Abstract

Effect of UV irradiation on Energy Homeostasis of Hairless Rats and Mice

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Ultraviolet (UV) light is an electromagnetic radiation with wavelengths ranged from 200 nm~400 nm, and human skin is continuously exposed to UV light.

Previous studies suggest that lipid metabolism in human subcutaneous (SC) fat tissues can be regulated by UV. In particular, UV radiation significantly reduces the lipids synthesis and lipolysis in the human SC fat tissues. Accordingly, this excess of energy derived from reduced SC fat is supposed to move to other

organs instead of being accumulated in SC fat tissues. In this study, changes in energy homeostasis induced by UV irradiation were examined in major organs which participate in energy homeostasis of hairless rats and mice *in vivo*.

Fifteen week-old rats (*Rattus rattus)* were separated into two groups, and 6 week-old mice (*Mus musculus*) were divided into two groups as well. One group was irradiated with UV light, and the other group was sham-irradiated for 8 weeks (3 times / week), (UV dose; 200~600 mJ/cm^2, total amount of UV radiation; 6000~8000 mJ/cm^2). After 8-week UV irradiation, serum, dorsal and abdominal skin including SC fat tissue, visceral fat, liver, and muscle tissues were obtained from seventeen rats and twenty mice.

Although, there was no significant difference in food intake between two groups, the average body weight at 8 weeks was slightly decreased in the UV-irradiated group in rats [1]. There was no significant difference in food intake and body weight between two groups in mice.

To investigate changes of lipid contents in major organs, we determined levels of triglyceride (TG), total cholesterol, and glucose by fluorescent enzymatic assay. Besides to identify their regulation factors, the expression of lipogenic enzymes, such as fatty acid synthase (FAS), and sterol regulated element-binding protein (SREBP)-1c and (SREBP)-2 were quantified. Moreover, expressions of glucose transporter type 4 (GLUT-4), a glucose metabolism gene, Fas receptor (TNF- **α** receptor), and suppressor of cytokine signal (SOCS)-3,

which are known to mediate inflammation and insulin resistance, were also examined by real-time quantitative polymerase chain reaction (PCR) or Western blot analysis.

Overall, this study demonstrated that UV irradiation affects impaired SC fat storage ability. The UV-irradiated rats showed a decreased tendency of SC fat accumulation in back and abdomen skin. In contrast, other energy metabolism in visceral fat and liver tissues tended to be enhanced in UV-irradiated group as compared to sham-irradiated one. Similarly, whereas SC fat from abdominal skin of UV-irradiated mice tended to decrease, their visceral fat increased after exposure to UV.

In conclusion, this study suggests that excess energy which could not be stored in SC fat tissues due to UV-induced impairment of SC fat storage could be stored in other organs, such as visceral fat or liver, probably through dynamic regulation of enzymes and transcription factors associated with glucose and lipids metabolism in those organs.

- Keyword:

Lipogenesis

Abbreviations

lipogenesis, lipolysis,

triglyceride (TG),

fatty acid synthase (FAS),

sterol regulated element-binding protein (SREBP)-1c and (SREBP)-2

glucose transporter type 4 (Glut)-4,

Fas receptor (TNF-α receptor)

suppressor of cytokine signal (SOCS)-3,

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Introduction

All living organisms need energy so that our body keeps delicate balance between food intake and expenditure. This energy balance is a foundation of biological homeostasis of energy in living systems [2]. Recently it is now increasingly evident that adipose tissue is one of the key regulators of energy balance to maintenance of energy between food intake and energy expenditure [3].

Adipose tissue is now recognized as a complicated organ with important endocrine and metabolic functions and also has various characteristics depending on its anatomical distribution. Besides, adipose tissue cross-talks with other body organs [4-6]. There are usually classified 2 types of fat, one is subcutaneous (SC) fat, which is portioned about 85 % in our body, and the other is visceral fat. Previous study showed that stored fat plays distinct roles in adipocyte function and metabolism according to their specific locations (depots) in human body [6]. In particular, increased visceral fat induces the accumulation of ectopic fat which can contribute to insulin resistance and metabolic syndrome [4]. In the contrast, increased SC fat is associated with a decreased risk of diseases such as metabolic syndromes [7-14]. Decreased storage capability of SC fat with aging leads to the untoward body composition characterized by a decreases of SC fat and an increase of visceral fat [13-19].

Human skin exposed continuously to UV radiation shows premature and accelerated skin aging characterized by formation of deep wrinkles, so-called photoaging. Photoaged skin is thicker skin thickness than intrinsically aged one [10]. Although UVB/A cannot penetrates into SC fat [21], it can trigger undesirable changes in body fat composition as well as induction of detrimental responses in epidermis and dermis [22,23]. In addition it is also reported that single acute UV irradiation of human skin showed decrease of free fatty acid (FFA), triglyceride (TG) levels in the SC fat, as well as low levels of lipogenic enzymes, lipogenic and adipogenic transcription activators. Therefore, UV may influence the underlying SC fat accumulation, which might result from inhibition of lipids synthesis [21].

The total fat mass in adipose tissue is strongly associated with the balance between lipid synthesis and lipolysis. Interestingly, not only expressions of lipogenic genes were decreased but those of lipid breakdown genes were also decreased by UV irradiation [21, 24]. Based on this result, we examined metabolism homeostasis of extra energy derived from decrease of lipids synthesis and lipids breakdown.

Each organ is closely associated with others to keep energy homeostasis.

First, in adipose tissue, generally food intake increases glucose uptake through GLUT-4 as well as stimulation of SREBP expression increase lipids storage.

Abnormal lipids storage in adipose tissue is associated with various kinds of metabolic disease such as diabetes, fatty liver, and insulin resistance.

Next, liver is well known as one of the most important organ in energy metabolism. Increased glucose also stimulates SREBP in this organ, and this induces synthesis of FFA and TG. Besides, glucose uptake also stimulates beta-oxidation in mitochondria and peroxisome. Sometimes, this beta-oxidation can induce excessive ROS production, and resultant liver damage associated with enhanced inflammatory gene expression. For example, Fas receptor (TNF- **α** receptor) and suppressor of cytokine signal (SOCS)-3 are linked between inflammation and insulin resistance [25].

In muscle, increased glucose in a body can induce FA oxidation, and increases insulin resistance via GLUT-4 [26].

Here, we investigated direct or indirect effect of chronic UV irradiation in energy homeostasis in back and abdomen skin, visceral fat, liver, and muscle tissues of hairless rats and mice *in vivo*.

Materials and methods

Animals and UV irradiation

Fifteen-week-old female albino hairless rats were obtained from Bio Genomics, Inc. (Seoul, Korea) and six-week-old female albino hairless mice were obtained Bio Orient, Inc. (Seoul, Korea). Animals were acclimated for 1 week prior to the study and had free access to food and water. All experimental protocols were approved by the Committee for Animal Care and Use at Seoul National University. For UV exposure, F75/85W/UV21 fluorescent sunlamps with an emission spectrum between 275 and 380 nm (peak at 310–315 nm) served as the UV source [27]. A Kodacil filter (TA401/407; Kodak Co., Rochester, NY) was mounted 2 cm in front of the UV tube for removal of wavelengths below 290 nm (UVC). Irradiation intensity at the skin surface was measured using a UV meter (model 585100; Waldmann Co., Villingen-chwenningen,Germany) [1]. The irradiation intensity 30 cm from the light source was 1.0 mW/cm^2. We initially measured the minimal erythema dose (MED) on the dorsal skin of mice. MED is defined as the minimum amount of radiation required to produce an erythema with sharp margins after 24 h. UV was exposed to the dorsal skin of hairless rats and mice in 2 MED (1 MED = 200 mJ/cm^2).

Rats and mice were divided into two groups; (i) sham-irradiated rat and mice,

(ii) UV-irradiated rat and mice. At baseline, there were 9 rats and 10 mice in each group, but one rat in UV irradiated group died during the study and was dropped out. The UV-irradiated rats and mice were exposed to UV from 1 MED (200 mJ/cm^2) to 3.0 MED (600 mJ/cm^2) three times a week for 8 weeks, and total amount of the UV irradiation was 30 or 40 MED (6000 or 8000 mJ/cm^2). In addition we also examined the UV-irradiated mice were exposed to UV from three times a week for 12 weeks, and total amount of the UV irradiation was 50 MED (10000 mJ/cm^2) for the supplement data. All animals were scarified at 48 h after final irradiation. Serum, abdomen and dorsal side of skin, visceral fat, liver, and muscle specimens were harvested [28].

Determination of TG, total cholesterol, HDL cholesterol, LDL cholesterol and glucose contents

Back skin, abdomen skin, visceral fat, liver, and muscle tissues were obtained and homogenized with 0.9% NaCl/ 1% Triton X-100. In addition serum were obtained from mice and separated by centrifuge (3000 rpm, 20 min). Then, TG, total cholesterol, and glucose, HDL cholesterol and LDL cholesterol contents were determined by using respective standard enzymatic assay kits: Cleantech TG-S kit, total cholesterol kit, glucose kit and HDL kit (*Asan pharmacy Ltd., Korea*). LDL contents were calculated by a widely using numerical expression; LDL cholesterol = Total cholesterol-TG/5-HDL cholesterol. [21, and 29]. These

contents were normalized to the protein contents, which were determined by the Bradford method in case of tissues (Bio-Rad, Hercules, CA) [24].

Messenger RNA expression level analysis

Total RNA was prepared from each organ tissue using Trizol method (Life Technologies, Rockville, MD) and 1 μg of total RNA was converted to complementary DNA using the First Strand cDNA Synthesis Kit (MBL Fermentas, Vilnius, Lithuania) according to the manufacturer's instruction. To quantitatively estimate the mRNA expression of each gene, polymerase chain reaction (PCR) was performed on a 7500 Real-time PCR system (Applied Biosystems, Foster City, CA) using SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) according to the manufacturer's instruction [30]. Primer information is shown in Table 1. The PCR conditions were 50℃ for 2 minutes, 95℃ for 2 minutes, followed by 40 cycles at 95℃ for 15 seconds, and 60℃ for 1 minute. The data are presented as relative mRNA level in gene expression normalized to 36B4.

Western blot analysis

Each organ tissue was obtained and homogenized, and proteins were extracted using RIPA buffer (Millipore, Billerica, MA) containing protease inhibitor cocktail (Roche, Indianapolis, IN, USA), phosphatase inhibitor cocktail (Roche,

Indianapolis, IN, USA), 5 mM phenyl-methylsulphonyl fluoride, and 1 mM dithiothreitol (Roche, Indianapolis, IN, USA) [21]. Equal amounts (50 μg) of protein were loaded, transferred, and analyzed. The proteins were loaded onto 10% SDS/acrylamide gels, and then electrophoretically transferred to polyvinylidene difluoride (PVDF) membrane. PVDF membranes were subsequently blocked with blocking buffer (5% nonfat dry milk, 1% Tween-20; in 20 mM TBS, pH 7.6) for 1 h at room temperature. Membranes were then incubated with a rabbit polyclonal antibody against SREBP-1 (Santa Cruz, CA). As a control, the corresponding a goat polyclonal antibody agaist β-actin (Santa Cruz, CA) was used. Blotting proteins were visualized by enhanced chemiluminescence (Amersham, Buckinghamshire, England). The relative signal strengths as normalized by β-actin were quantified using a densitometric program (TINA 2.0; Raytest, Straubenhardt, Germany) [28].

Statistics

Each individually expressed level was selected only mid-range press and then average statistical significance determined by Student's T-test. Results were presented as mean values ± standard error of the mean (SEM). All reported *P* values are two-tailed, and significance was accepted at $P<0.05$.

Table 1. Polymerase chain reaction primers, predicted size of PCR products, and gene bank number

Gene name	Primer sequence	Size	Gene ID
r36B4	Forward-5'-AAA GGGTCCTGGCTTTGTCT-3' Reverse-5'-CTTCCTTTGCTTCGACCT-3'	173	NM_022302
rFAS	Forward-5'-TGATGGGGCTGGTACCCGCA-3' Reverse-5'-ACCACCGGAGCCCGAGTGAA-3'	170	NM_017332
rSREBP-1c	Forward-5'-GGAGCCATGGATTGCACATTTGAAG-3' Reverse-5'-AGGAGCCCAGAGAAGCAGGAGA-3'	175	NM_213329
rSREBP-2	Forward-5'-CGCCCAGCATACCGCAAGGT-3' Reverse-5'-TGGCAGTGGCTCGTTCTCGC-3'	175	NM_001033694
rGLUT-4	Forward-5'-TCGGCAGCGAGTGACRGGAACA-3' Reverse-5'-CCAGAGCGRAGTGAGGGTGCCT-3'	196	NM_009204
rFas receptor	Forward-5'-GCTGATCCTCCCCGGTTTGGC-3' Reverse-5'-CCGGGATCTTGTGCTGCCGAG-3'	212	NM_031841
rSOCS-3	Forward-5'-ACCACTACATGCCGCCCCCA-3' Reverse-5'-TCGGCTCAGTACCAGCGGGA-3'	167	NM_053565
m36B4	Forward-5'-TGG GCTCCAAGCAGATGC-3' Reverse-5'-GGCTTCGCTGGCTCCCAC-3'	409	NM_00747
mFAS	Forward-5'-TGAGCCTCACTGCCATCC-3' Reverse-5'-GTGGGGGCAATTCCTTCC-3'	400	NM_007988
mSREBP-1c	Forward-5'-GCAGGTCCCAGTTGTATTGCAGCC-3' Reverse-5'-CAGGAGCCAGGGTGCTGATGC-3'	119	NM_011408
mGLUT-4	Forward-5'-AGCAGGTCCCAGTTGTATTGCAGC-3' Reverse-5'-AGGAGCCAGGGTGCTGATGC-3'	280	NM_010568
mFas receptor	Forward-5'-GGCAGGTCTACTTTGGAGTCAT-3' Reverse-5'-ACATTCGAGGCTCCAGTGAATTCGG-3'	307	NM_013693
mSOCS-3	Forward-5'-ACCTTCAGCTCCAAAAGCGA-3' Reverse-5'-TAGGTTCTTGGTCCCCGACT-3'	210	NM_007707

Result

Part 1. UV irradiation affects lipids and glucose contents of hairless rats and mice.

1-1. Changes in the amount of diet intakes, body weights and liver weights in UV-irradiated and sham-irradiated mice.

Six-week-old female albino hairless mice were divided into two groups at baseline and each group was sham-irradiated (n=10) or UV-irradiated (n=10; total UV dose, 8000mJ/cm^2; 40MED). The body weights and amount of diet intake were measured in each week to figure out any differences in body weight or diet intake. Diet intakes for each week and total sum of diet intake for 8 weeks from two groups were shown in Fig. 1A. Total diet intake for 8 weeks were 687±1.0 g (sham-irradiated group) and 686±1.4 g (UV-irradiated group).

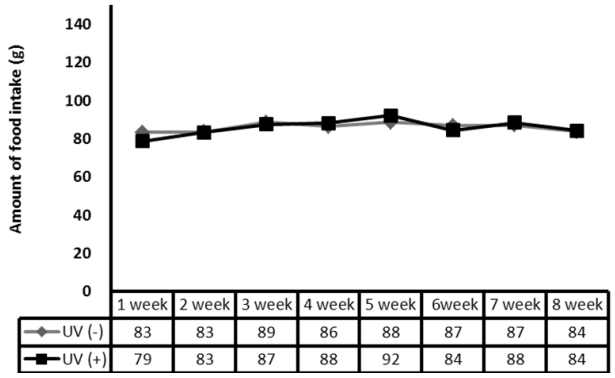
These results suggested that there was no significant difference in diet intake between two groups (Fig. 1A). Body weights of each mouse measured in each week and before sacrifice were shown in Fig. 1B. The average body weights of mice before sacrifice were 27.4 ± 0.5 g (sham-irradiated group) and 28.2 ± 0.4 g (UV-irradiated group). These results suggested that there was no significant difference in body weight between two groups (Fig. 1B).

Lastly, the trimmed liver weights were measured and compared between two groups (Fig. 1C). No significant difference in liver weight was also found between these two groups.

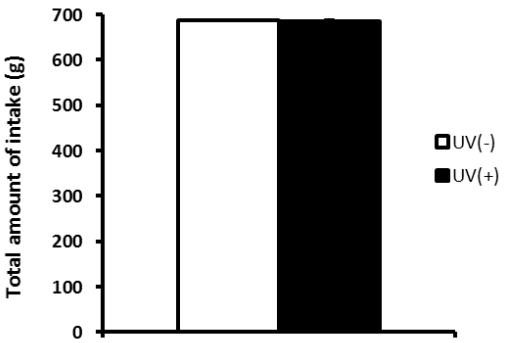
In the previous study, diet intake, body weight, and liver weight were observed in hairless rat experiment. Similar to mouse experiment, 15-week-old female albino hairless rats were divided into two groups at baseline and each group was sham-irradiated (n=9) or UV-irradiated (n=9; total UV dose, 6000mJ/cm^2; 30 MED). One rat in UV-irradiated group which died during UV irradiation was excluded for data analysis. There was no significant difference in total diet intake and liver weight between two groups [1]. However, the average body weight of rats in UV-irradiated group before sacrifice was slightly less than that in sham-irradiated group (p=0.011) [1].

In this study, I further analyzed several tissue samples from those UV- and sham-irradiated rats for energy homeostasis regulation, in parallel with mouse experiment.

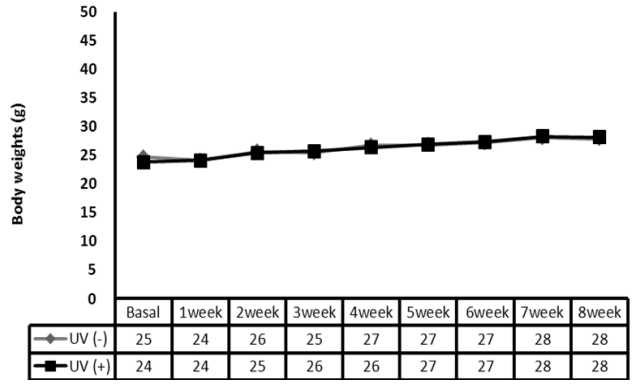
(A)



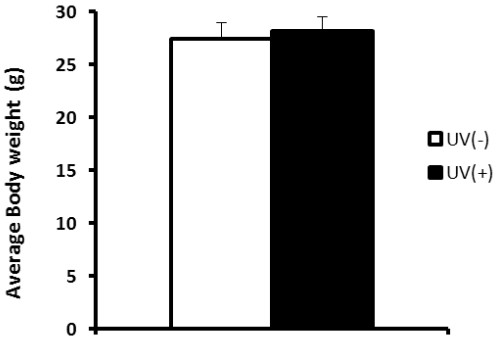
	1 week	2 week	3 week	4 week	5 week	6week	7 week	8 week
UV (-)	83	83	89	86	88	87	87	84
UV (+)	79	83	87	88	92	84	88	84



(B)



	Basal	1week	2week	3week	4week	5week	6week	7week	8week
UV (-)	25	24	26	25	27	27	27	28	28
UV (+)	24	24	25	26	26	27	27	28	28



(C)

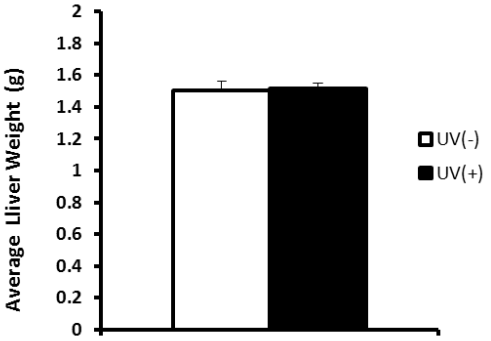


Figure 1. Amount of diet intake, body weights and liver weights in sham-irradiated or UV-irradiated hairless mice

The hairless mice were divided into two groups at baseline, and each group (n=10) was sham-irradiated or UV-irradiated for 8 weeks (total UV dose, 8000 mJ/cm^2; 40 MED). (A) Amounts of diet intakes (g) for each week and total amounts of diet intake. (B) Body weights (g) for each week. (C) Liver weights at sacrifice. Each value is shown as mean ± standard error of the mean (SEM) (n=10). UV(-); sham-irradiated group, UV(+); UV-irradiated group.

1-2. UV-irradiated mice showed higher TG contents than sham-irradiated mice in serum.

Decreased storage capability of SC fat with increasing age leads to the body composition changes by a decrease of SC fat and an increase of visceral fat [13-19]. In this study, the research hypothesis was that excess energy due to UV-induced impaired SC fat storage capability in dorsal and abdomen skin flows to the other organs, such as visceral fat or liver. At first, to identify UV irradiation effect on lipids and glucose movement between major energy regulatory organs, and serum levels of TG, total cholesterol, HDL cholesterol, LDL cholesterol, and glucose were measured after sacrifice in sham-irradiated and UV-irradiated mice. The TG content in serum from UV-irradiated mice was significantly increased, compared to sham-irradiated mice (32% increases; Fig. 2). Total cholesterol content in serum showed no difference between two groups (Fig. 2). UV-irradiated group showed no differences in serum HDL cholesterol and higher serum LDL cholesterol contents, (6% decreases and 14% increase, respectively), compared to sham-irradiated group, but those were not statistically significant (Fig. 2). Glucose contents tended to increase in serum from UV-irradiated mice (16% increases; Fig. 2).

The prior rats study also examined lipid profiles such as TG, total cholesterol, HDL cholesterol, and LDL cholesterol. In that study, there was no significant

difference in all lipid profiles between two groups, though TG and total cholesterol levels in 8-week-serum from UV-irradiated rats tended to increase [1].

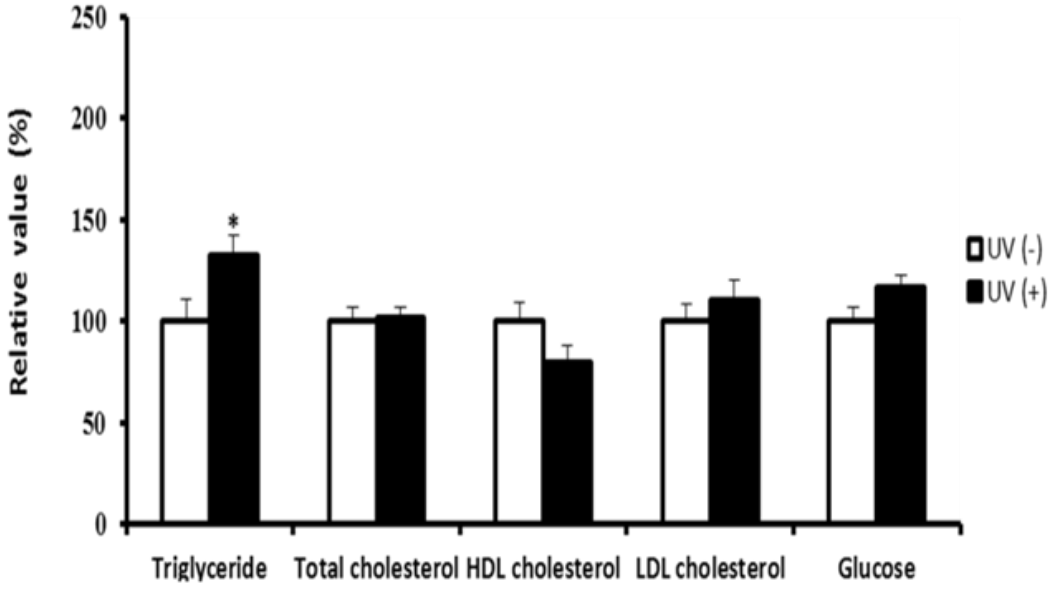


Figure 2. Determination of lipids and glucose profiles of mice serum

Mice were divided into two groups at baseline, and each group (n=10) was sham-irradiated or UV-irradiated for 8 weeks (total UV dose, 8000 mJ/cm^2; 40 MED). At sacrifice, serum was prepared from mice, and lipid and glucose contents in serum were quantified by respective assay . Relative value (%) of serum contents of triglyceride, total cholesterol, HDL cholesterol, LDL cholesterol, and glucose from sham-irradiated and UV-irradiated mice are shown as mean ± SEM. * P<0.05, and versus the sham-irradiated group. UV (-); sham-irradiated group, UV (+); UV-irradiated group.

1-3. UV-irradiated rats and mice showed lower TG contents in dorsal skin including SC fat than sham-irradiated counterparts.

SC fat is important organs to determine movement of extra energy. Recently our group reported acute UV irradiation affects decreased SC fat storage capability in human skin [21]. To identify changes of TG, total cholesterol, and glucose levels in major energy regulatory organs following chronic UV irradiation, I first determined each lipid and glucose level in dorsal skin tissues, including SC fat, from rats and mice, using commercial TG, total cholesterol, and glucose level measuring kits.

UV-irradiated rats tended to show lower TG and glucose contents in dorsal skin than sham-irradiated ones (11% and 15% decreases, respectively; Fig. 3A).

Furthermore, in dorsal skin tissues from UV-irradiated mice, TG contents also showed a decreased tendency (16% decrease), and total cholesterol contents showed a significant decrease, compared to sham-irradiated mice (30% decrease; Fig. 3B). However, glucose content tended to show higher in UV-irradiated group than sham-irradiated group (28% increase; Fig. 3B).

These results suggest that dorsal skin from UV-irradiated rats and mice tended to show lower TG contents, which is consistent with the previous finding that SC fat volume was statistically less in UV-irradiated rats [1].

(A) (B)

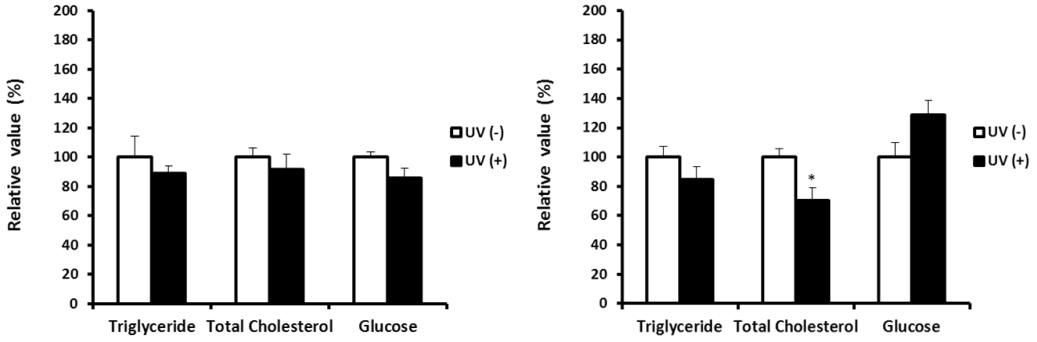


Figure 3. Determination of triglyceride, total cholesterol, and glucose contents in isolated dorsal skin tissues of rats and mice

(A) Rats and (B) mice were divided into two groups at baseline, and each group was sham-irradiated or UV-irradiated (total UV dose, 6000 and 8000 mJ/cm^2; 30 and 40 MED) for rats (n=9) and mice (n=10), respectively. One rat in UV-irradiated group which died in the study was excluded for data analysis. These animals were sacrificed at 48 h after the final irradiation and dorsal skin tissues were obtained and homogenized. Then triglyceride, total cholesterol, and glucose were determined by a fluorescent enzymatic method. Values are shown as mean ± SEM. * P<0.05, and ** P <0.01 versus the sham-irradiated control group. UV (-); sham-irradiated group, UV(+); UV-irradiated group.

1-4. UV-irradiated group tended to show lower TG and total cholesterol contents, showed significantly decrease in glucose levels in abdomen skin including SC fat from rats and showed significantly lower TG and total cholesterol contents in mice abdomen skin.

This study suggests that impaired storage capability of dorsal skin including SC fat is associated with decreased local lipid levels in response to UV irradiation. Since energy metabolism is under systemic control, and UV might affect indirectly the other tissues that were not irradiated directly. Therefore, I investigated changes of TG, total cholesterol, and glucose levels in abdomen skin tissues following chronic UV irradiation.

UV-irradiated group tended to show lower TG contents in rats and exhibited significantly decreased TG contents in mice, compared to sham-irradiated group (9% and 39% decreases, respectively; Fig. 4A and 4B). Total cholesterol contents were also decreased in UV irradiated rats and significantly decreased in UV-irradiated mice (23 % and 29% decreases, respectively; Fig. 4A and 4B). Although glucose contents were significantly decreased in UV-irradiated group in rats, those from mice showed no difference between two groups (34% and 3% decreases, respectively; Fig. 4A and 4B).

These results suggest that not only dorsal skin including SC fats but also abdomen skin including SC fats from UV-irradiated group tended to display

decreased TG and total cholesterol contents in both rats and mice, implying that UV irradiation effect on lipid content regulation seems to systemically.

(A) (B)

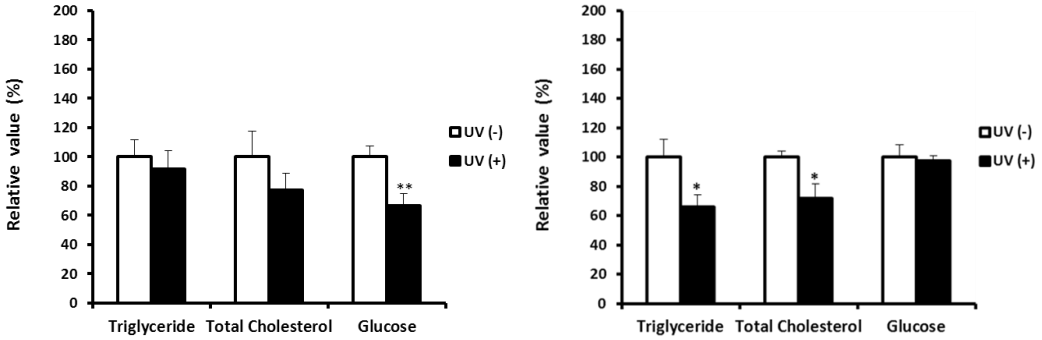


Figure 4. Determination of triglyceride, total cholesterol, and glucose contents in isolated abdomen skin tissues of rats and mice

(A) Rats and (B) mice were divided into two groups at baseline, and each group was sham-irradiated or UV-irradiated (total UV dose, 6000 and 8000 mJ/cm^2; 30 and 40 MED) for rats (n=9) and mice (n=10), respectively. One rat in UV-irradiated group which died in the study was excluded for data analysis. These animals were sacrificed at 48 h after the final irradiation and abdomen skin tissues were obtained and homogenized. Then triglyceride, total cholesterol, and glucose were determined by a fluorescent enzymatic method. Values are shown as mean ± SEM. * P<0.05, and ** P <0.01 versus the sham-irradiated control group. UV (-); sham-irradiated group, UV(+); UV-irradiated group.

1-5. UV-irradiated rats and mice tended to show higher TG and glucose contents in isolated visceral fat.

Visceral fat function is not merely for the storage fat, but secretes various adipokines, so that it is strongly associated with insulin resistance. To identify UV irradiation-induced changes of TG, total cholesterol, and glucose levels induced by UV irradiation, we next determined each level in isolated visceral fat from rats and mice by using a TG, total cholesterol, glucose level measuring kits.

UV irradiated rats tended to show higher TG, and glucose in isolated visceral fat tissues (15%, and 10% increases, respectively; Fig. 5A). Total cholesterol contents showed no differences between the two groups which were displayed only 7% increase in visceral fat after UV irradiation (Fig. 5A).

UV-irradiated mice tended to show higher TG, total cholesterol and glucose contents in isolated visceral fat tissues (17%, 17% and 10% increases, respectively; Fig. 5B.

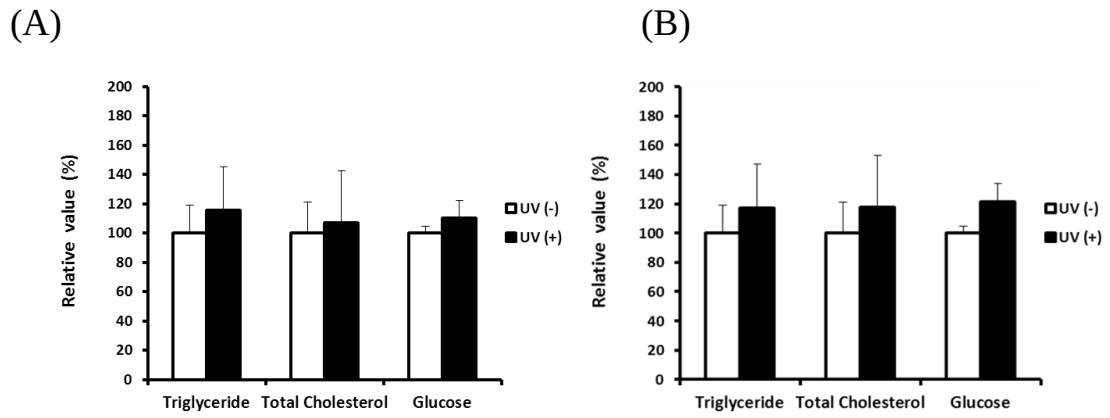


Figure 5. Determination of triglyceride, total cholesterol, and glucose contents in isolated visceral fat of rats and mice

(A) Rats and (B) mice were divided into two groups at baseline, and each group was sham-irradiated or UV-irradiated (total UV dose, 6000 and 8000 mJ/cm^2; 30 and 40 MED) for rats (n=9) and mice (n=10), respectively. One rat in UV-irradiated group which died in the study was excluded for data analysis. These animals were sacrificed at 48 h after the final irradiation and visceral fat tissues were obtained and homogenized. Then triglyceride, total cholesterol, and glucose were determined by a fluorescent enzymatic method. Values are shown as mean ± SEM. * P<0.05, and ** P <0.01 versus the sham-irradiated control group. UV (-); sham-irradiated group, UV(+); UV-irradiated group.

1-6. UV-irradiated rats showed significantly higher TG and glucose contents and tended to show higher total cholesterol contents in isolated liver tissues, whereas UV-irradiated mice had a tendency of lower TG, total cholesterol, and glucose contents in isolated liver tissues.

Increased glucose is associated with FFA and TG synthesis in liver, so that liver is well known organs associated with energy homeostasis. In addition, it is widely known as fatty liver can be induced by severe stress [23]. Therefore, to identify changes of TG, total cholesterol, and glucose levels in isolated liver tissues following chronic UV irradiation, we determined each level in isolated liver tissues from rats and mice by using TG, total cholesterol, glucose level measuring kits.

UV-irradiated rats showed significantly increased levels of TG, and glucose contents in isolated liver tissues, compared to sham-irradiated ones (13% and 16% increases, respectively; Fig. 6A). Total cholesterol contents showed a slight increase in isolated liver tissues from UV-irradiated rats (11% increases; Fig 6A).

However, lower levels of TG, total cholesterol and glucose contents in isolated liver tissues were observed in mice after UV irradiation (26%, 30% and 26% decreases, respectively; Fig. 6B). It will be discussed in following supplementary data (Fig. 11, 12 and13) and discussion part.

(A) **(B)**

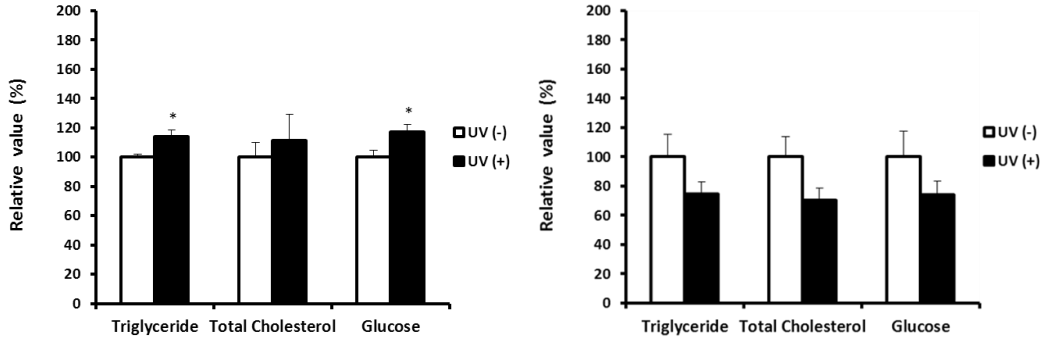


Figure 6. Determination of triglyceride, total cholesterol, and glucose contents in isolated liver of rats and mice

(A) Rats and (B) mice were divided into two groups at baseline, and each group was sham-irradiated or UV-irradiated (total UV dose, 6000 and 8000 mJ/cm^2; 30 and 40 MED) for rats (n=9) and mice (n=10), respectively. One rat in UV-irradiated group which died in the study was excluded for data analysis. These animals were sacrificed at 48 h after the final irradiation and liver tissues were obtained and homogenized. Then triglyceride, total cholesterol, and glucose were determined by a fluorescent enzymatic method. Values are shown as mean ± SEM. * P<0.05, and ** P <0.01 versus the sham-irradiated control group. UV (-); sham-irradiated group, UV(+); UV-irradiated group.

1-7. UV-irradiated group tended to show higher TG in muscle tissues from both rats and mice, whereas glucose contents significantly increased in muscle from UV-irradiated rats, and total cholesterol contents were significantly decreased in muscle from UV-irradiated mice.

In muscle, glucose uptake is mediated via glucose transporter, and this increased glucose can be converted into glycogen and can induce FA oxidation. To identify UV irradiation-induced changes of TG, total cholesterol, and glucose levels in major energy regulatory organs in rats and mice, I determined each level in their isolated muscle tissues in rats and mice using a TG, total cholesterol, glucose level measuring kits.

UV irradiation had a tendency to up-regulate TG contents in rat muscle (26% increase; Fig. 7A). Meanwhile, rats showed no difference in total cholesterol levels in muscle tissues in response to chronic UV exposure (1% decrease; Fig. 7A). Furthermore, glucose contents were significantly increased in isolated muscle tissues from UV-irradiated rats (25% increase; Fig. 7A).

The muscle of UV irradiated mice showed a little higher TG (11% increase; Fig. 7B), but significantly lower total cholesterol contents than those of sham-irradiated mice (44% decrease, Fig. 7B). Besides, glucose contents in mouse muscle tissues showed no difference between UV and sham-irradiated groups (4% increase; Fig. 7B).

(A) **(B)**

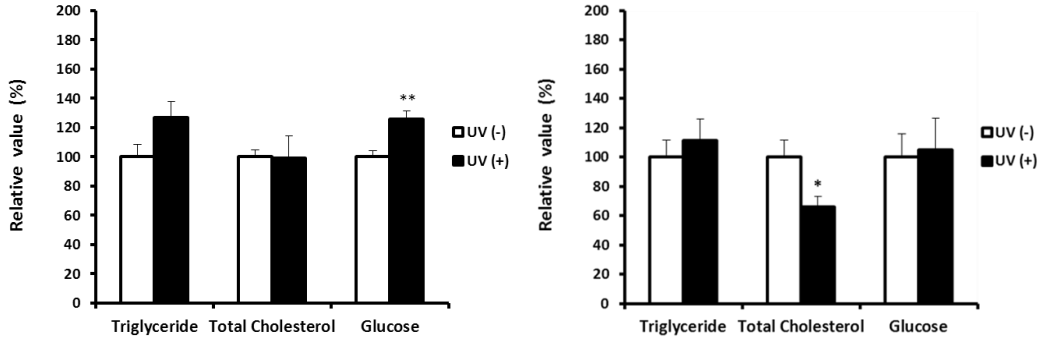


Figure 7. Determination of triglyceride, total cholesterol, and glucose contents in isolated muscle of rats and mice

(A) Rats and (B) mice were divided into two groups at baseline, and each group was sham-irradiated or UV-irradiated (total UV dose, 6000 and 8000 mJ/cm^2; 30 and 40 MED) for rats (n=9) and mice (n=10), respectively. One rat in UV-irradiated group which died in the study was excluded for data analysis. These animals were sacrificed at 48 h after the final irradiation and msucle tissues were obtained and homogenized. Then triglyceride, total cholesterol, and glucose were determined by a fluorescent enzymatic method. Values are shown as mean ± SEM. * P<0.05, and ** P <0.01 versus the sham-irradiated control group. UV (-); sham-irradiated group, UV(+); UV-irradiated group.

Part 2. UV irradiation affects expression of genes related to lipogenesis and inflammation in hairless rats

2-1. Dorsal skin from UV-irradiated rats tended to show lower FAS mRNA levels, and showed significantly decrease in FAS mRNA levels in UV-irradiated mice. Moreover, SREBP-1 protein levels showed significant decreases in dorsal skin from UV-irradiated rats.

Previously, I examined changes in lipid and glucose profiles following chronic UV irradiation. To identify association of these consequences with lipid synthesis, mRNA levels of lipogenic genes such as FAS, and SREBP-1c in dorsal skin were quantified. There is increased evidence that some components of the insulin resistance syndrome are related to inflammatory markers [31]. Thus I examined expression levels of Fas receptor in dorsal skin from rats, which is an important mediator of inflammation. SREBP-1c showed no differences between two groups in rats. FAS and Glut-4 mRNA tended to decrease in dorsal skin from UV-irradiated rats (Fig. 8A), and SREBP-1 protein levels significantly decreased in UV-irradiated group (Fig. 8B). FAS mRNA levels showed significantly decreased in UV-irradiated mice (Fig. 8C). In addition, Fas receptor showed an increased tendency in dorsal skin from UV-

irradiated rats (Fig. 8A).

These data showed correlation with decreased tendency of TG contents in UV-irradiated dorsal skin including SC fat from rats and mice. Besides, increased expression of Fas receptor might be induced by inflammation from UV exposure in rats.

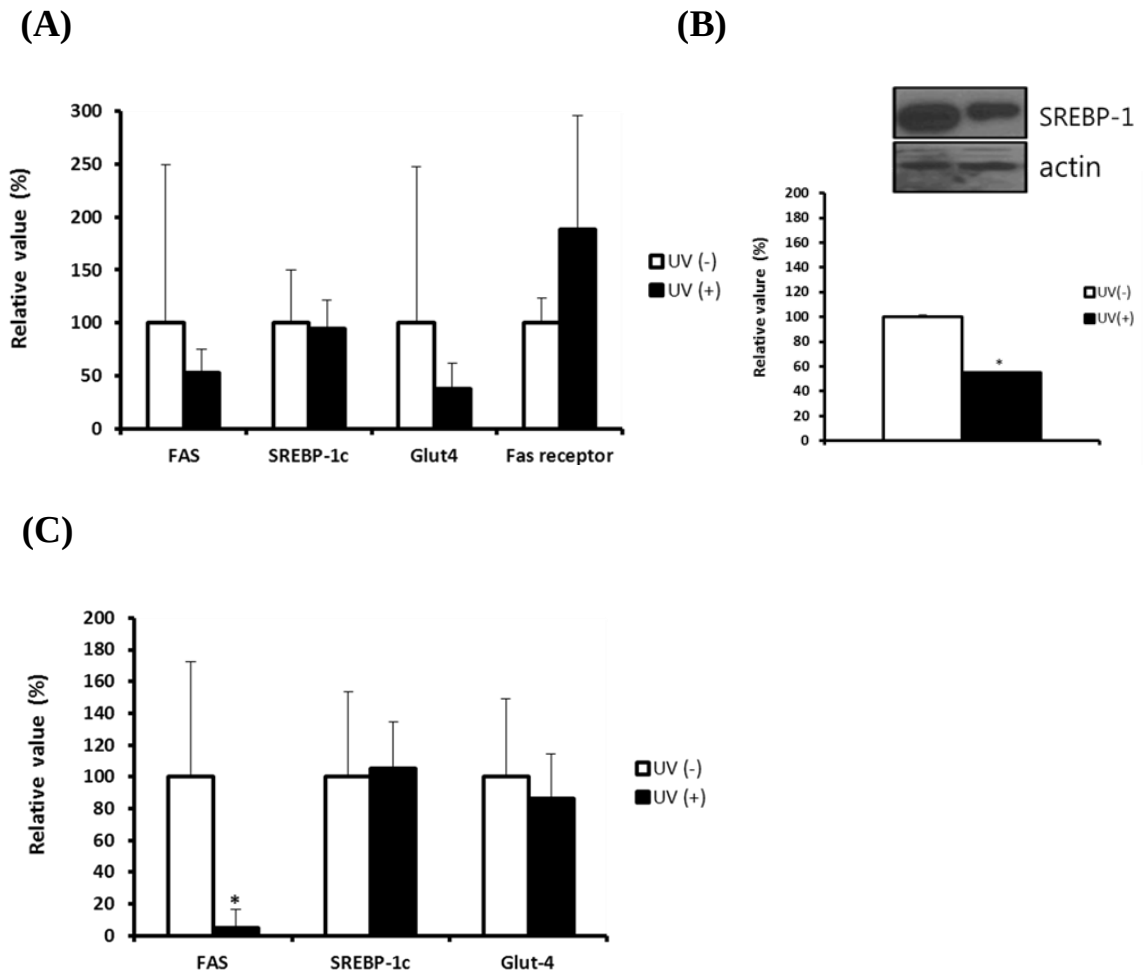


Figure 8. Expression of FAS, SREBP-1c, and GLUT-4 related to lipogenesis, and Fas receptor related to inflammation, and SREBP-1 protein levels in isolated dorsal tissues of rats and mice

Expression of FAS, SREBP-1c, GLUT-4, and Fas receptor related to lipogenesis and inflammation were detected by using quantitative PCR in isolated dorsal skin tissues from rats and mice.

Moreover, levels of SREBP-1 were determined by Western blot and subject to subsequent densitometry analysis in dorsal skin tissues from rats. SREBP-1 was

normalized by the corresponding β-actin level. Genes expressions levels and protein levels are shown as relative values (%) and mean±SEM. *P<0.05 *versus* the sham-irradiated group. (A) mRNA expression level analysis, (B) Western blot analysis.

2-2. UV-irradiated rats showed significantly decreased levels of FAS and SREBP-2 mRNA and a decreased tendency of SREBP-1c, Glut-4 mRNA and SREBP-1 protein. Moreover, FAS, SREBP-1c, Glut-4 tended to decrease in UV-irradiated mice.

Previously, this study examined changes of lipid and glucose contents in abdominal skin from rats and mice following chronic UV irradiation. To identify association of these consequences with lipid synthesis, mRNA levels of lipogenic genes such as FAS and SREBP-1c were quantified in abdominal skin from rats and mice. To investigate changes of inflammatory gene by UV-irradiation, we examined expression levels of Fas receptor and SOCS-3.

The abdomen skin from UV-irradiated rats showed significantly decreased FAS and SREBP-2 expression levels, and tended to exhibit decreased SREBP-1c and Glut-4 expression levels in rats (Fig. 9A). UV-irradiated group showed decreased tendency of FAS, SREBP-1c, and Glut-4 mRNA in mice as well (Fig. 9C). In addition this data showed no differences of SREBP-1 protein levels in rats (Fig. 9B). However, Fas receptor (Fig. 9A) and SOCS-3 (Fig. 9C) tended to show increased expressions in abdomen skin tissues from UV-irradiated rats and mice.

Not only decreased tendency of TG contents and total cholesterol (Fig. 4) were observed, but also significantly decreased expression of FAS was seen in abdominal skin of the UV-irradiated rats (Fig. 9A).

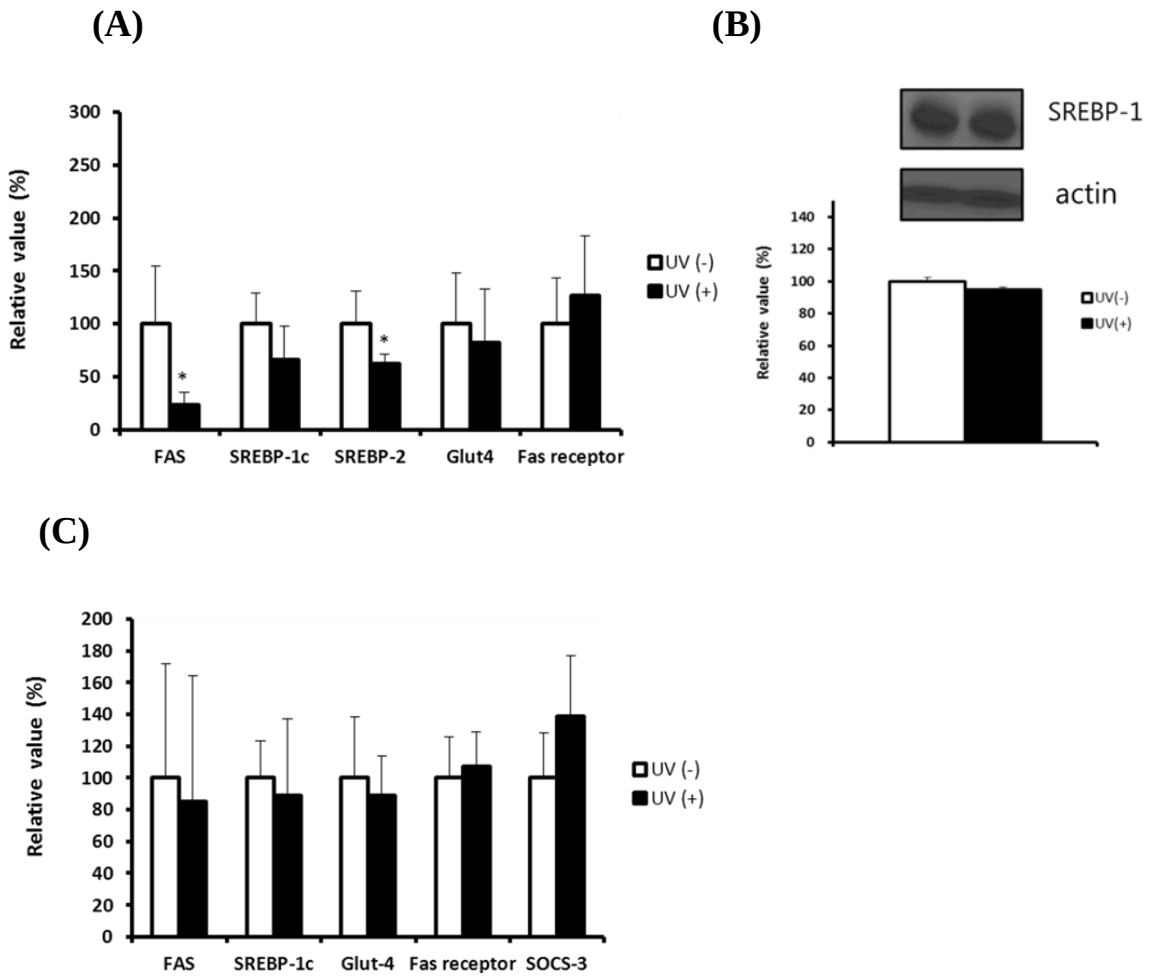


Figure 9. Expression of FAS, SREBP-1c, SREBP-2, and GLUT-4 related to lipogenesis, and Fas receptor, SOCS-3 related to inflammation, and SREBP-1 protein levels in abdominal skin of rats and mice

Expression of FAS, SREBP-1c, SREBP-2, GLUT-4, Fas receptor and SOCS-3 related to lipogenesis and inflammation were detected by using quantitative PCR in abdominal skin tissues from rats and mice.

The levels of SREBP-1 protein were determined by Western blot and subsequent densitometry analyses in abdominal skin tissues from rats. SREBP-1 was normalized by the corresponding β-actin level. Values are shown as relative values (%) and mean±SEM. *P<0.05 *versus* the sham-irradiated control group. (A) mRNA expression level analysis, (B) Western blot analysis.

2-3. Glut-4 and Fas receptor mRNA significantly increases in UV-irradiated rats. Moreover, SREBP-1c and Glut-4 gene expression tended to increase in visceral fat tissues from UV-irradiated mice.

Previously, I determined changes of lipid and glucose profiles along chronic UV irradiation. To identify association of these consequences with lipid metabolism, mRNA levels of Glut4 was detected and lipogenic protein levels of SREBP-1 was quantified in visceral fat from rats. To elucidate inflammatory gene expressions which can cause insulin resistance syndrome, we also examined expression levels of Fas receptor associated with inflammation.

As a result, UV-irradiated visceral fat from rats showed significantly increased expressions of Glut-4 and Fas receptor (Fig. 10A). In addition, SREBP-1c and Glut-4 showed increased tendency in UV-irradiated mice, as well (Fig. 10C). Glucose tended to show increased contents in UV-irradiated visceral fat in mice (Fig. 5). This data had a correlation between increased tendency of Glut-4 and glucose contents in UV-irradiated visceral fat from rat. SREBP-1 protein levels showed a slight increased tendency in visceral fat tissues from UV-irradiated rats (Fig. 10B). Besides Fas receptor showed significantly increased expressions in UV-irradiated visceral fat tissues of rats (Fig. 10A).

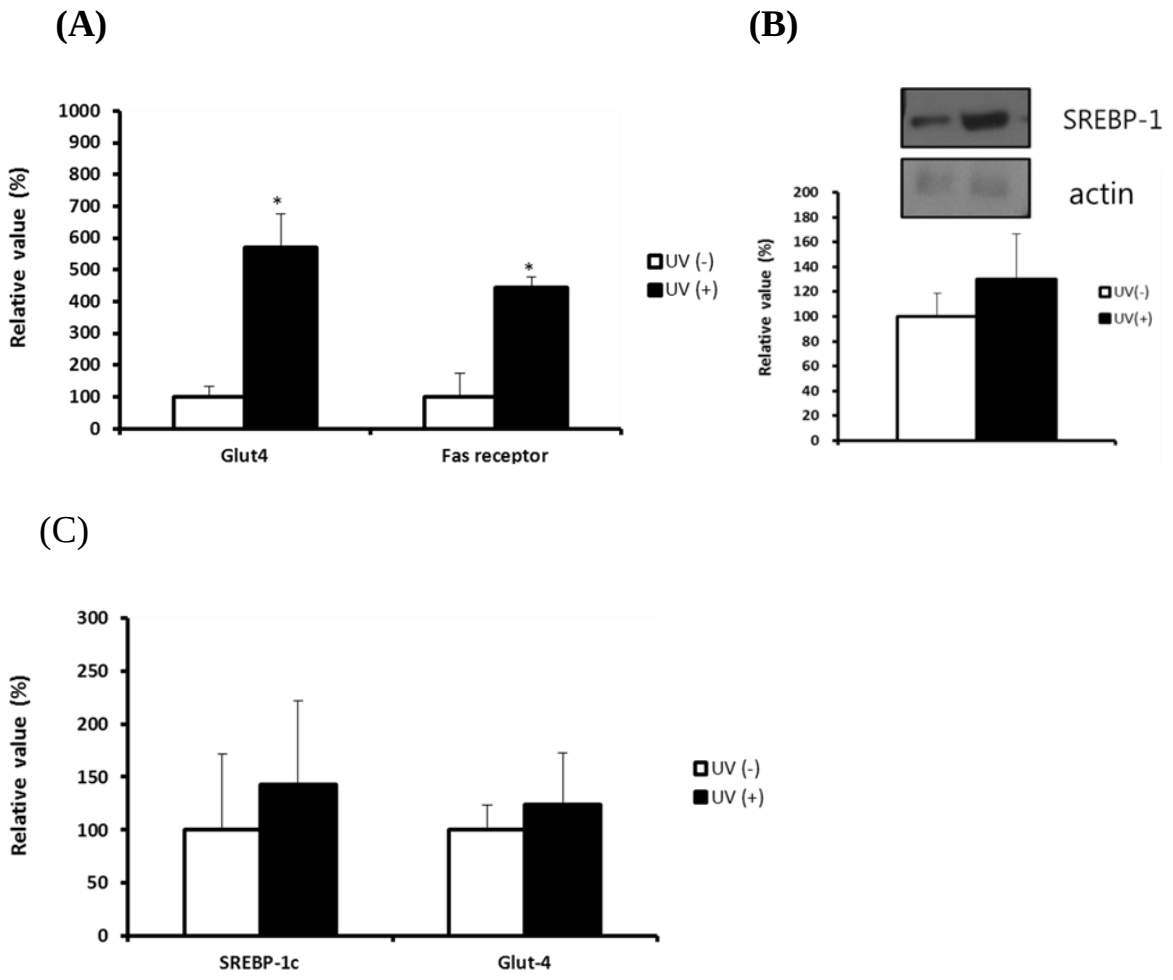


Figure 10. Expression of SREBP-1c, and Glut-4 related to lipogenesis, and Fas receptor related to inflammation, and SREBP-1 protein levels in isolated visceral fat tissues of rats and mice.

Expression of SREBP-1c, Glut-4 related to lipogenesis and Fas receptor related to inflammation were detected by using quantitative PCR in isolated visceral fat tissues from rats and mice.

The levels of SREBP-1 were determined by Western blot and subsequent densitometry analyses in isolated visceral fat tissues from rats. SREBP-1 was normalized by the corresponding β-actin level. Values are shown as relative

values (%) and mean±SEM. *P<0.05 *versus* the sham-irradiated control groups**.**

(A) mRNA expression level analysis, (B) Western blot analysis.

2-4. Whereas UV-irradiated rat group tended to increase FAS, SREBP-1c, Glut-4, and Fas receptror, UV-irradiated mice group tended to decrease those mRNA related to lipogenesis in isolated liver except for SOCS-3.

Previously, this study examined changes in lipid and glucose profiles in UV-irradiated rats compared to sham-irradiated ones. To identify association of these consequences with lipid metabolism, mRNA levels of lipogenic genes such as FAS, SREBP-1c, and SREBP-2 in liver were quantified. In addition, glucose transporter Glut-4 and Fas receptor, inflammatory gene markers, were also detected.

FAS, SREBP-1c, Glut-4 mRNA levels tended to increase in liver tissues from UV-irradiated rats (Fig 11A), and SREBP-1 protein were significantly increased in UV-irradiated group (Fig. 11B).

However, mice data indicated that FAS tended to decrease in liver tissues from UV-irradiated mice, and SREBP-1c showed statistically decreased mRNA levels in UV-irradiate mice (Fig. 11C). As this study showed, UV-irradiated liver tended to show increased lipids contents in rats while decreased tendency in UV-irradiated liver tissues from mice (Fig 6B). Unlike UV treated mice for 8 weeks (Fig 6B and Fig 11C), significant higher contents of TG (Fig. 12A) and FAS, and SREBP-1c mRNA levels were detected UV- treated mice in 12 weeks (Fig. 12B). We will run back over these results on the discussion.

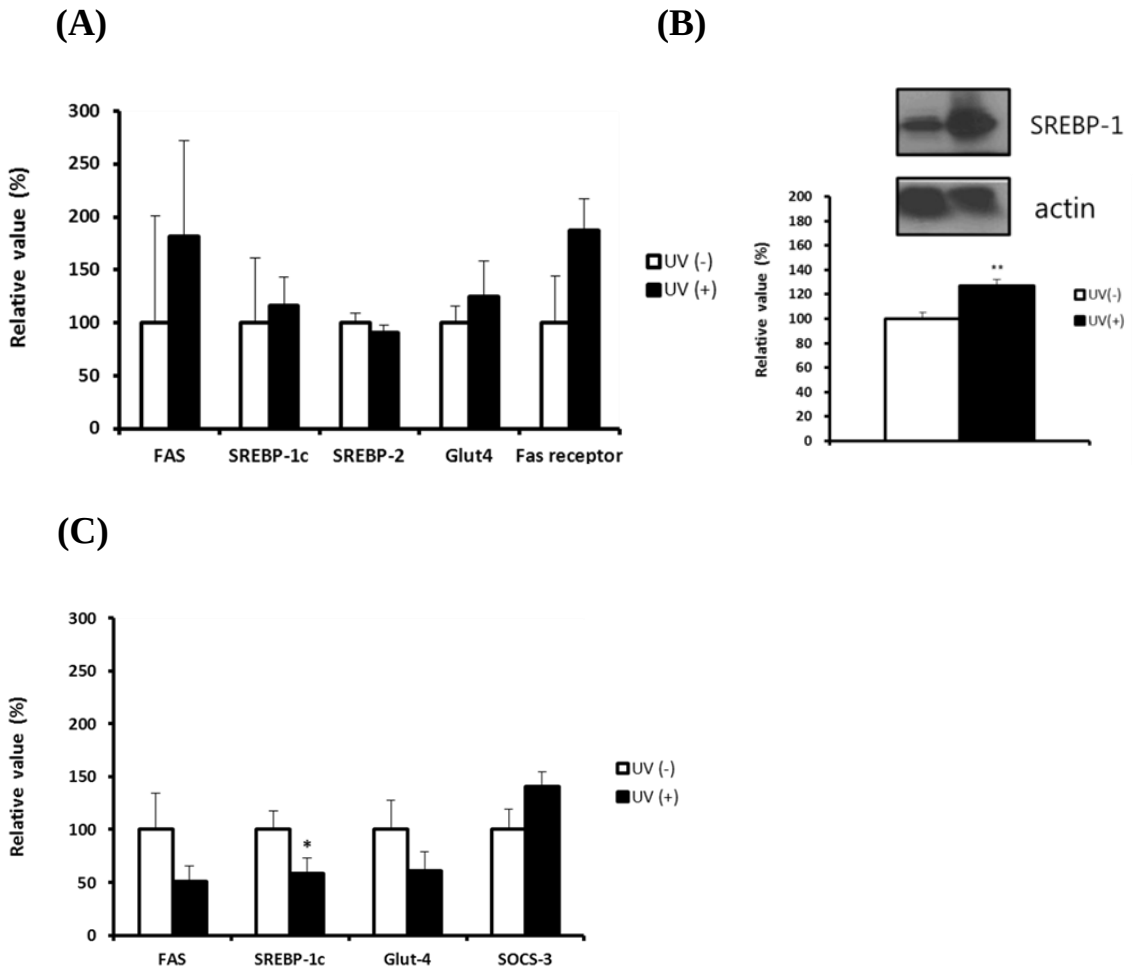


Figure 11. Expression of FAS, SREBP-1c, SREBP-2, and GLUT-4 related to lipogenesis, and Fas receptor, SOCS-3 related to inflammation, and SREBP-1 protein levels in isolated liver tissues of rats and mice.

Expression of FAS, SREBP-1c, SREBP-2, GLUT-4, Fas receptor, and SOCS-3 related to lipogenesis and inflammation were detected by using quantitative PCR in isolated liver tissues from rats and mice.

SREBP-1 level was determined by Western blot and subsequent densitometry analysis in isolated liver tissues from rats. SREBP-1 was normalized by the corresponding β-actin level. Values are shown as relative values (%) and mean± SEM. *P<0.05, and ** P<0.01 *verse* the control group. (A) mRNA expression level analysis, (B) Western blot analysis.

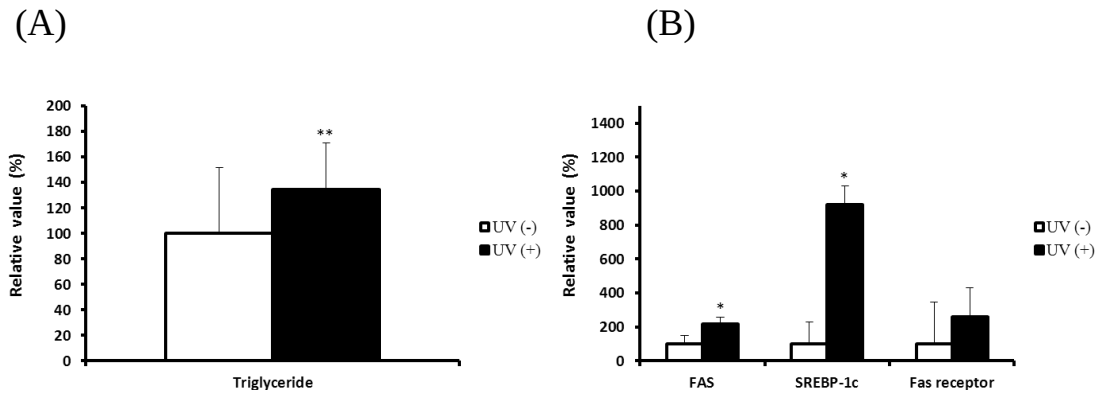


Figure 12. Determination of TG contents and FAS, SREBP-1c, and Fas receptor gene expression in isolated liver tissues of 12weeks UV treated mice.

Mice were divided into two groups at baseline and each group was sham-irradiated or UV-irradiated during 12 weeks (total UV dose, 10000 mJ/cm^2; 50 MED). These animals were sacrificed at 48 h after the final irradiation and liver tissues were obtained and homogenized. Then triglyceride was determined by a fluorescent enzymatic method. Expression of FAS, SREBP-1c, and Fas receptor related to lipogenesis and inflammation in liver tissues were also detected in mice. Values are shown as mean ± SEM. * $P<0.05$ versus the sham-irradiated control group. (A) TG contents, (B) mRNA expression level analysis.

2-5. FAS, SREBP-1c, SREBP-2, and GLUT-4 tended to decrease and SREBP-1 protein levels showed significantly decreased pattern in UV-irradiated muscle tissues of rats.

Previously, we investigated changes in lipid and glucose levels in UV- and sham-irradiated group from rat. To figure out the association with changes of lipid and glucose contents and mRNA levels of lipogenic genes such as FAS, SREBP-1c, SREBP-2, Glut-4, and Fas receptor related to inflammation in muscle were quantified.

FAS, SREBP-1c, SREBP-2, and Glut-4 mRNA tended to decrease in muscle tissues from UV-irradiated rats (Fig. 13A) and SREBP-1 protein levels significantly decreased in UV-irradiated group compared to sham-irradiated ones (Fig. 13B). TG and glucose contents tended to increase in muscle from UV-irradiated rats (Fig. 7A). Although glucose contents were significantly increased, Glut-4 expressions showed decreased tendency in muscle from UV-irradiated rats (Fig 12A).

In addition, Fas receptor showed an increased tendency in muscle tissues from UV-irradiated group of rats (Fig. 13A).

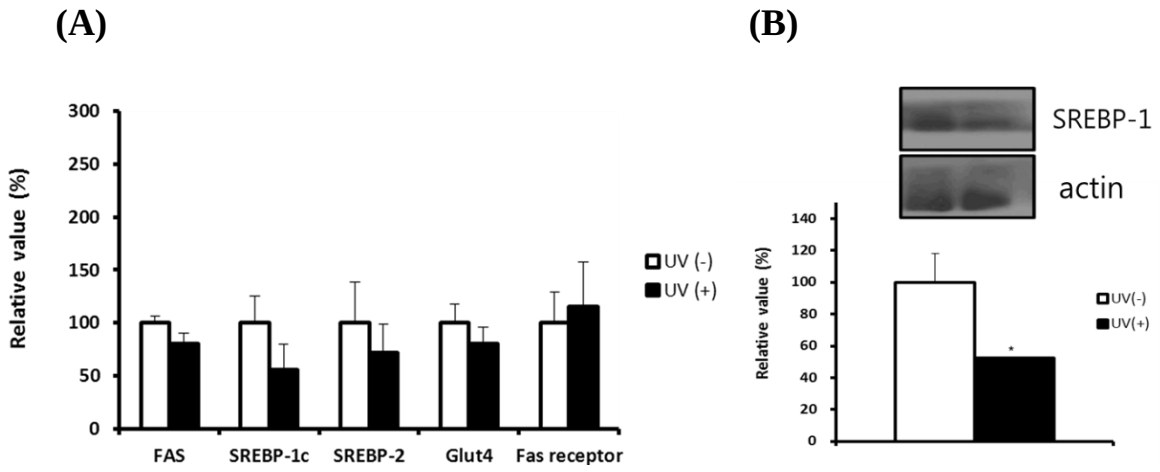


Figure 13. Expression of FAS, SREBP-1c, SREBP-2, and GLUT-4 related to lipogenesis, and Fas receptor related to inflammation and SREBP-1 protein levels in isolated muscle tissues of rats.

Expression of FAS, SREBP-1c, SREBP-2, GLUT-4, and Fas receptor related to lipogenesis and inflammation were detected by using quantitative PCR in isolated muscle tissues in rats.

Quantify UV-induced proteins related to lipid metabolism, levels of SREBP-1 was determined by Western blot and subsequent densitometry analysis. SREBP-1 was normalized by the corresponding β-actin level. Genes expressions levels and protein levels are shown mid-range press as relative values (%) and mean± SEM. *P<0.05 *verse* the control group. (A) mRNA expression level analysis, (B) Western blot analysis

Discussion

Aging is classified into intrinsic aging and photoaging. Intrinsic aging is characterized by a general decline in cellular function. That means ultimately aging can affect whole body homeostasis. As a consequence, this malfunction of body homeostasis can induces various types of intrinsic aging-associated chronic diseases such as cardiovascular diseases, hypertension, and diabetes [31]. In contrast, influence of photoaging on energy homeostasis has been rarely reported.

Recently we reported that the novel effects of UV irradiation in lipid metabolism in human SC fat tissue [21]. In this study, I examined direct or indirect effects of chronic UV irradiation on energy homeostasis using hairless rats and mice. This study revealed that impaired storage of excess energy into SC fat tissue can induce changes in lipid and glucose contents, and alter associated metabolic regulation in internal organs such as visceral fat, liver, and muscle.

Lipids composition and contents are important factors to keep a healthy body in terms of metabolic homeostasis. Despite they are important to keep our health, excessive and uncontrolled fat accumulation, especially for visceral fat, may do harm to our body [32]. Here, I determined lipid and glucose contents in major energy regulatory organ tissues, and also examined changes of gene

expression levels related to lipid and glucose metabolism in hairless rats and mice. In UV-irradiated rats, back and abdomen skin showed a tendency of decreased lipid and glucose contents whereas visceral fat and liver exhibited increased lipid and glucose contents. In muscle from UV-irradiated rats TG and glucose contents were increased. Surprisingly, although abdominal skin was indirectly affected by UV, the changes in abdominal skin showed similar and more enhanced pattern than in dorsal skin.

Moreover, UV-irradiated group tended to show decreased lipogenic gene expressions in underlying SC fat tissues, while in other organs these lipogenic gene expressions increased.

Therefore, TG contents and lipogenic and lipolytic genes expression levels showed practical correlations in rats. Decreased TG contents were consistent with decreased lipogenic gene expression patterns in back and abdominal skin. On the contrary, visceral fat and liver showed increased lipogenic gene expressions as well as increased TG contents in UV-irradiated group in rats.

Lipid metabolisms in mammalian cells are controlled by a family of endoplasmic reticulum (ER) membrane-associated transcription factors comprising SREBPs. SREBPs consist of SREBP-1a, SREBP-1c, and SREBP-2 [33]. SREBPs activate specific genes involved in fatty acid synthesis, TG synthesis, cholesterol synthesis, endocytosis of LDLs, and glucose metabolism [34]. Recently one group reported that increased SREBP-1c led to mixed insulin

resistance and sensitivity in livers of lipodystrophic and ob/ob mice [35]. In addition, the formation of SREBP-1c is known to be controlled at multiple levels in response to changes in levels of oxysterols, insulin/glucose and polyunsaturated fatty acid. As precise regulation of SREBP can have important clinical implications in metabolic diseases such as hyperlipidemia and diabetes [36], expression levels of SREBP were investigated. In back and abdomen skin FAS, SREBP-1c and SREBP-2 tended to decrease following chronic UV irradiation in rats. On the other hand, SREBP-1c was slightly increased in liver from UV-irradiated group, and SREBP-2 showed slightly increased pattern in liver as well. Therefore, UV-induced lipid accumulation via enhanced SREBP expression in visceral fat and liver may be related to the dysregulation of energy metabolism in rats. As I mentioned before, 8week-irradiated-mice showed decreased tendency of TG contents and lipogenic gene levels in the liver of UV-irradiated from mice (Fig. 12). In particular, decreased expressions of lipogenic genes can be attributed to decreased TG contents in UV-irradiated group. However, 12week-irradiated-mice showed more enhanced patterns of TG contents and lipogenic gene levels as well was previous rat study (Fig. 13).

Many researchers have reported that inflammation causes insulin resistance [37,38]. One group reported that the actions of insulin on lipid metabolism were both time and tissue dependent [39]. In addition, another group reported that chronic subclinical inflammation is a part of the insulin resistance syndrome

[40]. In this study, I also investigated inflammatory gene expressions such as Fas receptor, and SOCS-3, which are associated with insulin resistance, and detected an increased expression of those genes in all major energy regulatory organs of UV exposure group. Chronic UV exposure can possibly induce inflammation associated insulin resistance. It is suggesting that UV might induce insulin resistance caused by metabolic changes in various organs.

In this study, I investigated the effects of UV irradiation on energy metabolism homeostasis with normal diet-fed hairless rats and mice. Taken together, major organs participated in lipid and glucose metabolism might be affected by UV exposure. For further study, I want to examine combined effects of dietary interventions such as high fat diet and UV irradiation based on patterns of energy flow in this study.

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