DNA AND CELL BIOLOGY
Volume 26, Number 9, 2007

Pp. 627–639
DOI: 10.1089/dna.2007.0617

Transcriptome of Primary Adipocytes from Obese Women in Response to a Novel Hydroxycitric Acid–Based Dietary Supplement

Sashwati Roy,[1] Hiral Shah,[1] Cameron Rink,[1] Savita Khanna,[1] Debasis Bagchi,[2] Manashi Bagchi,[2] and Chandan K. Sen[1]

Background: Obesity is a global public health problem. Traditional herbal medicines may have some potential in managing obesity. The dried fruit rind of *Garcinia cambogia*, also known as Malabar tamarind, is a unique source of (-)-hydroxycitric acid (HCA), which exhibits a distinct sour taste and has been safely used for centuries in Southeastern Asia to make meals more filling. Recently it has been demonstrated that when taken orally, a novel, highly soluble calcium/potassium salt of HCA (HCA-SX) is safe and bioavailable in the human plasma. Although HCA-SX seems to be conditionally effective in weight management in experimental animals and in humans, its mechanism of action remains unclear. *Methods*: In this study, subcutaneous preadipocytes collected from obese women with body mass index $>25\,kg/m^2$ were differentiated to adipocytes for 2 weeks in culture. The effects of low-dose HCA-SX on lipid metabolism and on the adipocyte transcriptome were tested. HCA-SX augmented isoproterenol- and 3-isobutyryl-1-methylxanthine-induced lipolysis. Using oil red O, the production of lipid storage droplets by the cultured mature human adipocytes was visualized and enumerated. *Results*: HCA-SX caused droplet dispersion facilitating lipase action on the lipids. HCA-SX markedly induced leptin expression in the adipocytes. In the microarray analyses, a total of 54,676 probe sets were screened. HCA-SX resulted in significant down-regulation of 348, and induction of 366 fat- and obesity-related genes. HCA-SX induced transactivation of hypoxia inducible factor (HIF), a novel approach in the management of obesity. *Conclusion*: Taken together, the net effects support the antilipolytic and antiadipogenic effects of HCA-SX. Further human studies are warranted.

Introduction

Obesity is a global public health problem, with about 315 million people worldwide estimated to fall into the WHO-defined obesity categories with a body mass index (BMI) of $30\,kg/m^2$ or above (Caterson and Gill, 2002). In the United States, in 1999, 27% of adults had a BMI $>30\,kg/m^2$, almost double the prevalence of 20 years earlier. The estimated mortality from obesity-related diseases in the United States is approximately 300,000 annually and growing. In the future, mortality related to obesity is expected to exceed that of smoking (O'Brien and Dixon, 2002). The quality of life of the obese is markedly reduced while the costs to the health care systems have markedly increased. In spite of the rising number of people who are overweight and obese, medical treatment of obesity is currently where medical treatment of hypertension was in the 1950s, with only two Food and Drug Administration (FDA)–approved medications for chronic weight loss and maintenance, sibutramine and orlistat (Schurgin and Siegel, 2003). Over the past decade, there has been a tremendous increase in the understanding of the molecular and neural mechanisms that control food intake and body weight, yet the incidence of obesity is rising at an alarming rate (Zigman and Elmquist, 2003).

Botanical dietary supplements often contain complex mixtures of phytochemicals that have additive or synergistic interactions. For example, the tea catechins include a group of related compounds with effects that are demonstrable beyond those that are seen with epigallocatechin gallate, the most potent catechin alone. Traditional herbal medicines may have some potential in obesity management. Several ethnobotanical studies have identified herbal treatments for diabetes, and similar surveys, termed "bio-prospecting," have identified potential herbal treatments for obesity as well (Heber, 2003). Beyond increasing thermogenesis, there are other biological rationales for the actions of several different alternative medical and herbal approaches to weight loss. For example, several supplements and herbs claim to result in nutrient

[1]Laboratory of Molecular Medicine and the Microarray Core Facility, Department of Surgery, Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, Ohio.
[2]School of Pharmacy and Health Professions, Creighton University Medical Center, Omaha, Nebraska.

partitioning so that ingested calories will be directed to muscle, rather than fat. These include an herb (*Garcinia cambogia*), and a lipid, which is the product of bacterial metabolism (conjugated linoleic acid). Moreover, a series of approaches attempt to physically affect gastric satiety by filling the stomach. Fiber swells after ingestion and has been found to result in increased satiety (Heber, 2003).

Although the market is replete with natural products aimed at weight management, scientific evidence examining such products are scanty at best. Although Americans spend $30 billion per year on weight-loss aids, the adequacy of our regulatory and monitoring capability as a society is a matter of concern (Spake, 2001; Heber, 2003). When the significance of scientific evidence is dwarfed by money or politics, the net result is exposure of the general public to unsafe dietary supplements (Wolfe, 2003). A classical example of such case is Ephedra, a popular natural weight-loss ingredient known to be associated with life-threatening side effects (Anonymous, 2003; Meadows, 2003; Shekelle *et al*., 2003; Wolfe, 2003). Recently, the FDA has issued a consumer alert indicating plans to prohibit the sale of Ephedra-containing dietary supplements in the United States (www.fda.gov/oc/initiatives/ephedra/december2003/advisory.html). Scientific investigations of herbal and alternative therapies represent a potentially important source for new discoveries in obesity treatment and prevention (Heber, 2003). Herbal products for weight reduction in motivated patients may be effective to treat clinically significant obesity. The consistency, safety, and efficacy of a bioavailable active herbal product remain important factors in the consideration of such therapy (Sindler, 2001).

The dried fruit rind of *G. cambogia* (family Guttiferae), also known as Malabar tamarind, is a unique source of (-)-hydroxycitric acid (HCA), which exhibits a distinct sour taste and has been used for centuries in Southeastern Asia to make meals more filling (Sergio, 1988). Recently it has been demonstrated that when taken orally, a novel, highly soluble calcium/potassium salt of HCA (HCA-SX) is safe (Shara *et al*., 2003, 2004) and bioavailable in the human plasma as studied by gas chromatography mass spectrometry (GC-MS) (Loe *et al*., 2001). Although HCA-SX has been observed to be conditionally effective in weight management in experimental animals (Shara *et al*., 2003) and in humans (Mattes and Bormann, 2000; Westerterp-Plantenga and Kovacs, 2002; Preuss *et al*., 2004), its mechanism of action remains to be understood. Recently, we have noted that HCA-SX is effective in restricting body weight gain in adult rats. Functional characterization of HCA-SX-sensitive genes revealed that up-regulation of genes encoding serotonin receptors represents a distinct effect of dietary HCA-SX supplementation (Roy *et al*., 2004). In this study, subcutaneous preadipocytes collected from women with BMI $> 25\,kg/m^2$ were differentiated to adipocytes for 2 weeks in culture. Using this experimental system, we tested the effects of low-dose HCA-SX on lipid metabolism and on the adipocyte transcriptome.

Materials and Methods

HCA-SX

A natural, highly water-soluble calcium-potassium salt of 60% HCA extract from *G. cambogia*, commercially known as Super Citrimax HCA-600-SXS (HCA-SX), was obtained from InterHealth Nutraceuticals, Benicia, CA (Ohia *et al*., 2001, 2002; Shara *et al*., 2003, 2004; Preuss *et al*., 2004). HCA-SX samples were stored in a dry, cool place at room temperature (18–25°C). HCA-SX contains 60% HCA acid in its free form, 1.0% HCA in its lactone form, 10% calcium, 15% potassium, 0.5% sodium, 0.05% total phytosterols, 0.3% total protein, 4.5% moisture, and 8.5% soluble dietary fiber (by difference). HCA-SX also contains 0.1% magnesium, 0.03% iron, and trace amounts of manganese, copper, zinc, selenium, total fat, and total sugar. HCA-SX provides approximately 150 calories per 100 g (Shara *et al*., 2004).

Cells and cell culture

Human primary subcutaneous cultured adipocytes were from Zen-Bio (Chapel Hill, NC). The cells were obtained from healthy nondiabetic females ($n = 6$; average BMI, 26.98). Differentiated adipocytes were maintained in AM-1 media (Zen-Bio) as recommended by Zen-Bio. For treatment, HCA-SX was dissolved in cell culture media and then applied to the cells.

Lipolysis assay

Adipocytes cultured in 96-well plates were treated with HCA-SX (0.5 mg/mL). Glycerol released in the media at 5 or 24 h after the treatment was measured using a commercially available human adipocyte lipolysis assay kit (Zen-Bio). Because of absence of glycerokinase activity in adipocytes, assessing lipolytic activity by the measurement of glycerol released into the medium is a standard protocol. The measurement principle of glycerol using the kit is as follows: glycerol released to the medium is phosphorylated by adenosine triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5′-diphosphate in the reaction catalyzed by glycerol kinase. G-1-P is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide (H_2O_2). A quinoneimine dye is produced by the peroxidase catalyzed coupling of 4-aminoantipyrine and sodium N-ethyl-N-(3-sulfopropyl)m-anisidine with H_2O_2, which has an absorbance maximum at 540 nm. The increase in absorbance at 540 nm is directly proportional to glycerol concentration of the sample. Isoproterenol is a β-adrenergic agonist, whereas 3-isobutyryl-1-methylxanthine (IBMX) is an inhibitor of cyclic AMP (cAMP) phosphodiesterases. The main lipolytic pathway involves β-agonists, which activate β-adrenergic receptors *via* the intracellular G proteins in adipocytes. This leads to the activation of adenylate cyclase, which then increases cAMP levels. Elevated cAMP acts as a second messenger to activate hormone sensitive lipase (HSL). HSL, the rate-limiting enzyme regulating adipocyte lipolysis, then catalyzes the hydrolysis of triglycerides and results in the release of glycerol and free fatty acid (FFA). Phosphodiesterases are enzymes that hydrolyze cAMP to 5′-AMP (5′ adenosine monophosphate). This action results in a decrease in lipolysis. Phosphodiesterase (PDE) inhibitors increase intracellular cAMP levels and thus increase lipolysis.

Oil red O staining

Oil red O (Sigma, St. Louis, MO) working solution (0.5% wt/v) was prepared in isopropanol, followed by dilution in water (40 mL of water and 60 mL of stock). Prior to staining, the cells were fixed with 10% buffered formalin. The cells were stained and then washed with water. Representative images of oil red O–stained cells were taken using an inverted micro-

scope (AxioVert 200M, Zeiss, Thornwood, NY). The large oil droplets (as shown in Fig. 1) in each image were counted using the Axiovision (Zeiss) software.

ELISA

Leptin and vascular endothelial growth factor (VEGF) levels were detected from culture media using commercially available ELISA kits (R&D systems, Minneapolis, MN) as per manufacturer's instructions.

Cell viability

The viability of cells was assessed by measuring lactate dehydrogenase (LDH) leakage from cells to media using *in vitro* toxicology assay kit from Sigma (St. Louis, MO). Media from treated or control cells were collected and centrifuged at 5500 rpm for 10 min in cold. After centrifugation, the supernatant (media only) was transferred to new tubes. The pellet containing detached cells were lysed using lysis buffer [0.5% v/v Triton X-100 in phosphate-buffered saline (PBS)]. Attached cells were washed twice with ice-cold PBS and lysed using lysis buffer as mentioned above. Cell viability was determined using the following formula: Viability = LDH activ-activity of cells in monolayer/total LDH activity (i.e., LDH activity of cells in monolayer + LDH activity of detached cells + LDH activity in the cell culture media).

GeneChip™ probe array analysis

RNA extraction and target preparation were performed as described previously (Roy *et al.*, 2003, 2006a, 2006b; Rink *et al.*, 2006). To assess the quality of the labeled targets, the samples were hybridized for 16 h at 45°C to GeneChip test arrays. Satisfactory samples were hybridized to the human genome arrays (U133 Plus 2.0). The arrays were washed, stained with streptavidin-phycoerythrin, and were then scanned with the high-resolution GeneChip scanner 3000 (Affymetrix, Santa Clara, CA) in our own facilities. To allow for statistical treatment, data were collected from three experiments. Raw data were collected using the GeneChip Operating Software (GCOS; Affymetrix). Human genome (U133 2.0) chips were used for the assay. A detailed analysis scheme has been illustrated in Figure 2. The.cel files were normalized with GCRMA approach using ArrayAssist® Expression version 4.0 (Stratagene, La Jolla, CA). Fat-specific probes were identified using Netaffx (Affymetrix). This subset was isolated from the main subset using R 2.2.1 software. dChip [*t*-test, $p < 0.05$; median false discovery rate (FDR) <5%] software was used to identify significantly up- or down-regulated genes as described previously (Roy *et al.*, 2003, 2006a, 2006b; Rink *et al.*, 2006). A comprehensive list of genes that were up- or down-regulated greater than 1.2 folds following HCA-SX treatment has been presented in Tables 1 and 2.

Reverse-transcription (RT) and real-time polymerase chain reaction (PCR) analysis

Expression levels of candidate genes' [elongation of very-long-chain fatty acids (ELOVL3); epoxide hydrolase 2, cytoplasmic (EPHX2); peroxisomal trans-2-enoyl-CoA reductase (PECR); perilipin (PLIN); peroxisome proliferative activated receptor γ, coactivator 1α (PPARGC1A); lipase, endothelial

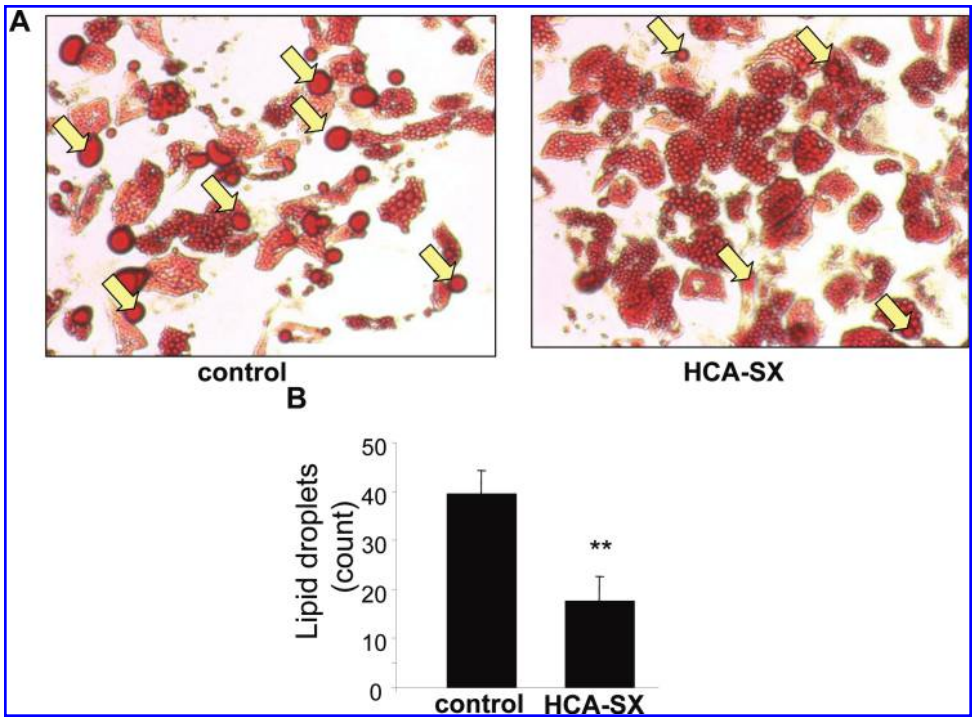

FIG. 1. HCA-SX treatment inhibited lipid droplet formation. Human adipocyte cells were treated with 0.5 mg/mL HCA-SX or untreated (control). Oil red O staining was performed 96 h after HCA-SX treatment, and culture plates were imaged. (**A**) Images of oil red O–stained cultures with or without HCA-SX treatment. Large lipid droplets (shown with yellow arrows) were counted. (**B**) Quantification of the lipid droplet counts. Data shown are mean ± SD ($n = 4$); $p < 0.01$ significantly different compared to control group.

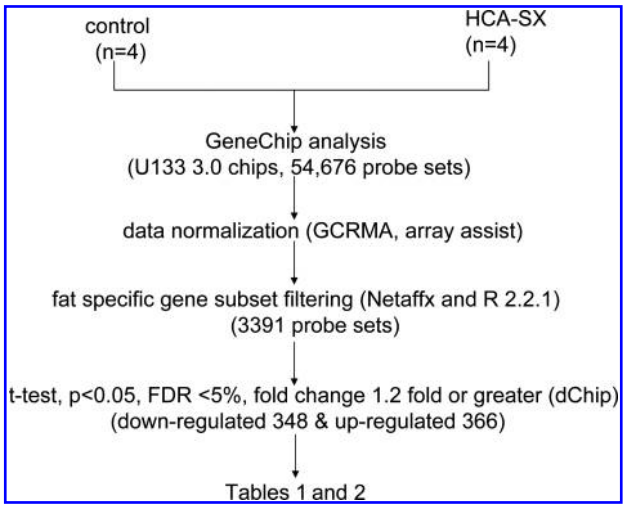

FIG. 2. GeneChip data analysis scheme. Approach to identify HCA-SX-sensitive genes where human adipocyte cells were treated with 0.5 mg/mL SCM ($n = 4$) or untreated (control, $n = 4$). RNA was extracted from both groups and was analyzed using the GeneChip microarray platform. Human genome (U133 2.0) chips were used for the assay. The.cel files were normalized with GCRMA approach using ArrayAssist software. Fat-specific probe sets were identified using Netaffx. This subset was isolated from the main subset using R 2.2.1 software. dChip (*t*-test, $p < 0.05$; median FDR < 5%; fold change: 1.2 or greater) software was used to identify significantly up- or down-regulated genes in the fat subset. FDR, false discovery rate.

(LIPG); matrix metallopeptidase (MMP); lipase hormone-sensitive (LIPE); plasminogen activator tissue (PLAT)] mRNAs were independently determined using RT and real-time PCR as described previously (Rink *et al*., 2006) In brief, total RNA (5 μg) was reverse transcribed into cDNA using oligo-dT primer and Superscript II. RT-generated DNA was quantified by real-time PCR assay using double-stranded DNA-binding dye SYBR Green-I as described previously (Rink *et al*., 2006). Individual gene data were quantified using relative quantification method and external standards. Data were normalized to house keeping gene β-actin (Rink *et al*., 2006). Individual gene primer sequences are listed in Table 3. VEGF primers were commercially obtained (Superarray Biosciences, Frederick, MD).

HRE-luciferase reporter assay

HIF activation was measured using a HIF response element-luciferase (HRE-Luc) promoter–reporter construct. In this assay, HIF activation results in binding of HIF with the promoter region containing five HRE sites. Such binding drives the reporter (luciferase) activity. Therefore, luciferase activity is assayed as a measure of HIF-1 activation (Khanna *et al*., 2006). To increase efficiency of HRE-Luc promoter–reporter construct expression in adipocytes, HRE-Luc adenovirus was constructed by our laboratory using Gateway *in vitro* recombination technology (Invitrogen, Carlsbad, CA). The adenovirus was generated by our laboratory by excising the 5-HRE-CMV mp-Luc fragment from pEF-5HRE CMV mp-Luc+ plasmid that was provided to us by Dr. M. Dewhirst (Duke University). The fragment was inserted into entry vector pENTR11 (Gateway Cloning; Invitrogen). pENTR-5HRE-Luc was recombined *in vitro* with destination vector (pAd-PlL-DEST) using Clonase II (Invitrogen). Correct clones were selected and sequence verified. Plasmid DNA of the correct clone was linearized with PacI, and adenovirus particles were generated at the Viral Vector Core Facility (Children's Research Institute, Columbus, OH). Validation of 5HRE-Luc adenovirus was performed by treating the ad.HRE-Luc infected adipocytes with desferrioxamine, a known inducer of HIF. To study effect of HCA-SX on HIF activation, the adipocytes were infected with ad.HRE-Luc for 48 h followed by treatment with HCA-SX (0.5 mg/mL). After 48 h of HCA-SX treatment, cells luciferase activity was determined from cells using a commercial assay kit (Stratagene) according to the manufacturer's protocol. Luminescence was measured using a LB 9507 luminometer (EG and G Berthold; Bad Wildbad, Germany).

Data presentation and analysis

Data are shown as mean ± SD. Difference between means was tested using Student's *t*-test. Difference between means was considered significant at $p < 0.05$.

Results

Defective lipolysis causes human obesity (Langin *et al*., 2005). Thus, approaches to induce lipolysis of cultured adipocytes are of interest. Two commonly used experimental inducers of lipolysis include (i) isoproterenol, a nonselective β-adrenergic receptor agonist (Ren *et al*., 2006), and (ii) IBMX, an inhibitor of cAMP phosphodiesterase activity (Marcinkiewicz *et al*., 2006). HCA-SX potentiated lipolysis induced by isoproterenol as well as by IBMX (Fig. 3). Potentiation of lipolysis mobilizes triacylglycerol stored in adipose tissue releasing fatty acids that may be used by other tissues. Neutral lipid is stored in spherical organelles called lipid storage droplets that are bound by a coat of proteins. Lipid formation and droplet accumulation go hand in hand. Using oil red O, we visualized and enumerated the production of lipid storage droplets by the cultured mature human adipocytes (Fig. 1A). In the HCA-SX-treated cells, droplet dispersion was noted to be significantly more than in the nontreated cells (Fig. 1B). This observation suggests that HCA-SX treatment influenced the expression of proteins that are known to coat the lipid droplets. Dispersion and loss of droplet size is known to be associated with lipolysis (Cohen *et al*., 2004) consistent with the observation that HCA-SX potentiates lipolysis. Reduction of droplet size is noted during fat loss as seen during weight loss and exercise (He *et al*., 2004). Leptin has been shown to reduce body fat *in vivo* by stimulating lipolysis (Rodriguez *et al*., 2003). Leptin also inhibits lipogenesis *in vivo*. We tested the effect of HCA-SX treatment on leptin protein expression by the human adipocytes. At the concentration used, HCA-SX markedly induced leptin expression in the adipocytes while not compromising cell viability (Fig. 4).

To obtain a comprehensive understanding of the genome-wide effects of low-dose HCA-SX treatment on human adipocytes, we conducted a DNA microarray analysis. As indicated in Figure 4, HCA-SX treatment did not cause any loss of cell viability. Data were collected from four independent experi-

Table 1. Genes (Fat and Obesity Specific) Down-Regulated[a] Following HCA-SX Treatment

Probe ID	*Symbol*	*Description*	*Mean*	*SD*
209368_at	EPHX2	Epoxide hydrolase 2, cytoplasmic	−1.73	0.02
217478_s_at	HLA-DMA	Major histocompatibility complex, class II, DM α	−1.59	0.08
217167_x_at			−1.49	0.24
213855_s_at	LIPE	Lipase, hormone-sensitive	−1.46	0.36
215039_at			−1.45	0.05
222330_at			−1.39	0.17
213992_at	COL4A6	Collagen, type IV, α 6	−1.39	0.13
205960_at	PDK4	Pyruvate dehydrogenase kinase, isoenzyme 4	−1.37	0.28
218730_s_at	OGN	Osteoglycin (osteoinductive factor, mimecan)	−1.36	0.14
200637_s_at	PTPRF	Protein tyrosine phosphatase, receptor type, F	−1.36	0.04
211652_s_at	LBP	Lipopolysaccharide-binding protein	−1.35	0.07
209301_at	CA2	Carbonic anhydrase II	−1.34	0.10
234513_at	ELOVL3	Elongation of very-long-chain fatty acids (FEN1/Elo2, SUR4/Elo3, yeast)-like 3	−1.34	0.12
222722_at	OGN	Osteoglycin (osteoinductive factor, mimecan)	−1.34	0.12
219616_at	FLJ21963	FLJ21963 protein	−1.34	0.08
211819_s_at	SORBS1	Sorbin and SH3 domain containing 1	−1.33	0.19
205220_at	HM74	Putative chemokine receptor	−1.31	0.05
214461_at	LBP	Lipopolysaccharide-binding protein	−1.29	0.06
216627_s_at	B4GALT1	UDP-Gal: βGlcNAc β 1,4-galactosyltransferase, polypeptide 1	−1.26	0.11
205530_at	ETFDH	Electron-transferring flavoprotein dehydrogenase	−1.26	0.04
221142_s_at	PECR	Peroxisomal trans-2-enoyl-CoA reductase	−1.25	0.06
242736_at			−1.25	0.15
33494_at	ETFDH	Electron-transferring flavoprotein dehydrogenase	−1.25	0.03
201981_at	PAPPA	Pregnancy-associated plasma protein A	−1.24	0.08
210096_at	CYP4B1	Cytochrome P450, family 4, subfamily B, polypeptide 1	−1.24	0.01
219181_at	LIPG	Lipase, endothelial	−1.24	0.11
204971_at	CSTA	Cystatin A (stefin A)	−1.24	0.05
219195_at	PPARGC1A	Peroxisome proliferative activated receptor γ, coactivator 1α	−1.24	0.01
202800_at	SLC1A3	Solute carrier family 1 (glial high affinity glutamate transporter), member 3	−1.23	0.02
217279_x_at	MMP14	Matrix metalloproteinase 14 (membrane-inserted)	−1.23	0.07
231678_s_at	ADH4	Alcohol dehydrogenase 4 (class II), pi polypeptide	−1.23	0.03
200635_s_at	PTPRF	Protein tyrosine phosphatase, receptor type, F	−1.23	0.05
229222_at	FLJ21963	FLJ21963 protein	−1.23	0.02
236432_at			−1.23	0.09
205913_at	PLIN	Perilipin	−1.22	0.02
202447_at	DECR1	2,4-Dienoyl CoA reductase 1, mitochondrial	−1.22	0.02
203682_s_at	IVD	Isovaleryl Coenzyme A dehydrogenase	−1.22	0.06
206069_s_at	ACADL	Acyl-Coenzyme A dehydrogenase, long chain	−1.21	0.13
211577_s_at	IGF1	Insulin-like growth factor 1 (somatomedin C)	−1.21	0.10
203295_s_at	ATP1A2	ATPase, Na^+/K^+ transporting, α 2 (+) polypeptide	−1.20	0.04
210070_s_at	CPT1B	Carnitine palmitoyltransferase 1B (muscle)	−1.20	0.11
203424_s_at	IGFBP5	Insulin-like growth factor binding protein 5	−1.20	0.10
210145_at	PLA2G4A	Phospholipase A2, group IVA (cytosolic, calcium-dependent)	−1.20	0.02

Data presented indicate fold change (mean ± SD) in expression of HCS-SX-treated genes compared to corresponding control samples. Probe ID, Affymetrix probe identification. Data correspond to Figure 5 cluster A.

[a] $p < 0.05$; FDR < 5%; fold change = 1.2 or greater.

ments so that appropriate data treatment could be possible. The data analysis design is illustrated in Figure 2 and was modeled on the basis of our previously published study (Rink *et al*., 2006). A total of 54,676 probe sets were screened. Treatment of adipocytes with HCA-SX resulted in altered expression profile of fat- and obesity-related genes as demonstrated in Figure 5. HCA-SX treatment resulted in a statistically significant effect that included down-regulation of 348, and induction of 366 fat- and obesity-related genes. An itemized list of the affected genes is presented in Tables 1 and 2. dChip-assisted visualization of the raw data collected from each case illustrated that the observed effect was very tight between experiments (Fig. 5).

To validate our DNA microarray results, the expression of select HCA-SX sensitive genes was independently determined using real-time PCR (Table 3). Using this approach, we typically find that the microarray approach underestimates the magnitude of change while reliably detecting the direction of change (Roy *et al*., 2003). We observed a consistent trend in the current study. Several HCA-SX-sensitive genes were selected for real-time PCR verification of microarray outcome. Results of the microarray experiment shed critical light on the

Table 2. Genes (Fat and Obesity Specific) Up-Regulated[a] Following HCS-SX Treatment

Probe ID	*Symbol*	*Description*	*Mean*	*SD*
205828_at	MMP3	Matrix metalloproteinase 3 (stromelysin 1, progelatinase)	1.64	0.12
201860_s_at	PLAT	Plasminogen activator, tissue	1.63	0.03
205680_at	MMP10	Matrix metalloproteinase 10 (stromelysin 2)	1.50	0.17
202859_x_at	IL8	Interleukin 8	1.46	0.03
223216_x_at	PBF	Papillomavirus regulatory factor PRF-1	1.44	0.11
214063_s_at	TF	Transferrin	1.39	0.03
206561_s_at	AKR1B10	Aldo-keto reductase family 1, member B10 (aldose reductase)	1.38	0.06
203213_at	CDC2	Cell division cycle 2, G1 to S and G2 to M	1.38	0.08
203400_s_at	TF	Transferrin	1.38	0.02
242255_at	KIAA0982	KIAA0982 protein	1.36	0.13
205439_at	GSTT2	Glutathione S-transferase theta 2	1.36	0.13
242775_at			1.34	0.03
221123_x_at	PBF	Papillomavirus regulatory factor PRF-1	1.33	0.08
210559_s_at	CDC2	Cell division cycle 2, G1 to S and G2 to M	1.29	0.05
232693_s_at	PBF	Papillomavirus regulatory factor PRF-1	1.29	0.07
242605_at	DCN	Decorin	1.27	0.05
203214_x_at	CDC2	Cell division cycle 2, G1 to S and G2 to M	1.26	0.14
211478_s_at	DPP4	Dipeptidylpeptidase 4 (CD26, adenosine deaminase complexing protein 2)	1.26	0.02
203027_s_at	MVD	Mevalonate (diphospho) decarboxylase	1.26	0.02
211506_s_at			1.26	0.12
228729_at	CCNB1	Cyclin B1	1.25	0.14
219049_at	ChGn	Chondroitin β1,4 N-acetylgalactosaminyltransferase	1.25	0.03
222536_s_at	DKFZp434K1210	Hypothetical protein DKFZp434K1210	1.25	0.11
218499_at	MST4	Mst3 and SOK1-related kinase	1.24	0.01
228825_at	LTB4DH	Leukotriene B4 12-hydroxydehydrogenase	1.24	0.05
210868_s_at	ELOVL6	ELOVL family member 6, elongation of long-chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	1.24	0.02
211892_s_at	PTGIS	Prostaglandin I2 (prostacyclin) synthase	1.23	0.13
210050_at	TPI1	Triosephosphate isomerase 1	1.23	0.02
201250_s_at	SLC2A1	Solute carrier family 2 (facilitated glucose transporter), member 1	1.23	0.03
207092_at	LEP	Leptin (obesity homolog, mouse)	1.22	0.03
236474_at			1.22	0.05
222856_at	APLN	Apelin	1.22	0.03
204256_at	ELOVL6	ELOVL family member 6, elongation of long-chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	1.21	0.01
230657_at	CLOCK	Clock homolog (mouse)	1.21	0.09
223557_s_at	TMEFF2	Transmembrane protein with EGF-like and two follistatin-like domains 2	1.20	0.13
222077_s_at	RACGAP1	Rac GTPase activating protein 1	1.20	0.01
202936_s_at	SOX9	SRY (sex-determining region Y)-box 9 (campomelic dysplasia, autosomal sex-reversal)	1.20	0.03

Data presented indicate fold change (mean ± SD) in expression of HCA-SX-treated genes compared to corresponding control samples. Probe ID, Affymetrix probe identification. Data correspond to Figure 5 cluster A.

[a] $p < 0.05$; FDR <5%; fold change = 1.2 or greater.

mechanism of action of HCA-SX. One of the genes down-regulated by HCA-SX was PLIN (Fig. 6). The PLINs are the most abundant proteins coating the surfaces of lipid droplets in adipocytes, which are found at lower levels surrounding lipid droplets in steroidogenic cells. PLINs drive triacylglycerol storage in adipocytes (Garcia *et al.*, 2003). HCA-SX-induced suppression in the expression of PLIN is likely to be responsible for the observed lipid droplet dispersion caused by HCA-SX (Fig. 1). PPARGC1 is a transcriptional coactivator protein that plays a critical role in the maintenance of glucose, lipid, and energy homeostasis, and are likely involved in the pathogenic conditions, such as obesity. HCA-SX treatment down-regulated PPARGC1 expression in adipocytes. Increased visceral adiposity is significantly correlated with elevated plasma levels of LIPG. LIPG has substantial phospholipase activity, but less triglyceride lipase activity. Overexpression of endothelial lipase undesirably reduces plasma high-density lipoprotein cholesterol levels in animal models. High-density lipoprotein hydrolysis by endothelial lipase activates PPARα and causes inflammation. HCA-SX treatment decreased the expression of LIPG in adipocytes (Fig. 6). Very little is known about the *in vivo* regulation of mammalian fatty

Table 3. Primers Used for Real-Time PCR

mRNA	*Primer sequence 5′ to 3′*
h_ELOVL3	CTCTGGTCCTTCTGCCTTGCA
	TTGATGAAGCACACGGTTTGC
h_EPHX2	GTGACCGGAATCCAGCTTCTCAATA
	CCAAGAATACCAACTCTCGGGAAAT
h_LEP	CACCAAAACCCTCATCAAGACAATT
	CCAGTGTCTGGTCCATCTTGGATAA
h_LIPG	CAGAGGGACGGCTGGAAGATAA
	CGACGGAGAGGTAGCATCCTTC
h_LIPE	GCTCAACAAGAAGCTGAATCAACA
	TTGTTAGAAATCCCAGCTCTGTCAA
h_MMP1	GGGCTTGAAGCTGCTTACGAATT
	CAGCATCGATATGCTTCACAGTTCT
h_MMP3	GGCTGTATGAAGGAGAGGCTGATAT
	CCTTTGTCCATTGTTCATCATCATC
h_MMP10	ATTTTGGCCCTCTCTTCCATCATAT
	CATCAATTTTCCTTATGGTTGGAGG
h_PECR	CGGAATGAGGAGGAGGTGAATAA
	CGTCAGGTTGGTCTCAAGCACA
h_PLIN	GCTTGGACCACCTGGAGGAA
	GGGTGGAGATGGTGTCCTTCAGC
h_PLAT	AGAGGAGCCAGATCTTACCAAGTGA
	GCGCAGCCATGACTGATGTT
h_PPARA	GGACAAGGCCTCAGGCTATCATTA
	CGACAGAAAGGCACTTGTGAAAT
h_PPARGC1A	CCACCCACCACTCCTCCTCATAA
	GGCCCTTTCTTGGTGGAGTTATT

acid chain elongation enzymes, as well as the role of specific fatty acid chain length in cellular responses and developmental processes. The ELOVL3 gene product participates in the formation of specific neutral lipids. ELOVL3 has now been recognized as an important facilitator of adipogenesis by catalyzing the synthesis of saturated very-long-chain fatty acids and triglyceride formation in adipose tissue. We noted that in adipocytes, HCA-SX treatment significantly down-regulated ELOVL3 gene expression (Fig. 6). Human soluble epoxide hydrolase metabolizes a variety of epoxides to the corresponding vicinal diols. Arachidonic and linoleic acid epoxides are thought to be endogenous substrates for human soluble epoxide hydrolase. Cytochrome P450 epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids, which are converted to dihydroxyeicosatrienoic acids by soluble epoxide hydrolase, such as EPHX2. Genetic variation in EPHX2 is

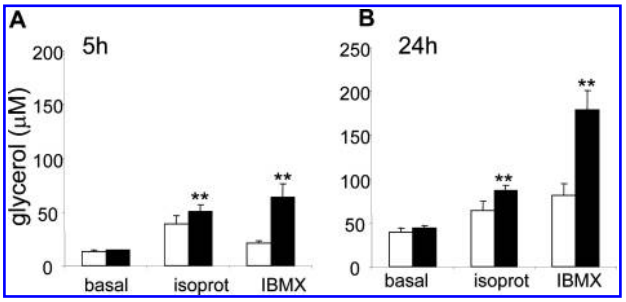

FIG. 3. HCA-SX augmented isoproterenol- and IBMX-stimulated lipolysis in human adipocytes. Human adipocyte cells were treated with 0.5 mg/mL HCA-SX (filled bars) or untreated (control, open bars). Cells were simultaneously treated with either isoproterenol (1 μM) or 3-isobutyl-1-methylxanthine (IBMX, 100 μM) to stimulate lipolysis. Lipolysis was assayed by measuring glycerol content in media either 5 h (**A**) or 24 h (**B**) after HCA-SX, isoproterenol, or IBMX treatments. Data shown are mean ± SD ($n = 6$); *$p <$ 0.05 and **$p < 0.0001$ significant compared to matched untreated controls.

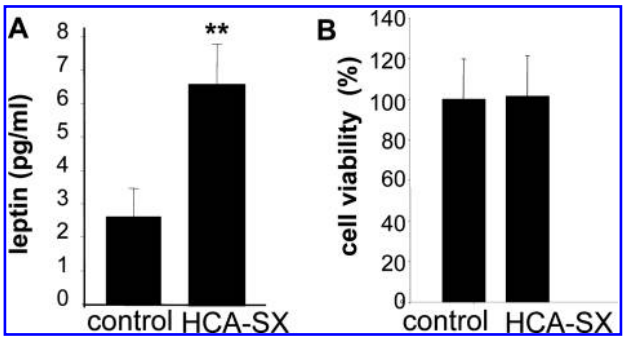

FIG. 4. The effect of HCA-SX on leptin secretion and cell viability. Human adipocyte cells were treated with 0.5 mg/mL HCA-SX or untreated (control) for 48 h. (**A**) Leptin levels in the culture media were measured using a solid-phase ELISA method. Data ($n = 4$) shown are mean ± SD; *$p < 0.01$ significantly different compared to control group. (**B**) Effect of HCA-SX treatment on cell viability was determined using the standard LDH leakage assay. Data presented as % compared to control (untreated) group. Data are mean ± SD, $n = 4$.

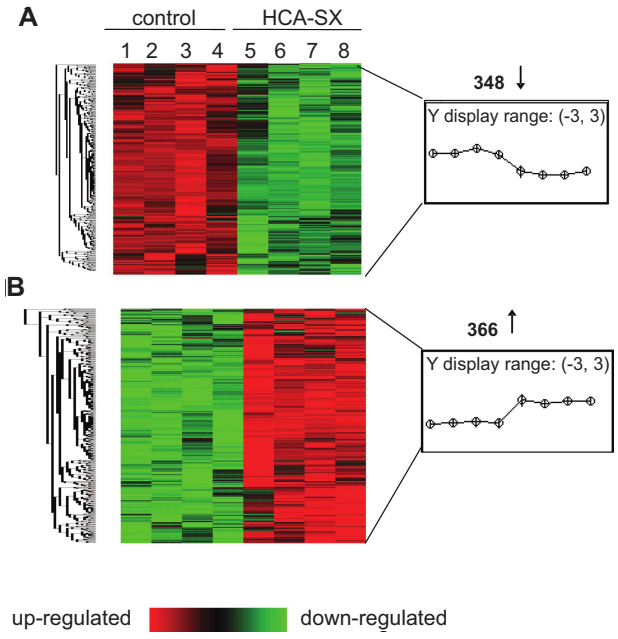

FIG. 5. Cluster images illustrating genes sensitive to HCA-SX. For a clear graphic display of HCA-SX-sensitive genes (as described in Fig. 2), the genes that significantly ($p < 0.05$) changed between control and treated groups were selected and subjected to hierarchical clustering using the dChip software. *Red* to *green* gradation in color represents higher to lower expression signal. Down-regulated (**A**) or up-regulated (**B**) genes in HCA-SX-treated group as compared to control.

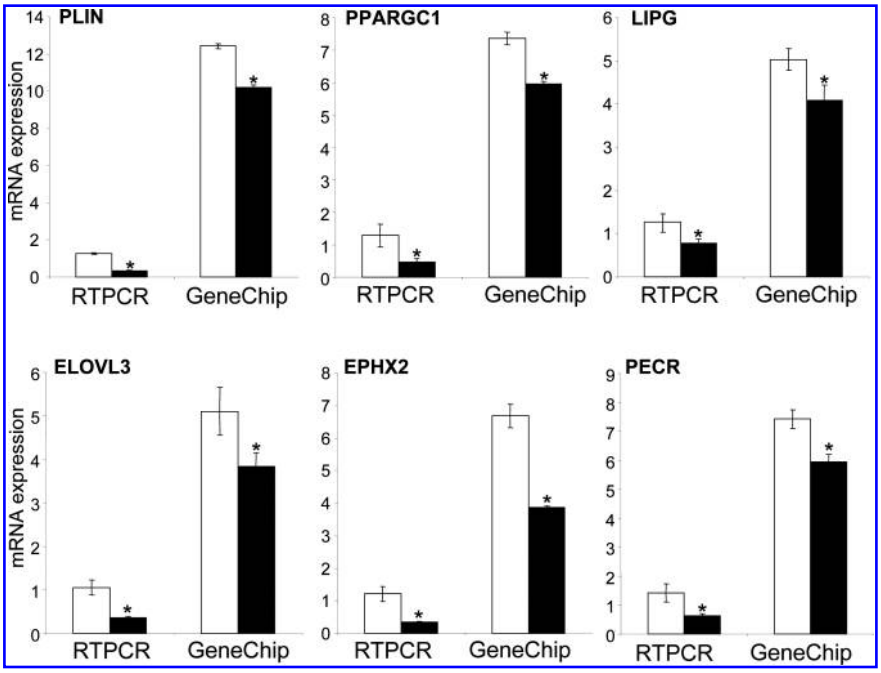

FIG. 6. Real-time PCR validation of HCA-SX-induced down-regulated genes. Expression levels of selected genes identified using GeneChip analysis were independently determined using real-time PCR. For comparison, the real-time PCR data (normalized to β-actin, a housekeeping gene) were proportionately adjusted to fit to the scale with GeneChip expression values (normalized using global scaling approach). Down-regulated genes were selected from Table 1. ELOVL3, elongation of very-long-chain fatty acids; EPHX2, epoxide hydrolase 2, cytoplasmic; PECR, peroxisomal trans-2-enoyl-CoA reductase; PLIN, perilipin; PPARGC1A, peroxisome proliferative activated receptor γ, coactivator 1α; LIPG, lipase, endothelial; LIPE, lipase, hormone-sensitive.

significantly associated with risk of incident coronary heart disease in Caucasians, implicating EPHX2 as a potential cardiovascular disease-susceptibility gene. HCA-SX treatment significantly lowered EPHX2 expression in human adipocytes (Fig. 6).

Phytanic acid is a derivative of the phytol side chain of chlorophyll. It appears in humans following the ingestion of fat-containing foods and is present in human blood at a low micromolar concentration. It may activate retinoid X receptors (RXR) or peroxisome proliferator-activated receptor (PPAR) α *in vitro*. Phytanic acid induces the adipocyte differentiation of 3T3-L1 cells in culture, as assessed by accumulation of lipid droplets and induction of the aP2 mRNA marker. In human preadipocytes in primary culture, phytanic acid also induced adipocyte differentiation. Phytol is a naturally occurring precursor of phytanic acid. The last step in the conversion of phytol to phytanoyl-CoA is the reduction of phytanoyl-CoA mediated by a PECR (Gloerich *et al*., 2006). Thus, PECR catalyzes the chain elongation of fatty acids in peroxisomes. Expression of PECR in adipocytes was down-regulated in response to HCA-SX treatment (Fig. 6).

Among the genes that were induced in adipocytes in response to HCA-SX treatment, members of the MMP family, leptin, and PLAT were prominent (Fig. 7). In the context of obesity and related disorders, leptin has numerous positive functions, including fat oxidation, weight-loss, and cardioprotection. The significant induction of leptin gene and protein by HCA-SX is of outstanding significance (Figs. 4 and 7). MMP plays specific roles in tissue remodeling. For example, angiogenesis is known to feed adipogenesis and growth of adipose mass. MMP-10 is a secreted endoproteinase that degrades the extracellular matrix and disrupts the vasculature. Treatment of adipocytes with HCA-SX potently induced the expression of MMP-10 (Fig. 7). MMP-1 and MMP-10 are important for fibrillar collagen and proteoglycan turnover. Proteoglycan turnover is directly implicated in adipocyte degradation and weight loss. MMP-3 and MMP-10 are also known as stromelysin 1 and 2. Lower stromelysin levels have been linked to obesity. HCA-SX significantly induced the expression of both stromelysin 1 and 2 in adipocytes (Fig. 7). MMP-3 activates MMP-1 (Sasaki *et al*., 2006). Consistently, in addition to inducing MMP-3, HCA-SX up-regulated the expression of MMP-1 in the adipocytes examined (Fig. 7). PLAT (EC 3.4.21.68) is a serine protease that activates the proenzyme plasminogen to plasmin, which in turn is responsible for fibrinolytic activity. PLAT is synthesized in vascular endothelial cells as a single polypeptide chain. Tissue-type plasminogen activator regulates fibrinolysis and is considered to be a primary endogenous defense mechanism against thrombosis. Adiposity is associated with an increased risk of atherothrombotic events. Overweight and obesity are associated with profound endothelial fibrinolytic dysfunction. Regular aerobic exercise can increase the capacity of PLAT release. Treatment of human adipocytes from obese women resulted in significant induction of PLAT (Fig. 7).

In adipocytes, HIF is activated by norepinephrine, a potent inducer of lipolysis (Nikami *et al*., 2005). HIF is also known to transactivate the human leptin gene promoter (Grosfeld *et al*., 2002). In this study, HCA-SX was noted to potentiate lipolysis and induce leptin expression. Because the mechanism of

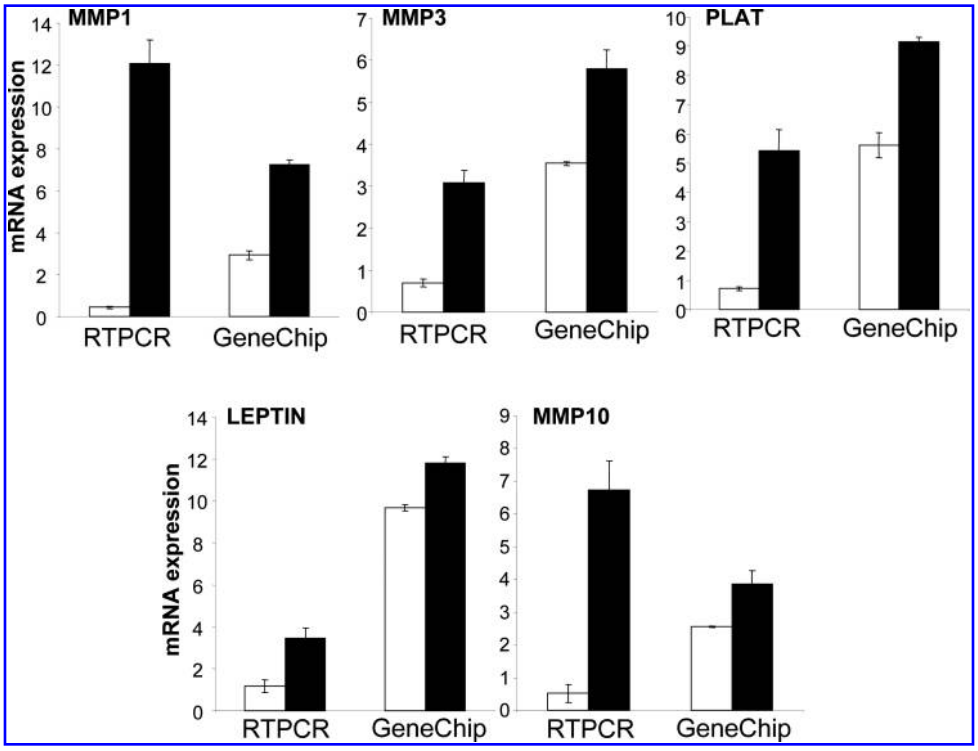

FIG. 7. Real-time PCR validation of HCA-SX-induced up-regulated genes. For description of approach, see legend of Figure 6. Up-regulated genes were selected from Table 2. MMP, matrix metallopeptidase; PLAT, plasminogen activator, tissue.

action of HIF closely matched the outcomes in response to HCA-SX, we sought to test the hypothesis that HCA-SX causes HIF transactivation. Using a HIF reporter assay construct, we noted that HCA-SX treatment significantly increased HIF transactivation. Desferrioxamine was used as a positive control (Fig. 8A). To test whether HCA-SX-induced HIF transactivation was effective in turning on HIF-driven genes, the expression of VEGF was studied. Consistent with the effects on inducing HIF transactivation, HCA-SX induced both VEGF gene and protein expression (Fig. 8B).

Discussion

A large number of herbal dietary supplements, available in the marketplace, that are aimed at weight management lack credible scientific evidence supporting safety, efficacy, and underlying mechanisms. Although it remains to be proven in large-scale clinical trials that herbal supplements represent a safe and efficient tool against obesity, public interest in these solutions is substantial. The fact that obesity is under strong genetic control has been well established (Liu *et al*., 2003). Thus, investigating the genetic basis of the effects of herbal supplements on weight management represents a powerful tool to identify underlying mechanisms and to determine possible health safety–related issues associated with the consumption of the specific supplement. DNA microarray is specifically suited to address genome-wide changes in gene expression profile in response to any given therapeutic regimen (Liu-Stratton *et al*., 2004). (-)-HCA, a natural plant extract from the dried fruit rind of *G. cambogia*, has been reported to promote body fat loss in humans without stimulating the central nervous system. The level of effectiveness of *G. cambogia* extract is typically attributed solely to HCA. Typically, HCA used in dietary weight-loss supplements is bound to calcium, which results in a poorly soluble (<50%) and less bioavailable form. Conversely, the structural characteristics of a novel Ca^{2+}/K^{+}–bound (-)-HCA salt make it completely water soluble, as well as bioavailable (Downs *et al*., 2005). Among the herbal supplements used for body weight reduction, HCA has been found to be particularly safe, and therefore further research has been recommended (Lenz and Hamilton, 2004; Pittler *et al*., 2005). In a recent study, we observed that at doses relevant for human consumption, dietary HCA-SX significantly limited weight gain (Roy *et al*., 2004). The current study represents the first effort to investigate the effects of a widely consumed herbal supplement, HCA-SX, on the transcriptome of human adipocytes.

In mature adipocytes, triglyceride is stored within lipid droplets, which are coated with the protein PLIN, which functions to regulate lipolysis by controlling lipase access to the droplet in a hormone-regulated fashion (Gross *et al*., 2006). Consistent with the observation that HCA-SX augmented induced-lipolysis, HCA-SX facilitated lipolysis by inducing the dispersion of lipid droplets. These results suggest that HCA-SX may have compromised the ability of the lipid droplets to guard themselves against lipases. Indeed, the genome-screen approach shed critical light explaining the mechanism of action. Mobilization of stored triglyceride is controlled by interactions among intracellular lipases and the PLIN protein coat that safeguards lipid storage droplets. Treatment of adipocytes with HCA-SX significantly lowered PLIN expression. This is expected to make the lipid droplets more vulnerable to lipase-dependent lipolysis. Indeed, HCA-SX was observed to cause the dispersion of lipid droplet dispersion consistent

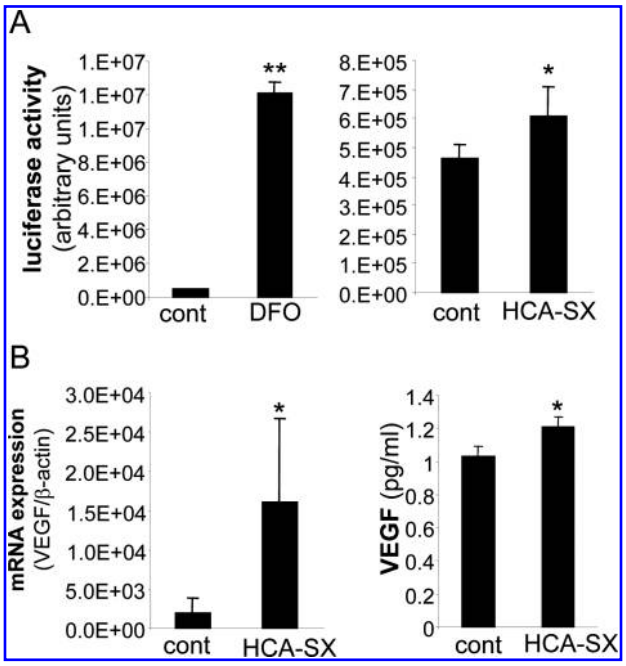

FIG. 8. HCA-SX induced HIF-1 transactivation and increased the expression of HIF-driven gene VEGF. (**A**) Human adipocyte cells were infected with adenovirus carrying a HRE promoter–luciferase reporter construct. *Right*: 24 h after the adenovirus infection, the cells were either treated with HCA-SX (0.5 mg/mL) or not treated (control). Luciferase activity was measured 48 h after the HCA-SX treatment. Data are mean ± SD ($n = 6$); *$p < 0.05$ compared to control. *Left*: Positive control. Desferrioximine (DFO, 50 μM, 24 h) was used as a known activator of HIF. (**B**) *Left*: VEGF mRNA expression. mRNA was quantified using real-time PCR (data normalized using β-actin as a housekeeping gene). *Right*: VEGF protein levels. In cell culture media as determined by ELISA. Data are mean ± SD ($n = 3$), *$p < 0.05$ compared to control.

with its ability to stimulate lipolysis by this mechanism of action. This observation is consistent with a previous observation reporting that the extract of *G. cambogia* inhibited lipid droplet accumulation in fat cells (Hasegawa, 2001). PPARGC1A has emerged as a master regulator of mitochondrial biogenesis (Leary and Shoubridge, 2003; Moyes, 2003; St-Pierre *et al*., 2003). PPARGC1A is induced during mitochondrial adaptations associated with tissue growth (Irrcher *et al*., 2003). Mitochondrial biogenesis is known to accompany adipogenesis to complement ATP and acetyl-CoA required for lipogenesis (Kim *et al*., 2004a). HCA-SX-induced down-regulation of PPARGC1A is consistent with the antiadipogenic property of this herbal weight-loss supplement.

A direct functional relationship between elongation of fatty acids and their incorporation into the adipocyte subcellular membranes has been suggested (Giacobino and Chmelar, 1977). A significant amount of the fatty acids synthesized by the cytosolic enzyme complex fatty acid synthase or taken up by the diet is further elongated into very long-chain fatty acids in a four-step reaction cycle by membrane-bound enzymes predominantly located in the endoplasmic reticulum. Members of the ELOVL gene family encode for elongases, which perform the first regulatory step (condensation) in the elongation cycle in mammals. This family of enzymes consists of at least six members in mouse and human, which are believed to carry out substrate-specific elongation with fatty acids of different lengths and degrees of unsaturation. ELOVL3 is involved in the elongation of saturated and monounsaturated very-long-chain fatty acids and, therefore, supports adipogenesis (Westerberg *et al*., 2006). During adipogenesis, ELOVL3 gene expression is markedly induced (Westerberg *et al*., 2006). Like ELOVL3, PECR catalyzes the chain elongation of fatty acids. In this case, the elongation of fatty acids happens in peroxisomes (Gloerich *et al*., 2006). HCA-SX-induced down-regulation of ELOVL3 and PECR in the human adipocyte is consistent with the antiadipogenic function of the herbal supplement. That HCA-SX may have antiadipogenic function is also supported by its effect on EPHX2. Human soluble epoxide hydrolase metabolizes a variety of epoxides to the corresponding vicinal diols. Increased soluble epoxide hydrolases are associated with impaired mesenteric artery dilator function in obese Zucker rats (Zhao *et al*., 2005). Cytochrome P450 epoxygenases metabolize arachidonic acid to epoxyeicosatrienoic acids, which are converted to dihydroxyeicosatrienoic acids by soluble epoxide hydrolase, such as EPHX2. The dihydroxyeicosatrienoic acids may support adipogenesis by enhancing blood supply to the tissue by means of its vasodilatory role. Dihydroxyeicosatrienoic acids may also function as an endogenous activator of PPARα.

Leptin is a recently isolated versatile 16 kDa circulating peptide hormone that is primarily synthesized and secreted by adipocytes. One of the major functions of this hormone is the control of energy balance by binding to receptors in the hypothalamus, leading to reduction in food intake, elevation in temperature, and energy expenditure (Paracchini *et al*., 2005). Originally known as the satiety factor, leptin is an attractive candidate for the treatment of obesity as it is an endogenous protein that has been demonstrated to have potent effects on body weight and adiposity in rodents. Leptin inhibits lipogenesis in adipocytes (Ramsay, 2003). Leptin has been successfully used in the treatment of leptin-deficient obese patients. Strategies aiming at enhancing leptin delivery systems are thought to be useful in treating obesity (Bell-Anderson and Bryson, 2004). Thus, the inducing effect of HCA-SX on leptin expression by human adipocytes is encouraging for the treatment of obesity.

The extracellular matrix is critical for all aspects of vascular biology. In concert with supporting cells, endothelial cells assemble a laminin-rich basement membrane matrix that provides structural and organizational stability. MMP-10 is a secreted endoproteinase that degrades the extracellular matrix and exerts antiangiogenic functions by disrupting the vasculature (Chang *et al*., 2006). Repression of MMP-10 facilitates blood vessel development (Chang *et al*., 2006). MMP-1 is an interstitial collagenase that plays a pivotal role in degradation of interstitial collagen types I, II, and III. In addition, MMP-1 degrades proteoglycans. Collagen is transcribed during adipogenesis and supports triglyceride accumulation in adipocytes. HCA-SX-induced expression of collagenase is consistent with the antiadipogenic function of the dietary supplement. Stromelysin, a member of the metalloproteinase gene family, plays a crucial role in the turnover of connective tissues. MMP-3 and MMP-10 are also known as stromelysin 1 and 2. Lower stromelysin levels have been linked to obesity (Lijnen *et al*., 2002; Maquoi *et al*., 2003). When MMP-3$^{-/-}$ and wild-type controls were kept on a high-fat diet for 15 weeks,

MMP-3$^{-/-}$ mice were hyperphagic and gained more weight than the MMP-3$^{+/+}$ mice. At the time of sacrifice, the body weight of the MMP-3$^{-/-}$ mice was significantly higher than that of the corresponding control mice, as was the weight of the isolated subcutaneous and gonadal deposits. Significant adipocyte hypertrophy was observed in the gonadal fat of MMP-3$^{-/-}$ mice. Thus, in a murine model of nutritionally induced obesity, MMP-3 impairs adipose tissue development, possibly by affecting food intake and/or adipose tissue–related angiogenesis (Maquoi *et al*., 2003). MMP-3 activates MMP-1. PLAT is a secreted serine protease that converts the proenzyme plasminogen to plasmin, a fibrinolytic enzyme. Plasminogen is synthesized as a single chain, which is cleaved by PLAT into the two-chain disulfide-linked plasmin. This enzyme plays a role in cell migration and tissue remodeling. PLAT represents the primary endogenous defense mechanism against thrombosis. Adiposity is associated with an increased risk of atherothrombotic events. Overweight and obesity are associated with profound endothelial fibrinolytic dysfunction. Induction of PLAT by HCA-SX may be viewed as a favorable response in that respect. Regular aerobic exercise can increase the capacity of PLAT release (Van Guilder *et al*., 2005).

Inducers of HIF activity are thought to be effective in inhibiting adipogenesis and in controlling obesity (Yun *et al*., 2002; Wada *et al*., 2006). HIF-stabilizing technologies represent a novel therapeutic strategy to manage obesity (www.fibrogen.com). Our observation that HCA-SX is an inducer of HIF transactivation is therefore of practical significance. Russians with Chuvash syndrome, a genetic mutation that leads to persistent HIF stabilization, show the impact of HIF system on metabolism. The incidence of obesity and the mean BMI in people with Chuvash syndrome are significantly lower than in their spouses, who are expected to have similar life styles and eating habits (Gordeuk and Prchal, 2006).

Regulation of adipocyte function by herbal supplements has provided encouraging results. *Hibiscus sabdariffa* specifically regulates adipogenic differentiation of 3T3-L1 cells at the cellular and molecular levels. Hibiscus extract inhibits adipocyte differentiation of 3T3-L1 preadipocytes induced by insulin, dexamethasone, and isobutylmethylxanthine. Hibiscus also blocked the cytoplasmic lipid accumulation. Hibiscus extract significantly attenuated the expression of key adipogenic transcription factors, including CCAAT element–binding protein (C/EBP) α and PPARγ (Kim *et al*., 2003). Of note, HCA-SX content in Hibiscus extract is high (Hida *et al*., 2005). Compared to other herbals in the weight-loss claim category, *Garcinia* seems to have a better safety record (Burdock *et al*., 2005; Pittler *et al*., 2005). (-)-HCA is a potent inhibitor of ATP citrate lyase, which catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. 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 (Jena *et al*., 2002). Chronic (-)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice (Ishihara *et al*., 2000). Consistent with findings of the current study, *Garcinia* extract has been noted to inhibit lipid droplet accumulation in fat cells (Hasegawa, 2001; Kim *et al*., 2004b). Flavonoids from *G. cambogia* exerted hypolipidemic activity in rats (Koshy and Vijayalakshmi, 2001; Mahendran and Devi, 2001). Extract of *G. cambogia* was found to enhance satiety via a serotonin-mediated pathway (Kaur and Kulkarni, 2001; Ohia *et al*., 2001; Roy *et al*., 2004). In addition, the extract improved glucose metabolism (Hayamizu *et al*., 2003). When treated over a period of 90 days, HCA-SX caused reduction in body weight in rats (Shara *et al*., 2003). Preliminary studies testing the effects of HCA-SX in humans are promising (Preuss *et al*., 2004, 2005). While the sum of current evidence in favor of HCA-SX is clearly encouraging, it is important to recognize that the bulk of that data comes from the study of rodents or rodent cell lines. The comprehensive screening performed in the current study should provide directed guidance in generating new hypotheses to explain the mechanism of action of HCA-SX. Additional studies testing the weight-loss claims of HCA-SX on human material and population are warranted.

References

Anonymous (2003). Working to get ephedra banned. Consum. Rep. **68,** 6.

Bell-Anderson, K.S., and Bryson, J.M. (2004). Leptin as a potential treatment for obesity: progress to date. Treat. Endocrinol. **3,** 11–18.

Burdock, G., Bagchi, M., and Bagchi, D. (2005). Garcinia cambogia toxicity is misleading. Food Chem. Toxicol. **43,** 1683–1684 (author reply 1685–1686).

Caterson, I.D., and Gill, T.P. (2002). Obesity: epidemiology and possible prevention. Best Pract. Res. Clin. Endocrinol. Metab. **16,** 595–610.

Chang, S., Young, B.D., Li, S., Qi, X., Richardson, J.A., and Olson, E.N. (2006). Histone deacetylase 7 maintains vascular integrity by repressing matrix metalloproteinase 10. Cell **126,** 321–334.

Cohen, A.W., Razani, B., Schubert, W., Williams, T.M., Wang, X.B., Iyengar, P., Brasaemle, D.L., Scherer, P.E., and Lisanti, M.P. (2004). Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. Diabetes **53,** 1261–1270.

Downs, B.W., Bagchi, M., Subbaraju, G.V., Shara, M.A., Preuss, H.G., and Bagchi, D. (2005). Bioefficacy of a novel calcium-potassium salt of (-)-hydroxycitric acid. Mutat. Res. **579,** 149–162.

Garcia, A., Sekowski, A., Subramanian, V., and Brasaemle, D.L. (2003). The central domain is required to target and anchor perilipin A to lipid droplets. J. Biol. Chem. **278,** 625–635.

Giacobino, J.P., and Chmelar, M. (1977). The role of chain elongation systems in the supplying of fatty acids to the adipocyte membrane lipids. Biochim. Biophys. Acta **487,** 269–276.

Gloerich, J., Ruiter, J.P., Van Den Brink, D.M., Ofman, R., Ferdinandusse, S., and Wanders, R.J. (2006). Peroxisomal trans-2-enoyl-CoA reductase is involved in phytol degradation. FEBS Lett. **580,** 2092–2096.

Gordeuk, V.R., and Prchal, J.T. (2006). Vascular complications in Chuvash polycythemia. Semin. Thromb. Hemost. **32,** 289–294.

Grosfeld, A., Andre, J., Hauguel-De Mouzon, S., Berra, E., Pouyssegur, J., and Guerre-Millo, M. (2002). Hypoxia-inducible factor 1 transactivates the human leptin gene promoter. J. Biol. Chem. **277,** 42953–42957.

Gross, D.N., Miyoshi, H., Hosaka, T., Zhang, H.H., Pino, E.C., Souza, S., Obin, M., Greenberg, A.S., and Pilch, P.F. (2006). Dynamics of lipid droplet-associated proteins during hormonally stimulated lipolysis in engineered adipocytes: stabilization and lipid droplet binding of adipocyte differentiation-related protein/adipophilin. Mol. Endocrinol. **20,** 459–466.

Hasegawa, N. (2001). Garcinia extract inhibits lipid droplet accumulation without affecting adipose conversion in 3T3-L1 cells. Phytother. Res. **15,** 172–173.

Hayamizu, K., Hirakawa, H., Oikawa, D., Nakanishi, T., Takagi, T., Tachibana, T., and Furuse, M. (2003). Effect of Garcinia cambogia extract on serum leptin and insulin in mice. Fitoterapia **74,** 267–273.

He, J., Goodpaster, B.H., and Kelley, D.E. (2004). Effects of weight loss and physical activity on muscle lipid content and droplet size. Obes. Res. **12,** 761–769.

Heber, D. (2003). Herbal preparations for obesity: are they useful? Prim. Care **30,** 441–463.

Hida, H., Yamada, T., and Yamada, Y. (2005). Production of hydroxycitric acid by microorganisms. Biosci. Biotechnol. Biochem. **69,** 1555–1561.

Irrcher, I., Adhihetty, P.J., Sheehan, T., Joseph, A.M., and Hood, D.A. (2003). PPARgamma coactivator-1alpha expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am. J. Physiol. Cell. Physiol. **284,** 1669–1677.

Ishihara, K., Oyaizu, S., Onuki, K., Lim, K., and Fushiki, T. (2000). Chronic (-)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice. J. Nutr. **130,** 2990–2995.

Jena, B.S., Jayaprakasha, G.K., Singh, R.P., and Sakariah, K.K. (2002). Chemistry and biochemistry of (-)-hydroxycitric acid from Garcinia. J. Agric. Food Chem. **50,** 10–22.

Kaur, G., and Kulkarni, S.K. (2001). Investigations on possible serotonergic involvement in effects of OB-200G (polyherbal preparation) on food intake in female mice. Eur. J. Nutr. **40,** 127–133.

Khanna, S., Roy, S., Maurer, M., Ratan, R.R., and Sen, C.K. (2006). Oxygen-sensitive reset of hypoxia-inducible factor transactivation response: prolyl hydroxylases tune the biological normoxic set point. Free Radic. Biol. Med. **40,** 2147–2154.

Kim, B.W., Choo, H.J., Lee, J.W., Kim, J.H., and Ko, Y.G. (2004a). Extracellular ATP is generated by ATP synthase complex in adipocyte lipid rafts. Exp. Mol. Med. **36,** 476–485.

Kim, M.S., Kim, J.K., Kim, H.J., Moon, S.R., Shin, B.C., Park, K.W., Yang, H.O., Kim, S.M., and Park, R. (2003). Hibiscus extract inhibits the lipid droplet accumulation and adipogenic transcription factors expression of 3T3-L1 preadipocytes. J. Altern. Complement. Med. **9,** 499–504.

Kim, M.S., Kim, J.K., Kwon, D.Y., and Park, R. (2004b). Anti-adipogenic effects of Garcinia extract on the lipid droplet accumulation and the expression of transcription factor. Biofactors **22,** 193–196.

Koshy, A.S., and Vijayalakshmi, N.R. (2001). Impact of certain flavonoids on lipid profiles—potential action of Garcinia cambogia flavonoids. Phytother. Res. **15,** 395–400.

Langin, D., Dicker, A., Tavernier, G., Hoffstedt, J., Mairal, A., Ryden, M., Arner, E., Sicard, A., Jenkins, C.M., Viguerie, N., Van Harmelen, V., Gross, R.W., Holm, C., and Arner, P. (2005). Adipocyte lipases and defect of lipolysis in human obesity. Diabetes **54,** 3190–3197.

Leary, S.C., and Shoubridge, E.A. (2003). Mitochondrial biogenesis: which part of "NO" do we understand? Bioessays **25,** 538–541.

Lenz, T.L., and Hamilton, W.R. (2004). Supplemental products used for weight loss. J. Am. Pharm. Assoc. (Wash) **44,** 59–67 (quiz 67–58).

Lijnen, H.R., Van, H.B., Frederix, L., Rio, M.C., and Collen, D. (2002). Adipocyte hypertrophy in stromelysin-3 deficient mice with nutritionally induced obesity. Thromb. Haemost. **87,** 530–535.

Liu, Y.J., Araujo, S., Recker, R.R., and Deng, H.W. (2003). Molecular and genetic mechanisms of obesity: implications for future management. Curr. Mol. Med. **3,** 325–340.

Liu-Stratton, Y., Roy, S., and Sen, C.K. (2004). DNA microarray technology in nutraceutical and food safety. Toxicol. Lett. **150,** 29–42.

Loe, Y.C., Bergeron, N., Rodriguez, N., and Schwarz, J.M. (2001). Gas chromatography/mass spectrometry method to quantify blood hydroxycitrate concentration. Anal. Biochem. **292,** 148–154.

Mahendran, P., and Devi, C.S. (2001). Effect of Garcinia cambogia extract on lipids and lipoprotein composition in dexamethasone administered rats. Indian J. Physiol. Pharmacol. **45,** 345–350.

Maquoi, E., Demeulemeester, D., Voros, G., Collen, D., and Lijnen, H.R. (2003). Enhanced nutritionally induced adipose tissue development in mice with stromelysin-1 gene inactivation. Thromb. Haemost. **89,** 696–704.

Marcinkiewicz, A., Gauthier, D., Garcia, A., and Brasaemle, D.L. (2006). The phosphorylation of serine 492 of perilipin a directs lipid droplet fragmentation and dispersion. J. Biol. Chem. **281,** 11901–11909.

Mattes, R.D., and Bormann, L. (2000). Effects of (-)-hydroxycitric acid on appetitive variables. Physiol. Behav. **71,** 87–94.

Meadows, M. (2003). Public health officials' caution against ephedra use. Health officials caution consumers against using dietary supplements containing ephedra. The stimulant can have dangerous effects on the nervous system and heart. FDA Consum. **37,** 8–9.

Moyes, C.D. (2003). Controlling muscle mitochondrial content. J. Exp. Biol. **206,** 4385–4391.

Nikami, H., Nedergaard, J., and Fredriksson, J.M. (2005). Norepinephrine but not hypoxia stimulates HIF-1alpha gene expression in brown adipocytes. Biochem. Biophys. Res. Commun. **337,** 121–126.

O'brien, P.E., and Dixon, J.B. (2002). The extent of the problem of obesity. Am. J. Surg. **184,** 4S–8S.

Ohia, S.E., Awe, S.O., Leday, A.M., Opere, C.A., and Bagchi, D. (2001). Effect of hydroxycitric acid on serotonin release from isolated rat brain cortex. Res. Commun. Mol. Pathol. Pharmacol. **109,** 210–216.

Ohia, S.E., Opere, C.A., Leday, A.M., Bagchi, M., Bagchi, D., and Stohs, S.J. (2002). Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). Mol. Cell. Biochem. **238,** 89–103.

Paracchini, V., Pedotti, P., and Taioli, E. (2005). Genetics of leptin and obesity: a HuGE review. Am. J. Epidemiol. **162,** 101–114.

Pittler, M.H., Schmidt, K., and Ernst, E. (2005). Adverse events of herbal food supplements for body weight reduction: systematic review. Obes. Rev. **6,** 93–111.

Preuss, H.G., Bagchi, D., Bagchi, M., Rao, C.V.S., Dey, D.K., and Satyanarayana, S. (2004). Effects of a natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and Gymnema sylvestre extract on weight loss. Diabetes Obes. Metab. **6,** 171–180.

Preuss, H.G., Garis, R.I., Bramble, J.D., Bagchi, D., Bagchi, M., Rao, C.V., and Satyanarayana, S. (2005). Efficacy of a novel calcium/potassium salt of (-)-hydroxycitric acid in weight control. Int. J. Clin. Pharmacol. Res. **25,** 133–144.

Ramsay, T.G. (2003). Porcine leptin inhibits lipogenesis in porcine adipocytes. J. Anim. Sci. **81,** 3008–3017.

Ren, T., He, J., Jiang, H., Zu, L., Pu, S., Guo, X., and Xu, G. (2006). Metformin reduces lipolysis in primary rat adipocytes stimulated by tumor necrosis factor-alpha or isoproterenol. J. Mol. Endocrinol. **37,** 175–183.

Rink, C., Roy, S., Khanna, S., Rink, T., Bagchi, D., and Sen, C.K. (2006). Transcriptome of the subcutaneous adipose tissue in response to oral supplementation of type 2 Leprdb obese diabetic mice with niacin-bound chromium. Physiol. Genomics **27,** 370–379.

Rodriguez, V.M., Macarulla, M.T., Echevarria, E., and Portillo, M.P. (2003). Lipolysis induced by leptin in rat adipose tissue from different anatomical locations. Eur. J. Nutr. **42,** 149–153.

Roy, S., Khanna, S., Krishnaraju, A.V., Subbaraju, G.V., Yasmin, T., Bagchi, D., and Sen, C.K. (2006a). Regulation of vascular responses to inflammation: inducible matrix metalloproteinase-3 expression in human microvascular endothelial cells is sensitive to antiinflammatory Boswellia. Antioxid Redox Signal **8,** 653–660.

Roy, S., Khanna, S., Kuhn, D.E., Rink, C., Williams, W.T., Zweier, J.L., and Sen, C.K. (2006b). Transcriptome analysis of the ischemia-reperfused remodeling myocardium: temporal changes in inflammation and extracellular matrix. Physiol. Genomics. **25,** 364–374.

Roy, S., Khanna, S., Wallace, W.A., Lappalainen, J., Rink, C., Cardounel, A.J., Zweier, J.L., and Sen, C.K. (2003). Characterization of perceived hyperoxia in isolated primary cardiac fibroblasts and in the reoxygenated heart. J. Biol. Chem. **278,** 47129–47135.

Roy, S., Rink, C., Khanna, S., Phillips, C., Bagchi, D., Bagchi, M., and Sen, C.K. (2004). Body weight and abdominal fat gene expression profile in response to a novel hydroxycitric acid-based dietary supplement. Gene Expr. **11,** 251–262.

Sasaki, K., Takagi, M., Konttinen, Y.T., Sasaki, A., Tamaki, Y., Ogino, T., Santavirta, S., and Salo, J. (2007). Upregulation of matrix metalloproteinase (MMP)-1 and its activator MMP-3 of human osteoblast by uniaxial cyclic stimulation. J. Biomed. Mater. Res. B Appl. Biomater. **80,** 491–498.

Schurgin, S., and Siegel, R.D. (2003). Pharmacotherapy of obesity: an update. Nutr. Clin. Care **6,** 27–37.

Sergio, W. (1988). A natural food, the Malabar Tamarind, may be effective in the treatment of obesity. Med. Hypotheses **27,** 39–40.

Shara, M., Ohia, S.E., Schmidt, R.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., and Stohs, S.J. (2004). Physico-chemical properties of a novel (-)-hydroxycitric acid extract and its effect on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days. Mol. Cell. Biochem. **260,** 171–186.

Shara, M., Ohia, S.E., Yasmin, T., Zardetto-Smith, A., Kincaid, A., Bagchi, M., Chatterjee, A., Bagchi, D., and Stohs, S.J. (2003). Dose- and time-dependent effects of a novel (-)-hydroxycitric acid extract on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological data over a period of 90 days. Mol. Cell. Biochem. **254,** 339–346.

Shekelle, P.G., Hardy, M.L., Morton, S.C., Maglione, M., Mojica, W.A., Suttorp, M.J., Rhodes, S.L., Jungvig, L., and Gagne, J. (2003). Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis [comment]. JAMA **289,** 1537–1545.

Sindler, B.H. (2001). Herbal therapy for management of obesity: observations from a clinical endocrinology practice [comment]. Endocr. Pract. **7,** 443–447.

Spake, A. (2001). Natural hazards. Tonic or toxic? Americans are gobbling up nature's remedies for everything from obesity to depression. US News World Rep. **130,** 42–49.

St-Pierre, J., Lin, J., Krauss, S., Tarr, P.T., Yang, R., Newgard, C.B., and Spiegelman, B.M. (2003). Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. J. Biol. Chem. **278,** 26597–26603.

Van Guilder, G.P., Hoetzer, G.L., Smith, D.T., Irmiger, H.M., Greiner, J.J., Stauffer, B.L., and Desouza, C.A. (2005). Endothelial t-PA release is impaired in overweight and obese adults but can be improved with regular aerobic exercise. Am. J. Physiol. Endocrinol. Metab. **289,** E807–E813.

Wada, T., Shimba, S., and Tezuka, M. (2006). Transcriptional regulation of the hypoxia inducible factor-2alpha (HIF-2alpha) gene during adipose differentiation in 3T3-L1 cells. Biol. Pharm. Bull. **29,** 49–54.

Westerberg, R., Mansson, J.E., Golozoubova, V., Shabalina, I.G., Backlund, E.C., Tvrdik, P., Retterstol, K., Capecchi, M.R., and Jacobsson, A. (2006). ELOVL3 is an important component for early onset of lipid recruitment in brown adipose tissue. J. Biol. Chem. **281,** 4958–4968.

Westerterp-Plantenga, M.S., and Kovacs, E.M. (2002). The effect of (-)-hydroxycitrate on energy intake and satiety in overweight humans. Int. J. Obes. Relat. Metab. Disord. **26,** 870–872.

Wolfe, S.M. (2003). Medicine. Ephedra—scientific evidence versus money/politics [comment][erratum appears in Science, 2003 May 21;300(5621):905]. Science **300,** 437.

Yun, Z., Maecker, H.L., Johnson, R.S., and Giaccia, A.J. (2002). Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. Dev. Cell. **2,** 331–341.

Zhao, X., Dey, A., Romanko, O.P., Stepp, D.W., Wang, M.H., Zhou, Y., Jin, L., Pollock, J.S., Webb, R.C., and Imig, J.D. (2005). Decreased epoxygenase and increased epoxide hydrolase expression in the mesenteric artery of obese Zucker rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. **288,** R188–R196.

Zigman, J.M., and Elmquist, J.K. (2003). Mini review: from anorexia to obesity—the yin and yang of body weight control. Endocrinology **144,** 3749–3756.

Address reprint requests to:
Chandan K. Sen
DHLRI Microarray Facility
512 Davis Heart and Lung Research Institute
The Ohio State University Medical Center
473 W. 12th Avenue
Columbus, OH 43210

E-mail: chandan.sen@osumc.edu

Received for publication May 9, 2007; accepted May 16, 2007.

This article has been cited by:

1. Andrea Maia-Landim, Juan M. Ramírez, Carolina Lancho, María S. Poblador, José L. Lancho. 2021. Garcinia cambogia and Glucomannan reduce weight, change body composition and ameliorate lipid and glucose blood profiles in overweight/obese patients. *Journal of Herbal Medicine* 100424. [Crossref]
2. Longlong Li, Huihui Zhang, Yao Yao, Zhongmiao Yang, Haitian Ma. 2019. (–)-Hydroxycitric Acid Suppresses Lipid Droplet Accumulation and Accelerates Energy Metabolism via Activation of the Adiponectin-AMPK Signaling Pathway in Broiler Chickens. *Journal of Agricultural and Food Chemistry* **67**:11, 3188-3197. [Crossref]
3. Ramgopal Mopuri, Md. Shahidul Islam. 2017. Medicinal plants and phytochemicals with anti-obesogenic potentials: A review. *Biomedicine & Pharmacotherapy* **89**, 1442-1452. [Crossref]
4. Ryoiti Kiyama. 2017. DNA Microarray-Based Screening and Characterization of Traditional Chinese Medicine. *Microarrays* **6**:1, 4. [Crossref]
5. Rajinder Raina, Dilip M. Mondhe, Jitendra K. Malik, Ramesh C. Gupta. Garcinia cambogia 669-680. [Crossref]
6. Amitava Das, Chandan K. Sen. NutrimiRomics 53-60. [Crossref]
7. Li Hua, Kang Ji-Hyun, Han Jong-Min, Cho Moon-Hee, Chung Young-Jin, Park Ki Hun, Shin Dong-Ha, Park Ho-Yong, Choi Myung-Sook, Jeong Tae-Sook. 2015. Anti-Obesity Effects of Soy Leaf via Regulation of Adipogenic Transcription Factors and Fat Oxidation in Diet-Induced Obese Mice and 3T3-L1 Adipocytes. *Journal of Medicinal Food* **18**:8, 899-908. [Abstract] [Full Text] [PDF] [PDF Plus]
8. Subhadip Ghatak, Yuk Cheung Chan, Savita Khanna, Jaideep Banerjee, Jessica Weist, Sashwati Roy, Chandan K Sen. 2015. Barrier Function of the Repaired Skin Is Disrupted Following Arrest of Dicer in Keratinocytes. *Molecular Therapy* **23**:7, 1201-1210. [Crossref]
9. Guanxing Liu, Ningning Han, Jing Han, Di Chen, Jian Kang, Haitian Ma. 2015. Garcinia Cambogia Extracts Prevented Fat Accumulation via Adiponectin-AMPK Signaling Pathway in Developing Obesity Rats. *Food Science and Technology Research* **21**:6, 835-845. [Crossref]
10. Li Oon Chuah, Wan Yong Ho, Boon Kee Beh, Swee Keong Yeap. 2013. Updates on Antiobesity Effect of Garcinia Origin (–)-HCA. *Evidence-Based Complementary and Alternative Medicine* **2013**, 1-17. [Crossref]
11. Fabiola Márquez, Nancy Babio, Mònica Bulló, J. Salas-Salvadó. 2012. Evaluation of the Safety and Efficacy of Hydroxycitric Acid or Garcinia cambogia Extracts in Humans. *Critical Reviews in Food Science and Nutrition* **52**:7, 585-594. [Crossref]
12. Ji-Eun Kim, Seon-Min Jeon, Ki Hun Park, Woo Song Lee, Tae-Sook Jeong, Robin A McGregor, Myung-Sook Choi. 2011. Does Glycine max leaves or Garcinia Cambogiapromote weight-loss or lower plasma cholesterol in overweight individuals: a randomized control trial. *Nutrition Journal* **10**:1. . [Crossref]
13. SD Anton. 2011. Investigations of botanicals on food intake, satiety, weight loss and oxidative stress: study protocol of a double-blind, placebo-controlled, crossover study. *Journal of Chinese Integrative Medicine* **9**:11, 1190-1198. [Crossref]
14. Kenichi Tamama, Haruhisa Kawasaki, Svetoslava S. Kerpedjieva, Jianjun Guan, Ramesh K. Ganju, Chandan K. Sen. 2011. Differential roles of hypoxia inducible factor subunits in multipotential stromal cells under hypoxic condition. *Journal of Cellular Biochemistry* **112**:3, 804-817. [Crossref]
15. Yuk Cheung Chan, Savita Khanna, Sashwati Roy, Chandan K. Sen. 2011. miR-200b Targets Ets-1 and Is Down-regulated by Hypoxia to Induce Angiogenic Response of Endothelial Cells. *Journal of Biological Chemistry* **286**:3, 2047-2056. [Crossref]
16. Sidney J. Stohs, Francis C. Lau, Doun Kim, Seung Un Kim, Manashi Bagchi, Debasis Bagchi. 2010. Safety assessment of a calcium-potassium salt of (–)-hydroxycitric acid. *Toxicology Mechanisms and Methods* **20**:9, 515-525. [Crossref]
17. . Monographs of Herbal Principles 41-372. [Crossref]
18. Blandine Gatta, Christine Zuberbuehler, Myrtha Arnold, Roberte Aubert, Wolfgang Langhans, Didier Chapelot. 2009. Acute effects of pharmacological modifications of fatty acid metabolism on human satiety. *British Journal of Nutrition* **101**:12, 1867-1877. [Crossref]
19. Savita Khanna, Han-A Park, Chandan K. Sen, Trimurtulu Golakoti, Krishanu Sengupta, Somepalli Venkateswarlu, Sashwati Roy. 2009. Neuroprotective and Antiinflammatory Properties of a Novel Demethylated Curcuminoid. *Antioxidants & Redox Signaling* **11**:3, 449-468. [Abstract] [Full Text] [PDF] [PDF Plus]
20. Savita Khanna, Han-A Park, Chandan K. Sen, Trimurtulu Golakoti, Krishanu Sengupta, Somepalli Venkateswarlu, SASHWATI ROY. 2008. Neuroprotective and anti-inflammatory properties of a novel demethylated curcuminoid. *Antioxidants & Redox Signaling* **0**:ja, 080910041331150. [Crossref]

21. N. S. Deshmukh, M. Bagchi, T. Yasmin, D. Bagchi. 2008. Safety of a Novel Calcium/Potassium Saltof (-)-Hydroxycitric Acid (HCA-SX): II.Developmental Toxicity Study in Rats. *Toxicology Mechanisms and Methods* **18**:5, 443-451. [Crossref]
22. N. S. Deshmukh, M. Bagchi, T. Yasmin, D. Bagchi. 2008. Safety of a Novel Calcium/Potassium Salt of Hydroxycitric Acid (HCA-SX): I. Two-Generation Reproduction Toxicity Study. *Toxicology Mechanisms and Methods* **18**:5, 433-442. [Crossref]