

REVIEWS

Chemistry and Biochemistry of (–)-Hydroxycitric Acid from
Garcinia

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(–)-Hydroxycitric acid [(–)-HCA] is the principal acid of fruit rinds of *Garcinia cambogia*, *Garcinia indica*, and *Garcinia atroviridis*. (–)-HCA was shown to be a potent inhibitor of ATP citrate lyase (EC 4.1.3.8), which catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA: citrate + ATP + CoA → acetyl-CoA + ADP + P_i + oxaloacetate. The inhibition of this reaction limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis during a lipogenic diet, that is, a diet high in carbohydrates. Extensive animal studies indicated that (–)-HCA suppresses the fatty acid synthesis, lipogenesis, food intake, and induced weight loss. In vitro studies revealed the inhibitions of fatty acid synthesis and lipogenesis from various precursors. However, a few clinical studies have shown controversial findings. This review explores the literature on a number of topics: the source of (–)-HCA; the discovery of (–)-HCA; the isolation, stereochemistry, properties, methods of estimation, and derivatives of (–)-HCA; and its biochemistry, which includes inhibition of the citrate cleavage enzyme, effects on fatty acid synthesis and lipogenesis, effects on ketogenesis, other biological effects, possible modes of action on the reduction of food intake, promotion of glycogenesis, gluconeogenesis, and lipid oxidation, (–)-HCA as weight-controlling agent, and some possible concerns about (–)-HCA, which provides a coherent presentation of scattered literature on (–)-HCA and its plausible mechanism of action and is provocative of further research.

Keywords: *Garcinia cambogia*; *Garcinia indica*; *Garcinia atroviridis*; (–)-hydroxycitric acid; ATP:citrate lyase; fatty acid synthesis; lipogenesis; appetite; antiobesity

INTRODUCTION

Garcinia (family: Guttiferae) is a large genus of polygamous trees or shrubs, distributed in tropical Asia, Africa, and Polynesia. It consists of 180 species, of which ~30 species are found in India (1). For the past several years, small and complex molecules have been isolated from the various species of *Garcinia*, which include xanthenes and xanthone derivatives (2–4). However, the isolation of (–)-hydroxycitric acid [(–)-HCA] from a few species of *Garcinia* and its biological properties have attracted the attention of biochemists and health practitioners.

The physiological and biochemical effects of (–)-HCA have been studied extensively for its unique regulatory effect on fatty acid synthesis, lipogenesis, appetite, and weight loss. The derivatives of (–)-HCA have been incorporated into a wide range of pharmaceutical preparations in combination with other ingredients for the claimed purpose of enhancing weight loss, cardioprotection, correcting conditions of lipid abnormalities, and endurance in exercise (5–22).

In the present review attempts have been made to pool, analyze, and summarize the available information on the chemical, physiological, and biochemical aspects of (–)-HCA.

SOURCES OF (–)-HCA

(–)-HCA is found in the fruit rinds of certain species of *Garcinia*, which include *G. cambogia*, *G. indica*, and *G. atroviridis* (23–25). These species thrive prolifically on the Indian subcontinent and in western Sri Lanka (1, 26).

G. cambogia is a small or medium-sized tree with a rounded crown and horizontal or drooping branches; its leaves are dark green and shiny, elliptic obovate, 2–5 in. long and 1–3 in. broad; its fruits are ovoid, 2 in. in diameter, yellow or red when ripe with six to eight grooves, and the fruits have six to eight seeds surrounded by a succulent aril. The tree is found commonly in the evergreen forests of Western Ghats, from Konkan southward to Travancore, and in the Shola forests of Nilgiris up to an altitude of 6000 ft. It flowers during the hot season, and fruits ripen during the rainy season. The seeds of *G. cambogia* contain 31% edible fat.

G. indica is a slender evergreen tree with drooping branches; its leaves are ovate or oblong lanceolate, 2.5–3.5 in. long and 1–1.5 in. broad, dark green above and pale beneath; its fruits are globose or spherical, 1–1.5 in. diameter, dark purple when ripe and enclosing five to eight large seeds. The tree is found in the tropical rain forests of Western Ghats, from Konkan southward to Mysore, Coorg, and Wynaad. It flowers in November–February, and fruits ripen in April–May. The root is astringent. The seeds of the fruit have edible fat, commercially known as Kokam butter

G. atroviridis is a moderate-sized graceful tree, 30–50 ft high, found in the northeastern districts of upper Assam. Leaves are 6–9 in. long and 2–3 in. broad, thickly coriaceous, glabrous, abruptly acuminate, and base contracted; its flowers are terminal, the female flower being solitary and large. The fruits are orange yellow, subglobose (3–4 in. across), fluted with a firmly textured outer rind and a rather thin and translucent pulp surrounding the seeds (1).

The fruits of *G. cambogia* are too acidic to be eaten raw. A decoction of the fruit rind is given for rheumatism and bowel complaints. It is also employed in veterinary medicine as a rinse for the diseases of the mouth in cattle. However, the fruit of *G. indica* has an agreeable flavor and a sweetish acid taste. It is used as a garnish to give an acid flavor to curries and also for preparing syrups during hot months. The fruit is also anthelmintic and useful in piles, dysentery, tumor, pains, and heart complaints. Syrup from the fruit juice is given in bilious affections. The fruits of both species are valued for their dried rinds, which are used as a condiment for flavoring curries in place of tamarind or lemon. In Ceylon the dried fruit rinds of *G. cambogia* are used along with salt in the curing of fish (1). The fruit rinds of *G. atroviridis* are also too acidic to be eaten raw, but the taste is excellent when stewed with sugar. In Malay, the sun-dried rinds of under-ripe fruits are sold as a sour relish for use in curries in place of tamarind and for the dressing of fish. The fruit is also used as a fixative with alum in the dyeing of silk. A decoction from leaves and roots is used in the treatment of earache (1).

CHEMISTRY OF (–)-HCA

Discovery of (–)-HCA. The dried rind of the fruit of *G. cambogia* popularly known as “Malabar tamarind” is extensively used all over the west coast of South India for culinary purposes and commercially for “Colombo curing” of fish (23, 27, 28). The organic acids present in the fruit have been held responsible for the bacteriostatic effect of the pickling medium by lowering the pH (27). The fruit contains ~30% acid calculated as citric acid on a dry weight basis (28). The organic acids present have been mistakenly identified as tartaric acid and citric acids (27, 29). The aqueous extracts of the fruit showed two predominant acid spots on paper chromatograms run on different solvents, which were very near to tartaric and citric acids, but there was always a significant small difference in the R_f values in all solvent systems. Analysis of the fruit juice by use of an ion exchange column as described by Palmer (30) showed the largest peak to be the citric acid region, but the acids failed the cream of tartar test for tartaric acid and the pentabromoacetone test for citric acid (28).

Lewis and Neelakantan (23) isolated the principal acid in the fruit rinds of *G. cambogia* and identified it as (–)-HCA on the basis of chemical and spectroscopic studies. Identification and separation of the hydroxycitric acid on Whatman No. 1 paper were performed using *n*-butanol/acetic acid/water (4:1:5) and *n*-propanol/formic acid/water (4:1:5). The spots were identified by spraying with 5% metavanadate. Upon saponification of the

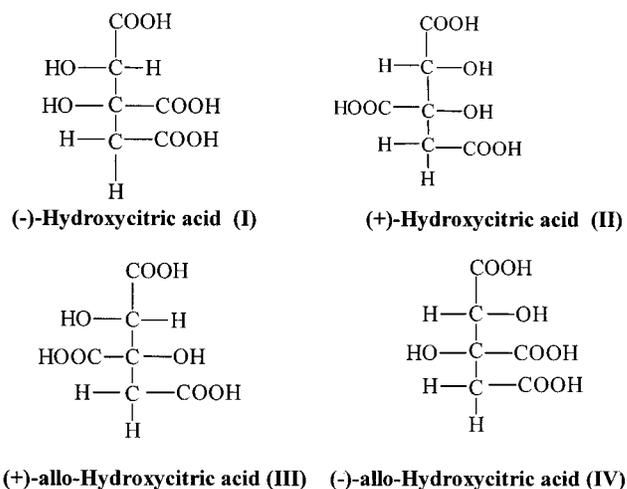


Figure 1. Structures of hydroxycitric acid isomers.

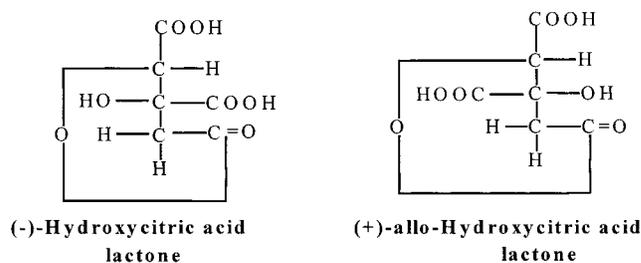


Figure 2. Structures of hydroxycitric acid lactones.

acid mixture with excess alkali and passing it through a column of ion exchange resin (Zeocarb 215), the eluate showed only one lower spot ($R_f = 0.34$) corresponding to the free (–)-HCA. On concentration the eluate gave only one upper spot ($R_f = 0.46$) corresponding to the lactone. Fruit extracts showed two predominant acid spots on chromatograms with two different solvent systems. On titration of this material with alkali, using phenolphthalein, two different end-points were obtained, in the cold and after boiling, showing the characteristics of lactones. The two spots on chromatograms were identified as hydroxycitric acid and its lactone (Figures 1 and 2). It was thus clear that the two spots on the chromatograms are those of γ -hydroxy acid and its lactone and not those of tartaric acid and citric acids.

Isolation. Lewis and Neelakantan (23) isolated this (–)-HCA on a large scale from the dried rinds of *G. cambogia*. The method consisted of extracting the acid by cooking the raw material with water under pressure (10 lb/in.² for 15 min). The extract was concentrated, and pectin was removed by alcohol precipitation. The clear filtrate was neutralized with alkali, passed through cation exchange resin for recovery of the acid, which was concentrated and dried. The crude dried mass was extracted with ether and recrystallized to give small needle-shaped crystals of lactone. Lewis (24) reported another method for the isolation of (–)-HCA from *G. cambogia* using acetone. The acetone extract was concentrated and the acid taken up in water. The aqueous solution yielded the lactone on evaporation.

Moffett et al. (31) have developed a process for the aqueous extraction of (–)-HCA from *Garcinia* rinds. The extract was loaded onto an anion exchange column for adsorption of (–)-HCA, and it was eluted with sodium/potassium hydroxide for release of (–)-HCA. The extract was passed through a cation exchange column to yield a free acid. Guthrie and Kierstead (9, 10) and Moffett et al. (32) have reported the preparation of (–)-HCA concentrate from *Garcinia* rinds with 23–54% (–)-HCA and 6–20% lactone.

Table 1. Comparison of Physical Properties of HCA and Lactones from *Garcinia* and *Hibiscus* (23)

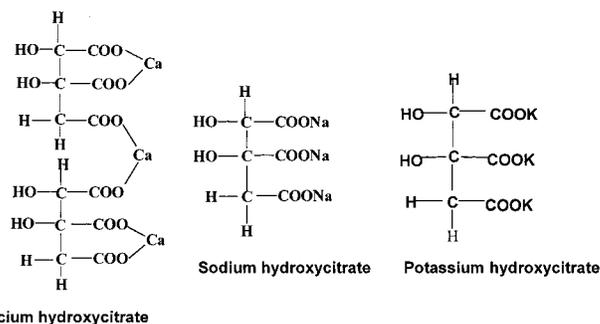
property	<i>Garcinia</i>		<i>Hibiscus</i>	
	free acid	lactone	free acid	lactone
mp (°C)		178	183	
[α] _D ²⁰ (deg)	-20	100	122	31
crystal shape		needles		needles
hygroscopicity		slight		high
solubility		high in alcohol and water; fair in ether		high in water and alcohol; slight in ether
paper chromatogr (<i>R</i> _f)				
butanol/formic acid/H ₂ O	0.24	0.42	0.15	0.39
propanol/acetic acid/H ₂ O	0.26	0.36	0.35	0.26
metavandate spray (5%)	yellow	reddish orange	yellow	yellow

Stereochemistry. Hydroxycitric acid (1,2-dihydroxypropane-1,2,3-tricarboxylic acid) has two asymmetric centers; hence, two pairs of diastereoisomers or four different isomers are possible (Figure 1). Martius and Maue (33) have synthesized the four possible stereoisomers of hydroxycitrate. One of these isomers occurs in *Garcinia* (Figure 1, I) and another in *Hibiscus* species (Figure 1, II) (24). The absolute configurations of the hydroxycitric acid lactones, hibiscus acid and garcinia acid, were determined to be (2*S*,3*R*)- and (2*S*,3*S*)-2-hydroxycitric acid-2,5-lactone, respectively (Figure 2). The absolute configuration is determined from Hudson's lactone rule, optical rotatory dispersion curves, circular dichroism curves, and calculation of partial molar rotations (34). Glusker et al. (35, 36) have reported the structure and absolute configuration of the calcium hydroxycitrate and (-)-HCA lactone by X-ray crystallography. Stallings et al. (37) have reported the crystal structures of the ethylenediamine salts of diastereoisomeric hydroxycitrates.

Properties of (-)-HCA and Lactone. The physical properties of (-)-HCA and lactones (Figure 2) from *Garcinia* and *Hibiscus* are presented in Table 1. The equivalent weight of pure lactone is 69, as determined by alkali titration or silver salt decomposition. The IR spectra of the ethyl ester showed ester and hydroxyl groups at 5.41–5.76 and 2.74–2.79 μ , respectively (23). The structure of the (-)-HCA lactone was further established by IR and ¹H NMR spectroscopy. The (-)-HCA lactone displayed strong IR bands at 3200, 1760, and 1680 cm⁻¹. ¹H NMR spectra of the (-)-HCA lactone showed two protons at the γ -carbon, which give an AB quartet at δ 2.53 and δ 2.74 with $J = 17.1$ Hz, and one proton at the α -carbon showing a singlet at δ 5.15 (38).

Estimation of (-)-HCA. Free (-)-HCA leads to the formation of (-)-HCA lactone during concentration and evaporation. The presence of minor organic acids such as citric acid, tartaric acid, and malic acid in the *Garcinia* fruits and the lack of official methods for the assay of (-)-HCA have created confusion and disagreement among analysts (39). The existing method for the determination of (-)-HCA content in *G. cambogia* extract involves acid–base titration, which gives the total acidity of the extract (40). However, in this method the concentrations of (-)-HCA and lactone cannot be estimated separately.

Lowenstein and Brunengraber (25) have estimated the hydroxycitrate content of the fruit of *G. cambogia* by gas chromatography (GC). GC estimation involves the conversion of acid to volatile silyl derivative. They used an OV-17 GC column (3 m \times 3 mm i.d.). The column was run at 145 °C using nitrogen as the carrier gas (40 mL/min) with an injection port temperature of 250 °C and a detector temperature of 300 °C. (-)-HCA lactone is the major constituent of the extract,

**Figure 3.** Structures of hydroxycitric acid derivatives.

and the recrystallized compound contains <0.5% of impurities. Silylation requires completely dried samples, but (-)-HCA has a tendency for lactonization during drying; because of the highly hygroscopic nature of (-)-HCA, it is rather difficult to dry the sample. For this reason, the free (-)-HCA content cannot be estimated.

Recently, Jayaprakasha and Sakariah (38, 41, 42) have developed high-performance liquid chromatography (HPLC) methods for the determination of organic acids in the fruits of *G. cambogia*, commercial samples of *G. cambogia* extracts, and leaves and rinds of *G. indica*. In these HPLC methods dilute extracts can be quantified without concentration, drying, and derivatization. Here, the advantage is that the (-)-HCA and its lactone can be quantified separately.

Hydroxycitric Acid Derivatives. The fruit rinds of *G. cambogia* and *G. indica* contain 20–30% (-)-HCA. It is thus the prime source of (-)-HCA (23). (-)-HCA is susceptible to lactonization during evaporation and concentration. Hence, stable derivatives of (-)-HCA, namely, lactone, ester (23, 43), sodium and potassium salts of (-)-HCA (24), and calcium salt (44), were prepared (Figure 3). In commercial samples of *G. cambogia* extracts, (-)-HCA is present as its calcium salt for the reasons of stability. Free (-)-HCA can easily be generated from the *G. cambogia* extract samples, for further analysis, by passing an aqueous solution of the calcium salt through a cation exchange resin. Majeed et al. (18) have reported the preparation of potassium hydroxycitrate from *Garcinia*. It involves the extraction of (-)-HCA from *Garcinia* fruit using alkyl alcohol, and the combined extract was treated with potassium hydroxide and refluxed to form potassium hydroxycitrate precipitate. Balasubramanyam et al. (45) have reported the preparation of a new soluble metal double salt of group IA and IIA of (-)-HCA. It involves the aqueous extraction of (-)-HCA and treating the extract with different metal hydroxides and metal chlorides to get a double metal salt. Ibnu Saud et al. (46) have reported the isolation of *Garcinia* acid from the fresh or dried rinds of the fruits of *G. cambogia*, *G. indica*, and *G. atroviridis*. It involves four to five extractions of *Garcinia* fruits with boiling water for 20 h. The combined extract was concentrated and treated with methanol to remove the pectin and filtered. The filtrate was treated with aqueous sodium hydroxide at 80 °C to obtain sodium hydroxycitrate.

BIOCHEMISTRY OF (-)-HCA

Inhibition of Citrate Cleavage Enzyme by (-)-HCA. Citrate cleavage enzyme, that is, ATP:citrate lyase (ATP:citrate oxaloacetate lyase, EC 4.1.3.8), catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. Watson et al. (47) encountered the powerful inhibition of ATP:citrate oxaloacetate lyase by (-)-HCA with purified enzyme from rat liver. In that experiment (-)-HCA had a much greater affinity

for the purified enzyme than the natural substrate, that is, citrate, and the K_i of (–)-HCA for citrate cleavage enzyme was between 0.2 and 0.6 μM depending on the conditions (47, 48). Later on, Cheema-Dhadli et al. (49) found inhibition of citrate cleavage enzyme by both free (–)-HCA ($K_i = 8 \mu\text{M}$) and (–)-HCA lactone ($K_i = 50\text{--}100 \mu\text{M}$). The lactone of (–)-HCA was found to be a very less effective inhibitor of citrate cleavage enzyme. In rat brain synaptosomes, (–)-HCA was shown to be a potent inhibitor of citrate cleavage enzyme (50). Sullivan et al. (51) and Stallings et al. (37), in similar studies, observed that of four isomers of HCA (Figure 1), (–)-HCA was the only potent inhibitor of ATP:citrate lyase. (–)-HCA was found to inhibit partially purified ATP:citrate lyase from human liver competitively with respect to citrate ($K_i = 3 \times 10^{-4} \text{ M}$) (52).

The discovery of the powerful inhibition of citrate cleavage enzyme by (–)-HCA provides a valuable tool for the study of the metabolic role of citrate cleavage reaction (47).

The biological effect of (–)-HCA stems from the inhibition of extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. The ultimate source of all the carbon atoms of fatty acids is acetyl-CoA, formed in the mitochondria by the oxidative decarboxylation of pyruvate, the oxidative degradation of some of the amino acids, or the β -oxidation of long-chain fatty acids. The synthesis of fatty acids is maximal when carbohydrate is abundant and the level of fatty acids is low. The conversion of carbohydrate into fat involves the oxidation of pyruvate to acetyl-CoA. Fatty acids are synthesized in the cytosol, whereas acetyl-CoA is formed from pyruvate in mitochondria. For fatty acid biosynthesis, the acetyl group of acetyl-CoA must be transferred from the mitochondria to the cytosol. Acetyl-CoA as such cannot pass out of the mitochondria into the cytosol. The barrier to acetyl-CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane. Citrate is formed in the mitochondrial matrix by the condensation of acetyl-CoA with oxaloacetate. When present at high levels, citrate is transported to cytosol, via the tricarboxylate transport system. In cytosol, acetyl-CoA is regenerated from citrate by the ATP:citrate lyase, which catalyzes the following reaction: citrate + ATP + CoA \rightarrow acetyl-CoA + ADP + P_i + oxaloacetate (53–57). ATP:citrate lyase is widely distributed in animal tissues (58). ATP:citrate lyase has been suggested to play a physiological role in lipogenesis from carbohydrate (59) and gluconeogenesis (60). The changes in activity of citrate cleavage enzyme correlate with changes in the rate of fatty acid synthesis and provide evidence for the involvement of the citrate cleavage reaction in fatty acid synthesis (61, 62). The activity of citrate cleavage enzyme varies in accordance with the nutritional status of the animal. Thus, during starvation or when fed on a high-fat diet, the enzyme levels fall drastically and on feeding of a high-carbohydrate diet elevated levels of ATP:citrate lyase were detected (61, 63). Evidence also exists demonstrating that >80% of the extramitochondrial acetyl-CoA produced from pyruvate in rat liver mitochondria is supplied via ATP:citrate lyase (64).

Effect of (–)-HCA on Fatty Acid Synthesis and Lipogenesis. (–)-HCA being a potent inhibitor of ATP:citrate lyase, which catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA, limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis (65–68). Many studies demonstrated both in vitro and in vivo that (–)-HCA suppresses the de novo fatty acid synthesis and lipogenesis.

In a cell-free system, consisting of particle-free cytoplasm and mitochondria prepared from rat liver, (–)-HCA was shown

to inhibit fatty acid synthesis from citrate and also from [^{14}C]-alanine by measuring the incorporation of $^3\text{H}_2\text{O}$ and ^{14}C (53). Sullivan et al. (65) studied the effect of stereoisomers of hydroxycitrate (Figure 1) on the rate of lipogenesis in rat liver. They found that only (–)-HCA significantly decreased the conversion of [^{14}C]citrate into lipid in the liver high-speed supernatant and the conversion of [^{14}C]alanine into lipid in vivo.

Beynen and Geelen (69) observed the inhibition of fatty acid synthesis from glucose by (–)-HCA in isolated hepatocytes, but it did not affect fatty acid synthesis from acetate; rather, (–)-HCA was shown to stimulate fatty acid synthesis from acetate (70). This effect was observed both in the isolated perfused rat liver and in the homogenates of this organ and was assumed to be due to an activation of acetyl-CoA carboxylase in analogy to other tricarboxylates (71, 72). Moreover, the findings of Hackenschmidt et al. (73) showed that (–)-HCA is a positive effector of acetyl-CoA carboxylase and similarity of (–)-HCA and citrate activation of enzyme activity was apparent in the time for maximal stimulation. It was concluded that (–)-HCA acts as an inhibitor of lipogenesis only if cytoplasmic acetyl-CoA is produced by citrate cleavage enzyme reaction, but it will activate fatty acid synthesis wherever an alternate source of acetyl-CoA, for example, acetate, is available.

The increase in lipogenesis in brown adipose in response to glucose feeding was inhibited by (–)-HCA (74). In starved rats, (–)-HCA did not inhibit the basal rates of brown fat lipogenesis and glucose utilization in response to insulin. As basal rates of lipogenesis were not inhibited by (–)-HCA, it is suggested that acetate may be a lipogenic substrate for brown fat in starvation (75). Smith (76) reported that rabbit adipose tissue has a low capacity for de novo fatty acid synthesis from glucose but a high capacity for synthesis from pyruvate and acetate. Experiments with (–)-HCA have demonstrated that citrate cleavage enzyme is an obligatory enzyme in lipogenesis from pyruvate and that the lipogenic system of rabbit adipose tissue resembles that of a ruminant in that it is adapted to utilize acetate rather than glucose.

(–)-HCA partially inhibited the incorporation of ^3H from $^3\text{H}_2\text{O}$ and ^{14}C from [U- ^{14}C]lactate into saponifiable fatty acid in the hepatocytes from fed rats, but incorporation of ^{14}C from [^{14}C]proline was completely inhibited. However, similar complete inhibition of incorporation of ^{14}C from [^{14}C]proline was observed in fed rats, glycogen-depleted hepatocytes, or hepatocytes from starved rats (77). In another observation, (–)-HCA depressed lipogenesis in hepatocytes from fed rats incubated with lactate plus pyruvate by ~51% but had little effect on lipogenesis in glycogen-depleted hepatocytes similarly incubated. There was a parallel inhibition of incorporation of ^{14}C from [U- ^{14}C]lactate to fatty acids, and lipogenesis was measured with $^3\text{H}_2\text{O}$ in each case. Thus, depletion of glycogen or conceivably the process of glycogen depletion causes a change in the rate-limiting step(s) for lipogenesis from lactate (78).

(–)-Hydroxycitrate markedly reduced tritiated water incorporation into fatty acid by lung tissue slices (79). Starving rats for 72 h markedly reduced in vivo $^3\text{H}_2\text{O}$ incorporation into pulmonary lipids. Intraperitoneal injection of (–)-HCA prior to $^3\text{H}_2\text{O}$ administration also markedly reduced isotope incorporation into pulmonary lipid fractions of fed rats. It has been concluded that in vivo synthesis of fatty acids was affected by the nutritional state of the animal and citrate appears to be a significant source of cytoplasmic acetyl-CoA for the de novo pulmonary lipogenesis in the fed rats (80). Sheehan and Yeh (81) observed that (–)-HCA inhibited fatty acid synthesis in neonatal rat lung from glucose, pyruvate, and β -hydroxybutyrate

by 88, 70, and 60%, respectively, but had no effect on that from acetoacetate. Lipogenesis from β -hydroxybutyrate involves both the cytoplasmic and citrate pathways. Crabtree et al. (82) observed that the fluxes of butyrate to acetate and fatty acids in rat hepatocytes were inhibited to a similar extent by (-)-HCA with no significant effect on butyrate uptake. Because butyrate is activated only in the mitochondria of rat liver (83) and (-)-HCA did not significantly inhibit butyrate uptake, this parallel inhibition of the flux of butyrate to acetate and fatty acids strongly suggests that the production of acetate occurs in the cytoplasm. (-)-HCA could not be expected to inhibit the production of acetate via the mitochondrial hydrolase. Experiments with (-)-HCA indicate that the major route for conversion of leucine carbon to lipid in rat mammary gland acini is via citrate translocation from the mitochondria (84). Mathias et al. (85) reported that in hepatocytes isolated from female rats, meal-fed a high-glucose diet, (-)-HCA depressed the incorporation of $^3\text{H}_2\text{O}$, [^{14}C]alanine, [$2\text{-}^{14}\text{C}$]leucine into fatty acids and cholesterol.

Hood et al. (86) have shown that (-)-HCA reduced the synthesis of fatty acids from lactate and glucose in bovine adipose tissue and rat adipose tissue, respectively, and suggested that the conversion of lactate to fatty acids probably occurs by way of citrate. In the (-)-HCA-treated rat liver cells, there was a decrease in the formation of phospholipids and triglycerides from lactate (87). Vicario and Medina (88) have also reported the inhibition of lipogenesis from lactate in rat brain by (-)-HCA, and the transfer of lactate carbons through the mitochondrial membrane is accompanied by the translocation of citrate.

(-)-HCA inhibited lipogenesis in rat brain slices (89, 90) and in freshly isolated mouse preputial gland (91). (-)-HCA equivalently reduced the biosynthesis of triglycerides, phospholipids, cholesterol, diglycerides, cholesteryl esters, and free fatty acids in isolated liver cells from normal and hyperlipidemic rats (92). (-)-HCA decreased the rate of cholesterol production in hepatocytes from fed rats by interfering with the flow of substrate into the sterol biosynthetic pathway (93). Measurement of the weight of desmosterol produced during its biosynthesis in the presence of tritiated water and triparanol has permitted a direct determination of the relative flux of carbon and tritium (the H/C ratio) into sterol in hepatocytes. The H/C ratio, which increased with the time of incubation, was shown to decrease with (-)-HCA. The depression of biosynthesis of long-chain fatty acids, 3- β -hydroxysterol, and cholesterol by (-)-HCA was confirmed in a perfused rat liver system (70, 94, 95). The inhibition of fatty acid synthesis by (-)-HCA was associated with the decreased rate of choline incorporation into desaturated phosphatidylcholine in fetal rat lung (96). Berkhout et al. (97) tested (-)-HCA in Hep G2 cells for effects on cholesterol homeostasis. After 2.5 and 18 h of incubation with (-)-HCA at a concentration of ≥ 0.5 mM, incorporation of [$1,5\text{-}^{14}\text{C}$]citrate into fatty acids and cholesterol was strongly inhibited, which may reflect an effective inhibition of ATP:citrate lyase. Preincubation with a higher concentration of (-)-HCA increased the cellular low-density lipoprotein (LDL) receptor activity as determined by the receptor-mediated association and degradation. Measurements of receptor-mediated binding versus LDL concentration suggested that this increase might be due to an increase in the number of LDL receptors. A simultaneous increase of the enzyme levels of 3-hydroxy-3-methylglutaryl-CoA (HMG) reductase was also observed in the same condition. These results suggest that the increase in HMG-CoA reductase and LDL receptor are initiated by the decreased flux of carbon

units in cholesterol synthetic pathway, owing to inhibition of ATP citrate lyase.

Hildebrandt et al. (98) studied the utilization and preferred metabolic pathway of ketone bodies for the synthesis of lipids by freshly isolated Morris Hepatoma 777 cells, which are known to actively convert ketone bodies to cholesterol and fatty acid. On the basis of the results with (-)-HCA, these authors found that the metabolic pathway for acetoacetate conversion to lipids is exclusively cytoplasmic, whereas that for 3-hydroxybutyrate involves both extra- and intramitochondrial compartments. Acetyl-L-carnitine is known as a modulator of metabolic function. Lligona-Trulla et al. (99) have studied lipogenesis in different cell lines and in rat hepatocytes and observed that the flux of acetyl-L-carnitine (ALC) to lipid was increased, not decreased, by (-)-HCA. In contrast, this inhibitor dramatically decreased the flux of glucose to lipid. These results indicate that the flux of acetyl-L-carnitine to lipid can bypass citrate and utilize cytosolic acetyl-CoA synthesis, and it was shown that when (-)-HCA was used, the capacity of ALC to supply acetyl units for de novo lipogenesis could be increased significantly. Because ALC provides acetyl units for metabolic activities without the cost of ATP hydrolysis, this increased capacity may also represent a mechanism by which ALC can provide acetyl units in metabolically compromised situations. In insulin-differentiated 3T3L1 cells, (-)-HCA was shown to inhibit lipogenesis and stimulate lipolysis (100).

Incorporation of ^3H from $^3\text{H}_2\text{O}$ was used to measure the rate of fatty acid synthesis in rat liver. In vivo inhibition of the rate of fatty acid synthesis in the rat liver was demonstrated after intraperitoneal/intravenous administration of (-)-HCA (101). The in vivo rate of lipogenesis was markedly decreased from [^{14}C]alanine following the administration of (-)-HCA either intraperitoneally or intravenously. Fatty acid and cholesterol syntheses were significantly inhibited by the oral administration of (-)-HCA only when the compound was given before the feeding period (65). The oral administration of (-)-HCA to rats significantly depressed the in vivo lipogenic rates in a dose-dependent manner in the liver, adipose tissue, and small intestine and caused significant reductions in body-weight gain, food consumption, and total body lipid (66, 67). (-)-Hydroxycitric acid and (+)-*allo*-hydroxycitric acid were also investigated (102) for their effects on in vivo lipid synthesis under conditions of either high-carbohydrate feeding or 24 h fasting, and it was found that in fed rat, (-)-HCA significantly reduced the incorporation of labeled H_2O and alanine into fatty acids and cholesterol. An increased rate of incorporation of labeled H_2O into fatty acids but no change in cholesterol synthesis in the fasted rat suggested that (-)-HCA might be an activator of acetyl-CoA carboxylase. (+)-*allo*-Hydroxycitric acid was ineffective in modulating the rates of fatty acid synthesis under either nutritional condition. Both (-)-hydroxycitric acid and (+)-*allo*-hydroxycitric acid were shown to be in vitro activators of acetyl-CoA carboxylase, the former being a stronger activator. Thus, stereospecificity of the hydroxycitrate isomers was demonstrated in both the inhibition and stimulation of fatty acid synthesis possibly occurring at the level of acetyl-CoA carboxylase. Because the increase of citrate level could have allosterically activated acetyl-CoA carboxylase (103), this activation of acetyl-CoA carboxylase may be due to the increased level of citrate caused by the inhibition of citrate cleavage enzyme with (-)-HCA.

In mature rat, the gold thioglucose-induced obese mouse, and the ventromedial hypothalamic lesioned obese rat, food intake, body-weight gain, and depression of body lipid levels were

reduced significantly by the chronic administration of (–)-HCA (104). Chee et al. (105) have observed species-specific response to (–)-HCA. Acute administration of (–)-HCA caused the depression of *in vivo* rates of fatty acid synthesis in the livers of chickens and rats. Chickens appeared to be more sensitive to the inhibitory effects of (–)-HCA than did the rats. Oral administration of (–)-HCA significantly reduced the rate of *in vivo* hepatic fatty acids and cholesterol synthesis, and there was a reduction in serum triglyceride and cholesterol levels in normolipidemic rats. It was also observed that (–)-HCA significantly reduced the hypertriglyceridemia and hyperlipogenesis in genetically obese Zucker rats, fructose-treated rats with elevated triglyceride, and Triton-treated rats (92, 106). Young Zucker lean (Fa/–) and obese (fa/fa) female rats were fed with (–)-HCA, and decreases in body weight, food intake, percent of body fat, and fat cell size in the lean rats were observed (107). In the obese rats food intake and body weight were reduced, but the percent of body fat remained unchanged and maintained a fat cell size equivalent to that of their obese controls. This study indicates that the obese rats, despite a substantial reduction in body weight produced by (–)-HCA, still defend their obese body composition. Rao and Sakariah (108) observed that inclusion of (–)-HCA in the lipogenic diet resulted in significant reduction in food intake, body weight, epididymal fat, and serum triglyceride in the albino rats and also a decrease in the feed efficiency ratio. The decreases in food intake, body-weight gain, and feed efficiency ratio brought about by (–)-HCA were dependent on the content of this compound in the diet. The study encouraged exploration of the efficacy of (–)-HCA in the control of obesity and hypertriglyceridemia.

Effect of (–)-HCA on Ketogenesis. Brunengraber et al. (95) reported that ketogenesis by livers of fed rats perfused without free fatty acid is strongly inhibited by (–)-HCA. It seems that such ketogenesis occurs via an extramitochondrial pathway that depends on the citrate cleavage reaction for its supply of extramitochondrial acetyl-CoA, which operates in carbohydrate-fed animals (109). However, the same parameter was not inhibited by (–)-HCA when livers from starved rats were perfused with oleate. On the other hand, the ketogenesis increased somewhat by (–)-HCA when livers from fed rats were perfused with oleate. The intramitochondrial pathway predominates either in starved animals or when the concentration of fatty acid is high or both. In the case of the rumen epithelium of sheep, the absence of any significant cytoplasmic component was emphasized by (–)-HCA, which should inhibit ketogenesis if ATP:citrate lyase plays any function in the process (110), but there was no such inhibition, lending support to the theory that ketogenesis proceeds exclusively through the mitochondrial pathway.

Other Biological Effects of (–)-HCA. (–)-HCA did not affect the rate of oxygen consumption by rat brain synaptosomes, and the activities of fatty acid synthase, carnitine acetyltransferase, glucose 6-phosphate-dehydrogenase, and acetyl-CoA synthetase but inhibited the activities of isocitrate dehydrogenase, malate dehydrogenase (decarboxylating), and aconitate hydratase at millimolar concentrations (50). (–)-HCA blocked dexamethasone stimulation of cytidyltransferase but not of fatty acid synthase in lung tissue (111).

Brunengraber et al. (95) observed that in rat liver, the inhibition of fatty acid synthesis by (–)-HCA was associated with an increase in the tissue content of glucose 6-phosphate and fructose 6-phosphate and a diminution in glycolytic intermediates from fructosebisphosphate to phosphoenolpyru-

vate. Presumably, the citrate content is elevated in cytoplasm in the presence of (–)-HCA. This can be expected to result in a reduced activity of phosphofructokinase because citrate is well-known to act as an inhibitor of this enzyme (112–114). It has also been seen that AMP contents drop in the presence of (–)-HCA. This can also be expected to reduce the activity of phosphofructokinase, because AMP is an activator of this enzyme (115). The inhibition of phosphofructokinase by (–)-HCA in rat hepatocytes has also been reported by McCune et al. (116). It can be concluded that in rat liver, the inhibition of phosphofructokinase by (–)-HCA leads to the accumulation of glucose 6-phosphate and fructose 6-phosphate and the decrease of glycolytic intermediates beyond fructosebisphosphate as the reaction catalyzed by phosphofructokinase in glycolysis is irreversible and controls the glycolysis.

Chronic metabolic acidosis increases proximal tubular citrate uptake, causing hypocitraturia associated with an increase in cortical ATP:citrate lyase activity and protein abundance. Hypokalemia, which causes only intracellular acidosis, also caused hypocitraturia and increased renal cortical ATP:citrate lyase activity. Inhibition of this enzyme with (–)-HCA significantly abated hypocitraturia and increased urinary citrate excretion in chronic metabolic acidosis and in K⁺ depletion (117). These results suggest an important role of ATP:citrate lyase in proximal tubular citrate metabolism. (–)-HCA has been reported to alter the pyruvate metabolism by tumorigenic cells (118). Pyruvate consumption by tumor cells declined, but the mean percentage of oxidation increased with (–)-HCA.

Possible Mode of Action on Reduction in Food Intake. The chronic oral administration of (–)-HCA to growing rats caused a reduction in body-weight gain, food consumption, and total body lipids. However, administration of equimolar amounts of citrate did not alter weight gain, appetite, or body lipids, and there was no increase in liver size or liver lipid content with either treatment. Paired feeding studies demonstrated that the reduction in food intake accounted for the decrease in weight gain and body lipid observed with (–)-HCA treatment, and this decrease in calorie intake was not responsible for the drug-induced depression of hepatic lipogenesis (66, 67). Rao and Sakariah (108), in their studies, suggested that the reduction in appetite in (–)-HCA-fed rats seems to be a specific effect of (–)-HCA ingestion and not due to alterations of taste as the diet fed to control group contained citrate at a level equivalent to that of (–)-HCA in the experimental diet. In view of the close structural relationship of (–)-HCA to citrate, it is reasonable to believe that (–)-HCA does not affect the taste of the food. Panksepp et al. (119) evaluated the capacity of various salts of (–)-HCA to produce conditioned rejection of a 0.25% saccharin solution and concluded that the food intake was reduced by (–)-HCA only during the first hour following administration of the drug and the magnitude of appetite rejection did not correspond to the degree of conditioned rejection, lending support to the conclusion that the food intake reduction was not merely a consequence of aversive effects of the drug.

A number of complex factors have been implicated in the appetite regulation and feeding behavior (67). Glucose utilization rates (120), unidentified humoral factor(s) in blood from satiated rats (121), enterogastrone (122) and other gastrointestinal hormones, plasma and brain tryptophan, brain serotonin interrelationships (123–127), and brain catecholamines (128) have all been suggested to play their role in the regulation of feeding behavior. Because (–)-HCA was demonstrated to inhibit fatty acid and cholesterol synthesis, presumably through a reduction

in acetyl-CoA pool in lipogenic tissues (65), the same effect would be expected in any cell possessing ATP:citrate lyase. Ricny and Tucek (129) have observed that the availability of acetyl-CoA had a decisive influence on both the rate of synthesis of acetylcholine and its steady-state concentration with glucose as the metabolic substrate in rat brain slices. Acetylcholine released by the phrenic nerve was measured in the isolated perfused rat hemidiaphragm; when (–)-HCA was added to the perfusate, the release of acetylcholine was stabilized at a level 40% below the control value (130). (–)-HCA was reported to inhibit the synthesis of acetylcholine in rat brain slices (90, 131, 132). These results suggested that citrate transport and subsequent cleavage give rise to acetyl-CoA as the principal precursor of acetylcholine synthesis. Sterling et al. (133) reported that in rat brain cortex, (–)-HCA reduced the incorporation of [³H]-glucose and [¹⁴C]- β -hydroxybutyrate to the acetyl moiety of acetylcholine. They suggested that in adult rats, β -hydroxybutyrate could contribute to the acetyl moiety of acetylcholine, possibly via the citrate cleavage pathway. Ricny and Tucek (134) observed the effects of (–)-HCA on the concentration of acetyl-CoA and acetylcholine in the tissue and on the release of acetylcholine into the media were investigated in experiments on slices of rat caudate nuclei incubated in different media. With glucose as the main metabolic substrate, (–)-HCA diminished the concentration of acetyl-CoA in all of the media used. ATP:citrate lyase appears to provide about one-third of acetyl-CoA used for the synthesis of acetylcholine. Gibson and Peterson (135) have shown that the (–)-HCA-reduced incorporation of [U-¹⁴C]glucose into acetylcholine was greater in septal than in hippocampal slices, indicating that acetylcholine metabolism varies regionally. Recently, Szutowicz et al. (136) reported that in Ca²⁺-activated rat synaptosomes, both synaptosomal acetyl-CoA and acetylcholine syntheses were suppressed by 27 and 29%, respectively, by 1 mM (–)-HCA. The impairment of acetylcholine synthesis with (–)-HCA was also reported in the brain stem of neonatal rat (137). All of these *in vitro* studies revealed that (–)-HCA can depress acetylcholine synthesis in nerve tissues, although so far there is no evidence of reduced synthesis of acetylcholine in intact animals with the administration of (–)-HCA. Sullivan et al. (67) suggested that if (–)-HCA could penetrate the blood–brain barrier, then the depression of acetylcholine levels or rate of turnover in the brain resulting from a decreased precursor pool could affect cholinergic receptor systems that might be involved in feeding behavior.

Fatty acid synthesis regulates fatty acid oxidation by a well-characterized mechanism (138, 139). In this paradigm, malonyl-CoA levels rise during fatty acid synthesis and result in inhibition of carnitine palmitoyl transferase 1 (CPT 1) mediated uptake of fatty acids into the mitochondria. This results in elevation of cytoplasmic long-chain fatty acyl (LCFA)-CoAs and diacylglycerol molecules that may play a role in signaling, which leads to the proposal that malonyl-CoA levels act as a signal of the availability of physiological fuels (140). One such proposal for malonyl-CoA is the mediation of nutrient-stimulated secretion in the beta cells. Glucose-sensing neurons, which regulate feeding in the hypothalamus, share many features with the beta cells, including expression of glucokinase and the adenosine-sensitive potassium channel (141). The studies of Chen et al. (142) showed that (–)-HCA profoundly inhibited the glucose-stimulated insulin secretion (GSIS) from beta cells and also provided evidence for the pivotal role of malonyl-CoA suppression of CPT 1 with attending elevation of the cytosolic LCFA-CoA concentration in GSIS from the normal pancreatic

beta cell. Moreover, Saha et al. (103) reported that inhibition of ATP:citrate lyase by (–)-HCA markedly diminished the malonyl-CoA level, indicating that citrate was the major substrate for the malonyl-CoA precursor, that is, cytosolic acetyl-CoA, and also there is sufficient evidence that (–)-HCA inhibits ATP:citrate lyase (47–51), limiting the pool of cytosolic acetyl-CoA, the precursor of malonyl-CoA. This type of regulation of malonyl-CoA level may affect the signaling of fuel status in hypothalamic neurons regulating feeding behavior, lending support that (–)-HCA may represent a biochemical target for the control of appetite/feeding behavior and body weight. Thus, (–)-HCA seems to act at the metabolic level and not directly at the central nervous system as classical appetite depressants do.

(–)-HCA May Promote Glycogenesis, Gluconeogenesis, and Lipid Oxidation. The inhibition of ATP:citrate lyase by (–)-HCA causes less dietary carbohydrate to be utilized for the synthesis of fatty acids, resulting in more glycogen storage in the liver and muscles. Sullivan et al. (143) reported an increase in the rate of *in vivo* hepatic glycogen synthesis with the administration of (–)-HCA. Sullivan et al. (144) and Sullivan and Green (145) proposed that (–)-HCA acted primarily through its effects on the appetite, possibly involving increases in glycogen levels. Even though the administration of (–)-HCA increased the hepatic glycogen contents in rats, Hellerstein and Xie (146) demonstrated that neither hepatic glycogen nor the hexosephosphates are involved in the food-intake suppressive effects of (–)-HCA. It seems the increased hepatic fatty acid oxidation leading to increased levels of acetyl-CoA and ATP plays some role in this effect.

In a well-fed state, the hepatic carnitine levels are far too low to activate CPT 1. The rate-limiting enzyme for hepatic lipid oxidation, CPT 1, is activated by exogenous carnitine and inhibited by malonyl-CoA. The lipogenesis inhibitor (–)-HCA can decrease the production of malonyl-CoA in hepatocytes by potent inhibition of ATP:citrate lyase and may activate CPT 1, which can facilitate lipid oxidation. Earlier studies demonstrated that (–)-HCA can reduce body-weight and fat accumulation in growing rats, owing to the reduction in appetite. From the above views McCarty (147) hypothesized that joint administration of (–)-HCA and carnitine should therefore promote hepatic lipid oxidation, gluconeogenesis, and satiety. In a recent study, joint administration of pyruvate, (–)-HCA, and carnitine to obese subjects was associated with a remarkable rate of body-fat loss and thermogenesis, strongly suggestive of uncoupled fatty acid oxidation (148). The reduction of free fatty acids by stimulating hepatic oxidation with (–)-HCA and carnitine may ameliorate risk factors associated with abdominal obesity (149).

Sustained aerobic exercise requires a severalfold increase in hepatic glucose output. An increasing proportion of this elevated glucose output must be provided by gluconeogenesis. Thus, preadministration of (–)-HCA may aid endurance during postabsorptive aerobic exercise by promoting gluconeogenesis. Carnitine and bioactive chromium may potentiate this benefit. The utility of this technique may be greatest in exercise regimens designed to promote weight loss (150). The ability of exercise to selectively promote fat oxidation should be optimized if exercise is done postabsorptively (preferably during morning fasting metabolism), if (–)-HCA and carnitine are administered prior to exercise, if the exercise regimen is of moderate intensity and prolonged duration, and if no calories are ingested for several hours following exercise (151).

Kriketos et al. (152) did not detect any effect of (–)-HCA administration on lipid oxidation, respiratory quotient (RQ), and

energy expenditure in humans during either exercise or moderately intense exercise. However, Ishihara et al. (153) demonstrated that chronic administration of (–)-HCA to mice lowered the RQ and increased lipid oxidation during both resting and exercising conditions and increased the endurance in exercise. These results indicated that the enhancement of endurance exercise by chronically administered (–)-HCA in mice might have occurred by the attenuation of glycogen consumption caused by the promotion of lipid oxidation during exercise.

(–)-HCA AS A WEIGHT-CONTROLLING AGENT

Weight gain occurs when the limited capacity for storing glycogen in the liver and muscles is attained, and beyond this point excess glucose is converted into fat and stored in fat cells throughout the body. (–)-HCA exerts its antiobesity effect by inhibiting ATP:citrate lyase, consequently inhibiting the cleavage of citrate to oxaloacetate and acetyl-CoA, a key molecule, which plays a critical role in energy storage as fat. Now, instead of wasting energy to synthesize fat, the energy is diverted to the production of glycogen in the liver and muscles. This slows the production of fatty acids, cholesterol, and triglycerides with the net effect of reduced fat production and storage (154).

Preliminary research based on laboratory and animal experiments suggests that (–)-HCA may be a useful weight loss aid (101, 102). (–)-HCA has been demonstrated in the laboratory to reduce the conversion of carbohydrates into stored fat by inhibiting certain enzyme processes (49, 65). Animal research also indicated that (–)-HCA suppresses appetite and food intake to induce weight loss (66, 67, 104, 107, 108).

Heymsfield et al. (155) stated in their publication that although (–)-HCA appears to be a promising experimental weight control agent, studies in humans are limited (156–160) and results have been contradictory. Supporting evidence of human (–)-HCA efficacy for weight control is based largely on studies with small sample sizes (157, 158), studies that failed to include a placebo-treated group (156), and use of inaccurate measures of body lipid change (158). Even though the effectiveness of (–)-HCA is unclear, at least 14 separate (–)-HCA-containing products are sold over-the-counter to consumers (161). To overcome limitations of the above studies and examine the effectiveness of (–)-HCA for weight loss and fat mass reduction, Heymsfield and his colleagues at Obesity Research Center, University College of Physicians and Surgeons, New York, designed their investigation with rigorous controlled trials. Subjects were randomized to receive either active herbal compound [1500 mg of (–)-HCA per day] or placebo. Both groups were prescribed a high-fiber, low-energy diet, and the treatment period was 12 weeks. Body weight was evaluated every week, and fat mass was measured at weeks 0 and 12. On the basis of the results obtained from these experiments, Heymsfield et al. (155) concluded that a prospective double-blind study failed to detect either weight loss or fat-mobilizing effects of (–)-HCA beyond those of a placebo.

Rebuttal letters from Vladimir Badmaev et al. of Sabinsa Corp. (a manufacturer of standardized *G. cambogia* extract); James L. Schaller, Birchrunville, PA; and Fabio Firenzuoli and Luigi Gori, St. Joseph Hospital, Empoli, Italy, published in the July 21, 1999, issue of the *Journal of the American Medical Association* were critical of Heymsfield's studies. The letters are summarized below:

- The results are contradictory to the positive results reported in several (–)-HCA clinical studies (156–160, 162), and it cannot be concluded from this single study that (–)-HCA is ineffective.

- Previous studies and their own preclinical research (163) suggest that for (–)-HCA to effectively inhibit fat formation and body-weight gain, it needs to be administered with a simple carbohydrate-rich (lipogenic) diet.

- The co-administration of (–)-HCA with a high-fiber diet may have inhibited the gastrointestinal absorption of (–)-HCA. This issue becomes critical with (–)-HCA; its reported efficacy in inhibiting intracellular enzyme ATP:citrate lyase depends entirely on the presence of (–)-HCA inside the target cell. The significance of (–)-HCA availability in the cytosol of the target cell for inhibiting lipid synthesis or ATP citrate lyase was confirmed (164).

- The low-energy diet used in the study by Heymsfield et al. negated the utility of (–)-HCA. The study of Heymsfield et al. failed to evaluate (–)-HCA's ability to reduce food intake by virtue of its energy-restricted protocol.

- The dose of (–)-HCA used in the study was low compared with that used in earlier animal studies (66, 67).

- Excess calcium used to stabilize the (–)-HCA molecule, common among products, reduces solubility and hinders bioavailability. A soluble *Garcinia* powder and liquid extract containing a lactone from (–)-HCA was compared with a calcium-type *Garcinia* powder administered in food to rats, and the liquid extract was proven to be more effective (165). Because blood (–)-HCA levels were not measured in the study, it was not known whether bioavailability was ensured in the studies of Heymsfield et al. (155).

SOME POSSIBLE CONCERNS ABOUT (–)-HCA

Animal studies indicate that (–)-HCA is no more toxic than citric acid itself, which is present in many foods in addition to being a normal intracellular compound. Also, (–)-HCA is a component of a natural product, which has been used in Indian cuisine as well as for medicinal purposes (1). Cloutre and Rosenbaum (154) pointed out that (–)-HCA has extremely low levels of toxicity. For example, recent oral toxicity studies performed at Wil Research Laboratories in Ashland, OH, found that 5000 mg/kg of body weight of (–)-HCA resulted in no visible symptoms of toxicity or deaths in laboratory animals. This is roughly equivalent to 350 g or 233 times the dosage of 1.5 g/day of (–)-HCA that might be consumed by an average-sized person. Because *G. cambogia* has a long history of usage as a flavoring agent, preservative, and herbal tonic and there are no reports of toxicity regarding traditional use of the *Garcinia* extract, it is highly unlikely that there may be any possible negative effect that may occur due to excess intake; the possibility of bowel intolerance can be easily reversed by simply reducing the dosage. However, this problem has not been reported in animal studies at the levels that were necessary to reduce appetite. However, despite its inherent safety, (–)-HCA, like any other diet product, is not recommended for certain groups of people. (–)-HCA has impacts on the body's production of fatty acids and cholesterol; therefore, it may directly influence the production of sterols, thus restricting the production of steroid hormones. As pregnancy is a time of extreme sensitivity to steroid hormones, products containing (–)-HCA should not be recommended during pregnancy. Likewise, women who are breast-feeding should also avoid (–)-HCA. Although experience with fruit sources of (–)-HCA shows that they are not dangerous to young children, they are advised not to consume (–)-HCA in large amounts for extended periods.

One concern is about the time of administration and dosage considerations of (–)-HCA for its efficacy. Sullivan et al. (65) demonstrated that the effects of (–)-HCA in animals depend

on the time of administration in relation to a meal, with (–)-HCA maximally effective when administered 30–60 min prior to feeding. Most of the clinical studies including that of Heymsfield et al. (155) administered (–)-HCA ~30 min prior to meals, which is the lower end of the maximally effective range. However, the administration of (–)-HCA postabsorptively in the studies of Kriketos et al. (152) showed a trend toward a lower RQ during (–)-HCA phase, although it was not statistically significant. Even though this study did not support the hypothesis that (–)-HCA alters the short-term rate of fat oxidation, the data certainly do not rule out its possibility. Another related concern is that (–)-HCA provided in divided doses was found to be more effective than the same amount given as single dose (67). In weight-loss protocols human doses ranging between 750 and 1500 mg/day of (–)-HCA (156–160) are the extreme lower end of the in vivo dose response range established by Sullivan et al. (66).

As discussed earlier, the administration of (–)-HCA increases the rate of gluconeogenesis and ketone bodies formation (166), which can be deleterious in subjects with type-2 diabetes. In this situation, we can consider the hypothesis of McCarty (167) that excessive exposure of tissues to fatty acids is likely to be the chief cause of the various dysfunctions that lead to sustained hyperglycemia in type-2 diabetes. Disinhibition of hepatic fatty acid oxidation and inhibition of fatty acid synthesis with (–)-HCA and carnitine, as suggested earlier (147), have considerable potential as a new weight-loss strategy, but diabetics run the risk of further enhancing excessive hepatic gluconeogenesis. McCarty (147) hypothesized that the clinical utility of metformin in diabetes is probably traceable to inhibition of gluconeogenesis, and its use as an adjunct to (–)-HCA/carnitine treatment of obesity in diabetics deserves evaluation, particularly as metformin therapy itself tends to reduce body weight. Furthermore, metformin therapy will not impede the activation of fatty acid oxidation by (–)-HCA/carnitine and is likely to potentiate the appetite-suppressant and thermogenic benefits of this strategy. Indeed, because metformin has been reported to lower body weight and improve cardiovascular risk factors in obese nondiabetics, a broader application of an (–)-HCA/carnitine/metformin therapy for obesity can be contemplated.

(–)-HCA may have deleterious effects on insulin sensitivity. Many researchers have suggested that malonyl-CoA has an important role in transmitting the insulin signal in the cells (140, 168, 169). It is well-known that (–)-HCA prevents the production of acetyl-CoA and subsequently malonyl-CoA (103); therefore, preventing its production may impair insulin responsiveness in some (or all) tissues. Thus, (–)-HCA is likely to have some effect on insulin sensitivity that can lead to type-2 diabetes, that is, NIDDM.

A role of malonyl-CoA in glucose-stimulated insulin secretion (GSIS) has been defined and a rise in malonyl-CoA, which preceded the insulin secretion in clonal pancreatic beta cells, was evident after exposure to glucose (170). Sener and Malaisse (171) reported the failure of (–)-HCA in the range of 1.0–2.0 mM to affect the GSIS in glucose-stimulated intact pancreatic islets of rat. However, Chen et al. (142) explored the emerging concept of Prentki et al. (172) that malonyl-CoA generation with concomitant suppression of mitochondrial CPT 1 represents an important component of GSIS by pancreatic beta cells and observed that (–)-HCA profoundly inhibited GSIS and also provided more direct evidence for a pivotal role of malonyl-CoA suppression of CPT 1 with attendant elevation of the cytosolic long-chain acyl-CoA concentration in GSIS from the normal pancreatic beta cell. There may be another advantage

of (–)-HCA in that it inhibits GSIS, which helps to keep this fat-storing hormone in check.

High blood sugar levels suppress the burning of fatty acids for the availability of energy. Within the past few years, researchers have shown that high blood sugar levels generate malonyl-CoA, which is an inhibitor of carnitine palmitoyl transferase (CPT 1) that controls the transfer of long-chain fatty acyl (LCFA)-CoA into the mitochondria where these are oxidized. An increase in malonyl-CoA also results in the elevation of acyl-CoA, which triggers the release of insulin (140, 168, 169, 173–176). Saha et al. (103) observed that inhibition of ATP:citrate lyase with (–)-HCA markedly diminished the increase in malonyl-CoA in skeletal muscle, indicating that citrate was the major substrate for malonyl-CoA precursor and cytosolic acetyl-CoA. As CPT 1 is considered to be a rate-limiting factor in the burning of fatty acids (177), the trick of long-term permanent fat loss is to increase the activity of CPT 1. (–)-HCA can be used to increase the activity of CPT 1 as does L-carnitine and glucagon. Because research has shown that the conversion of carbohydrates into fat is prevented by (–)-HCA, a more important function of this incredible nutrient is its ability to increase the CPT 1 activity by decreasing the pool of acetyl-CoA, thus reducing the level of malonyl-CoA and raising the activity of CPT 1.

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