Stimulation of CPT-1 as a Means to Reduce Weight

BACKGROUND

Field of the Invention

This invention is directed to a method for development of therapeutics that selectively enhance fatty acid oxidation, increase energy production, and reduce adiposity while preserving lean mass, through the pharmacological stimulation of CPT-1 activity.

Review of Related Art

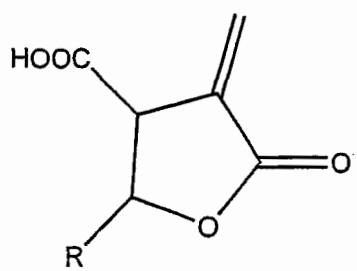
Cerulenin treatment of MCF-7 human breast cancer cells *in vitro* significantly inhibits fatty acid oxidation, probably through increased levels of malonyl-CoA (Loftus, et al. (2000) *Science*, **288:**2379-2381). C75 is a member of a family of α-methylene-γ-butyrolactones which are known inhibitors of fatty acid synthase (FAS) (Kuhajda, et al. (2000) *Proc. Natl. Acad Sci USA*, **97:**3450-3454). Treatment of mice with C75 leads to inhibition of hepatic fatty acid synthesis and high levels of malonyl-CoA (Loftus, et al. (2000); Pizer, et al. (2000) *Cancer Res.*, **60:**213-218). In the brain, C75 reduces the expression of hypothalamic neuropeptide-Y (NPY) leading to reversible inanition (Loftus, et al, 2000). During in vivo treatment of ob/ob mice with C75 there was profound loss of fat in the liver and peripheral tissues despite the increased levels of hepatic malonyl-CoA (Loftus, et al., 2000).

Malonyl-CoA is a potent inhibitor of fatty acid oxidation through its action as an inhibitor of carnitine-palmitoyl-transferase-1 (CPT-1) (Witters, et al. (1992) *J. Biol. Chem.*, **267:**2864-2867). CPT-1 enables the entry of long-chain acyl-CoA's into the mitochondria for fatty acid oxidation. When treated with FAS inhibitors, genetically and diet-induced obese mice undergo a selective and significant loss of adipose tissue despite the high levels of malonyl-CoA induced by FAS inhibition. Since malonyl-CoA is a potent inhibitor of fatty acid oxidation through its inhibition of carnitine palmitoyltransferase-1 (CPT-1, E.C. 2.3.1.21), the rapid and selective loss of adipose tissue was surprising. High systemic levels of malonyl-CoA would be expected to inhibit fatty acid oxidation leading instead to a selective loss of lean mass during C75 induced inanition.

SUMMARY OF THE INVENTION

It is an object of this invention to provide methods and compositions for inducing weight loss and maintaining optimum weight which do not require inhibition of fatty acid synthesis. This and other objects are met by one or more of the following embodiments.

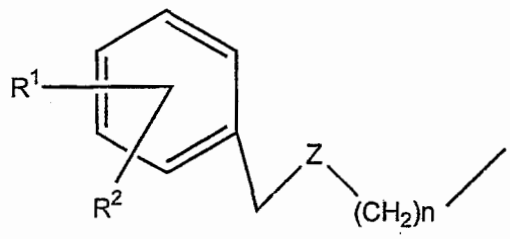
In one embodiment, this invention provides a method of inducing weight loss comprising administering an agent that stimulates carnitine palmitoyl transferase – 1 (CPT-1) activity to the patient in need, including human patients. In a preferred mode, the agent is administered in an amount sufficient to increase fatty acid oxidation. In another preferred mode, the agent is administered in an amount sufficient to antagonize malonyl CoA inhibition of CPT-1. In yet another preferred mode, the agent is administered in an amount sufficient to increase malonyl CoA level. In still another preferred mode, upon administration of the agent, malonyl CoA level is not substantially increased. Substantial increase in malonyl CoA level as contemplated herein is equivalent to about one-half the K_i for malonyl CoA inhibition of CPT-1. In yet another preferred mode, the agent which stimulates CPT-1 activity also inhibits fatty acid synthase (FAS). In an alternative mode, FAS is not significantly inhibited. Insignificant inhibition as contemplated herein is less that 15%, preferably less than 10%, and more preferably less than 5% inhibition. Methods for assay of FAS activity are disclosed in U.S. Patent No. 5,981,575, incorporated herein by reference. In preferred modes of the above embodiments, the agent which stimulates CPT-1 activity is not a compound of formula:



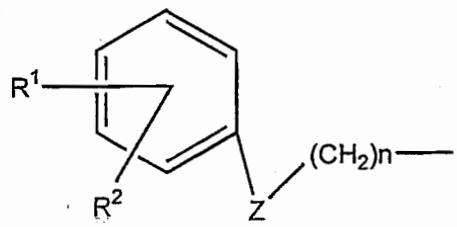
wherein R is a substitute selected from the group consisting of:

(a) saturated linear or branched alkyl groups having 3-18 carbon atoms,

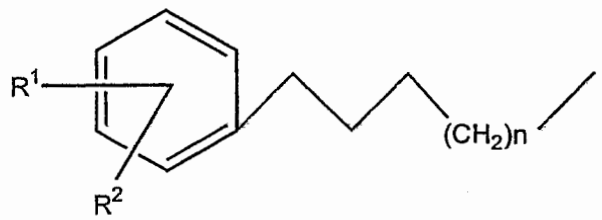
(b) unsaturated linear or branched alkyl groups having 3-18 carbon atoms,

(c) 

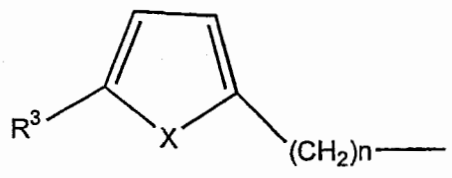
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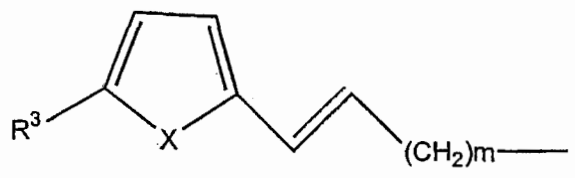
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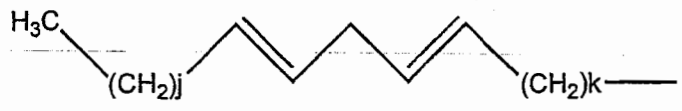
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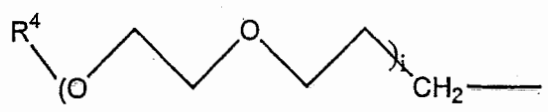
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(g) 

,

(h) 

; and

(i) 

wherein:

R^1 and R^2, the same or different, are H, CH_3, C_2H_5, C_3H_7, C_4H_9, CF_3, OCH_3, F, Cl, or Br;

R^3 is H, CH_3, C_2H_5, C_2H_5, C_4H_9, COOH, $COOCH_3$, $COOC_2H_5$, $COOC_2H_5$, or $COOC_4H_9$;

R^4 is H, CH_3, C_2H_5, C_3H_7, or C_4H_9;

X is NH, S, or O;

Z is CH_2, O, NH, or S;

i is 1 to 5;

j is 0 to 10;

k is 1 to 10;

m is 1-13; and

n is 1 to 15.

In another embodiment, this invention provides a method for stabilizing weight comprising chronic administration of an agent that stimulates CPT-1 activity in an amount that does not significantly inhibit FAS. In a preferred mode, the agent is administered in an amount sufficient to increase fatty acid oxidation. In another preferred mode, the agent is administered in an amount sufficient to antagonize malonyl CoA inhibition of CPT-1. In yet another preferred mode, the agent is administered in an amount sufficient to increase malonyl CoA level. In still another preferred mode, upon administration of the agent, malonyl CoA level is not substantially increased. Substantial increase in malonyl CoA level as contemplated herein is equivalent to about one-half the K_i for malonyl CoA inhibition of CPT-1.

In still another embodiment, this invention provides a method of screening for agents that induce weight loss, comprising determining whether a candidate weight loss agent stimulates CPT-1 activity; and selecting an agent that stimulates CPT-1 activity. Preferably, this method further comprises determining whether the candidate weight loss agent is an antagonist of malonyl CoA inhibition of CPT-1,

and candidate weight loss agents are selected that obviate malonyl CoA inhibition of CPT-1.

In yet another embodiment, this invention provides a therapeutic composition comprising an agent that stimulates CPT-1 activity and L-carnitine. Preferably, the therapeutic composition comprises an antagonist of malonyl CoA inhibition of CPT-1.

In still another embodiment, this invention provides a nutritional composition comprising nutritionally sufficient amounts of fats, carbohydrates and amino acids, said composition further comprising an antagonist of malonyl CoA inhibition of CPT-1 and L-carnitine. In one mode, the nutritional composition is adapted for parenteral administration.

To investigate the mechanism of action leading to the paradoxical reduction of fatty liver in the setting of high hepatic levels of malonyl-CoA during C75 treatment, the effect of C75 on CPT-1 activity was studied. Surprisingly, C75 and related compounds concomitantly stimulated CPT-1 activity and fatty acid oxidation *in vitro* while inhibiting FAS. In addition to its overall allosteric activation of CPT-1, C75 abrogated the inhibitory effect of malonyl-CoA on CPT-1 activity *in vitro*. As a consequence of increased fatty acid oxidation, C75 increased cellular ATP levels.

To test the effect of C75 on fatty acid oxidation *in vivo*, whole animal calorimetry was utilized to measure the respiratory exchange ratio (RER) in mice treated with C75. Following C75 therapy, the RER dropped within 2 h to the range of 0.7, indicative of fatty acid oxidation. This rate of RER decline was similar to food withdrawal from animals fed *ad libitum* with mouse chow. These studies indicate that, despite high hepatic levels of malonyl-CoA, C75 treated animals freely oxidized fatty acids.

These data suggest that C75 blocks the inhibitory action of malonyl-CoA on CPT-1 activity *in vivo* leading to a reduction in fatty liver and adipose mass during FAS inhibition. This invention describes a method to develop therapeutics that selectively reduce adiposity while preserving lean mass through the pharmacological stimulation of CPT-1 activity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the effect of C75 on fatty acid oxidation in MCF-7 cells, compared to the effect of Etomoxir.

Figure 2 shows concentration dependent stimulation of CPT-1 activity by C75 and inhibition by malonyl CoA.

Figure 3 shows reversible stimulation of CPT-1 by C75.

Figure 4 shows stimulation of CPT-1 by various C75 analogs.

Figure 5 shows concentration dependent enhancement of cellular ATP levels by C75 in MCF-7 cells.

Figure 6 shows concentration dependent stimulation of fatty acid oxidation by C75 in mouse adipocytes.

Figure 7 shows concentration dependent enhancement of cellular ATP levels by C75 in mouse adipocytes.

Figure 8 shows respiratory exchange ratio (RER) measured by indirect calorimetry for mice in the absence (A) and presence (B, C) of C75.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Inhibition of fatty acid synthase (FAS) *in vivo* has been shown to cause rapid and profound weight loss. Both cerulenin, a natural product, and C-75, a synthetic small-molecule, cause similar weight loss when administered intracerebroventricularly (i.c.v.) to rats. When treated systemically (e.g., intraperitoneally), C-75 causes more profound weight loss, even weight loss greater than starved animals. These data demonstrate a greater peripheral (non-CNS) effect on weight loss for C-75 than for cerulenin.

While studying the mechanism of action of this profound peripheral effect of C-75, the inventors have recently found that in addition to inhibition of FAS, C-75 and its family of α-methylene-γ-butyrolactones, directly stimulates carnitine palmitoyltransferase-1 (CPT-1) leading to increased mitochondrial fatty acid oxidation. Cerulenin, in contrast, leads to reduced CPT-1 activity and reduced fatty acid oxidation through generation of high malonyl-CoA levels from FAS inhibition.

C75 treatment of MCF-7 cells *in vitro* stimulated CPT-1 activity from 150-160%. There was also a concomitant increase in fatty acid oxidation. Among the C75 analogs, a carbon chain length of C6-C16 was optimum for CPT-1 stimulatory activity. In the presence of C75, malonyl-CoA is no longer able to inhibit CPT-1 activity, suggesting that in addition to its stimulatory effect, C75 also prevents malonyl-CoA inhibition of CPT-1. There is no detectable covalent interaction between CPT-1 and C75.

Thus, the peripheral (non-CNS) weight loss effect of C-75 is at least in part due to CPT-1 stimulation and increased fatty acid oxidation with concomitant fatty acid synthesis inhibition. These data identify a family of α-methylene-γ-butyrolactones as malonate or malonyl-CoA mimetics and CPT-1 as a target for weight loss therapeutics and. More broadly, our data suggest that other malonate or malonyl-CoA mimetics can be designed and synthesized to function as effective weight loss agents.

Data demonstrate that C-75 and its family of α-methylene-γ-butyrolactones directly interact with CPT-1 leading to increased CPT-1 enzymatic activity and fatty acid oxidation. The chemical structure of C75 and numerous analogs, as well as methods of synthesizing these analogs, are disclosed in U.S. Patent No. 5,981,575, which is incorporated herein by reference. The stimulatory effect of C75 is related to the length of the saturated carbon side chain, with the optimum length between 6-18 carbon atoms. With regard to the discussion of the present invention, C75 is the prototype agent for stimulation of CPT-1; reference to C75 hereinafter includes other suitable agents which stimulate CPT-1 activity, except where indicated otherwise by context.

In addition to its direct effect upon CPT-1, C-75 abolishes the inhibitory effect of malonyl-CoA on CPT-1 activity. Although C75 exhibits kinetic features of a slow-binding inhibitor with purified FAS (1), its interaction with CPT-1 appears rapid and competitive. Thus, the stimulatory effect of C75 upon fatty acid oxidation may be due to either its direct stimulation of CPT-1 activity, its interference of malonyl-CoA inhibition of CPT-1, or both. Interestingly, the effects of C75 are not restricted to murine CPT-1, as human CPT-1 was similarly affected. As a

consequence of increased fatty acid oxidation, C75 also increased ATP levels in both the human and murine cells.

The effect of C75 on fatty acid metabolism *in vivo* mirrored the alterations seen on a cellular level. C75 treatment of lean mice led to a profound and rapid increase in fatty acid oxidation, despite the high levels of malonyl-CoA generated by C75 *in vivo*. Thus, C75 and its family of α-methylene-γ-butyrolactones, appear to act as competitive agonists of CPT-1. This agonist activity of C75 appears to overcome inhibitory effects of malonyl CoA on the same enzyme. The increased fatty acid oxidation induced by C75 is an important mechanism accounting for marked reduction in adiposity seen during C75 treatment of mice.

In summary, this invention describes a method to develop therapeutics that selectively enhance fatty acid oxidation, increase energy production, and reduce adiposity while preserving lean mass, through the pharmacological stimulation of CPT-1 activity.

Formulation of therapeutic compositions containing C75 and/or other agents that stimulate CPT-1, and methods of administering such agents, are within the skill of the art, particularly in view of the description in U.S. Patent No. 5,981,575, the text of which is incorporated herein by reference.

Use of CPT-1 stimulating agents to increase energy production by administering the agents contemporaneously with fatty acids or compounds containing fatty acid residues is also within the skill of the art, particularly in view of the nutritional compositions disclosed in U.S. Patent No. 4,434,160, the text of which is incorporated herein by reference.

EXAMPLES

In order to facilitate a more complete understanding of the invention, a number of Examples are provided below. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only.

Example 1. Paradoxical Effects of a Fatty Acid Synthase Inhibitor.

Cerulenin, an FAS inhibitor, increases malonyl-CoA amount in MCF-7 cells (3). As a consequence of the massive increase in malonyl-CoA, cerulenin causes

inhibition of fatty acid oxidation through the malonyl-CoA inhibition of CPT-1 (Thupari, et al. (2001) *Biochem. Biophys. Res. Comm.*, **285:**217-223). Previously, it was shown that C75 treatment of MCF-7 cells resulted in a >5-fold increase in malonyl-CoA through C75 inhibition of fatty acid synthase (FAS) (3). The effect of C-75 on fatty acid oxidation was tested as follows.

Human breast cancer cell line MCF-7 was obtained from the American Type Culture Collection. 1 x 10^6 MCF-7 cells were plated in T-25 flasks in triplicate and incubated overnight at 37°C. Drugs were then added as indicated diluted from 5 mg/ml stock in DMSO. After 2 hours, medium with drugs was removed and cells were preincubated for 30 min. with 1.5 ml of the following buffer: 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, glucose 11 mM. After preincubation, 200 µl of assay buffer was added containing: 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, glucose 11 mM, 2.5 mM palmitate (containing with 10 µCi of [1-^{14}C]palmitate) bound to albumin, 0.8 mM L-carnitine, and cells were incubated at 37°C for 2 h. Following the incubation, 400 µl of benzethonium hydrochloride was added to the center well to collect released $^{14}CO_2$. Immediately, the reaction was stopped by adding 500 µl of 7% perchloric acid to the cells. The flasks with wells were then incubated for 2 h at 37°C after which the benzothonium hydrochloride was removed and counted for ^{14}C. Blanks were prepared by adding 500 µl of 7% perchloric acid to the cells prior to the incubation with the assay buffer for 2 h.

When cells were treated with C75 2 hours before fatty acid oxidation was measured, C75 treatment resulted in a 156% increase in fatty acid oxidation compared to the control (see Figure 1; p=0.0012, two-tailed t-test, Prism 3.0). In contrast, Etomoxir, a known inhibitor of fatty acid oxidation and non-competitive inhibitor of CPT-1, decreased fatty acid oxidation to 32% of control (p=0.0006, two-tailed t-test, Prism 3.0). C-75 treatment of MCF-7 cells repeatedly resulted in increased fatty acid oxidation with doses from 5-20 µg/ml.

Paradoxically, despite an increase in malonyl-CoA similar to that induced by cerulenin, C75 treatment increased rather than decreased fatty acid oxidation in

MCF-7 cells. This implies a direct effect of C75 upon carnitine palmitoyltransferase-1 (CPT-1).

Example 2. C75 Stimulates Activity of Human CPT-1.

CPT-1 activity was assayed in MCF-7 cells by the following published procedure: MCF-7 cells were plated in DMEM with 10% fetal bovine serum at 10^6 cells in 24-well plates in triplicate. Following overnight incubation at 37°C, the medium was removed and replaced with 700 µl of assay medium consisting of: 50 mM imidazole, 70 mM KCl, 80 mM sucrose, 1 mM EGTA, 2 mM $MgCl_2$, 1 mM DTT, 1 mM KCN, 1 mM ATP, 0.1% fatty acid free bovine serum albumin, 70 µM palmitoyl-CoA, 0.25 µCi [methyl-^{14}C]L-carnitine, 40 µg digitonin with or without 20 µM malonyl-CoA. After incubation for 6 minutes at 37°C, the reaction was stopped by the addition of 500 µl of ice-cold 4 M perchloric acid. Cells were then harvested and centrifuged at 13,000 x g for 5 min. The pellet was washed with 500 µl ice cold 2mM perchloric acid and centrifuged again. The resulting pellet was resuspended in 800 µl dH_2O and extracted with 150 µl of butanol. The butanol phase was counted by liquid scintillation and represents the acylcarnitine derivative.

MCF-7 cells were treated with C75 at 10 or 20 µg/mL for 1 hr before CPT-1 activity was assayed. The assay was performed with the C75 and malonyl-CoA concentrations indicated ("M" indicates malonyl-CoA at 20 uM). Malonyl-CoA treatment alone caused a 46% reduction in CPT-1 activity similar to the previous experiment (see Figure 2; p=0.02, two-tailed t-test, Prism 3.0). The level of malonyl-CoA inhibition of the CPT-1 activity is consistent with the activity of the liver isoform of CPT-1 in MCF-7 cells. The K_i of malonyl-CoA for the liver isoform of CPT-1 is ~ 7 µM while the K_i for the muscle isoform of CPT-1 is 0.07 µM. Thus, MCF-7 cells express predominantly the liver isoform of CPT-1 (consistent with the immunoblot analysis).

There was no statistically significant difference in CPT-1 activity between cells treated with C75 or C75 and malonyl-CoA (Figure 2). Thus, in the presence of C75, malonyl-CoA lost its inhibitory effect on CPT-1; conversely, C75 stimulation of CPT-1 occurred regardless of the presence of malonyl-CoA. Thus, in the presence of C75, the normal inhibitory activity of malonyl-CoA is lost. Malonyl-CoA

inhibition of CPT-1 activity demonstrated that C75 and related compounds were activating CPT-1 rather than CPT-2 activity which is not inhibitable by malonyl-CoA.

In a subsequent experiment (data in Figure 3), MCF-7 cells were untreated (left bar) or treated with C75 at 20 μg/ml for one hour before CPT-1 activity was measured (middle and right bars). During the 6 minutes of the CPT-1 assay, C75 was removed from the assay buffer and replaced with buffer (middle bar) or malonyl-CoA 20 μM was added (left & right bars). Malonyl-CoA treatment alone during the assay resulted in a ~70% inhibition of CPT-1 activity (left bar) (p=0.0045, two-tailed t-test, Prism 3.0). Prior C75 treatment with no C75 in the assay buffer resulted in CPT-1 activity of 158% of control (p=0.028, two-tailed t-test, Prism 3.0), similar to the results when C75 is kept in the assay buffer (see above experiment). However, when C75 is removed from the reaction buffer and malonyl-CoA is replaced, C75 stimulatory activity is lost (right bar). Thus, C-75 does not detectably bind covalently to CPT-1, and it is likely a competitive antagonist with malonyl-CoA. These data also suggest that C-75 interacts with CPT-1 at the malonyl-CoA binding site.

Example 3. Structure of Effective CPT-1 Stimulators.

Analogs of α-methylene-γ-butyrolactones differing only in the length of their saturated carbon 'tail' were prepare as described in US. Patent No. 5,981,575, incorporated herein by reference. C75 has an eight-carbon tail, C12 and C16 have tails of 12 and 16 carbons respectively. Cells were treated with C75 and C75 analogs at 20 μg/ml 1 hr before CPT-1 activity was measured. Malonyl-CoA was added only to the reaction buffer since the whole cell is impermeable to malonyl-CoA. C75 stimulated CPT-1 activity to 166% of control at a dose of 20 μg/ml (see Figure 4; p=0.0092, two-tailed t-test, Prism 3.0). C12 analog stimulated to 186% (p=0.0099, two-tailed t-test, Prism 3.0) and C16 analog stimulated to 138% of control (p=0.055, two-tailed t-test, Prism 3.0). Malonyl-CoA, an intracellular competitive inhibitor of CPT-1, reduced CPT-1 activity to 64% of control at 20 μM (p=0.023, two-tailed t-test, Prism 3.0). The optimum carbon chain length for CPT-1 activation is between 6 and 16 carbons.

Example 4. Enhanced Fatty Acid Oxidation from CPT-1 Stimulation Produces ATP.

As a consequence of increased fatty acid oxidation, ATP was elevated in MCF-7 cells following C75 treatment. 1 x 10^5 MCF-7 cells were plated in 96 well plates. Cells were treated with C75 or vehicle. After 2 hours, ATP was measured using a luciferase assay using the ATP Bioluminescence Kit CLS II (Roche) following the manufacturer's protocol. Plates were read by a Perkin Elmer Wallac Victor^2 1420 luminometer. C75 treatment at 10 μg/ml and 20 μg/ml both resulted in a significant increase in total cellular ATP (see Figure 5; $p=0.0001$; $p<0.0001$ compared to control, two-tailed t-test, Prism 3.0). Similar results were obtained after 1hr incubation with C75. Thus, cellular energy production increased as a result of C75 increasing fatty acid oxidation.

Example 5. C75 Stimulates Activity of Muscle Form CPT-1.

To expand the study of effects of C75 on fatty acid metabolism beyond cancer cell lines to normal adipocytes, differentiated (non-transformed) mouse NIH 3T3-L1 adipocytes were used in assays similar to those performed with the MCF-7 cells. 3T3-L1 cells were obtained from the American Type Culture Collection, and cells were cultured in DMEM with high glucose (4.5 g/l) (Gibco 12100-046) with 10% fetal calf serum and Biotin (Sigma B-4639) 0.008 g/L. Differentiation was initiated three days after the cells were confluent, when the standard culture medium was replaced with differentiation medium. The differentiation medium contained standard culture medium to which the following were added to achieve the final concentrations: methylisobutylxanthine (MIX) 0.5 mM, dexamethasone (DEX) 1 μM, and insulin 10 μg/ml. After 48 hrs, the differentiation medium was replaced with post-differentiation medium which contained insulin at the above concentration, without MIX and DEX. The differentiated cells were ready to be used for experiments in 7-10 days.

C75 increased CPT-1 activity and fatty acid metabolism in the NIH 3T3-L1 cells differentiated into adipocytes. One week post differentiation, cells were treated with either vehicle control or C75 for 2 hours at doses indicated below. CPT-1 activity, fatty acid oxidation, and total cellular ATP were measured as described in

Examples 2, 1, and 4. C75 treatment of 3T3-L1 adipocytes led to a 377% increase in CPT-1 activity above control ($p<0.0001$, two-tailed t-test, Prism 3.0). As a consequence of increased CPT-1 activity, C75 at doses of 20 μg/ ml or greater, significantly increased fatty acid oxidation (see Figure 6; 20 μg/ ml, $p=0.007$; <20 μg/ ml, $p<0.0001$; two-tailed t-test, Prism 3.0). Moreover, the increase in fatty acid oxidation led to significantly increased levels of ATP at C75 doses of 20 μg/ ml or greater (see Figure 7; 20 μg/ ml, $p=0.05$; 30 μg/ ml, $p<0.01$; 40 μg/ml, $p<0.0001$; two-tailed t-test, Prism 3.0). The enhanced fatty acid oxidation induced by C75 is likely responsible for the marked reduction in adipose tissue mass seen with C75 administration *in vivo*.

Example 6. *In vivo* Stimulation of CPT-1 Shifts Metabolism to Fatty Acid Oxidation.

In keeping with the C75 effect on both human and murine CPT-1 and fatty acid metabolism, C75 induces a profound and rapid stimulation of fatty acid oxidation in vivo. Mice were maintained in the Oxymax calorimeter (Oxymax Equal Flow System®, Columbus Instruments, Columbus, OH). Oxygen consumption and CO_2 production was measured in up to four mice simultaneously using the indirect calorimeter. Measurements were recorded every 15 minutes over the entire course of the experiments. The respiratory exchange ratio (RER) was calculated by the Oxymax® software version 5.9. RER is defined as the ratio of CO_2 to O_2 at any given time irrespective if equilibrium was reached. Mice were maintained on water and mouse chow *ad libitum*. In the control mice (Figure 8A), note the diurnal variation of RER indicating feeding and resting cycles of the animals. An RER of 1 is consistent with oxidation of carbohydrates while 0.7 indicates oxidation of fatty acids. Mice treated with C75 and maintained in the Oxymax calorimeter showed a rapid decrease in the respiratory exchange ratio (RER) to ~0.7 (Figure 8B). C75 treatment at 30 mg/kg disrupts the diurnal pattern of the control mice, showing a rapid drop in RER to complete oxidation of fatty acids within about 2 hours. Similarly, C75 treatment at 20 mg/kg shows a similar rate of drop of RER but without the prolonged effect (Figure 8C). Importantly, the rate of

decline of RER was similar to that observed for animals deprived of food (data not shown).

Despite the elevated levels of malonyl-CoA generated by C75 *in vivo*, C75 treatment led to a rapid, profound increase in fatty acid oxidation as measured by RER. Thus, C75 treated animals are able to significantly reduce adipose mass and reverse fatty liver, by allowing fatty acid oxidation to occur despite the high levels of malonyl-CoA generated during FAS inhibition *in vivo*.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in medicine, immunology, hybridoma technology, pharmacology, and/or related fields are intended to be within the scope of the following claims.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All such publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

1. Use of an agent that stimulates carnitine palmitoyl transferase-1 (CPT-1) activity in the manufacture of a medicament for the induction of weight loss.

2. The use of claim 1, wherein the medicament stimulates an increase in malonyl CoA levels.

3. The use of claim 1, wherein the medicament does not stimulate an increase in malonyl CoA levels.

4. The use of claim 1, wherein the medicament causes an increase in fatty acid oxidation.

5. Use of an agent that stimulates CPT-l activity in the manufacture of a medicament for stabilizing weight when chronically administered in an amount that does not significantly inhibit fatt,, acid synthase (FAS).

6. The use of claim 5, wherein the medicament stimulates an increase in malonyl CoA levels.

7. The use of claim 5, wherein the medicament does not stimulate an increase in malonyl CoA levels.

8. The use of claim 5, wherein the medicament causes an increase in fatty acid oxidation.

9. A method of screening for agents that induce weight loss, comprising determining whether a candidate weight loss agent stimulates CPT-1 activity; and selecting an agent that stimulates CPT-1 activity.

10. A therapeutic composition comprising an agent that stimulates CPT- 1 activity.

11. The therapeutic composition of claim 10, further comprising L-carnitine.

12. A nutritional composition comprising nutritionally sufficient amounts of fats, carbohydrates and amino acids, said composition further comprising L-carnitine and an antagonist of malonyl CoA inhibition of CPT-1.

13. The use of a compound that stimulates CPT-1 activity in a pharmaceutical composition.

14. The use of a compound that stimulates CPT-1 activity in a pharmaceutical composition, wherein said composition does not significantly inhibit FAS.

15. A therapeutic composition comprising L-carnitine and an a-methylene-(3-carboxy-y butyrolactone.

16. A therapeutic composition comprising L-carnitine and trans-tetrahydro-3-methylene-2 oxo-5-n-octyl-4-furancarboxylic acid.