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Basic nutritional investigation

Ingested cocoa can prevent high-fat diet–induced obesity by regulating the expression of genes for fatty acid metabolism

Naoko Matsui[a,*], Ryoichi Ito[a], Eisaku Nishimura, Ph.D.[a], Mariko Yoshikawa[a], Masatoshi Kato, Ph.D.[a], Masanori Kamei, Ph.D.[a], Haruki Shibata[b], Ichiro Matsumoto, Ph.D.[c], Keiko Abe, Ph.D.[c], and Shuichi Hashizume, Ph.D.[b]

[a] *Research Institute, Morinaga & Co., Ltd., Kanagawa, Japan*
[b] *Morinaga Institute of Biological Science, Kanagawa, Japan*
[c] *Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan*

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Abstract

Objective: We previously found that ingested cocoa decreased visceral adipose tissue weight in rat. To elucidate the molecular mechanisms of that effect, we carried out experiments aimed at analyzing biochemical parameters and gene expression profiles.

Methods: Rats were fed either of two high-fat diets, differing only in supplementation with real or mimetic cocoa. On day 21, body weights, mesenteric white adipose tissue weights, and concentrations of serum triacylglycerol were measured. To investigate the molecular mechanisms underlying the effects of cocoa on lipid metabolism and triacylglycerol accumulation, we examined gene expression profiles in liver and mesenteric white adipose tissues using the GeneChip microarray system.

Results: Final body weights and mesenteric white adipose tissue weights were significantly lower in rats fed the real cocoa diet than in those fed the mimetic cocoa diet ($P < 0.05$), and serum triacylglycerol concentrations tended to be lower in rats fed the real cocoa diet ($P = 0.072$). DNA microarray analysis showed that cocoa ingestion suppressed the expression of genes for enzymes involved in fatty acid synthesis in liver and white adipose tissues. In white adipose tissue, cocoa ingestion also decreased the expression of genes for fatty acid transport-relating molecules, whereas it upregulated the expression of genes for uncoupling protein-2 as a thermogenesis factor.

Conclusions: Ingested cocoa can prevent high-fat diet–induced obesity by modulating lipid metabolism, especially by decreasing fatty acid synthesis and transport systems, and enhancement of part of the thermogenesis mechanism in liver and white adipose tissue.

Keywords: Cocoa; Antiobesity; DNA microarray; Fatty acid synthesis; Fatty acid transport; Thermogenesis

Introduction

Cocoa has various effects that promote good health, including the improvement of mental relaxation [1] and concentration [2] and the prevention of dental caries in the case of cacao bean husk [3,4]. In particular, cacao liquor polyphenols exert potent effects in terms of antioxidant activity [5], inhibition of arteriosclerosis [6,7], modulation of immune function [8], suppression of gastric mucosal lesions [9], and antimutagenicity [10]. We previously reported on various favorable effects of cocoa, including its antibacterial effects on *Escherichia coli* O157:H7 and *Helicobacter pylori* [11–15], neutralization of lipopolysaccharides [16], improvements in bowel movement and reduction of stool odor [17], enhancement of wound healing [18], and mitigation of peripheral intolerance to cold [19]. While studying these effects, we found that visceral adipose tissue weight tended to be lower in rats fed a cocoa-containing diet than in those fed a cocoa-free diet. However, little is known about these antiobesity effects of cocoa and their underlying

* Corresponding author. Tel.: +81-45-571-2340; fax: +81-45-571-6109.

E-mail address: n-matsui-jj@morinaga.co.jp.

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Table 1
Compositions of the high-fat diets*

Component	Composition (g/kg diet)	
	HC diet	HF diet
Ash	35.000	35.000
Fiber	50.000	50.000
Fat	270.000	270.000
Carbohydrate	304.486	304.486
Protein	203.000	203.000
Vitamins and antioxidants	12.514	12.514
Cocoa powder	125.000	—
Mimetic cocoa	—	125.000
Total calories (kcal/g diet)†	4.91	4.91

HC, high-fat diet plus real cocoa; HF, high-fat diet plus mimetic cocoa.
* Rats were examined for effects of cocoa ingestion with high-fat diets. These diets were based the AIN-93G diet and beef tallow was added to provide a high-fat content. Compositions of real and mimetic cocoa are listed in Table 2.
† The energy contents of the diets were calculated as described in the text.

mechanisms. Although *n*-3 polyunsaturated fatty acids (FAs) in fish oil and lignans in sesame have been reported to be effective in the prevention of obesity, and their effects have been associated with changes in the expression of hepatic genes related to lipid metabolism [20–23], the mechanisms by which these dietary items control lipid metabolism have not been elucidated. In this study, we verified the antiobesity effects of cocoa in rats and examined the gene expression profiles in rat liver and mesenteric white adipose tissues (MES-WAT) by using the GeneChip microarray system to reveal the molecular mechanisms underlying the effects of cocoa on lipid metabolism and triacylglycerol (TG) accumulation.

Materials and methods

Animals

All animal experiments were conducted in accordance with Standards Relating to the Care and Management of Experimental Animals (notification 6, March 27, 1980 of the Prime Minister's Office, Japan). Six-week-old male Wistar rats (about 180 g) were purchased from CLEA Japan, Inc. (Kanagawa, Japan) and maintained on a normal laboratory diet CE-2 (CLEA) for 1 wk to stabilize metabolic conditions before starting the experiments. The rats were housed separately in plastic cages with mesh bottoms and kept at 24 ± 2°C and 50% humidity with a light cycle of 12 h of light (6:00 AM to 6:00 PM) and 12 h of dark (6:00 PM to 6:00 AM).

Diets

Twenty rats were assigned to two groups of 10 each alternately by order of body weight to minimize any differences between groups, and each group was fed for 21 d on the following diets. Rats in the test group were maintained on an AIN-93G–based high-fat diet containing 12.5% (w/w) cocoa powder (Pure Cocoa, Morinaga & Co., Ltd, Tokyo, Japan) (HC diet) and those in the control group were maintained on essentially the same diet except for supplementation with mimetic cocoa (HF diet) instead of real cocoa. The nutrient contents of HC and HF diets were adjusted to be identical (Table 1), with the mimetic cocoa made of purified major nutrients (Table 2). The two groups of rats were pair fed on the HC or HF diet to ensure equal energy intakes, with water provided ad libitum throughout. The amount of cocoa intake by the rat is equivalent to 10 cups of cocoa drink (50 g of cocoa powder) per day for adult humans on the basis of energy intake. The high-fat diets were prepared by modifying the cornstarch composition of the original AIN-93G diet formula, i.e., cornstarch (397.486 g/kg of diet) was replaced with a mixture consisting of beef tallow (200 g/kg of diet), cornstarch (72.486 g/kg of diet), and real or mimetic cocoa powder (125 g/kg of diet). The fat accounted for 49.5% total energy in these high-fat diets. Beef tallow was used to provide fat because its fatty acid composition is similar to one of cocoa fat. The energy content of the diets was calculated on the basis of 9 kcal/g for fat, 4 kcal/g for carbohydrate, 4 kcal/g for protein, 0 kcal/g for fiber (cellulose), and 0.1 kcal/g for cocoa fiber (lignin is the major component).

Nutritional and biochemical analysis

After pair feeding for 21 d, all rats were starved overnight and anesthetized with ether. Blood was withdrawn by cardiac puncture, and serum obtained after centrifugation (10 min, 3000 rpm) was stored at −20°C until analyzed. The rats were killed, and organs including liver, heart, both kidneys, spleen, right gastrocnemius muscle, and MES-WAT as a representative of visceral adipose tissues were dissected out immediately, weighed, quickly frozen in liq-

Table 2
Compositions of real and mimetic cocoa*

Component	Composition (g/kg diet)	
	Mimetic cocoa	Cocoa powder
Moisture	15	17
Ash	85	85
Fiber	280	280
Fat	230	228
Carbohydrate	150	150
Protein	240	240
Total calorie (kcal/g)†	3.63	3.64

* The mimetic cocoa was prepared with purified major nutrients, i.e., distilled water for moisture, AIN- 93G mineral mix for ash, cellulose for fiber, beef tallow for fat, corn starch for carbohydrate, and milk casein for protein.
† The energy contents of the diets were calculated as described in the text.

uid nitrogen, and stored at −80°C until used. Serum leptin concentrations were determined with a commercially available kit (Rat Leptin Enzyme-linked Immunosorbent Assay Kit, Morinaga Institute of Biological Science, Yokohama, Japan). Total and low-density lipoprotein cholesterols, TG, and free fatty acids in serum were determined enzymatically (SRL Inc., Tokyo, Japan). High-density lipoprotein cholesterol in serum was determined with a direct measurement (SRL Inc.).

Data are presented as mean ± standard deviation. Statistic analysis was performed with unpaired two-tailed Student's *t* tests. $P < 0.05$ was considered statistically significant.

DNA microarray procedure

Two representative rats were selected from the HC group and the HF group. These were selected to be as close as possible to the mean values of each group in terms of body weight gain; weight of the liver, MES-WAT, and right gastrocnemius muscle as proportions of whole body weight; and serum TG concentrations. These representative rats were designated HC-1, HC-2, HF-1, and HF-2 (Figure 1).

Total RNA was obtained from the liver and MES-WAT of selected rats by successive extractions with ISOGEN (Nippon Gene Co, Ltd, Toyama, Japan) and RNeasy Mini Kits (Qiagen K.K., Tokyo, Japan). The RNA was assessed for quality and quantity by agarose gel electrophoresis and ultraviolet spectrophotometry, respectively. DNA microarray analysis was performed according to the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA) as described elsewhere [24]. Briefly, double-stranded cDNA was synthesized from 10 μg of total RNA by reverse transcription with SuperScript Choice System (Invitrogen, Carlsbad, CA, USA). Biotinylated cRNA was transcribed from the double-stranded cDNA by T7 RNA polymerase reaction by using an RNA Transcript Labeling Kit (Enzo Biochem, Farmingdale, NY, USA), fragmented, and applied to GeneChips (Rat Genome U34A Array), which contained more than 8000 gene probes as detailed in the Expression Analysis Technical Manual (Affymetrix). After hybridization for 16 h at 45°C, the GeneChip was washed and labeled with R-phycoerythrin streptavidin using an Affymetrix Fluidics station 400. The fluorescent signal intensities were measured with an Affymetrix Scanner.

DNA microarray data analysis

The liver and MES-WAT were examined separately for cocoa-dependent increases and decreases in gene expression by the use of Affymetrix software (Microarray Suite 5.0), where fluorescent intensities of the relevant gene were compared between GeneChips for rats fed the HC and HF diets. The comparison was performed for all four combinations of HC and HF diet-fed rats; i.e., HC-1 versus HF-1, HC-1 versus HF-2, HC-2 versus HF-1, and HC-2 versus HF- 2 (Figure 1). The absolute signal intensity, which differed from one GeneChip to another, was standardized to facilitate direct comparisons by keeping the total fluorescent intensity constant. The indications of "increase" and "decrease" for cocoa-dependent changes in gene expression by "change call" of Affymetrix software were judged valid only when the change calls matched more than three data in four combinations. The indication was assessed as "no change" when the data matched less. Genes whose "detection call" in the higher fluorescent intensity group were recorded as "absent" or "marginal" were excluded from further considerations. The alteration ratios of the gene expression are represented as mean ± standard deviation of the four quotients. Quotients were calculated from the gene expression for the HC-fed rats divided by that of the HF-fed rats (Figure 1).

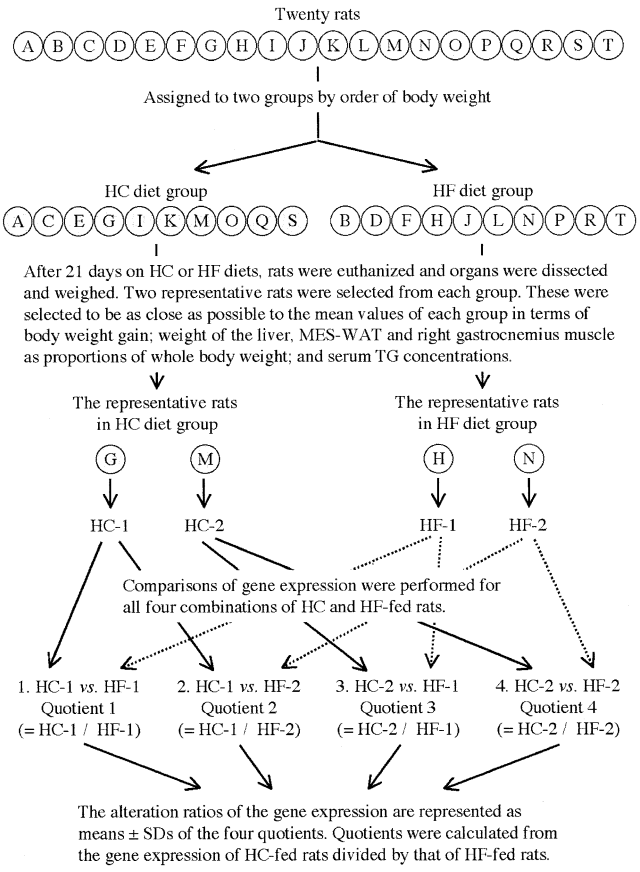

Fig. 1. Diagram of the experimental design. HC, high-fat diet plus real cocoa; HF, high-fat diet plus mimetic cocoa; MES-WAT, mesenteric white adipose tissue; SD, standard deviation; TG, triacylglycerol.

Results

Nutritional and biochemical analysis

Rats were fed either of two high-fat diets (HC and HF) for 21 d, and we measured biochemical parameters related to energy metabolism. The nutrient contents of HC and HF

diets were adjusted to be identical (Table 1), with the mimetic cocoa made of purified major nutrients (Table 2). For HC-diet rats compared with HF-diet rats, cocoa ingestion significantly decreased the weight gain in the entire body and the MES-WAT (Table 3 and Figure 2). MES-WAT was the only tissue that showed a significant cocoa-dependent decrease in weight among the tissues and organs examined (Table 3). Concerning serum parameters on lipid metabolism, TG concentrations were decreased to a large extent ($P = 0.072$) by cocoa ingestion (Table 3 and Figure 2). Total cholesterol concentrations increased in accordance with the increment of high-density lipoprotein cholesterol concentrations by cocoa ingestion (Table 3). Leptin concentrations, known to track in parallel with body fat mass [25], were also decreased to 75% of HF-fed rats by the ingestion of cocoa (data not shown). These results, showing significant improvements in physical and biochemical parameters in response to cocoa ingestion, strongly suggest that cocoa effectively prevented high-fat diet–induced obesity.

Gene expression profiles of lipid metabolism-relating enzymes in liver and MES-WAT

To investigate the molecular mechanisms underlying the effects of cocoa on lipid metabolism and TG accumulation, gene expression profiles in rat liver and MES-WAT were analyzed using DNA microarrays, with a focus on those genes relating to lipid metabolism and TG accumulation.

As presented in Tables 4 and 5, cocoa ingestion decreased the hepatic gene expression for tricarboxylate (citrate) transport protein, adenosine triphosphate citrate lyase, and fatty acid synthase. These results indicated that cocoa decreased gene expression of the FA synthesis system in the liver. In addition, cocoa produced extensive decreases in the expression of genes for cholesterol biosynthesis including squalene synthase, squalene epoxidase (monooxygenase), and 7- dehydrocholesterol reductase (Tables 4 and 5).

In MES-WAT, cocoa decreased the gene expression for molecules involved in FA transport, such as fatty acid translocase/CD36, fatty acid transporter as a shorter form of fatty acid translocase/CD36 [26], and fatty acid binding protein (Tables 4 and 6). Interestingly, the gene expression for peroxisome proliferator-activated receptor γ (PPAR-γ), which is the transcription factor inducing those FA transport-relating molecules [27–30], was also decreased. As in the liver, cocoa ingestion similarly decreased the expression of genes for enzymes involved in FA synthesis, such as fatty acid synthase, 3-oxoacyl (ketoacyl)-coenzyme A thiolase, and enoyl-coenzyme A hydratase (Table 6). It is notable that the expression of adipocyte determination and differentiation factor-1 (also called sterol regulatory element-1 binding protein-1c), which is known to be the transcription factor of

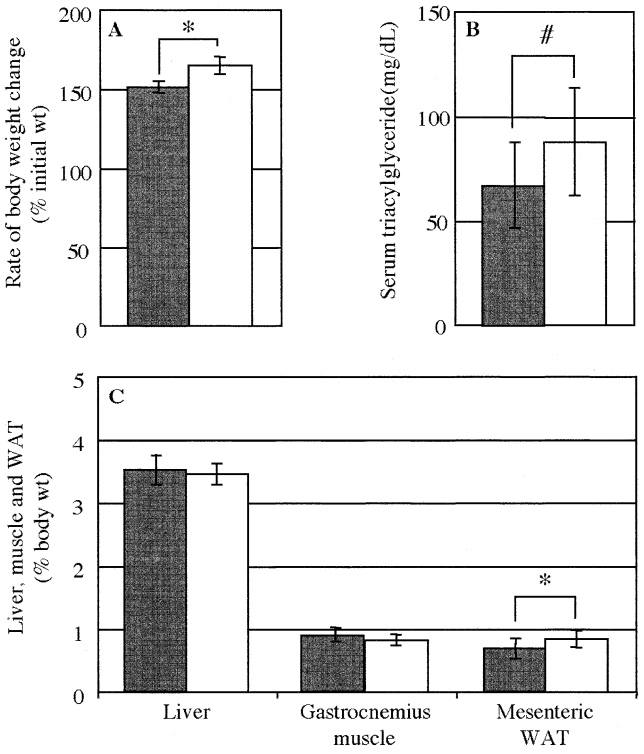

Fig. 2. Effects of cocoa ingestion on physical and biochemical parameters. Two groups of rats fed for 21 d on a high-fat diet plus real cocoa (solid bar) or a high-fat diet plus mimetic cocoa (open bar) were compared for whole-body weight gain (A), serum triacylglycerol concentration (B), and weight of the liver, right gastrocnemius muscle, and mesenteric white adipose tissue as expressed in percentage of body weight (C). Values are expressed as means ± standard deviations ($n = 10$). Statistical analysis was performed with unpaired two-tailed Student's t test. *$P < 0.05$; # $P = 0.072$. WAT, white adipose tissue.

Table 3
Effects of cocoa ingestion on physical and biochemical parameters in rats after 21 d of pair feeding on HC and HF diets, respectively*

Determination	HC group	HF group
Initial body weight (g)	207.1 ± 4.3	207.1 ± 5.0
Final body weight (g)	313.1 ± 9.2†	341.2 ± 10.4
Rate of body weight change (%initial weight)	151.2 ± 3.3†	164.8 ± 5.1
Caloric intake (kcal/d per rat)	86.8 ± 1.6	85.7 ± 1.9
Liver (%body weight)	3.525 ± 0.234	3.452 ± 0.161
Heart (%body weight)	0.327 ± 0.022†	0.297 ± 0.021
Kidneys (%body weight)	0.710 ± 0.038	0.682 ± 0.052
Spleen (%body weight)	0.186 ± 0.010	0.204 ± 0.026
Right gastrocnemius muscle (%body weight)	0.900 ± 0.103	0.820 ± 0.090
MES-WAT (%body weight)	0.683 ± 0.153†	0.838 ± 0.131
Serum triacylglycerol (mg/dL)	67.0 ± 20.3	87.8 ± 25.6
Serum free fatty acid (mEq/L)	0.699 ± 0.143	0.600 ± 0.123
Serum total cholesterol (mg/dL)	87.3 ± 12.3†	75.8 ± 7.4
Serum LDL cholesterol (mg/dL)	12.5 ± 2.5	11.2 ± 2.3
Serum HDL cholesterol (mg/dL)	31.4 ± 3.7†	27.9 ± 2.7

HC, high-fat diet plus real cocoa; HDL, high-density lipoprotein; HF, high-fat diet plus mimetic cocoa; LDL, low-density lipoprotein; MES-WAT, mesenteric white adipose tissue.

* Each value is expressed as mean ± standard deviation ($n = 10$). Statistical analysis was performed with unpaired two-tailed Student's t tests.

† $P < 0.05$.

Table 4
Numbers of genes whose expression in liver and MES-WAT was changed or unchanged: effect of HC diet versus that of HF diet*

	Decrease	Increase	No change	Not further considered†
A. Fatty acid synthesis enzymes (14 genes)				
Liver	4	0	7	3
MES-WAT	4	1	8	1
B. Fatty acid degradation and β-oxidation enzymes (27 genes)				
Liver	1	0	21	5
MES-WAT	6	0	16	4
C. Cholesterol biosynthesis and taurine metabolism enzymes (17 genes)				
Liver	6	0	7	4
MES-WAT	4	0	7	6
D. Transcriptional factors regulating lipid metabolism (9 genes)				
Liver	0	0	6	3
MES-WAT	5	0	1	3
E. Fatty acid transporter-relating molecules (25 genes)				
Liver	0	0	17	8
MES-WAT	9	1	5	10
F. Uncoupling proteins, leptin, and insulin (20 genes)				
Liver	0	0	14	6
MES-WAT	0	2	3	15

HC, high-fat diet plus real cocoa; HF, high-fat diet plus mimetic cocoa; MES-WAT, mesenteric white adipose tissue.
* The involved genes were classified into six categories (A–F) according to metabolic functions.
† The genes indicated to be "absent" and "marginal" by "detection call" in the higher fluorescent intensity group were excluded from further consideration.

genes related to FA synthesis, was also decreased [31] (Table 6). In contrast, the gene expression of uncoupling protein (UCP) 2 as a thermogenesis factor [32] was upregulated in HC-fed rats (Table 6). These results suggest that cocoa ingestion leads to gene expression to decrease TG accumulation in MES-WAT.

Because glucose, as well as FA, is a main respiratory substrate, we examined the expression of genes for enzymes involved in glucose metabolism and gluconeogenesis. However, cocoa ingestion did not affect the expression of these genes in the liver or MES-WAT (data not shown). These results indicate that cocoa ingestion produces its antiobesity effects primarily by affecting lipid metabolism, especially those enzymes involved in FA synthesis in the liver, and by modulating FA synthesis and transport systems in MES-WAT.

Discussion

We found that cocoa ingestion suppressed the high-fat diet–induced gain of body weights, weights of MES-WAT, and serum TG concentration in rats (Table 3 and Figure 2). The effects of cocoa ingestion in the liver and MES-WAT were studied using DNA microarrays, focusing especially on genes related to lipid metabolism and TG accumulation.

A main hepatic antiobesity mechanism works by suppressing FA synthesis and by activating FA oxidation [33]. However, we found that cocoa ingestion decreased the expression of genes for FA synthesis in the liver without activating the expression of genes for FA oxidation (Tables 4 and 5). It is therefore plausible that ingestion of cocoa in combination with other foods that can enhance FA oxidation might fortify the antiobesity effects of cocoa.

Table 5
Genes with remarkably altered expression in the rat liver after cocoa ingestion

Category*		Accession no.	Gene products	Alteration ratio†
A	↓	L12016	Tricarboxylate transport protein	0.67 ± 0.23
A	↓	J05210	Adenosine triphosphate citrate lyase	0.34 ± 0.13‡
A	↓	M76767	Fatty acid synthase	0.66 ± 0.12
B	↓	AB012933	Acyl–coenzyme A synthetase 5	0.78 ± 0.08
C	↓	M95591	Hepatic squalene synthetase	0.60 ± 0.07
C	↓	D37920	Squalene epoxidase	0.44 ± 0.06
C	↓	AB016800	7-Dehydrocholesterol reductase	0.56 ± 0.14‡
C	↓	AF003835	Isopentenyl diphosphate-dimethylallyl diphosphate isomerase	0.45 ± 0.12
C	↓	M64755	Cysteine sulfinic acid decarboxylase	0.43 ± 0.07

* Six categories (A–F) according to metabolic functions are shown in Table 4.
† The alteration ratio is represented as mean ± standard deviation of four quotients of gene expression of rats fed the high-fat diet plus real cocoa divided by that of rats fed the high-fat diet plus mimetic cocoa.
‡ Similar results were obtained with two different probes for different sites.

Table 6
Genes with remarkably altered expression in rat mesenteric adipose tissue after cocoa ingestion

Category*		Accession no.	Gene products	Alteration ratio†
A	↓	M76767	Fatty acid synthase	0.59 ± 0.13
A	↓	X05341	3-Oxoacyl-CoA thiolase	0.66 ± 0.18
A	↓	X15958	Mitochondrial enoyl-CoA hydratase	0.76 ± 0.07
A	↓	J02585	Liver stearyl-CoA desaturase	0.57 ± 0.11
A	↑	U67995	Stearyl-CoA desaturase 2	1.60 ± 0.50
B	↓	D43623	Carnitine palmitoyltransferase-I–like protein	0.52 ± 0.09‡
B	↓	J05470	Mitochondrial carnitine palmitoyltransferase-II	0.71 ± 0.11
B	↓	D30647	Very long-chain acyl-CoA dehydrogenase	0.75 ± 0.02
B	↓	D16479	Mitochondrial long-chain 3-ketoacyl-CoA thiolase β-subunit of mitochondrial trifunctional protein	0.83 ± 0.16
B	↓	X05341	3-Oxoacyl-CoA thiolase	0.66 ± 0.18
C	↓	M33648	Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase	0.61 ± 0.29‡
C	↓	M95591	Hepatic squalene synthetase	0.59 ± 0.09
C	↓	M64755	Cysteine sulfinic acid decarboxylase	0.76 ± 0.30
D	↓	X12752	DNA binding protein C/EBP-α	0.59 ± 0.08
D	↓	S77528	rNFIL-6 = C/EBP-related transcription factor (C/EBP-β)	0.51 ± 0.06
D	↓	X60769	silencer factor B = C/EBP-β	0.69 ± 0.05
D	↓	AB011365	PPAR-γ	0.59 ± 0.16
D	↓	L16995	add-1 = SREBP-1c	0.66 ± 0.11
E	↓	V01235	Liver fatty acid binding protein	0.21 ± 0.14
E	↓	K01180	Intestinal fatty acid binding protein	0.13 ± 0.09
E	↓	S52878	Intestinal 15-kDa protein = fatty acid–binding protein homolog	0.23 ± 0.19
E	↓	J02773	Low-molecular-weight fatty acid binding protein	0.77 ± 0.30
E	↓	AF072411	Fatty acid translocase/CD36	0.62 ± 0.14‡
E	↓	AB005743	Fatty acid transporter	0.63 ± 0.04‡
E	↓	J02597	Apolipoprotein A-I	0.37 ± 0.31
E	↑	S76779	Apolipoprotein E	1.50 ± 0.33
F	↑	AB010743	UCP2	1.25 ± 0.25
F	↑	AB005143	UCP2	1.53 ± 0.60

add-1, adipocyte determination and differentiation factor-1; C/EBP, CCAAT/enhancer binding protein; CoA, coenzyme A; HC, high-fat diet plus real cocoa; HF, high-fat diet plus mimetic cocoa; NFIL-6, nuclear factor interleukin-6; PPAR-γ, peroxisome proliferator-activated receptor-γ; SREBP, sterol regulatory element-1 binding protein.

* Six categories (A–F) according to metabolic functions are shown in Table 4.

† The alteration ratio is represented as mean ± standard deviation of four quotients of gene expressions of HC-fed rats divided by that of HF-fed rats.

‡ Similar results were obtained with two different probes for different sites.

Although WAT has few roles except for the storage of TG, cocoa ingestion altered the expression of various kinds of genes in this tissue (Tables 4 and 6). In MES-WAT, cocoa ingestion decreased the expression of genes for FA transport-relating molecules and their transcription factor, PPAR-γ (Table 6). Because FA is known to serve as a ligand for PPAR-γ [34], the simultaneous decrease in concentrations of PPAR-γ and serum TG suggests a causal relation between them. The decrease in PPAR-γ expression in response to cocoa ingestion might result from a decrease in serum TG by a feedback control mechanism, which leads to decreases in the expression of FA transport-relating molecules and thus decrease TG uptake into MES-WAT (Figure 3). Another rationale for the effectiveness of cocoa ingestion in the prevention of TG accumulation in WAT can be ascribed to the cascade suppression of adipocyte determination and differentiation factor-1 and then the FA synthesis system and to the enhancement of UCP2 (Figure 3). UCP2 is a homolog of UCP1 [32]. UCP1 has an important role in controlling non-shivering thermogenesis, and its mRNA expression is generally lower in obese than in normal-weight rodents [35]. Because the expression of UCP2 mRNA is increased in UCP1 knockout mice, it is believed that UCP2 also mediates thermogenesis [35]. Cocoa ingestion increased total cholesterol concentrations, especially high-density lipoprotein cholesterol, in serum, whereas gene expression for cholesterol biosynthesis was decreased in liver and MES-WAT. This suggests that the increase of serum cholesterol may be due to some factor other than cholesterol biosynthesis, e.g., reabsorption of cholesterol from the small intestine.

In conclusion, we postulate a mechanism for the anti-obesity effects of cocoa: that ingestion effectively prevents TG accumulation by suppression of FA synthesis in the

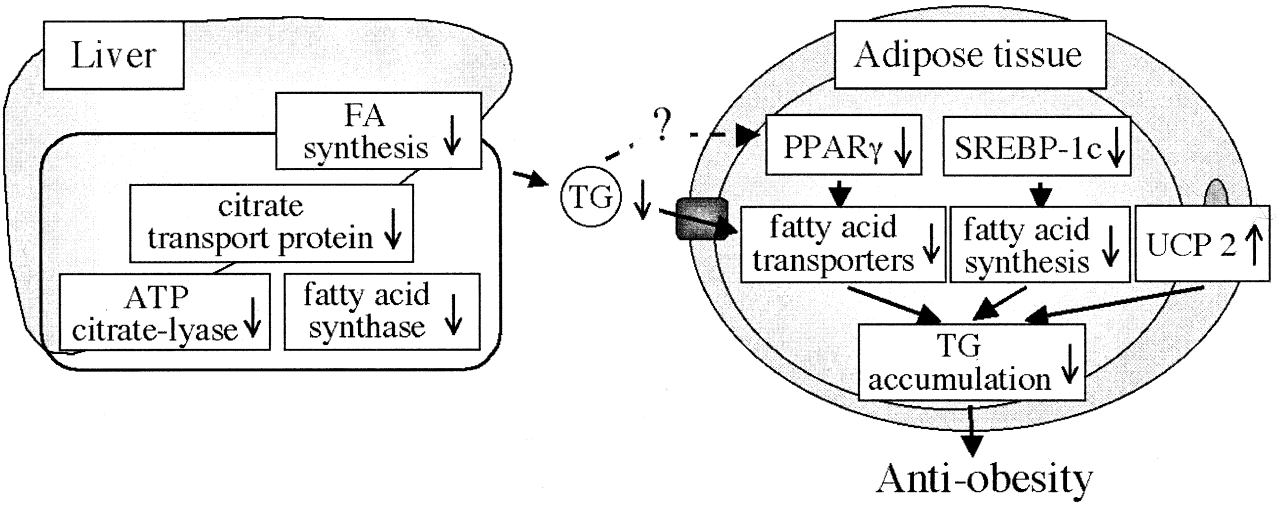

Fig. 3. A proposed model of the antiobesity effects induced by cocoa ingestion. ATP, adenosine triphosphate; FA, fatty acid; PPARγ, peroxisome proliferator-activated receptor-γ; SREBP, sterol regulatory element- 1 binding protein; TG, triacylglycerol; UCP 2, uncoupling protein-2.

liver and by suppression of FA synthesis and transport systems with concurrent activation of thermogenesis in MES-WAT (Figure 3). Further studies are needed to help reveal the molecular mechanisms underlying such effects.

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