UV Modulation of Subcutaneous Fat Metabolism

Eun Ju Kim[1,2,3], Yeon Kyung Kim[1,2,3], Ji Eun Kim[1,2,3], Sojeong Kim[1,2,3], Min-Kyoung Kim[1,2,3], Chi-Hyun Park[1,2,3] and Jin Ho Chung[1,2,3]

Adipose tissue is not a homogeneous organ. Visceral fat accumulation is associated with atherosclerosis and metabolic syndrome, but peripheral subcutaneous (SC) fat accumulation may be protective. Human skin is continuously exposed to UV light. UV can penetrate the epidermis and into the mid-dermis, but not into the SC fat tissue of human skin. However, we here show that SC fat tissue in chronically sun-damaged skin contains less fat than naturally aged skin, and even a single UV exposure of human skin reduced lipid synthesis in the underlying SC fat tissue through transcriptional regulation of the lipogenic enzymes, acetyl CoA carboxylase, fatty acid synthase, and stearoyl CoA desaturase, of their transcription activator sterol regulatory element-binding protein-1 (SREBP-1), and of two key adipogenic transcription factors, CCAAT/enhancer-binding protein α and peroxisome proliferator-activated receptor γ. The cytokines IL-6, IL-8, monocyte chemoattractant protein–3 (MCP–3), and placenta growth factor, produced by keratinocytes and fibroblasts in response to UV, may be responsible for the reduction of SC fat, and these cytokines, except MCP-3, may act by upregulation of suppressor of cytokine signaling-3 expression. Our data demonstrate the inhibitory effects of UV light on SC lipid synthesis and provide proof of concept for targeting cytokines for SC fat tissue modification.

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INTRODUCTION

Subcutaneous (SC) fat is a basic component of the skin. SC fat comprises around 85% of total body fat (Thomas *et al.*, 1998). The role of SC and visceral (VIS) fat, which was previously regarded as simple energy storage, was found to be different in relation to the occurrence of various diseases. An increase of VIS fat is associated with an increase in the risk for metabolic syndrome including insulin resistance (Carey *et al.*, 1997; Wang *et al.*, 2005), whereas an increase of SC fat improves insulin sensitivity and lowers the risk of related diseases (Tanko *et al.*, 2003; Tran *et al.*, 2008). With increasing age, the pattern of adiposity changes: SC fat is decreased, but intra-abdominal VIS fat is increased (Chumlea and Baumgartner, 1989; Hughes *et al.*, 2004). In elderly individuals, there is a progressive inability of the body to develop adequate SC adipose tissue mass to store lipid (Kotani *et al.*, 1994). This lipodystrophic nature of aged SC fat is characterized by a reduced ability of SC adipocytes to act as lipid storage sites and failure to adequately uptake circulating free fatty acids (FFAs; Lemieux, 2004; Uranga *et al.*, 2005; Despres and Lemieux, 2006). The subsequent accumulation of lipid within non-SC fat is strongly associated with several deleterious health outcomes, such as dyslipidemia (Lemieux, 2004), insulin resistance (Lewis *et al.*, 2002), and metabolic syndrome (Miranda *et al.*, 2005). Therefore, SC fat may have an important role in the maintenance of good health.

The fat tissue mass is regulated by alterations in both adipocyte number (adipogenesis) and cell size (lipogenesis; Prins and O'Rahilly, 1997). Adipogenesis is the process of development of mature fat cells from preadipocyte precursor cells and is controlled by two key transcription factors: CCAAT/enhancer-binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ) (Wu *et al.*, 1996; Zuo *et al.*, 2006). Lipogenesis is the process of lipid accumulation and is regulated by sterol regulatory element-binding protein-1 (SREBP-1), a transcription factor involved in lipid biosynthesis (Raghow *et al.*, 2008). The total fat accumulation in adipocytes is determined by the balance between lipid synthesis (lipogenesis) and lipid breakdown (lipolysis/fatty acid oxidation; Avram *et al.*, 2005a, b).

Human skin is continuously exposed to UV radiation, which causes premature and accelerated skin aging, called photoaging. Photoaged skin is characterized by coarse wrinkling and is thinner skin than intrinsically aged skin (Fisher *et al.*, 1997). UV radiation is divided into UVC (100–280 nm), UVB (280–320 nm), and UVA (320–400 nm). UVB/A penetrates into the epidermis and the dermis, and then triggers undesirable changes in body composition and induces detrimental responses (Krutmann, 2000; Makrantonaki and Zouboulis, 2007). Previously, we reported

[1]*Department of Dermatology, Seoul National University College of Medicine, Seoul, Korea;* [2]*Laboratory of Cutaneous Aging Research, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea and* [3]*Institute of Dermatological Science, Seoul National University, Seoul, Korea*

Correspondence: Jin Ho Chung, Department of Dermatology, Seoul National University Hospital, 28 Yongon-dong, Chongno-gu, Seoul 110-744, Korea. E-mail: jhchung@snu.ac.kr

Abbreviations: ACC, acetyl CoA carboxylase; C/EBPα, CCAAT/enhancer-binding protein α; FAS, fatty acid synthase; FFA, free fatty acid; MCP–3, monocyte chemoattractant protein–3; PlGF, placenta growth factor; PPARγ, peroxisome proliferator-activated receptor γ; SC, subcutaneous; SCD, stearoyl CoA desaturase; SOCS-3, suppressor of cytokine signaling-3; SREBP-1, sterol regulatory element-binding protein-1; TG, triglyceride; VIS, visceral

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such direct effect of UV on the lipid metabolism in the epidermis in human skin (Kim *et al.*, 2010). Recently, we also observed that the SC fat tissue of chronically sun-exposed skin of elderly subjects contained lower amounts of fat than that of sun-protected skin, even though UV cannot reach down to fat tissue directly. Therefore, UV may be an important environmental factor in determining the amount of SC fat in the skin. However, the influence of environmental stimuli, such as UV radiation, on SC adipose tissue in human skin remains unknown. We here show that single UV exposure to human skin and photoaging process reduce the lipid synthesis in the SC fat tissue. The cytokines, including IL-6, IL-8, monocyte chemoattractant protein–3 (MCP–3), and placenta growth factor (PlGF), secreted from the skin cells by UV are responsible for the alteration of SC fat functions.

RESULTS AND DISCUSSION

UV decreases lipid synthesis in SC fat tissues

Skin aging is associated with undesirable changes in body composition, such as damage to connective tissues that are further aggravated by the photoaging process (Makrantonaki and Zouboulis, 2007). As photoaging is caused by repeated exposure to UV radiation, we investigated whether chronic UV damage (photoaging) and acute UV exposure of human skin can lead to changes in the SC fat tissue. We found that the amounts of FFA and triglycerides (TGs) in the SC fat tissues of the photoaged forearm (sun-exposed skin) were significantly less than those in the buttocks (sun-protected skin) of the same elderly individuals (Figure 1a). On the other hand, the SC fat tissues from the forearm skin of young volunteers did not show significant decrease of lipid levels, compared with those from the buttock skin of the same volunteers (Supplementary Figure S1 online). Therefore, the decreased level of lipids in the photodamaged forearm SC fat tissues was not due to anatomical site differences, but to the chronic effects of UV irradiation.

To determine whether the reductions of these lipid levels are related to lipid synthesis, we evaluated the expression of lipogenic enzymes, such as acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl CoA desaturase (SCD). The expression of ACC, FAS, and SCD mRNA were significantly decreased in the photoaged forearm SC fat tissues compared with those from the buttocks of the same elderly volunteers (Figure 1b). The level of phospho-ACC, which is an inactive form, was not decreased, whereas the expression of total ACC protein was markedly decreased in the photoaged forearm fat tissues (Figure 1c). Furthermore, the mRNA expressions of SREBP-1c, C/EBPα, and PPARγ, known as global regulators of lipid synthesis (Eberle *et al.*, 2004) and adipocyte differentiation (Wu *et al.*, 1999), were also significantly decreased in the photoaged forearm fat tissues compared with those of the buttocks of the same elderly volunteers (Figure 1d).

Next, to examine whether the reduction of lipid accumulation in the SC fat tissues by photoaging process is provoked by UV stimulation, we measured the amounts of FFA and TG in UV-irradiated buttock fat tissues of young individuals. The buttock skin was acutely irradiated with 2 MED (minimal erythema dose) of UV and biopsied at 24, 48, and 72 hours after irradiation. Acute UV irradiation maximally decreased the levels of FFA and TG in the buttock SC fat tissues of human skin *in vivo* at 48 hours (Figure 1e). UV significantly decreased the mRNA expression of ACC, FAS, and SCD in the buttock SC fat tissues (Figure 1f). Western blot analysis demonstrated that UV irradiation decreased the total ACC expression and increased the level of phospho-ACC, which is an inactive form (Figure 1g). Acute UV also dramatically decreased the mRNA expression of the transcription factors, SREBP-1c, C/EBPα, and PPARγ, in the buttock SC fat tissues (Figure 1h). These results suggest that chronic repetitive UV exposure and acute single UV irradiation reduce lipid synthesis in the human SC fat tissue through downregulation of gene expression involved in lipogenesis and adipogenesis.

Moreover, we also found that the expression levels of genes involved in fatty acid oxidation, including acyl-CoA oxidase, carnitine palmitoyl transferase-1 and malonyl-CoA decarboxylase, and lipolysis, including hormone sensitive lipase, were also decreased in photoaged forearm SC tissues compared with buttock skin of the same individuals (Supplementary Figure S2a online), as well as in acutely UV-irradiated young buttock SC tissues (Supplementary Figure S2b online). Taken together, these data indicate that the reduction of fat content by the photoaging process and UV is due to decreased lipid synthesis rather than increased lipid breakdown.

Cytokines secreted from UV-irradiated keratinocytes or fibroblasts modulate SC adipocyte functions

UV cannot directly reach the SC fat tissue of human skin (Krutmann, 2000). Therefore, we hypothesized that soluble factors produced by epidermal keratinocytes or fibroblasts in the upper dermis after UV irradiation may have a role in UV modulation of SC fat metabolism. To test our hypothesis, differentiated human SC adipocytes were co-cultured with UV-irradiated keratinocytes or fibroblasts, or treated with conditioned media of UV-irradiated cells. The ACC, FAS, and SCD mRNA and the transcription factor SREBP-1c mRNA expression in the cultured human adipocytes were significantly decreased by co-culture with UV-irradiated keratinocytes (Figure 2a) or fibroblasts (Figure 2b). Furthermore, the treatment of the differentiated human SC adipocytes with the conditioned media of UV-irradiated keratinocytes reduced ACC, FAS, and SCD mRNA levels in a dose-dependent manner (Figure 2c). Also, the expressions of ACC, FAS, SCD, and SREBP-1c mRNA in the treatment group with 50% conditioned media of UV-irradiated keratinocytes/fibroblasts were more decreased than those in the treatment group with 50% conditioned media of non-UV-irradiated keratinocytes/fibroblasts (Supplementary Figure S3 online). These data suggest that UV irradiation of keratinocytes or fibroblasts can indirectly attenuate lipid synthesis in differentiated human SC adipocytes via soluble cytokines secreted from UV-irradiated skin cells.

To identify the cytokines responsible for UV modulation of SC fat metabolism, cytokine arrays were performed with the conditioned media of UV-irradiated keratinocytes or fibroblasts. The cytokines, IL-6, IL-8, MCP-3, and PlGF, were

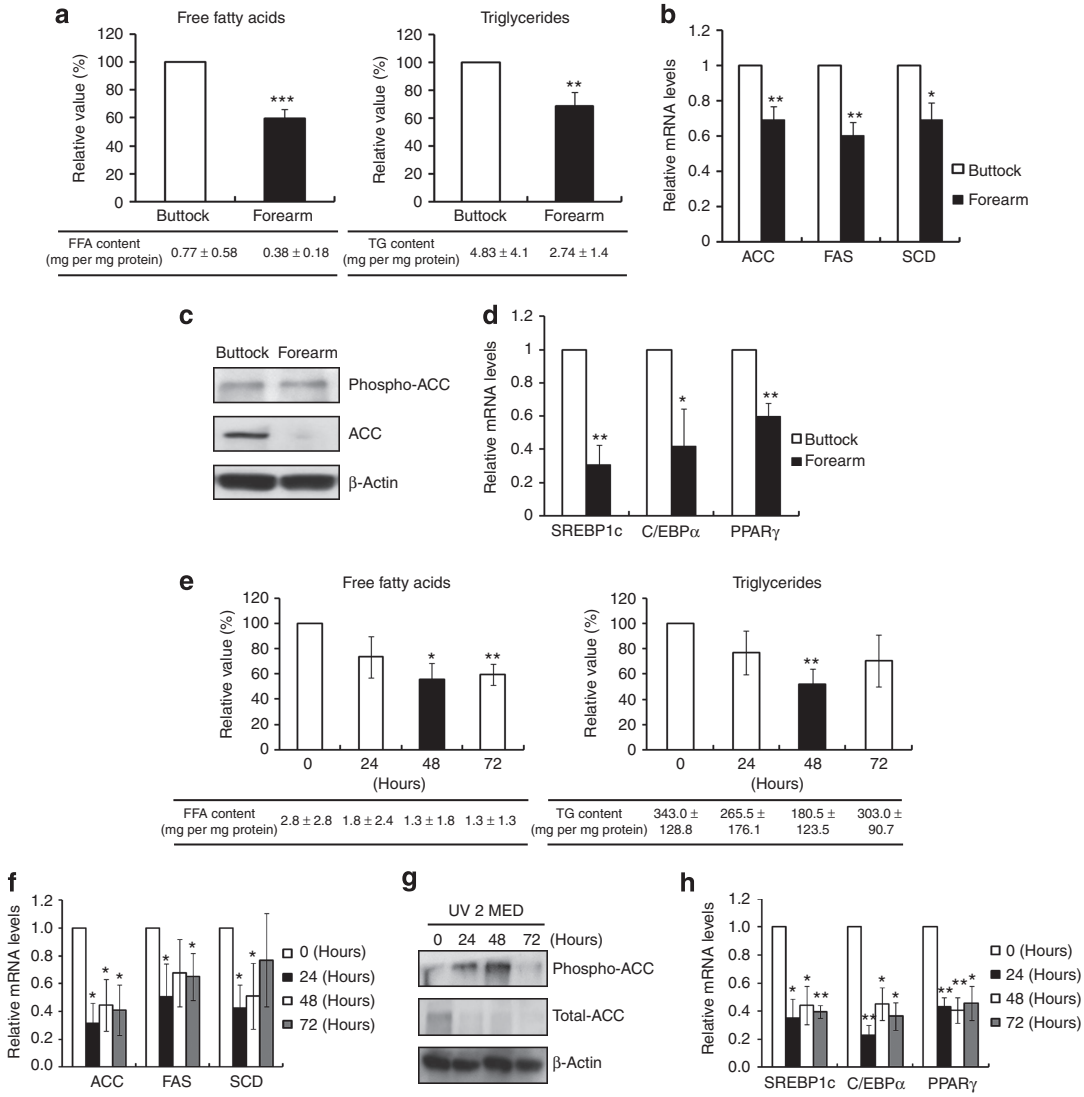

Figure 1. UV decreases lipid synthesis in subcutaneous (SC) fat tissues. Aged human (mean age 72.7 years; age range 70–75 years) buttock and forearm skin was obtained by punch biopsy and SC fat tissues were separated from the dermis. (**a**) Free fatty acid (FFA) and triglyceride (TG) contents were determined by a fluorescent enzymatic method. Data represent mean ± SEM ($n=7$). (**b**) Acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl CoA desaturase (SCD) mRNA levels were measured by real-time PCR ($n=4\sim5$). (**c**) Western blot analysis was performed using rabbit polyclonal antibody against ACC or phospho-ACC. (**d**) Sterol regulatory element-binding protein-1c (SREBP-1c), CCAAT/enhancer-binding protein α (C/EBPα), and peroxisome proliferator-activated receptor γ (PPARγ) mRNA levels were measured by real-time PCR ($n=4\sim5$). (**e**) Young human (mean age 26.5 years; age range 21–33 years) buttock skin was obtained by punch biopsy at the indicated time points after UV irradiation (2 MED (minimal erythema dose)) and SC fat tissues were separated from the dermis. FFA and TG contents were determined by a fluorescent enzymatic method. Data represent mean ± SEM ($n=6$). (**f**) ACC, FAS, and SCD mRNA levels were measured by real-time PCR ($n=3\sim5$). (**g**) Western blot analysis was performed using rabbit polyclonal antibodies against ACC or phospho-ACC. (**h**) SREBP-1c, C/EBPα, and PPARγ mRNA levels were measured by real-time PCR ($n=3\sim4$). All real-time PCR data represent mean ± SEM of the ratio between each gene and 36B4. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

greatly induced in the culture media of both UV-irradiated keratinocytes and fibroblasts. We then confirmed the UV-induced mRNA expression of these four cytokines by real-time PCR in both UV-irradiated keratinocytes (Supplementary Figure S4a online) and fibroblasts (Supplementary Figure S4b online). Next, we demonstrated that treatment of the differentiated human SC adipocytes with these cytokines reduced the TG content at certain concentrations (Figure 2d), as well as the mRNA expression of ACC (Figure 2e), FAS (Figure 2f), SCD (Figure 2g), and SREBP-1c (Figure 2h).

To confirm the role of these cytokines in the UV-induced inhibition of lipid synthesis, neutralizing antibodies for IL-6, IL-8, MCP-3, or PlGF were added to the co-culture system. The blocking of IL-6, IL-8, MCP-3, and PlGF activity in the culture media of keratinocytes (Supplementary Figure S5 online) and fibroblasts prevented the UV-induced inhibition of ACC (Figure 3a), FAS (Figure 3b), and SREBP-1c (Figure 3c) mRNA expression in differentiated human SC adipocytes. These results suggest that IL-6, IL-8, MCP-3, and PlGF, secreted from keratinocytes or fibroblasts after UV irradiation,

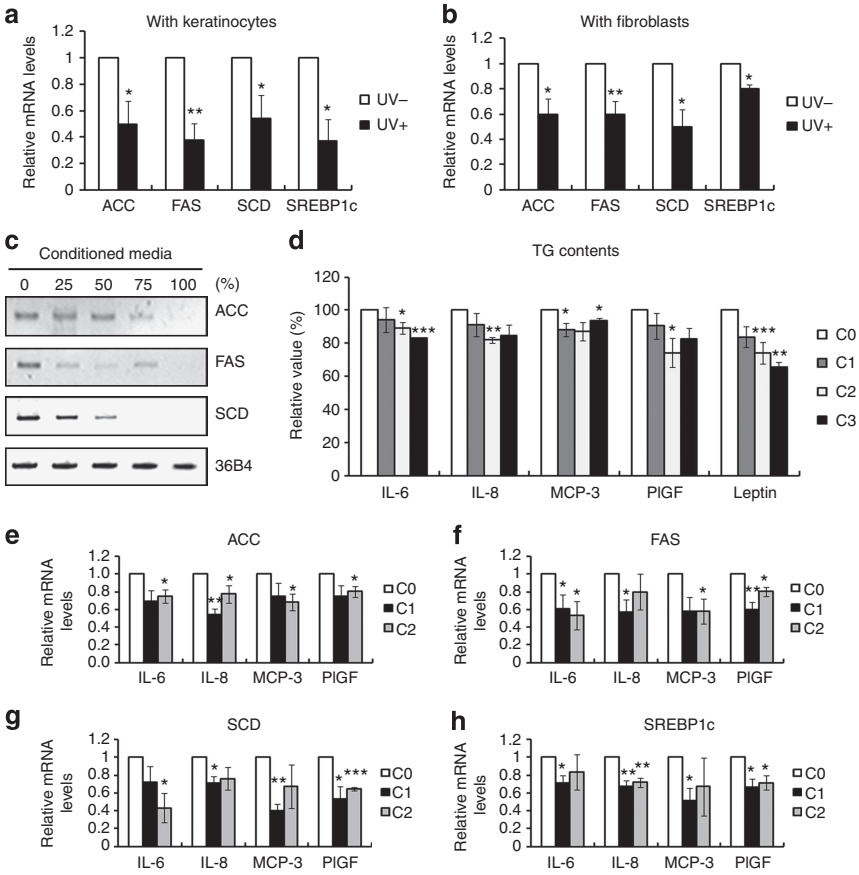

Figure 2. Cytokines secreted from UV-irradiated keratinocytes or fibroblasts modulate subcutaneous (SC) adipocyte functions. Differentiated human SC adipocytes (day 10) were co-cultured in serum-free DMEM with UV-irradiated (**a**) keratinocytes or (**b**) fibroblasts cultured in the inserted transwell, or (**c**) treated with different concentrations (0, 25, 50, 75, and 100%) of conditioned media of UV-irradiated keratinocytes. After 24 hours, acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl CoA desaturase (SCD), and sterol regulatory element-binding protein-1c (SREBP-1c) mRNA levels in the adipocytes were measured by real-time PCR or semi-quantitative PCR. Data represent mean ± SEM of the ratio between each gene and 36B4. $n = 3 \sim 5$; $^{*}P < 0.05$, $^{**}P < 0.01$. Differentiated human SC adipocytes (day 10) were treated with (C1, C2, and C3) or without (C0) various concentrations of recombinant cytokines, including IL-6, IL-8, monocyte chemoattractant protein–3 (MCP–3) (50, 100, and 200 μg ml^{-1}), and placenta growth factor (PlGF; 5, 10, and 20 μg ml^{-1}) for 24 hours. Recombinant leptin (10, 100, and 200 μg ml^{-1}) was used as a positive control. (**d**) Lipids were stained with oil red O and were isolated in isopropanol. The TG content was measured using a spectrophotometer at 518 nm. Data represent mean ± SEM ($n = 3 \sim 4$); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (**e**) ACC, (**f**) FAS, (**g**) SCD, and (**h**) SREBP-1c mRNA levels were measured by real-time PCR. Data represent mean ± SEM of the ratio between each gene and 36B4. $n = 3 \sim 5$; $^{*}P < 0.05$, $^{**}P < 0.01$.

may have important roles in the decreased synthesis of lipids in the SC fat tissue of the human skin.

UV irradiation inhibits lipid synthesis through Suppressor of cytokine signaling-3 induction caused by IL-6, IL-8, and PlGF secreted from UV-irradiated keratinocytes and fibroblasts

The suppressor of cytokine signaling (SOCS)-3 acts as a negative regulator of the insulin-signaling pathway by decreasing insulin receptor substrate 1 tyrosine phosphorylation (Emanuelli *et al.*, 2000; Lagathu *et al.*, 2003; Senn *et al.*, 2003). Insulin is known to be a key regulator of SREBP-1c gene expression, which is involved in *de novo* lipid biosynthesis (Raghow *et al.*, 2008). SREBP-1c induces the expression of PPARγ, which is considered to be the master regulator of adipogenesis (Farmer, 2006). We found that the expression of SOCS-3 was significantly increased in the photoaged forearm SC fat tissue (Figure 4a) compared with that in the naturally aged buttock of the same elderly individuals, as well as in the SC fat tissue of young buttock skin by acute UV irradiation (Figure 4b). We demonstrated that the blocking of IL-6, IL-8, and PlGF, but not MCP-3, activities in the culture media from keratinocytes (Supplementary Figure S6 online) and fibroblasts (Figure 4c) prevented the UV-induced upregulation of SOCS-3 mRNA expression in differentiated human SC adipocytes. Therefore, our data suggest that UV irradiation suppresses lipid accumulation in the underlying SC adipocytes through transcriptional inactivation of the SREBP-1c genes via SOCS-3 induction caused by IL-6, IL-8, and PlGF secreted from UV-irradiated keratinocytes and fibroblasts. Our suggestions are based on only neutralization analysis and further studies using other methods such as cytokine knockout or knockdown analysis are needed.

To our knowledge, this is previously unreported, to definitively demonstrate the inhibitory effects of UV on lipid synthesis in SC fat tissue of human skin. Single acute UV

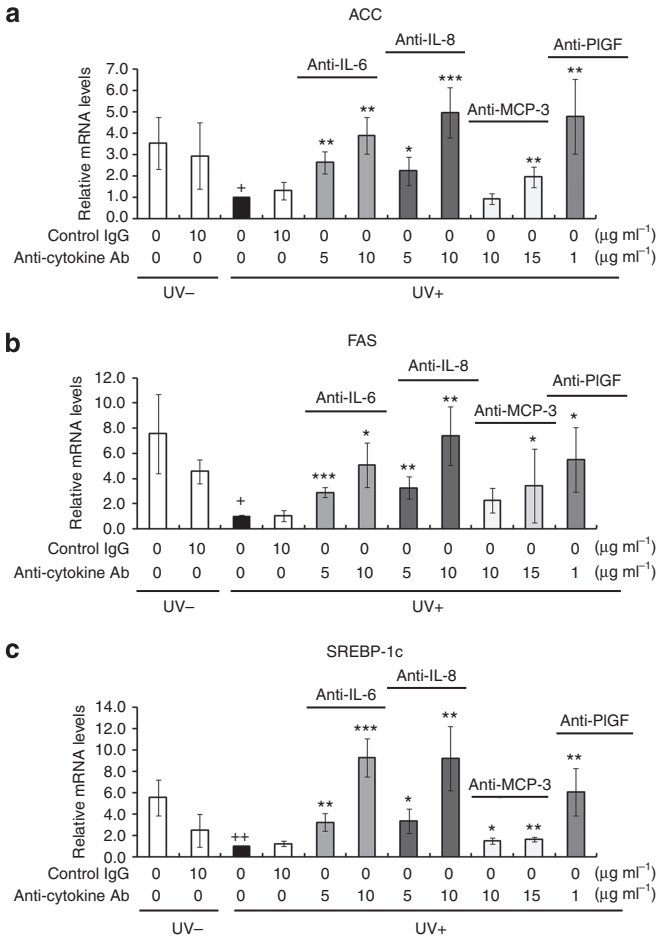

Figure 3. Neutralizing anti-IL-6, -IL-8, -monocyte chemoattractant protein–3 (MCP–3), or -placenta growth factor (PlGF) antibody prevents UV-induced decrease of acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and sterol regulatory element-binding protein-1 (SREBP)-1c mRNA. Differentiated human subcutaneous adipocytes (day 10) were co-cultured in serum-free DMEM with UV-irradiated fibroblasts cultured in the inserted transwell. Anti-IL-6, -IL-8, -MCP-3, or -PlGF neutralization antibody was added to the co-culture medium based on the ND_{50} provided by the manufacturer. The control IgG was added to the same concentration for control experiments. After 24 hours of incubation, the adipocytes in the lower wells were collected for gene expression experiments. (**a**) ACC, (**b**) FAS, and (**c**) SREBP-1c mRNA levels were measured by real-time PCR. Data represent mean ± SEM of the ratio between each gene and 36B4 ($n = 3 \sim 8$). $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$ versus the vehicle control group, $^{*}P<0.05$, $^{**}P<0.01$ versus the UV-irradiated group.

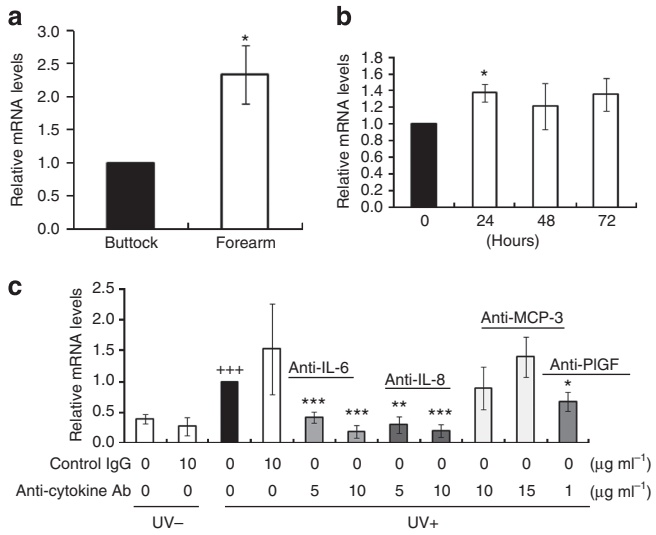

Figure 4. UV-induced IL-6, IL-8, and placenta growth factor (PlGF) mediate suppressor of cytokine signaling (SOCS)-3 expression in the subcutaneous (SC) fat tissue of human skin. (**a**) Aged human (mean age 72.7 years; age range 70–75 years) buttock and forearm skin from the same elderly individuals was obtained. (**b**) Young human (mean age 26.5 years; age range 21–33 years) buttock skin was obtained by punch biopsy at the indicated time points after UV irradiation (2 MED (minimal erythema dose)). SOCS-3 mRNA levels were measured by real-time PCR. Data represent mean ± SEM of the ratio between SOCS-3 gene and 36B4 ($n = 3$); $^{*}P<0.05$. (**c**) Differentiated human SC adipocytes (day 10) were co-cultured in serum-free DMEM with UV-irradiated fibroblasts cultured in the inserted transwell. Anti-IL-6, -IL-8, -monocyte chemoattractant protein–3 (MCP–3), or -PlGF neutralization antibody was added to the co-culture medium based on the ND_{50} provided by the manufacturer. The control IgG was added to the same concentration for control experiments. After 24 hours of incubation, the adipocytes in the lower wells were collected for gene expression experiments. SOCS-3 mRNA levels were measured by real-time PCR. Data represent mean ± SEM of the ratio between SOCS-3 gene and 36B4 ($n = 3 \sim 8$); $^{++}P<0.01$ versus the vehicle control group, $^{*}P<0.05$ versus the UV-irradiated group.

exposure of human skin decreases FFA and TG levels in the underlying SC fat tissue, and reduces the expression of the lipogenic enzymes, ACC, FAS, and SCD, their transcription activator SREBP-1c, and two key adipogenic transcription factors, C/EBPα, and PPARγ. IL-6, IL-8, MCP-3, and PlGF released from skin cells after UV irradiation mediates the inhibition of lipid synthesis in the SC fat tissues, which is located so deep under the skin that it cannot be directly exposed to UV. Under physiological conditions, human cells are exposed to UVB and UVA radiation. UVC (<280 nm) is completely absorbed by ozone in the stratosphere (Krutmann, 2000). UVA penetrates into the papillary area of the dermis, but UVB penetrates into the epidermis. Therefore, the effects of UVB and UVA on secretion of cytokines in keratinocytes or fibroblasts may be different. It is currently unclear which of the two UV wavelengths is more effective for the reduction of SC fat. Our results suggest that various environmental stimuli, such as UV, heat, or air pollutants, which could result in skin inflammation, may influence the underlying SC fat accumulation, resulting in less SC fat accumulation in exposed skin. It is a well-known fact that lipodystrophic change in SC fat is associated with a corresponding increase in VIS fat (Kotani *et al.*, 1994; Lewis *et al.*, 2002; Lemieux, 2004; Miranda *et al.*, 2005; Uranga *et al.*, 2005; Despres and Lemieux, 2006). As a consequence, the excess energy which is supposed to be stored in SC fat of sun-exposed skin may, instead, accumulate as non-SC fat in various internal organs, which is strongly associated with several deleterious health outcomes such as dyslipidemia and metabolic syndrome. Moreover, UV-induced SOCS-3, which is considered to be a key link between inflammation and insulin resistance (Chatterjee, 2010), may be related to the regulation of the lipid metabolism. Our data support a function for cytokines, originating from epidermis and dermis, in underlying SC lipid synthesis, and suggest that regulation of local cytokine

production in the skin may constitute a promising clinical strategy to modulate the underlying SC fat of human skin.

MATERIALS AND METHODS

Human studies

Seven elderly male (mean age 72.7 years, age range 70–75 years; body mass index range 19.5–22.8 $kg\,m^{-2}$) and five young male (mean age 30.2 years, age range 24–33 years; body mass index range 16.6–25.1 $kg\,m^{-2}$) Koreans without current or previous skin disease provided both sun-protected buttock and photodamaged extensor forearm skin samples. Another group of six young male volunteers (mean age 26.5 years, age range 21–33 years; body mass index range 18.6–23.3 $kg\,m^{-2}$) provided buttock skin samples. The buttock skin was exposed to UV light using a F75/85W/UV21 fluorescent lamp with an emission spectrum between 275 and 380 nm (peak at 310–315 nm) (Seo *et al.*, 2001). A Kodacel filter (TA401/407; Kodak, Rochester, NY) was used to remove wavelengths below 290 nm (UVC). The MED was determined 24 hours after irradiation. The MED usually ranged between 70 and 90 $mJ\,cm^{-2}$ for most Korean skin. The SC fat tissues were obtained from each subject by punch biopsy and SC fat tissues were separated from the dermis. This study was approved by the Institutional Review Board at Seoul National University Hospital, and all subjects provided written informed consent. The study was conducted according to the Principles of the Declaration of Helsinki.

Determination of free fatty acid and triglyceride content

SC fat tissues were obtained and homogenized, and the lipids were extracted with chloroform/methanol/water (1:2:0.8, v/v/v). FFAs and TG content were determined by a fluorescent enzymatic method with commercially available determination kits (Roche, Indianapolis, IN and BCS, Seoul, Korea, respectively) and normalized to the protein content. Proteins were determined by the Bradford method (Bio-Rad, Hercules, CA).

Western blot analysis

SC fat tissues were obtained and homogenized, and proteins were extracted using RIPA buffer (Millipore, Billerica, MA) containing complete protease, phosphatase inhibitor (Roche), 5 mM phenylmethylsulphonyl fluoride, and 1 mM dithiothreitol. Equal amounts (50 μg) of protein were loaded, transferred, and analyzed using rabbit polyclonal antibodies against ACC and phospho-ACC (Ser79) (Cell Signaling Technology, Beverly, MA), and a goat polyclonal antibody against β-actin (Santa Cruz Biotechnology, SantaCruz, CA).

Real-time and semi-quantitative PCR (RT-PCR)

Total RNA was prepared from separated SC fat tissues using the Trizol method (Life Technologies, Rockville, MD) and 1 μg of total RNA was converted to complementary DNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. To quantitatively estimate the mRNA expression of each gene, 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 instructions. Primer information is shown in Supplementary Table 1 online (Kim *et al.*, 2010). The PCR conditions were 50 °C for 2 minutes, 95 °C for 2 minutes, followed by 40 cycles at 95 °C for 15 seconds, and 60 °C for 1 minute. The data are presented as fold changes in gene expression normalized to 36B4. For the semi-quantitative PCR, 19–30 cycles of PCR were performed using the following parameters: 92 °C for 30 seconds, 55 °C for 30 seconds, and 72 °C for 30 seconds. The PCR products were electrophoresed on a 1.5% agarose gel.

Cell studies

SC adipose tissue was obtained from elective liposuction (mean age 33.7 years, age range 28–37 years, women, body mass index range 43–56 $kg\,m^{-2}$). Human preadipocytes were isolated as described (Halvorsen *et al.*, 2001). For differentiation, human preadipocytes were cultured in DMEM with 10% fetal bovine serum and antibiotics (100 U ml^{-1} penicillin and 100 mg ml^{-1} streptomycin). At confluence (day 0), differentiation was induced by adding 0.5 mM isobutylmethylxanthine, 1 μM dexamethasone, 10 μg ml^{-1} insulin, and 10 μg ml^{-1} rosiglitazone (PPARγ agonist, Sigma, St Louis, MO) for 2 days. Then the cells were allowed to differentiate by adding 10% fetal bovine serum and 10 μg ml^{-1} insulin, and the medium was changed every 2 days.

Human epidermal keratinocytes were isolated as described previously (Gilchrest, 1983). Human epidermal keratinocytes were used at the third or fourth passage. Primary human dermal fibroblasts were cultured in DMEM with 10% fetal bovine serum and antibiotics. Cultured human dermal fibroblasts were used at passages 6–10.

For co-culture experiments, differentiated human SC adipocytes (day 10) were co-cultured in serum-free DMEM with UV-irradiated keratinocytes or fibroblasts in the inserted transwells (Corning). After 24 hours, the adipocytes in the lower wells were collected. In addition, differentiated human SC adipocytes (day 10) were treated for 24 hours with different concentrations (0, 25, 50, 75, and 100%) of conditioned media of UV-irradiated keratinocytes or fibroblasts.

For all UV irradiation studies, Philips TL 20W/12RS fluorescent sun lamps (Philips, Eindhoven, The Netherlands) with an emission spectrum between 275 and 380 nm (peak, 310–315 nm) were used as the UV source. The power output distribution of the UV emission spectrum was 10.2% UVC (275–290 nm), 53.3% UVB (290–320 nm), 25.3% UVA1 (320–340 nm), and 11.2% UVA2 (340–380 nm; Park *et al.*, 2006). A Kodacel filter (TA401/407; Kodak) was used to block UVC, which has wavelengths of <290 nm. UV strength was measured using a UV meter (Model 585100; Waldmann, Villingen-Schwenningen, Germany; Cho *et al.*, 2008).

Cytokine array

The culture media of UV-irradiated keratinocytes or fibroblasts were collected 24 hours post-irradiation. The cytokine array was performed with the RayBiotech Kit (RayBiotech, Norcross, GA).

Neutralization study

Neutralization antibodies, rabbit anti-IL-6 (Merck, Darmstadt, Germany), mouse anti-IL-8 (BD Biosciences, San Jose, CA), goat anti-MCP-3 (Abcam, Cambridge, UK), rabbit anti-PlGF (PromoKine, Heidelberg, Germany), and each control IgG were added to the co-culture medium (serum-free DMEM) based on the ND_{50} provided by the manufacturer.

Cytokine treatment

Differentiated human SC adipocytes (day 10) were cultured in serum-free DMEM with or without various concentrations of human

recombinant cytokines, IL-6, IL-8 (BioVision, Mountain View, CA), MCP-3 (50, 100, and 200 μg ml^{-1}, PeproTech, Rocky Hill, NJ), and PlGF (5, 10, and 20 μg ml^{-1}, BioVision), for 24 hours. Recombinant leptin (10, 100, and 200 μg ml^{-1}, BioVendor Laboratory Medicine, Heidelberg, Germany) was used as a positive control.

Statistical analysis

Data are presented as the means ± SEM. Significance was analyzed by the paired *t*-test or Student's *t*-test. Differences were considered significant when $P<0.05$.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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