pathologies related to IGF 1.

WO 95/05168 describes an oral medicament at controlled release comprising carnitine, which does not causes gastrointestinal irritation, for the treatment of the osteoporosis.

In Journal of Pharmaceutical Sciences Vol. 84; N° 4; April 1995 is described the use of long chain acyl carnitine ester in racemic form (palmitoyl-carnitine, miristoyl-carnitine and lauroyl-carnitine), as useful agent for the prevention of the osteoporosis.

In "Comptes rendus des séances de la Société de Biologie. Seance du 27 Juin 1964.- Tome CLVIII, n 6, 1964, p. 1410" is reported that in rat the use of D,L-carnitine increases the bone calcium.

In The Journal of Rheumatology 1999, 26; 2229-32 is described the use of carnitine, in subject adult, for the treatment of the hip fracture.

WO 02/49639

PCT/IT01/00614

4

In Revue Aggressologie 1963 – IV., 4, is described the use of carnitine during the growth or following fractures.

In Gerontologist, (9), 32-33, 1992 is reported that in mice treated with L-carnitine increase the production of osteocalcine (index of better calcification), while in the same animals the treatment with acetyl L-carnitine reduces the production of this marker during the formation of the bone matrix.

None of the above cited publications teach or suggest the use of isovaleryl L-carnitine for the preparation of a medicament for the FRACTURES treatment of osteoporosis.

Moreover, the cited publication on Gerontologist gives a severe technical prejudice on the use of a short chain alkanoyl L-carnitines for promoting the production of bone matrix; in fact it is reported that acetyl L-carnitine to reduces the production of osteocalcine.

~~It has surprisingly been found that isovaleryl L-carnitine promotes the formation and proliferation of osteoblasts and is a therapeutic agent useful for the prevention and treatment of osteoporosis and for the prevention and treatment of fractures during post-menopausal osteoporosis or secondary to elderly.~~

The experimental data, in the following reported, show that the compound according to the invention promotes cells proliferation and collagen production in human osteoblastic cells *in vitro*. In fact, isovaleryl L-carnitine shows to be more active than L-carnitine, or as active as, but a doses less than 10 or 20 times, respect to L-carnitine.

AMENDED SHEET

Is therefore object of the present invention the use of isovaleryl L-carnitine or a pharmacological acceptable salt thereof, for preparing a medicament useful for increasing the healing of fractures, ~~and useful for the prevention and treatment of~~
5 ~~osteoporosis.~~

For pharmaceutically acceptable salt of isovaleryl L-carnitine is intended any salts of this with an acid which does not give rise to undesirable toxic or side effects.

This acids are well known to the experts in pharmacy and
10 pharmaceutical technology.

Non-limiting examples of suitable salts include the chloride, bromide, orotate, acid aspartate, acid citrate, magnesium citrate, acid phosphate, fumarate, acid fumarate, magnesium fumarate, lactate, maleate, acid maleate, mucate, acid oxalate, pamoate, acid
15 pamoate, acid sulphate, glucose phosphate, tartrate, acid tartrate, magnesium tartrate, 2-amino etanesulfonate, magnesium 2-amino etanesulfonate, coline tartrate and trichloroacetate.

Preferred is isovaleryl L-carnitine fumarate.

The compound according to the present invention can be
20 administered to patients in need, either alone or in concomitance with vitamins, mineral salts and/or other known drugs useful for the treatment of ~~osteoporosis and fractures~~ during osteoporosis.

The following example illustrate the invention.

EXAMPLE

Preparation of the human osteoblasts

The differentiated human osteoblasts were prepared starting
5 from bone fragments obtained during surgical operation following
the procedure described in Calcif. Tissue Int. 62: 362-365, 1998;
and in Calcif. Tissue Int. 37: 453-460, 1985.

In short, bone fragments were undertaken to enzymatic
digestion with collagenase (1 mg/ml) diluted in phosphate buffer in
10 order to remove fibroblasts, haematic endothelial cells or bone
marrow cells, if any.

The fragments so cleaned were maintained in culture in
presence of a medium containing 20% of foetal calf serum (FCS) to
obtain osteoblasts population for explant.

15 The cells so obtained were characterised to verify their
osteoblastic phenotype then used for the experiments.

Cellular proliferation test

The cellular proliferation test was done growing the cells in 96
well plates, in medium without FCS, and with bovine serum albumin
20 (BSA).

The cells were exposed to increasing doses of L-carnitine
fumarate and isovaleryl L-carnitine fumarate. The positive control
group was constituted by cells growing in presence of FCS (15%).

Every 24 ore, for 5 days, samples from plates in culture were
25 fixed with a solution of paraformaldehyde at 3%, the cells were

stained with crystal-violet at 0,5%, washed to eliminate the excess of stain and a solution of sodium citrate 0,1 M in ethanol was added. The number of the cells contained in the wells was evaluated by colorimetric test at 540 nm.

5 The results obtained, reported in Figure 1, shows that isovaleryl L-carnitine fumarate promotes cells proliferation.

Moreover, the results obtained reported in Figure 1 (isovaleryl L-carnitine fumarate) in comparison with the results reported in Figure 2 (L-carnitine fumarate) shown that isovaleryl L-carnitine fumarate induces cells proliferation (Figure 1) in a more potent way, if compared with the cells proliferation induced by L-carnitine fumarate (Figure 2).

In fact, it is worthy of note that in Figure 1 isovaleryl L-carnitine fumarate at a dose (10^{-3} M) 10 times lower than that of L-carnitine fumarate (10^{-2} M) has the same cellular proliferation activity compared to the activity of L-carnitine fumarate (Figure 2). The statistical analysis of the results was made using the Student "t" test.

Production of collagen by incorporation of ^3H -proline

20 The collagen production represents a method for the evaluation of the osteoblast activity.

This production was evaluated on semiconfluent cells grown on 24 wells plates, in medium without serum (FCS) containing 1 μCi /well of ^3H -proline, both in the wells of the control and in the wells where were present the tested compounds. After 4 hours of

incubation in presence of the tracer the cells were lysed and undertaken to an extraction procedure (Journal of Bone and Mineral Research, 2000; Vol. 15(2); 188-97). The cells were washed with phosphate buffer (PBS) dissolved in 0,5 ml/well of sodium dodecyl sulphate at 10% and undergone to precipitation for 30 minutes at 4°C by the addition of sulphate trichloroacetic acid at 3%. The radioactivity of the cells, as index of the cells collagen synthesis, was evaluated with a beta-counter.

The results obtained reported in Figure 3 shown that isovaleryl L-carnitine fumarate increases the production of collagen in an higher way respect to the control sample, in which the tested compound was absent.

Moreover, the results obtained reported in Figure 3 shown that isovaleryl L-carnitine fumarate has a favouring activity for the production of collagen in a significant way respect to the same doses of L-carnitine fumarate. The statistical analysis of the results was made using the Student "t" test.

As above mentioned, the results obtained shown that the compound according to the present invention promotes in a statistical significant way the proliferation and the collagen production in human osteoblastic cells *in vitro* respect to the control samples, in which said compounds was absent. Moreover, isovaleryl L-carnitine shows to be more active in some experiments, and as active as, at doses at least 10 times lower, also respect to the reference compound (L-carnitine).

The statistical analysis of all the results was made using the Student "t" test.

The amount of the drug to be administered to patients in need will be decided by the physician on the base of the age, weight and
5 general conditions of the patient.

However, the unitary dosage to be administered will be about 0,5-3 g/day, preferably 1-2 g/day, in solid form, powder, granular or liposomic in pills, capsules, granulates, powder, vials for oral, parenteral, rectal or topic use.

10 These administration forms may further contain vitamins, mineral salts, and/or other known drugs useful for the treatment of the osteoporosis and fractures during osteoporosis.

WO 02/49639

PCT/IT01/00614

10

CLAIMS

1. Use of isovaleryl L-carnitine or a pharmaceutical acceptable salt
USEFUL FOR INCREASING THE HEALING OF FRACTURES.
thereof for preparing a medicament ~~for the prevention and~~

5 ~~treatment of osteoporosis.~~

OF ISOVALERYL L-CARNITINE OR A PHARMACEUTICAL ACCEPTABLE SALT THEREOF

2. Use ~~according to claim 1,~~ for the prevention and treatment of
fractures during osteoporosis.

3. Use according to claim 1, or 2, in which the pharmaceutical
acceptable salt is selected from the group consisting of: chloride,

10 bromide, orotate, acid aspartate, acid citrate, magnesium citrate,
acid phosphate, fumarate, acid fumarate, magnesium fumarate,
lactate, maleate, acid maleate, mucate, acid oxalate, pamoate,
acid pamoate, acid sulphate, glucose phosphate, tartrate, acid
tartrate, magnesium tartrate, 2-amino etanesulfonate,

15 ~~magnesium 2-amino etanesulfonate, coline tartrate and~~
trichloroacetate.

4. Use according to claims 1-3, in which the salt is fumarate.

5. Use according to any preceding claims further comprising
vitamins, mineral salts and/or other known drugs useful for the
20 treatment of osteoporosis and fractures during osteoporosis.

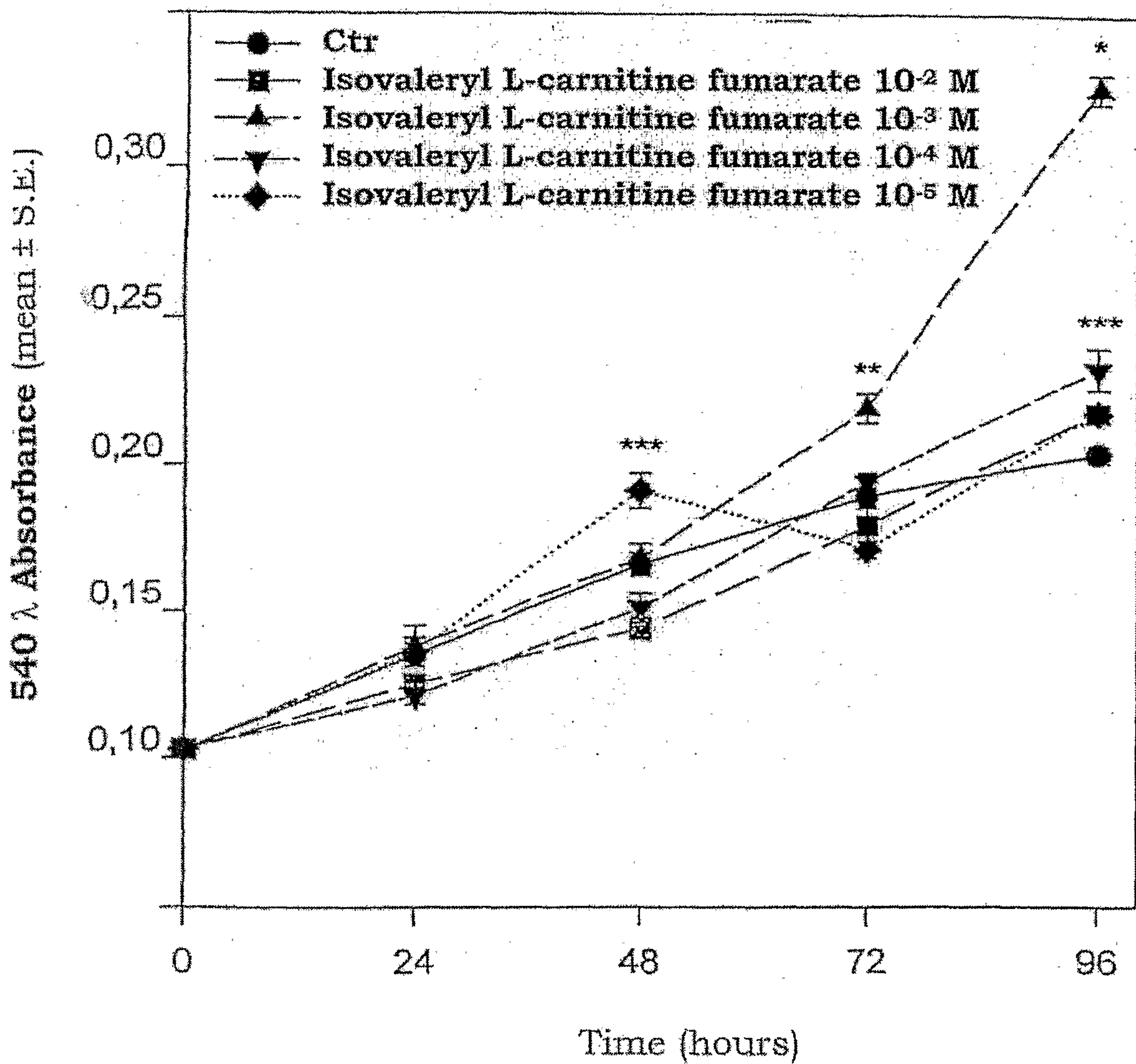
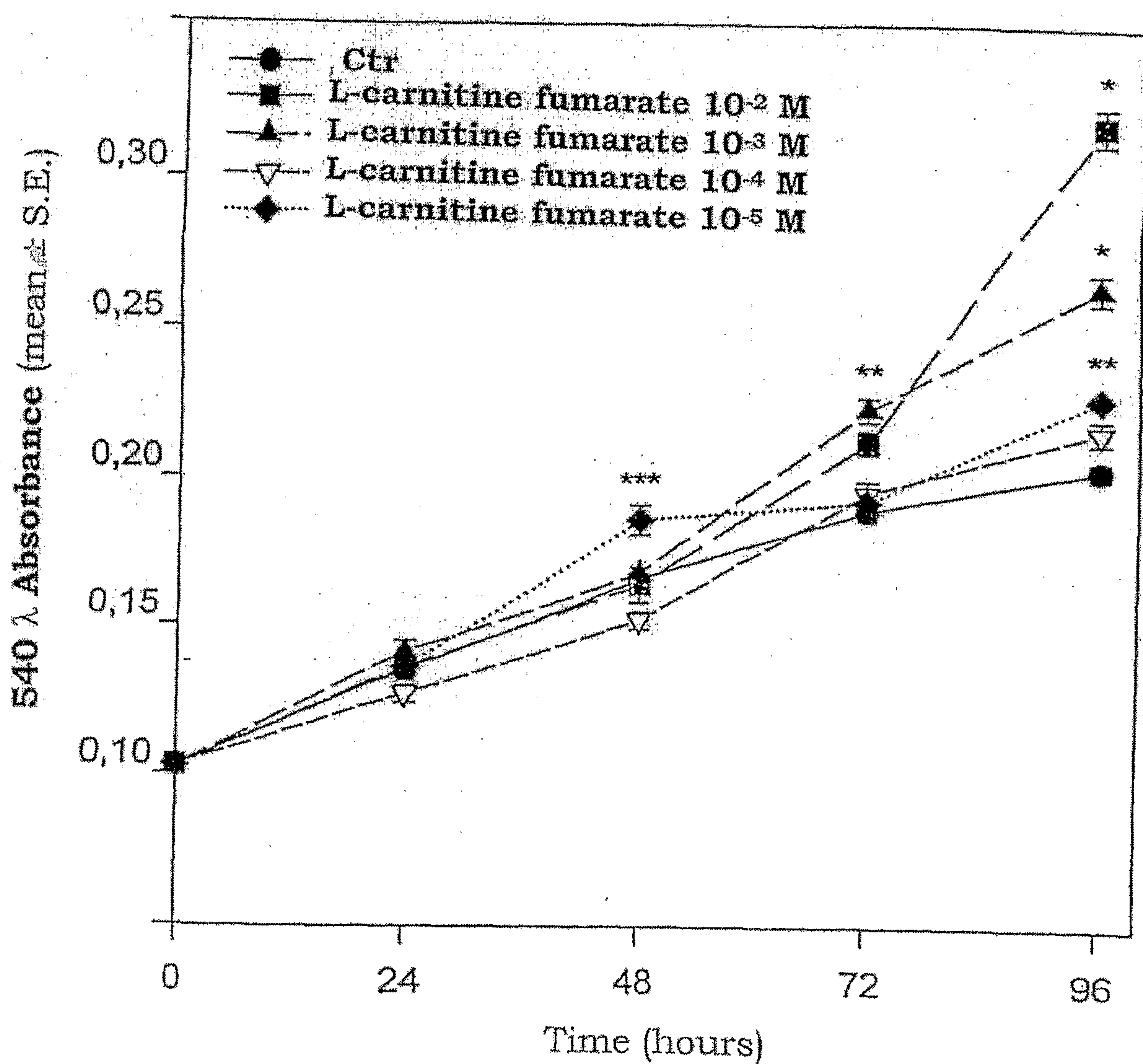
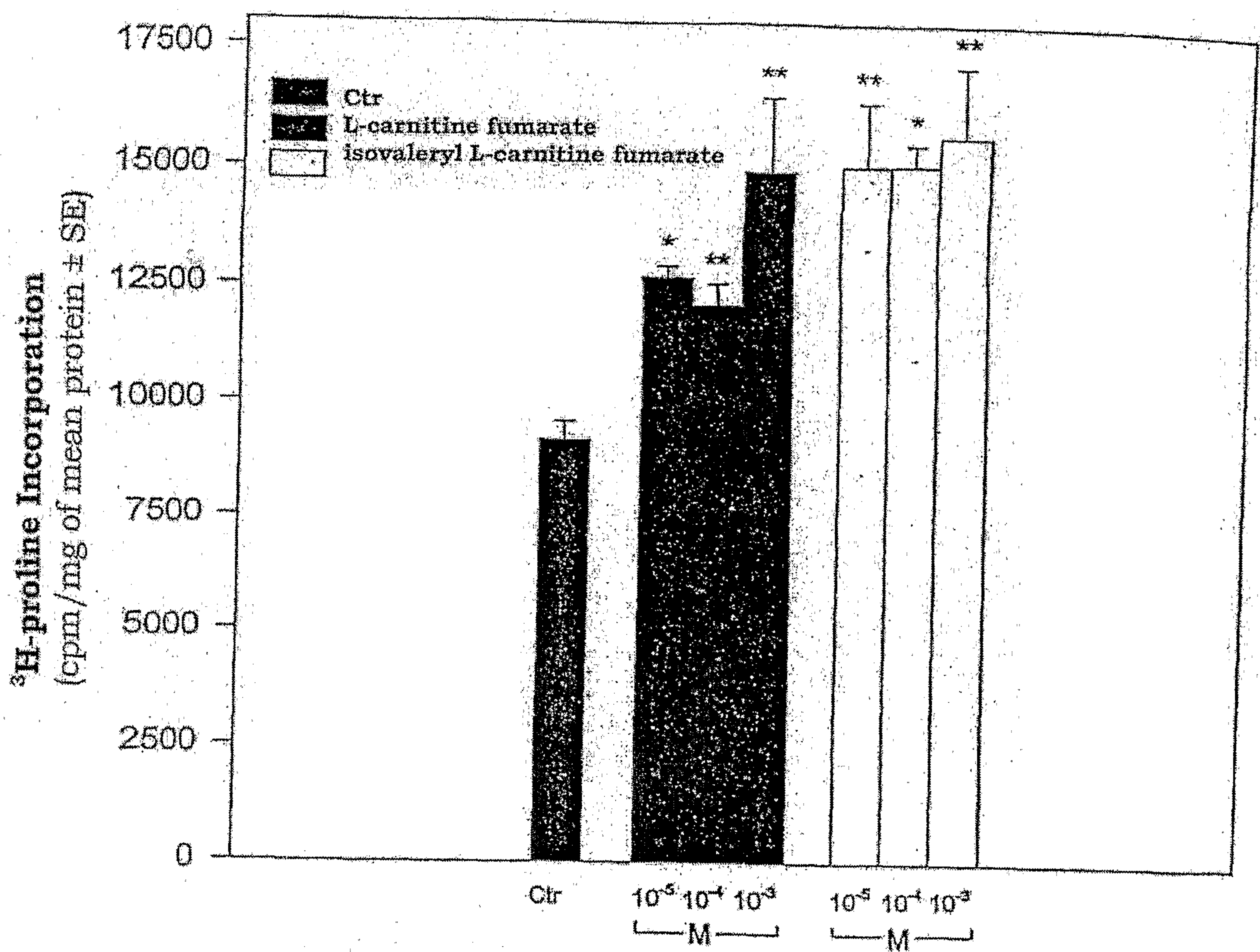
FIGURE 1**CELL PROLIFERATION**

FIGURE 2**CELL PROLIFERATION**

* $p < 0,0001$ vs. Ctr

** $p < 0,001$ vs. Ctr

*** $p < 0,04$ vs. Ctr

FIGURE 3 **^3H -proline incorporation**

* p < 0,001 vs. Ctr

** p < 0,02 vs. Ctr