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RESEARCH ARTICLE

A high-fat diet reduces ceramide synthesis by decreasing adiponectin levels and decreases lipid content by modulating *HMG-CoA* reductase and *CPT-1* mRNA expression in the skin

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Scope: Molecules involved in skin function are greatly affected by nutritional conditions. However, the mechanism linking high-fat (HF) diets with these alterations is not well understood. This study aimed to investigate the molecular changes in skin function that result from HF diets.

Methods and results: Sprague-Dawley rats were fed HF diets for 28 days. The skin levels of ceramide, lipids and mRNAs involved in lipid metabolism were evaluated using TLC, oil red O staining and quantitative PCR, respectively. The serum adiponectin concentration was determined by ELISA. HF diets led to reduced ceramide levels and lowered skin lipid content. They also decreased mRNA levels of *serine palmitoyltransferase* (*SPT*) and *3-hydroxy-3-methylglutaryl coenzyme A* (*HMG-CoA*) *reductase* in the skin and those of *peroxisome proliferator-activated receptor-α* (*PPAR-α*), which upregulates SPT and HMG-CoA reductase expression. The HF diets reduced the serum concentration of adiponectin, which acts upstream of PPAR-α. Finally, these diets led to increased mRNA levels of *carnitine palmitoyltransferase-1*, the rate-limiting enzyme that acts in β-oxidation.

Conclusion: Our study suggests that HF diets reduce ceramide and lipid synthesis in the skin by reducing levels of SPT and HMG-CoA reductase through lowered adiponectin and PPAR-α activity. Additionally, they decrease lipid content by enhancing β-oxidation.

Keywords:
Adiponectin / Ceramide / High-fat diet / Peroxisome proliferator-activated receptor-α / Serine palmitoyltransferase

1 Introduction

Skin lipids contain ceramides, fatty acids, and cholesterol, and play important roles in physiological responses such as cell growth, differentiation, energy metabolism, and signal transduction. They also act as a permeability barrier that prevents entry of toxic compounds and loss of water and electrolytes [1]. Thus, any disturbance in the metabolism of these lipids can lead to skin dysfunction [2, 3].

Lipid metabolism is controlled by several enzymes and transcription factors, including peroxisome proliferator-activated receptor-α (PPAR-α) and sterol regulatory element-binding proteins (SREBP-1c and SREBP-2). PPAR-α increases the mRNA level of *3-hydroxy-3-methylglutaryl coenzyme A* (*HMG-CoA*) *reductase*, which acts as the rate-limiting enzyme in cholesterol biosynthesis [4]. Furthermore, some researchers have shown that PPAR-α increased the ceramide content [5, 6]. Rivier et al. [5] reported that

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Abbreviations: CPT-1, carnitine palmitoyltransferase-1; **FAS,** fatty acid synthase; **HF,** high fat; **HMG-CoA reductase,** 3-hydroxy-3-methylglutaryl coenzyme A reductase; **PPAR-α,** peroxisome proliferator-activated receptor-α; **SPT,** serine palmitoyltransferase; **SREBP,** sterol regulatory element binding protein

the increase resulted from the enhancement of serine palmitoyltransferase (SPT) activity. SREBP-1c induces fatty acid synthesis, whereas SREBP-2 is primarily involved in stimulating cholesterol synthesis. Carnitine palmitoyltransferase-1 (CPT-1) localizes to the outer mitochondrial membrane and catalyzes the translocation of long-chain fatty acids into the mitochondrial matrix [7]. CPT-1 is the rate-limiting enzyme involved in β-oxidation, and enhances triglyceride hydrolysis [8].

These molecules, all involved in skin functionality, are greatly affected by nutritional conditions [9, 10]. Excessive consumption of high-fat (HF) foods remains one of the largest problems for the people of many countries. Excessive fat intake causes not only lifestyle-related diseases, but also skin-related diseases such as skin atrophy, the retardation of wound healing, and the exacerbation of bedsores [11–13]. We have shown that HF diets reduce the levels of type I tropocollagen and hyaluronan in the skin by decreasing the levels of transforming growth factor-β1, insulin-like growth factor-I, and adiponectin [14]. However, the molecular mechanism underlying the alteration of skin lipids by HF diets is not well understood. Therefore, we focused on ceramide, fatty acids, cholesterol, and molecules involved in skin lipid metabolism after the consumption of HF diets.

In this study, we examined the ceramide levels and lipid contents in the skin of rats fed HF diets of varying fat levels. The HF diets were designed for comparability to the amounts of dietary fats commonly consumed by humans [15]. To investigate the mechanism by which an HF diet regulates ceramide production and lipid contents, we measured the expression levels of *SPT*, *fatty acid synthase* (*FAS*), *HMG-CoA reductase*, *PPAR-α*, *SREBP-1c*, *SREBP-2*, and *CPT-1* genes by quantitative PCR. We also determined serum adiponectin concentrations by ELISA.

2 Materials and methods

2.1 Materials

A High-Capacity cDNA Reverse Transcription Kit, a TaqMan Universal PCR Master Mix core reagent kit, and TaqMan Gene Expression Assays were obtained from Applied Biosystems (Foster City, IN, USA). A mouse/rat adiponectin ELISA kit was purchased from Otsuka Pharmaceutical (Tokyo, Japan).

2.2 Animals and diets

Four-week-old male Sprague–Dawley rats (CLEA Japan, Tokyo, Japan) were individually housed in stainless-steel cages and kept in an animal room at 23–25°C and 50–56% humidity under a 12-h light cycle (lights on 8:00–20:00). The animals had free access to food and drinking water. After the animals were acclimated to a normal diet based on AIN-76 feed composition [16] for 1 wk, they were divided into four groups (control (CO; corn oil 5%), Lard 5, 15, and 25% groups) of five individuals and fed for 28 days. Lard-containing diets were prepared by replacing corn oil and sucrose with lard (Table 1). The percentages of fat energy/total energy in the CO diet and in the 5, 15, and 25% lard diets were 12, 12, 31, and 46%, respectively. On the final day of the experimental period, the animals were dissected under sodium pentobarbital anaesthetic (body weight, 5.8 mg/100 g). To determine adiponectin concentrations, serum was prepared by centrifuging blood samples (3000 rpm, 4°C, 20 min). To measure ceramide levels, stratum corneum specimens were obtained using a stripping procedure. To detect lipid content, skin was cut from the mid-dorsal region of each specimen and fixed with 10% neutral-buffered formalin. The remaining skin was rapidly frozen in liquid nitrogen and used for mRNA quantification. The animal experiments conformed to the guidelines for the maintenance and handling of experimental animals established by the Ethics Committee of the Tokyo University of Agriculture (permission number: 060071).

Table 1. Composition and energy values of experimental diets

Ingredients (g/kg diet)	Groups			
	CO	Lard 5%	Lard 15%	Lard 25%
Casein	200.0	200.0	200.0	200.0
DL-Methionine	3.0	3.0	3.0	3.0
Sucrose	500.0	500.0	400.0	300.0
Cornstarch	150.0	150.0	150.0	150.0
Cellulose powder	50.0	50.0	50.0	50.0
Mineral mixture[a)]	35.0	35.0	35.0	35.0
Vitamin mixture[a)]	10.0	10.0	10.0	10.0
Choline bitartrate	2.0	2.0	2.0	2.0
Corn oil	50.0	–	–	–
Lard	–	50.0	150.0	250.0
% Total energy				
Carbohydrates	67	67	50	37
Fat	12	12	31	46
Protein	21	21	18	16
Energy density (kJ/g diet)	16	16	18	20

a) Based on AIN-76 mixture (Oriental Yeast, Tokyo).

2.3 Quantification of ceramide

Total lipid in stratum corneum specimens was extracted with hexane–ethanol (19:1 v/v), filtered, evaporated to dryness at room temperature under a stream of nitrogen, and finally reconstituted in chloroform to a concentration of 20 mg/mL. The lipid samples (5 μL) were spotted onto a

thin-layer chromatography (TLC) plate and separated using chloroform/methanol/acetic acid (192:7:1 v/v/v) as the developing solvent. The plate was dried, sprayed with 16% (w/v) cupric sulfate pentahydrate in 8% (w/v) phosphoric acid, and heated to 150°C for 10 min, to visualize target ceramide bands. The ceramide level of each test group was quantified by using LAS-3000 (Fujifilm, Tokyo, Japan) and expressed as a relative value to that of the control.

2.4 Oil red O staining

The skin samples were fixed in 10% neutral-buffered formalin and frozen. The frozen sections (7 μm) were stained with oil red O and haematoxylin to detect lipid contents.

2.5 RNA extraction and quantitative PCR

Total RNA was extracted from the skin, as described by Chomczynski and Sacchi [17]. The reverse transcription reaction was performed using a High-capacity cDNA Reverse Transcription Kit. Hybridization primers were synthesized by Applied Biosystems. The amplification products obtained were detected using a TaqMan Universal PCR Master Mix core reagent kit. Oligonucleotides that specifically hybridized to proximate regions of the target cDNA were prepared. The target genes were analyzed, and the sequences of the respective primers and probes are listed in Table 2. The amplification products of *FAS*, *HMG-CoA reductase*, *PPAR-α*, *SREBP-1c*, *SREBP-2*, and *CPT-1* were detected using TaqMan Gene Expression Assays. The mRNA levels of *SPT*, *FAS*, *HMG-CoA reductase*, *PPAR-α*, *SREBP-1c*, *SREBP-2*, and *CPT-1* were measured by quantitative PCR using an ABI Prism 7300 apparatus (Applied Biosystems); mRNA levels were expressed as a relative value to those of β-actin. Amplifications were performed under the following conditions: 2 min at 50°C and 10 min at 95°C, followed by 50 cycles of 15 s at 95°C and 1 min at 60°C.

2.6 Analysis of serum adiponectin

The serum level of adiponectin was measured by ELISA according to the kit manufacturer's instructions.

2.7 Statistical analysis

Statistical analysis was performed by using the SPSS 15.0J software. The significant differences from the means among four groups were assessed by Tukey's multiple comparison test.

3 Results

3.1 Body weight and energy intake

Table 3 summarizes, compared with the CO group, the addition of dietary lard increased body weight and energy intake in a dose-dependent manner throughout the experimental period.

3.2 Effects of HF diets on the levels of ceramide and lipids in rat skin

Figure 1A shows the level of skin ceramide in HF diet-fed rats. The addition of lard reduced the level of ceramide in a dose-dependent manner; both the 15 and 25% lard groups had significantly decreased ceramide levels, as compared with the CO group. Oil red O staining showed that the rats in HF diet groups had markedly fewer lipid droplets compared with control animals (Fig. 1B and C). Histological examinations revealed a loss of lipid accumulation in rats fed an HF diet.

3.3 Effects of HF diets on lipid synthesis in rat skin

To determine whether the ceramide and lipid reductions were related to lipid synthesis, we measured the level of mRNA encoding three major lipogenic enzymes (SPT, FAS,

Table 2. Primer and probe sequences used for the quantification of gene expression

Target gene	Primer and Probe sequences (5′-3′)		GeneBank accession number
Rat β-actin	Forward Primer	CCCTGGCTCCTAGCACCAT	
	Reverse Primer	GATAGAGCCACCAATCCACACA	V01217
	Probe	VIC-AGATCATTGCTCCTCCTGAGCGCAAGT-TAMRA	
Rat SPT	Forward Primer	CAGTGCAGCCTGCTTTGCTA	
	Reverse Primer	GCCTTTCGAGGATTCTTTTGATC	XM341495
	Probe	FAM-CCAGAAAGGACTACAGGCATCACGCAG-TAMRA	
Rat HMG-CoA reductase	Forward Primer	AGAGAAAGGTGCGAAGTTCCTTAGT	
	Reverse Primer	CCATGAGGGTTTCCAGTTTGTAG	NM013134
	Probe	FAM-CAGTTGGTCAATGCTAAGCACATCCCAG-TAMRA	

Table 3. Effect of dietary fats on energy intake and initial and final body weights in rats

	Energy intake (kJ/day)	Initial body weight (g)	Final body weight (g)
CO	362±5[a]	147±4	375±16
Lard 5%	350±6[a]	147±1	374±11
Lard 15%	396±6[b]	147±3	405±17
Lard 25%	413±5[b]	148±2	419±16

Values are mean±SE ($n=5$). Values bearing different letters in the same column are significantly different at $p<0.05$.

and HMG-CoA reductase) and transcription factors (PPAR-α, SREBP-1c, and SREBP-2) in the skin. Levels of *SPT* mRNA were significantly decreased in the 15 and 25% lard groups (Fig. 2A). No significant differences in the mRNA level of *FAS* were observed in any of the test groups when compared with the CO group (Fig. 2B). The mRNA levels of *HMG-CoA reductase* in animals of the 15 and 25% lard groups were considerably lower than those in the CO group animals (Fig. 2C). The mRNA level of *PPAR-α* decreased in the lard groups – particularly in the 15 and 25% lard groups (Fig. 3A). No significant differences in mRNA levels of *SREBP-1c* (Fig. 3B) and *SREBP-2* (Fig. 3C) were observed.

3.4 Effects of HF diets on the serum adiponectin concentration

Our preliminary experiments showed that adiponectin enhanced *PPAR-α* mRNA expression in human dermal fibroblasts (data not shown). Therefore, we examined the effect of HF diets on the concentration of serum adiponectin. The concentrations of serum adiponectin in the 15 and 25% lard groups were substantially lower than those in the other groups (Fig. 4).

3.5 Effects of HF diets on the mRNA level of CPT-1 in skin of rats fed HF diets

To elucidate the mechanisms involved in reducing skin lipids, the effects of HF diets on *CPT-1* mRNA expression were investigated. The addition of lard upregulated the expression of *CPT-1* mRNA in a dose-dependent manner; diets containing 15 and 25% lard significantly enhanced transcription of the *CPT-1* gene when compared with the CO diets (Fig. 5).

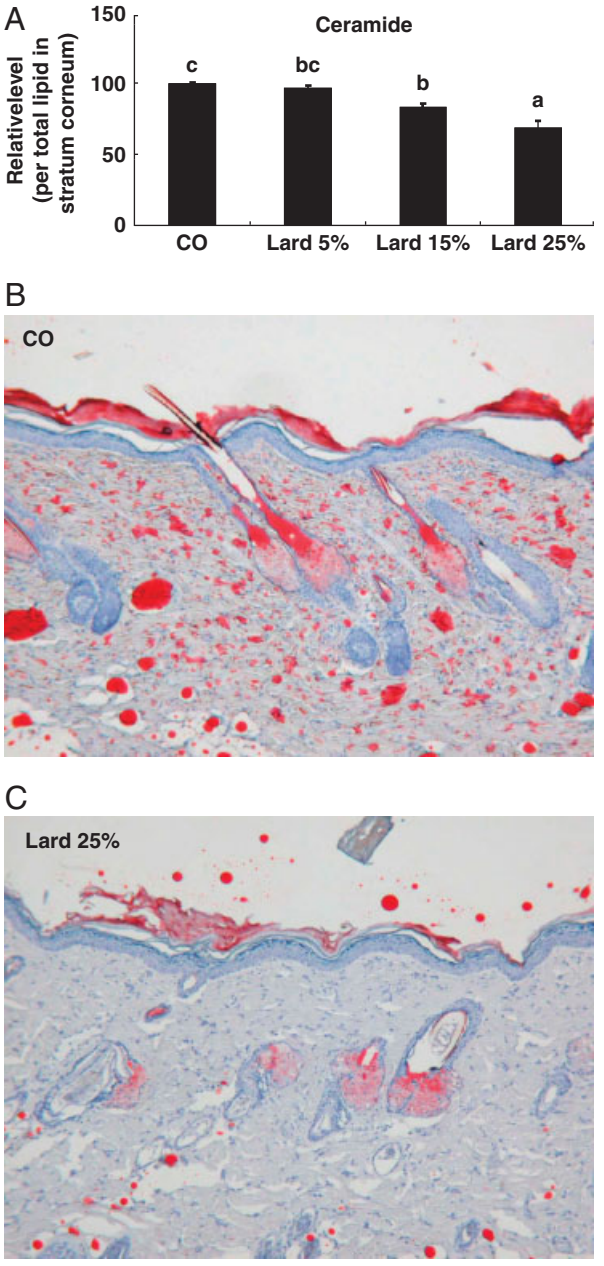

Figure 1. Effects of HF diets on the level of ceramide and lipids in the dorsal skin of rats. Ceramide levels (A) in rat skin were quantified by TLC. The ceramide level in the HF diet groups was expressed as a fold change relative to the band intensity in the control (CO) group. Bars are expressed as mean±SE ($n=5$). Bars without a common letter represent significant differences ($p<0.05$). The lipid contents in the skin of rats fed CO (B) and lard 25% (C) diets were evaluated by oil red O staining.

4 Discussion

It is well known that human skin diseases such as psoriasis, atopic dermatitis, and ichthyosis are often associated with a reduction in skin ceramide levels [2]. Fatty acids are efficiently accumulated in the form of triglycerides, which are major energy sources and important constituents of membrane lipids. They lipids often serve as cellular signaling molecules in the skin [3]. Haratake et al. [18] suggested

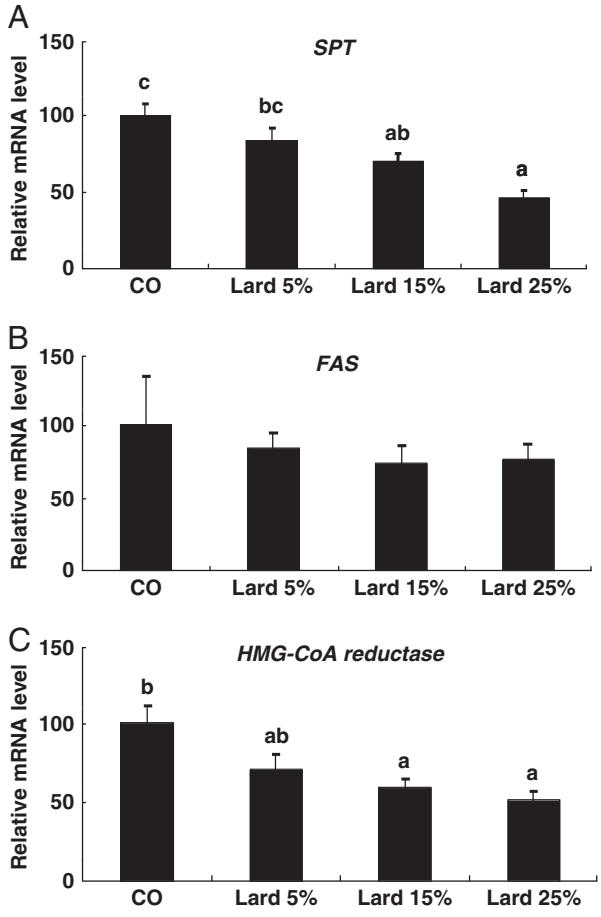

Figure 2. Effects of HF diets on the mRNA levels of *SPT*, *FAS*, and *HMG-CoA reductase* in the skin of rats. The mRNA levels of *SPT* (A), *FAS* (B), and *HMG-CoA reductase* (C) in rat skin were measured by quantitative PCR and expressed as a relative value to those of β-actin. Bars are given as mean ± SE ($n = 5$). Bars without a common letter represent significant differences ($p < 0.05$).

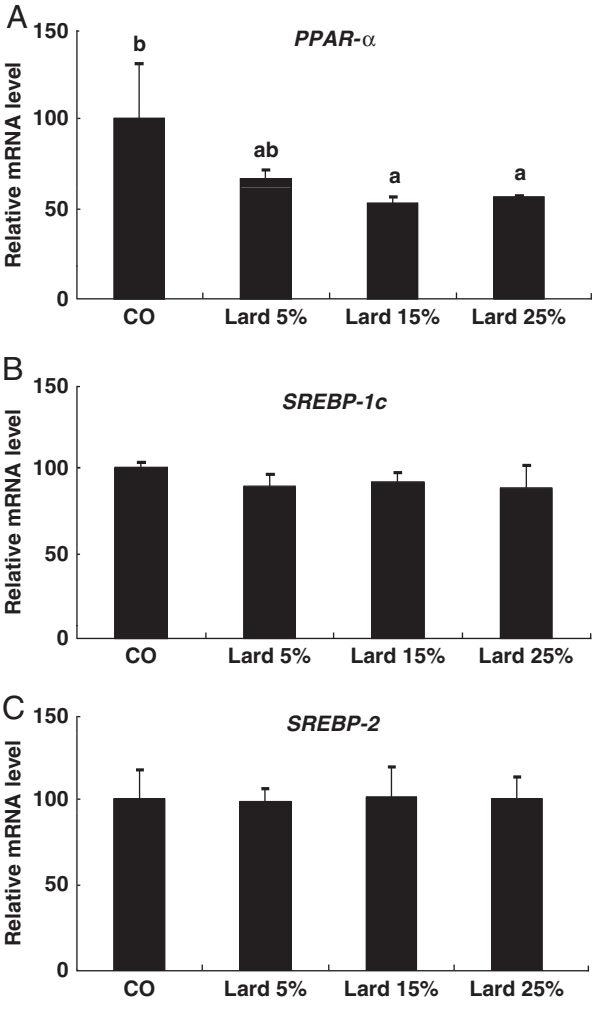

Figure 3. Effects of HF diets on the mRNA levels of *PPAR-α*, *SREBP-1c*, and *SREBP-2* in the skin of rats. The mRNA levels of *PPAR-α* (A), *SREBP-1c* (B), and *SREBP-2* (C) in rat skin were measured by quantitative PCR and expressed as a relative value to those of β-actin. Bars are given as mean ± SE ($n = 5$). Bars without a common letter represent significant differences ($p < 0.05$).

that stimulation of de novo cholesterol synthesis via an increase in the HMG-CoA reductase activity might impact the homeostatic function of the murine epidermal permeability barrier. Our data showed that HF diets reduced both the ceramide level and the lipid content of rat skin. Further, through histology studies, we found that there were considerably fewer lipid droplets in the HF diet groups than in the CO group. To clarify the mechanisms underlying these HF diet-related changes, we quantified the levels of skin mRNAs corresponding to key enzymes and transcription factors that are involved in ceramide, fatty acid, and cholesterol synthesis.

Increased ceramide synthesis is associated with the activation of SPT, the rate-limiting enzyme in de novo ceramide synthesis [19]. PPAR-α upregulates the synthesis of ceramide via the activation of SPT in skin [5]. In the present study, HF diets significantly reduced *SPT* and *PPAR-α* mRNA levels. Thus, HF diets probably reduce ceramide

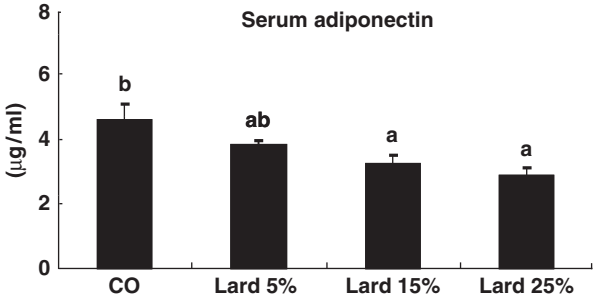

Figure 4. Effects of HF diets on serum adiponectin concentration in rats. Serum adiponectin levels in rats were measured by ELISA. Bars are given as mean ± SE ($n = 5$). Bars without a common letter represent significant differences ($p < 0.05$).

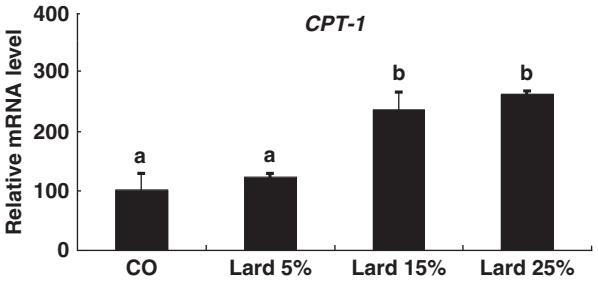

Figure 5. Effects of HF diets on the mRNA level of *CPT-1* in the skin of rats. The mRNA levels of *CPT-1* in rat skin were measured by quantitative PCR and expressed as a relative value to those of β-actin. Bars are given as mean ± SE ($n = 5$). Bars without a common letter represent significant differences ($p < 0.05$).

levels by depressing SPT activity through decreased PPAR-α expression in the skin.

SREBP-2 preferentially stimulates transcription of the genes involved in cholesterol synthesis. SREBP-2 and PPAR-α also regulate the level of HMG-CoA reductase, which acts as the rate-limiting enzyme in cholesterol biosynthesis. The association of PPAR-α with SREBP-2 expression, however, is still unclear. Our results show that HF diets significantly reduce *HMG-CoA reductase* and *PPAR-α* mRNA levels without affecting *SREBP-2* expression. These results show that in skin, PPAR-α has a greater effect on *HMG-CoA reductase* expression than SREBP-2 does. Therefore, it is likely that HF diets decrease the level of cholesterol in lipids by lowering HMG-CoA reductase expression through the PPAR-α pathway. No correlation was observed between *SREBP-2* and *HMG-CoA reductase* expression. SREBP-2, therefore, does not necessarily regulate HMG-CoA reductase in skin. The lack of a correlation between hepatic *SREBP-2* and *HMG-CoA reductase* expression has been reported previously [10].

Adiponectin is one of many adipocytokines secreted by adipose tissue. A significant negative correlation exists between the serum adiponectin concentration and the body fat mass, and the adiponectin concentration reduces with HF diets [20, 21]. The downstream signaling effects of adiponectin are mediated by activation of PPAR-α, which leads to increased levels of ceramide, cholesterol esters, and cholesterol sulfate in reconstructed skin. These effects lead to the activation of key enzymes involved in the synthesis of these lipid species [5]. We observed that adiponectin receptor (*AdipoR1*, *AdipoR2*) genes were expressed in rat skin and adiponectin promoted *PPAR-α* mRNA expression in human dermal fibroblasts (data not shown). Furthermore, Akazawa et al. [22] also reported that *AdipoR1* and *AdipoR2* mRNAs were expressed in cultured human dermal fibroblasts and keratinocytes. Fujita et al. [23] suggested that the interaction of adiponectin with adiponectin receptors enhanced PPAR-α activity through AMP-activated protein kinase pathway in cardiac fibroblasts. Taken together, our results suggest that HF diets reduce ceramide and lipid levels by depressing the expression of SPT and HMG-CoA reductase in the skin. This appears to be a result of lowered PPAR-α levels acting in the adiponectin-mediated signal transduction pathway.

SREBP-1c acts in the fatty acid synthetic pathway to regulate transcription of *FAS*, one of the key enzymes of lipogenesis [24]. In this study, consumption of HF diets did not affect gene expression of *FAS* and *SREBP-1c* in rat skin. These results suggest that HF diets do not regulate lipogenesis in the skin. We focused on the effects of HF diets on β-oxidation in skin. In other tissues, some researchers have shown that HF feeding increases mRNA expression and activity of CPT-1, the rate-limiting enzyme involved in β-oxidation [7, 25]. Oikawa et al. [26, 27] demonstrated that dietary fatty acids were easily transferred into the skin and that the properties of mouse skin were readily modified by variations in dietary fatty acid sources. Lard contains a high amount of palmitic acid and oleic acid, which have been shown to increase the transcription of *CPT-1* in a dose-dependent manner [28]. Therefore, it is likely that the palmitic acid and oleic acid components of lard were responsible for the observed increase in *CPT-1* mRNA expression. Taken together, these results indicate that the reduction in skin lipid contents after HF feeding may partially result from increased β-oxidation due to CPT-1 activation.

In this study, we measured the expression levels of *FAS* and *SREBP-1c* genes in liver by quantitative PCR. HF diet downregulated the expression of *FAS* mRNA in liver (data not shown). No significant differences in the mRNA level of *SREBP-1c* were observed in any of the test groups when compared with the CO group (data not shown). Furthermore, Hernández Vallejo et al. [29] have also shown similar findings to our results. Therefore, it is likely that lipid metabolism in skin is quite different from that in liver.

In summary, we have shown that HF diets lead to lowered adiponectin levels, which reduce ceramide synthesis and skin lipid content. Additionally, we have shown that upregulation of *CPT-1* gene expression and the resulting enhancement of β-oxidation can also lead to a decrease in skin lipid content. Our previous studies also show that HF diets reduce the levels of type I tropocollagen and hyaluronan in rat skin [14]. Taken together, our data suggest that HF diets can lead to rough and moistureless skin.

The authors have declared no conflict of interest.

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