J Nutr Health. 2024 Apr;57(2):171-184
https://doi.org/10.4163/jnh.2024.57.2.171
eISSN 2288-3959

JNH
Journal of Nutrition and Health

Research Article

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High-fat diet alters the thermogenic gene expression to β-agonists or 18-carbon fatty acids in adipocytes derived from the white and brown adipose tissue of mice

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OPEN ACCESS

Received: Nov 25, 2023
Revised: Mar 15, 2024
Accepted: Mar 18, 2024
Published online: Apr 17, 2024

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ABSTRACT

Purpose: Although activating thermogenic adipocytes is a promising strategy to reduce the risk of obesity and related metabolic disorders, emerging evidence suggests that it is difficult to induce adipocyte thermogenesis in obesity. Therefore, this study aimed to investigate the regulation of adipocyte thermogenesis in diet-induced obesity.
Methods: Adipose progenitor cells were isolated from the white and brown adipose tissues of control diet (CD) or high-fat diet (HFD) fed mice, and fully differentiated white and brown adipocytes were treated with β-agonists or 18-carbon fatty acids for β-adrenergic activation or peroxisome proliferator-activated receptor (PPAR) activation.
Results: Compared to the CD-fed mice, the expression of uncoupling protein 1 (*Ucp1*) was lower in the white adipose tissue of the HFD-fed mice; however, this was not observed in the brown adipose tissue. The expression of peroxisome proliferator-activated receptor gamma (*Pparg*) was lower in the brown adipose progenitor cells isolated from HFD-fed mice than in those isolated from the CD-fed mice. Norepinephrine (NE) treatment exerted lesser effect on peroxisome proliferator-activated receptor-γ coactivator (*Pgc1a)* upregulation in white adipocytes derived from HFD-fed mice than those derived from CD-fed mice. Regardless which 18-carbon fatty acids were treated, the expression levels of thermogenic genes including *Ucp1*, *Pgc1a*, and positive regulatory domain zinc finger region protein 16 (*Prdm16)* were higher in the white adipocytes derived from HFD-fed mice. Oleic acid (OLA) and γ-linolenic acid (GLA) upregulated *Pgc1a* expression in white adipocytes derived from HFD-fed mice. Brown adipocytes derived from HFD-fed mice had higher expression levels of *Pgc1a* and *Prdm16* compared to their counterparts.
Conclusion: These results indicate that diet-induced obesity may downregulate brown adipogenesis and NE-induced thermogenesis in white adipocytes. Also, HFD feeding may induce thermogenic gene expression in white and brown primary adipocytes, and OLA and GLA could augment the expression levels.

Keywords: high-fat diet; fatty acids; adrenergic beta-agonists; adipocytes; adipose-derived mesenchymal stromal cells

JNH
Journal of Nutrition and Health

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Funding
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1G1A1092356).

Conflict of Interest
There are no financial or other issues that might lead to conflict of interest.

Author Contributions
Conceptualization: Shin S; Formal analysis: Park S, Ock SA, Park YJ, Shin S; Funding acquisition: Shin S; Investigation: Park S, Ock SA, Park YJ, Lee YH, Park CY, Shin S; Methodology: Shin S; Supervision: Shin S; Writing – original draft: Shin S; Writing – review & editing: Park CY, Shin S.

INTRODUCTION

Whereas white adipocytes store energy, brown and beige adipocytes generate heat from the stored energy. Brown adipocytes express thermogenic genes even in basal conditions; however, beige adipocytes are activated under stimulation [1-3]. Therefore, activation of beige adipocytes exerts beneficial effects on body composition and metabolic health by increasing energy expenditure [4-6].

To induce thermogenesis in beige adipocytes, β-adrenergic signaling should be stimulated by β-agonists, such as norepinephrine (NE) and CL316,243 (CL). Under the activation by β-agonists, multiple transcription factors, including peroxisome proliferator-activated receptor (PPAR), are recruited and upregulate *Ucp1* and other thermogenic genes. As PPAR agonists, long-chain fatty acids (LCFA) are also able to induce thermogenesis [7-12]. We and others have reported that polyunsaturated fatty acids increased adipose thermogenesis compared to saturated fatty acids [7-9], and the high n-6:n-3 ratio of diets lowered thermogenesis and induced body fat accumulation [10,11].

However, multiple studies have shown that beige adipocytes are hard to be activated in obese animals and humans [4,5,13]. This may be because obesity alters the numbers and the functions of various kinds of cells found in adipose tissue, including mature adipocytes, macrophages, and adipose progenitor cells [14]. Obesity is reported to polarize adipose tissue macrophages from anti-inflammatory type to pro-inflammatory type, which increases adipose tissue inflammation [15]. Obesity also changes the characteristics of adipose progenitor cells, leading to more generation of white adipocytes, less generation of brown and beige adipocytes, and more whitening of beige adipocytes [5].

Nevertheless, the thermogenic effects of β-agonists and LCFA on adipocytes derived from obese animal models has not been determined yet. Therefore, we conducted this study to demonstrate if obesity changes the thermogenic response of primary adipocytes to β-agonists and 18-carbon fatty acids. We chose 18-carbon fatty acids as PPAR agonists in this study because they are the most common fatty acids found in human diets, such as dietary fats and oils, meat, and nuts, and have multiple isomers in terms of the number and the position of double bonds [9,10].

METHODS

Animals and diets

Four-week-old C57BL/6 male mice were fed a control diet (CD; 11% kcal fat; D132; SAFE Complete Care Competence, Rosenberg, Germany) or a high-fat diet (HFD; 60% kcal fat; D12492; Research diets, New Brunswick, NJ, USA) for 16 weeks (**Fig. 1A**). **Table 1** shows the composition of the experimental diets. At the end of the experimental period, the mice were euthanized, and white adipose tissue (WAT) and brown adipose tissue (BAT) were dissected. All animal procedures were approved and carried out in accordance with the Institutional Animal Care and Use Committee of the University of Suwon (No. USW-IACUC-2021-004).

Stromal vascular cell (SVC) isolation and differentiation

SVCs, containing adipose progenitor cells, were isolated from the inguinal subcutaneous WAT and BAT of the experimental mice, and grown in Dulbecco's modified Eagle's medium/

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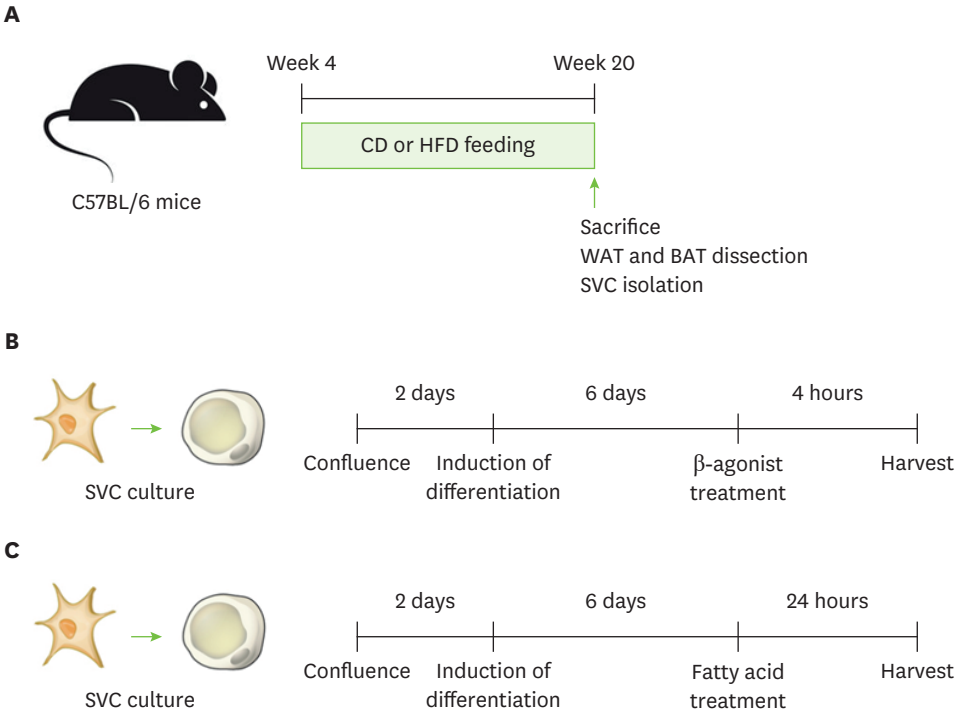

Fig. 1. Experimental design.
(A) C57BL/6 mice were fed CD or HFD for 16 weeks. (B, C) Isolated stromal vascular fraction from WAT and BAT of the mice were cultured and differentiated into mature adipocytes. Mature adipocytes were treated with (B) β-agonists for 4 hours or (C) 18-carbon fatty acids for 24 hours.
CD, control diet; HFD, high-fat diet; WAT, white adipose tissue; BAT, brown adipose tissue; SVC, stromal vascular cell.

Table 1. Composition of the experimental diets

Ingredients	Control diet[1)]		High-fat diet[2)]	
	g (%)	Kcal (%)	g (%)	Kcal (%)
Carbohydrate	55.4	67	26.4	20
Protein	18.6	22	26.2	20
Fat	4.1	11	34.9[3)]	60
Kcal/g	3.33		5.24	

[1)]D132, SAFE Complete Care Competence, Rosenberg, Germany.
[2)]D12492, Research diets, New Brunswick, NJ, USA.
[3)]Lard 245.00 g and soybean oil 25.00 g in the total 773.85 g of diet.

F12 (DMEM/F12; Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS; Corning Inc., Corning, NY, USA) and 1% penicillin and streptomycin (Sigma-Aldrich) at 37°C and 5% CO_2. Two days after confluence, cell differentiation was initiated with DMEM/F12 with 10% FBS, 1.7 μM insulin, 1 μM dexamethasone, 500 μM isobutylmethylxanthine, 1μM rosiglitazone, 17 μM pantothenic acid, and 33 μM biotin for 6 days (**Fig. 1B and C**).

β-agonist treatment

To induce thermogenesis by stimulating β-adrenergic signaling, 10 μM of NE (a non-selective β-adrenergic receptor agonist) or CL (a specific β_3-adrenergic receptor agonist) was treated for 4 hours on day 6 of differentiation (**Fig. 1B**).

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Fatty acid preparation

Eighteen carbon fatty acids, including stearic acid (STA; 18:0), oleic acid (OLA; 18:1, Δ9), linoleic acid (LNA; 18:2, Δ9,12), α-linolenic acid (ALA; 18:3, Δ9,12,15), γ-linolenic acid (GLA; 18:3, Δ6,9,12), and pinolenic acid (PLA; 18:3, Δ5,9,12; Cayman, Ann Arbor, MI, USA), were completely dissolved in 0.9% NaCl solution. Each solution was mixed with 20% bovine serum albumin (BSA) in 0.9% NaCl to make the final concentration of the fatty acids to 5 mM, and sterilized through a 0.2 μm syringe filter. To determine thermogenic effects of the fatty acids on mature adipocytes, fully differentiated SVC were treated with 50 μM of the fatty acids for 24 hours bound to BSA on day 6 of differentiation (**Fig. 1C**).

Quantitative real-time polymerase chain reaction (PCR)

Total RNA was extracted from the cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was synthesized from 1 μg of total RNA using the PrimeScript II 1st strand cDNA synthesis kit (Takara, Tokyo, Japan). The mRNA levels of *Cebpb*, *Cebpa*, *Pparg*, *Ucp1*, *Pgc1a*, and *Prdm16* were quantified using SYBR Green PCR Master Mix (Applied Biosystems, Waltham, MA, USA) by StepOnePlus Real-time PCR System (Applied Biosystems). The mRNA levels were normalized relative to 18S rRNA, and fold changes of gene expression were calculated by the ΔΔCt method. Specific primer sequences used are shown in **Table 2**.

Statistical analysis

Student's t-test or two-way analysis of variance with Duncan's post-hoc test was performed to determine significant differences among groups. Differences were considered statistically significant at $p < 0.05$. Data were presented as means ± SEM, and analyzed using SPSS version 24 (SPSS Inc., Chicago, IL, USA).

RESULTS

Body and tissue weight

HFD-fed mice had significantly higher body weight (1.47-fold), weight gain (2.46-fold), and WAT (3.60-fold) and BAT (1.84-fold) mass compared to CD-fed mice (**Table 3**), indicating that HFD feeding successfully induced obese phenotype in the mice.

Thermogenic gene expression in adipose tissues

To determine if HFD feeding alters thermogenic gene expression in whole adipose tissues, *Ucp1*, *Pgc1a*, and *Prdm16* expression levels were measured in WAT and BAT (**Fig. 2**). HFD-fed mice had significantly lower *Ucp1* expression and tended to have lower *Pgc1a* expression in WAT, but there was no difference in the expression of the genes in BAT. These data suggest that HFD may downregulate thermogenesis in WAT but not in BAT.

Table 2. Primer sequences used for quantitative real-time polymerase chain reaction

Genes	Forward primer	Reverse primer
18S	ATC CCT GAG AAG TTC CAG CA	CCT CTT GGT GAG GTC GAT GT
Cebpb	TTG ATG CAA TCC GGA TCA AAC G	CAG TTA CAC GTG TGT TGC GTC
Cebpa	AAT GGC AGT GTG CAC GTC TA	CCC CAG CCG TTA GTG AAG AG
Pparg	TTG ACC CAG AGC ATG GTG C	GAA GTT GGT GGG CCA GAA TG
Ucp1	GGG CCC TTG TAA ACA ACA AA	GTC GGT CCT TCC TTG GTG TA
Pgc1a	GTC CTT CCT CCA TGC CTG AC	GTG TGG TTT GCT GCA TGG TT
Prdm16	GGC TCA AGG AGG AGG AGA GA	AGG TCC GGG TCA GGT TCA TA

18S, 18S ribosomal RNA; *Cebpb*, CCAAT enhancer binding protein beta; *Cebpa*, CCAAT enhancer binding protein alpha; *Pparg*, Peroxisome proliferator-activated receptor gamma; *Ucp1*, Uncoupling protein 1; *Pgc1a*, Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *Prdm16*, PR domain containing 16.

https://doi.org/10.4163/jnh.2024.57.2.171

Table 3. Body and tissue weight of the mice fed experimental diets[1)]

Variables	Control diet	High-fat diet	Fold changes	p-value
Body weight at 4 wks (g)	19.78 ± 0.29	20.08 ± 0.32	1.02	0.512
Body weight at 20 wks (g)	28.98 ± 0.80	42.67 ± 2.18*	1.47	< 0.001
Body weight gain (g)	9.19 ± 0.84	22.59 ± 2.22*	2.46	< 0.001
WAT[2)] (%)	3.32 ± 0.27	11.96 ± 0.73*	3.60	< 0.001
BAT (%)	0.45 ± 0.03	0.83 ± 0.07*	1.84	< 0.001

Data are presented as means ± SEM (n = 6 for each group). Asterisks indicate significant differences at $p < 0.05$.
WAT, white adipose tissue; BAT, brown adipose tissue.
[1)]Four-week-old C57BL/6 mice were fed control (11% kcal fat) or high-fat (60% kcal fat) diets for 16 weeks.
[2)]WAT includes inguinal subcutaneous, epididymal, perirenal, and retroperitoneal WAT.

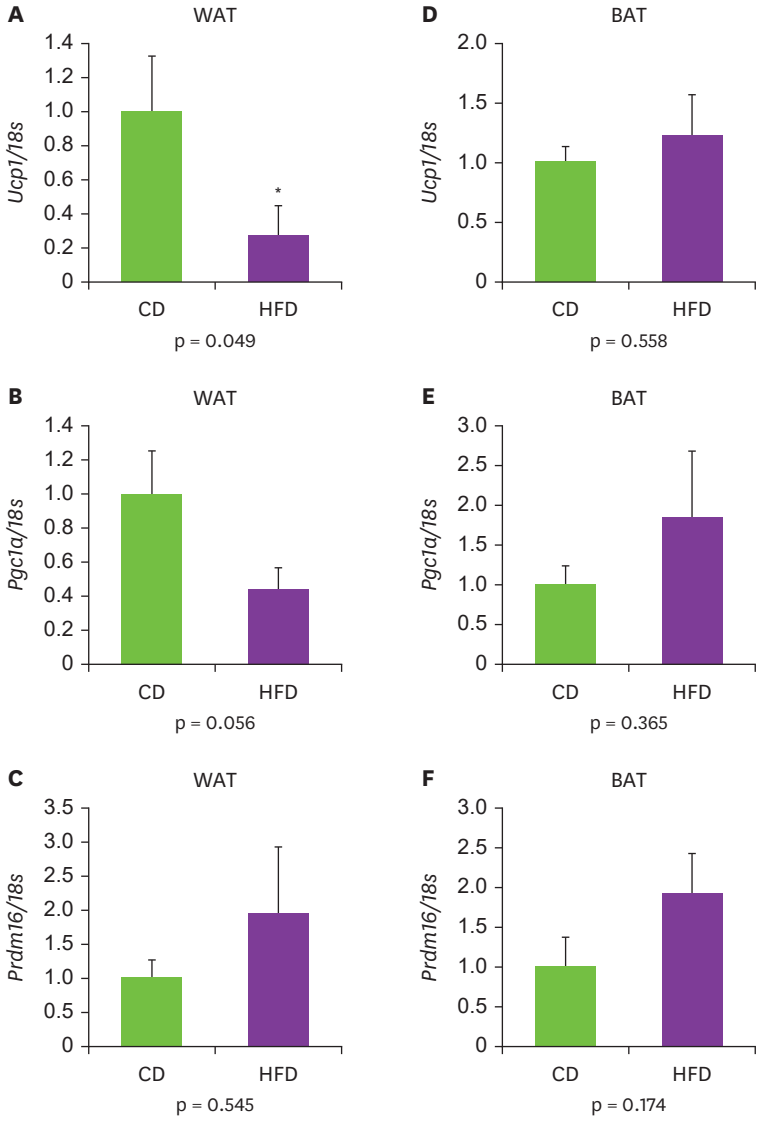

Fig. 2. Effects of HFD on thermogenic gene expression in WAT and BAT.
Ucp1, *Pgc1a*, and *Prdm16* expression in WAT (A-C) and BAT (D-F). Thermogenic gene expression was determined by quantitative real-time polymerase chain reaction with normalization relative to 18S rRNA. Data are presented as means ± SEM (n = 6). Asterisks indicate significant differences at $p < 0.05$ by Student's t-test.
CD, control diet; HFD, high-fat diet; WAT, white adipose tissue; BAT, brown adipose tissue.

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Adipogenic capability of SVCs

To determine if HFD feeding alters adipogenic capability of adipose progenitor cells, *Cebpb*, *Cebpa*, and *Pparg* mRNA expression of SVC isolated from WAT and BAT of CD- or HFD-fed mice was measured (**Fig. 3**). While HFD feeding tended to upregulate *Pparg* expression in WAT-derived SVC, it significantly downregulated *Pparg* expression in BAT-derived SVC. These data suggest that HFD-induced obesity may increase white adipogenesis but decrease brown adipogenesis.

Response of fully differentiated adipocytes to β-agonists

To determine if HFD feeding alters thermogenic response of adipocytes under β-adrenergic stimulation, β-agonists, including NE and CL, were treated on fully differentiated

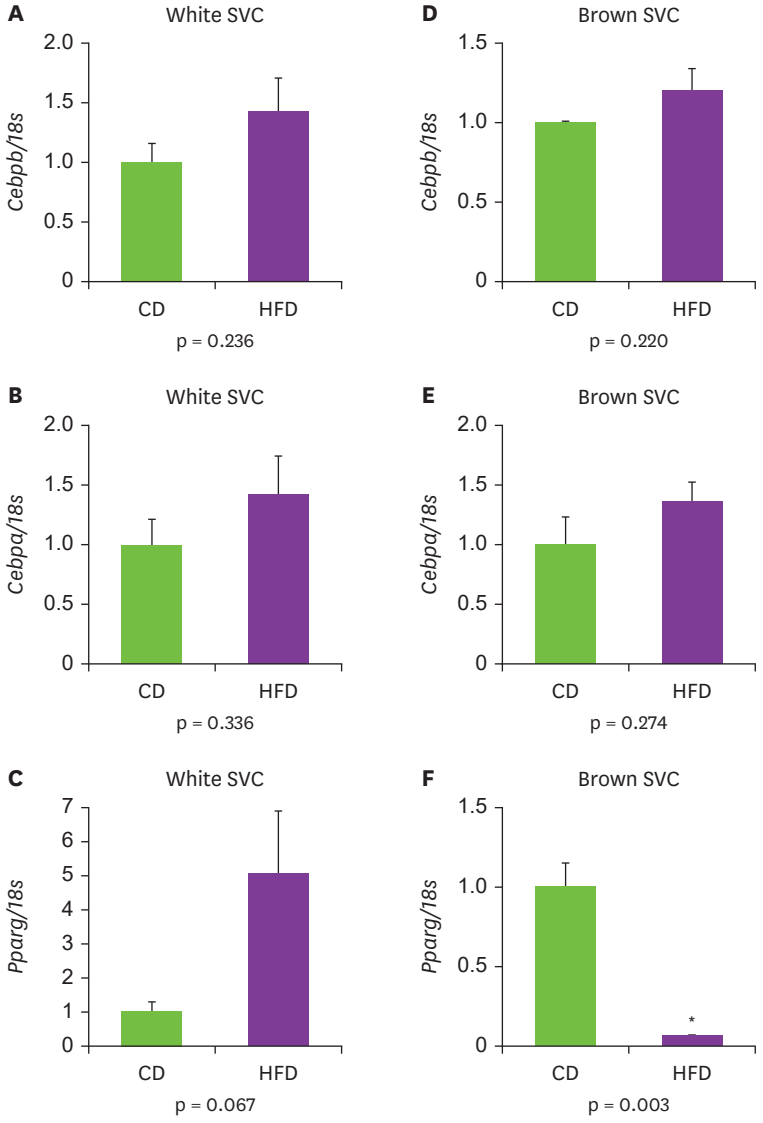

Fig. 3. Effects of HFD on adipogenic capability of SVCs.
Cebpb, *Cebpa*, and *Pparg* expression in SVCs isolated from WAT (A-C) and BAT (D-F). SVCs were isolated from subcutaneous WAT or BAT. Adipogenic gene expression mRNA expression was determined by quantitative real-time polymerase chain reaction with normalization relative to 18S rRNA. Data are presented as means ± SEM (n = 3). Asterisks indicate significant differences at $p < 0.05$ by Student's t-test.
SVC, stromal vascular cell; CD, control diet; HFD, high-fat diet; WAT, white adipose tissue; BAT, brown adipose tissue.

https://doi.org/10.4163/jnh.2024.57.2.171

subcutaneous WAT and BAT derived adipocytes, and thermogenic gene expression was measured (**Fig. 4**).

In white adipocytes, both NE and CL upregulated *Ucp1* mRNA expression regardless of the experimental diets fed to mice. However, NE increased *Pgc1a* mRNA expression only in white adipocytes derived from CD-fed mice. Both NE and CL downregulated *Prdm16* mRNA expression, but the degree of downregulation induced by NE was greater in white adipocytes derived from HFD-fed mice compared to those derived from CD-fed mice.

HFD feeding had no effect on the thermogenic response of brown adipocytes. By both NE and CL, *Ucp1* and *Pgc1a* mRNA expression were upregulated, but *Prdm16* mRNA expression was not altered by β-agonist treatment.

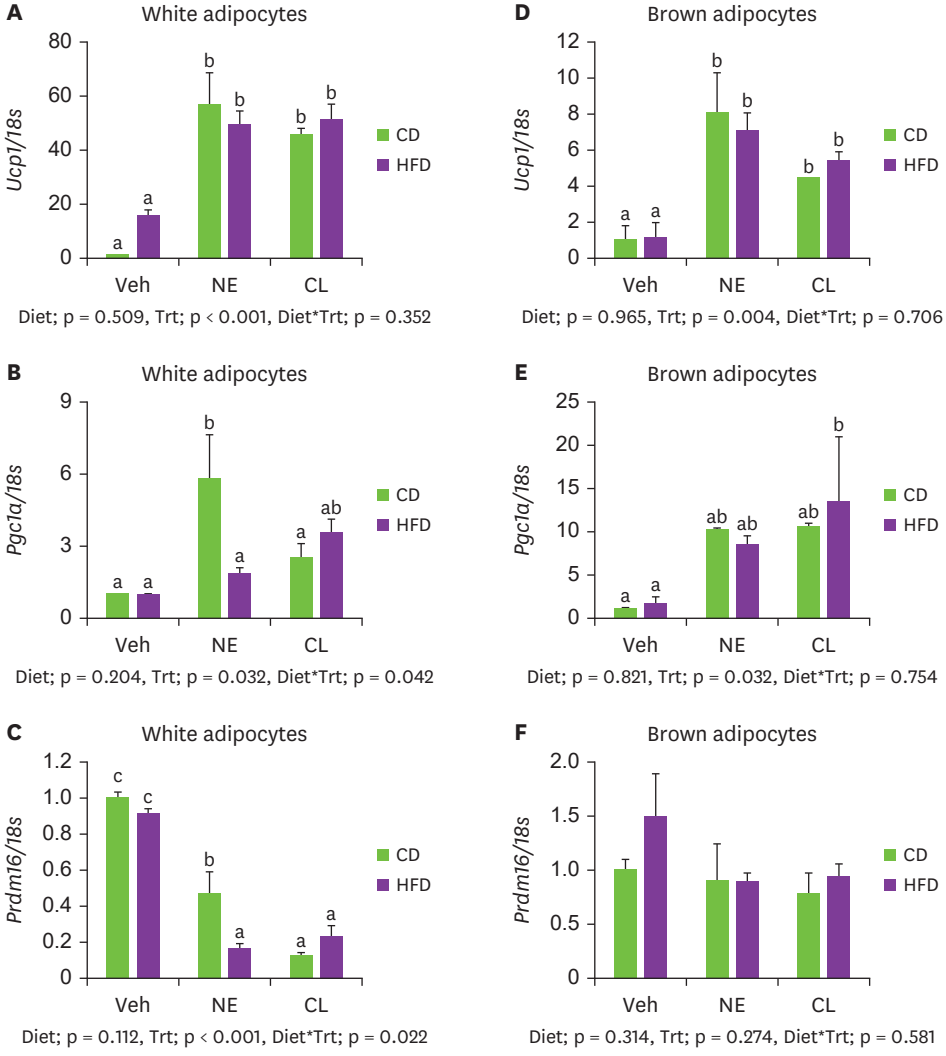

Fig. 4. Effects of HFD on thermogenic response of fully differentiated adipocytes to β-adrenergic agonists.
Ucp1, *Pgc1a*, and *Prdm16* expression in white (A-C) and brown adipocytes (D-F). Fully differentiated adipocytes were treated with 10 μM of NE or 10 μM of CL for 4 hours. Thermogenic gene expression was determined by quantitative real-time polymerase chain reaction with normalization relative to 18S rRNA. Data are presented as means ± SEM (n = 3). Different superscripts (a-c) indicate significant differences at $p < 0.05$ by two-way analysis of variance with Duncan's multiple comparison test. If a group has a common superscript with another group, it means they are not statistically different.
CD, control diet; HFD, high-fat diet; Veh, vehicle; NE, norepinephrine; CL, CL316,243; Diet, diet effect; Trt, β-agonist treatment effect; Diet*Trt, interaction effect.

https://doi.org/10.4163/jnh.2024.57.2.171

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Response of fully differentiated adipocytes to 18-carbon fatty acids

To determine if HFD feeding changes the thermogenic response of adipocytes under PPAR activation, 18-carbon fatty acids with the different number and the position of double bonds, including STA, OLA, LNA, ALA, GLA, and PLA, were treated on fully differentiated subcutaneous WAT and BAT derived adipocytes, and thermogenic gene expression was measured (**Figs. 5** and **6**).

HFD feeding was shown to upregulate *Ucp1* and *Prdm16* mRNA expression levels in white adipocytes compared to CD. In white primary adipocytes derived from HFD-fed mice, *Ucp1* mRNA expression was lowered by STA, OLA, LNA, GLA, and PLA, and OLA and GLA induced *Pgc1a* mRNA expression (**Fig. 5**).

OLA upregulated *Ucp1* mRNA expression in brown adipocytes derived from CD-fed mice, but not in those derived from HFD-fed mice. Brown adipocytes derived from HFD-fed mice had higher *Pgc1a* and *Prdm16* mRNA expression than those derived from CD-fed mice, but there was no effect of fatty acids on the expression of the genes (**Fig. 6**).

DISCUSSION

Although it has been reported that adipose thermogenesis is reduced in obesity [5], the effects of obesity on thermogenic response of adipocytes to β-adrenergic stimulation and PPAR activation are still not clear. Therefore, we aimed to examine the effects of β-agonists and 18-carbon fatty acids on primary white and brown adipocytes derived from HFD-induced obese mice.

In this study, *Ucp1* mRNA expression was downregulated by HFD feeding in WAT, but not in BAT. This is consistent with our previous study, in which diet-induced obesity did not induce the difference in the *Ucp1* expression in BAT, but *Ucp1* level in WAT was negatively correlated with body adiposity [10]. The lower *Ucp1* expression in WAT could be due to hypertrophic adipocytes, the main cell type found in WAT of obese mice. Diet-induced obesity promotes excessive white fat accumulation by hypertrophy as well as hyperplasia, and hypertrophic adipocytes secrete proinflammatory cytokines and have very low thermogenic function [16,17].

HFD also divergently modulated adipogenic capability of SVC isolated from WAT and BAT of mice by altering *Pparg* expression. *Pparg* plays a key role in the regulation of adipogenesis, and its target genes promote lipid accumulation in adipocytes during the terminal stage of differentiation [18]. Whereas HFD enhanced *Pparg* expression in SVC from WAT, it reduced the gene expression in those from BAT. This is consistent with previous reports showing that HFD feeding induces white adipogenesis [10,19,20] and inhibits brown adipogenesis [5]. The increase of BAT mass in HFD-fed mice is considered due to whitening of BAT, which impairs thermogenic function of BAT [21].

In white adipocytes derived from both CD- and HFD-fed mice, *Ucp1* mRNA expression was upregulated by NE and CL treatment. However, *Pgc1a* mRNA expression was not induced by neither NE nor CL in HFD-fed mice-derived white adipocytes although NE induced the gene more than 5-fold in CD-fed mice-derived cells. This indicates that HFD feeding could suppress adipose thermogenic response to β-adrenergic stimulation, and consistently, it has been reported that *Pgc1a* expression was lower and not upregulated by NE injection in visceral WAT of HFD-fed mice compared to low-fat diet-fed mice [10].

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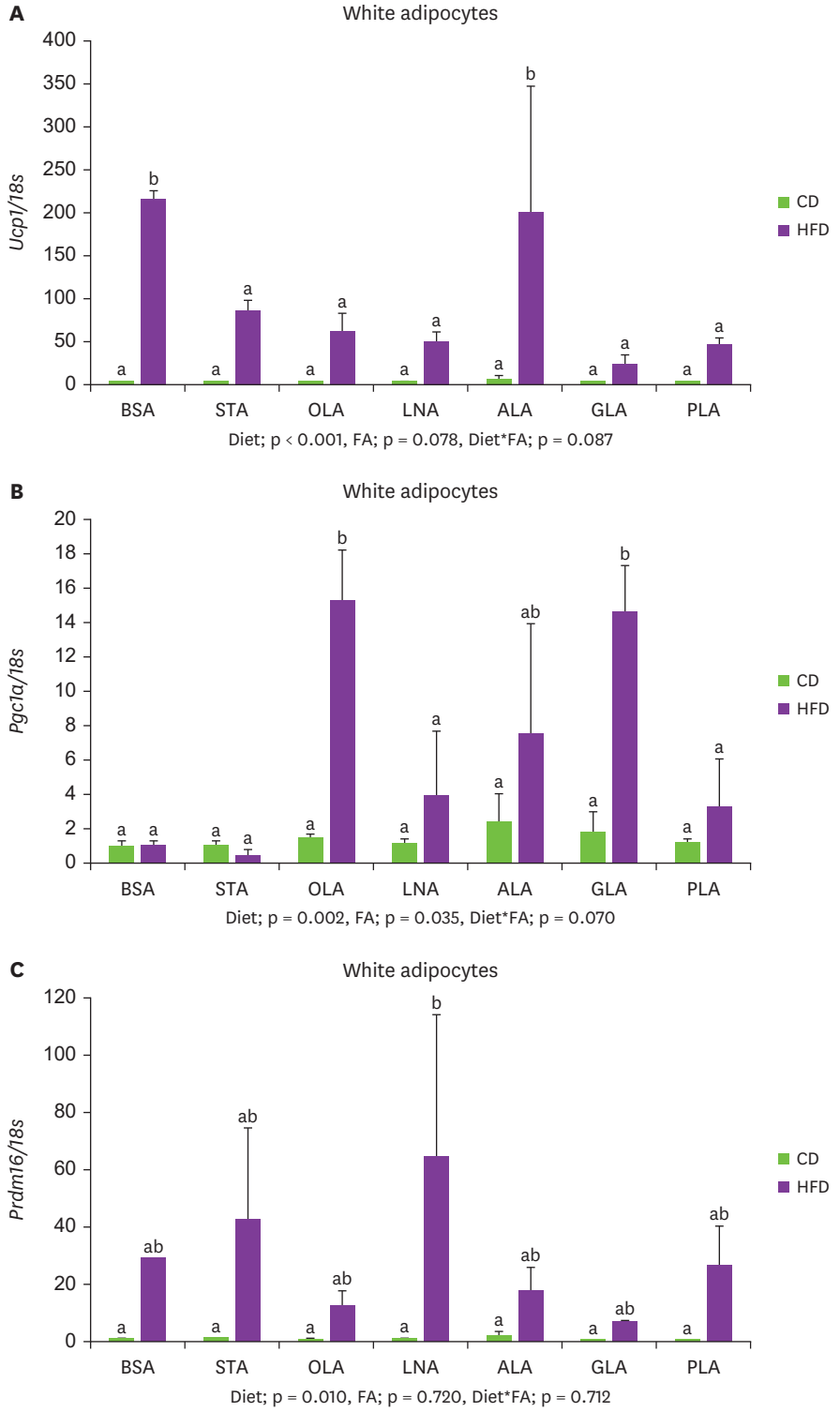

Fig. 5. Effects of HFD on thermogenic response of fully differentiated white adipocytes to 18-carbon fatty acids.
(A) *Ucp1*, (B) *Pgc1a*, and (C) *Prdm16*. Fully differentiated subcutaneous white adipose tissue derived adipocytes were treated with 50 μM of each fatty acid or BSA for 24 hours. Thermogenic gene expression was determined by quantitative real-time polymerase chain reaction with normalization relative to 18S rRNA. Data are presented as means ± SEM (n = 3). Different superscripts (a, b) indicate significant differences at $p < 0.05$ by two-way analysis of variance with Duncan's multiple comparison test. If a group has a common superscript with another group, it means they are not statistically different.
CD, control diet; HFD, high-fat diet; BSA, bovine serum albumin; STA, stearic acid; OLA, oleic acid; LNA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; PLA, pinolenic acid; Diet, diet effect; FA, 18-carbon fatty acid effect; Diet*Trt, interaction effect.

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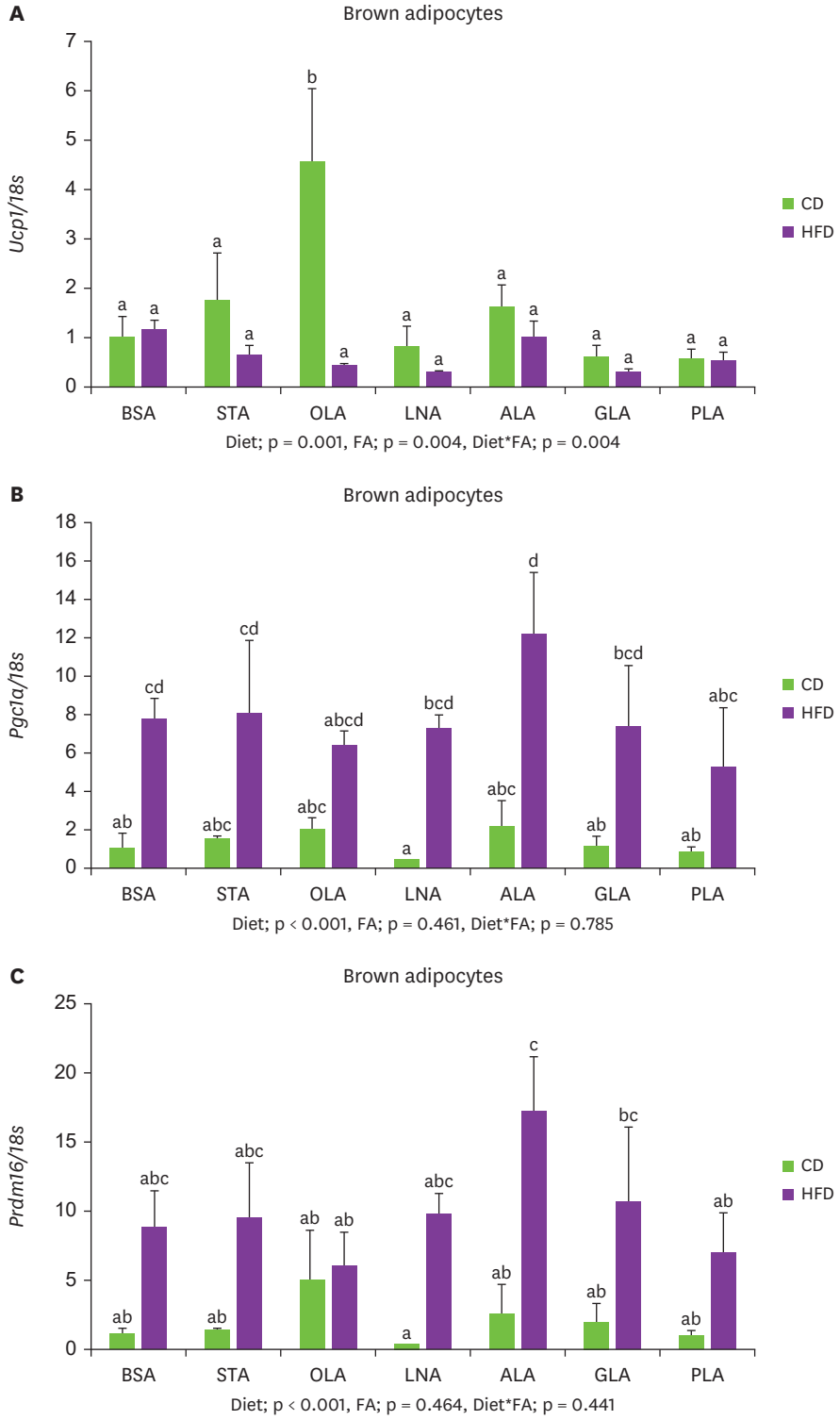

Fig. 6. Effects of HFD on thermogenic response of fully differentiated brown adipocytes to 18-carbon fatty acids.
(A) *Ucp1*, (B) *Pgc1a*, (C) *Prdm16*. Fully differentiated brown adipose tissue derived adipocytes were treated with 50 μM of each fatty acid or BSA for 24 hours. Thermogenic gene expression was determined by quantitative real-time polymerase chain reaction with normalization relative to 18S rRNA. Data are presented as means ± SEM (n = 3). Different superscripts (a-d) indicate significant differences at $p < 0.05$ by two-way analysis of variance with Duncan's multiple comparison test. If a group has a common superscript with another group, it means they are not statistically different.
CD, control diet; HFD, high-fat diet; BSA, bovine serum albumin; STA, stearic acid; OLA, oleic acid; LNA, linoleic acid; ALA, α-linolenic acid; GLA, γ-linolenic acid; PLA, pinolenic acid; Diet, diet effect; FA, 18-carbon fatty acid effect; Diet*Trt, interaction effect.

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Prdm16 mRNA expression was downregulated by both NE and CL in white adipocytes regardless of diets fed to mice. The downregulation of *Prdm16* is considered as negative feedback because adrenergic receptor desensitization occurs under β-agonist treatment to prevent an overstimulation of the signaling [22-24]. It is also thought that the negative feedback downregulated the expression of *Prdm16*, but not *Pgc1a*, since PRDM16 is activated earlier than PGC-1α in the β-adrenergic signaling pathway [25,26].

The basal level of *Ucp1* expression, without β-adrenergic stimulation, in white adipocytes derived from HFD-fed mice was higher than those derived from CD-fed mice although it was not statistically significant. Under the HFD, mice use fat as a main source of energy, which is demonstrated by the lower value of respiratory quotient (RQ) [10,27]. Since the RQ of glucose is 1 and that of fatty acids is close to 0.7, mice fed with HFD have lower RQ than those fed with a low-fat diet [10,27]. Since using fat as an energy source increases the systemic level of non-esterified fatty acids, genes related to fatty acid oxidation, such as *Ppara* and *Pgc1a*, are upregulated [10,19,20], and the proteins encoded by these genes upregulate thermogenic gene expression as transcription factors [28].

In white adipocytes, ALA treatment preserved HFD-induced *Ucp1* upregulation while other 18-carbon fatty acids suppressed the gene expression. Omega-3 fatty acids are known to stimulate thermogenic activity through multiple mechanisms [5]. Eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), which can be synthesized from ALA in the body, have been reported to activate PPAR [29,30], free fatty acid receptor 4 [31], and transient receptor potential vanilloid 1 [32] and to inhibit pattern recognition receptors activation [33] and pro-inflammatory eicosanoid formation [34]. In mice and rats, fish oil rich in EPA and DHA was shown to increase oxygen consumption rate and rectal temperature, which was accompanied with upregulation of adipose thermogenesis [31,32,35].

OLA and GLA upregulated *Pgc1a* expression in white adipocytes derived from HFD-fed mice. OLA has been reported to increase cAMP concentration and activate PKA, leading to PGC-1α activation [36], and multiple studies have reported the thermogenic effect of olive oil (OLA-rich oil) in animal models and human subjects. Compared to other 18-carbon fatty acid-rich oils, olive oil feeding was shown to increase BAT mass [20] and hypothalamic *Lepr* expression, which could promote thermogenesis [37], and lower body fat accumulation accompanied with the higher oxygen consumption rate in mice [10]. Olive oil-fed rats had higher *Ucp1*, *Ucp2*, and *Ucp3* mRNA levels in BAT [38], and healthy normal weight men consumed olive oil had higher energy expenditure [39]. Also, it was shown that *Ucp1* expression was enhanced by GLA in fully differentiated C3T10T1/2 adipocytes [9]. GLA-rich borage oil was also reported to upregulate *Ucp1* mRNA level in BAT of rats [8] and in subcutaneous WAT of mice [40]. The underlying mechanism of GLA is activation of PPARα and PPARγ [41,42]. These data indicate that distinct fatty acids with different number and position of double bond exerts divergent effects on adipose thermogenesis, and fatty acid composition of dietary fat may regulate the risk of obesity and its related metabolic disorders.

In brown adipocytes, no significant effect of diets was observed on thermogenic gene expression in response to β-agonists. Both β-agonists upregulated the expression of *Ucp1* and *Pgc1a* in the cells regardless of diets fed to mice. Although OLA upregulated *Ucp1* expression in brown adipocytes derived from CD-fed mice, no effect of OLA was shown in those derived from HFD-fed mice. Under the 18-carbon fatty acids or BSA treatment, brown adipocytes from HFD-fed mice had significantly higher *Pgc1a* and *Prdm16* expression than those from

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CD-fed mice, which is consistent with the data of white adipocytes. These data indicate that the increased level of fatty acids under HFD feeding could induce thermogenic gene expression in both white and brown adipose progenitor cells.

A limitation of this study includes that the adipogenic and thermogenic gene expression levels were exclusively measured at the mRNA levels. However, this study still provides the significant evidence regarding the response of adipocytes derived from obese animals to β-adrenergic stimulation and PPAR activation, and the genes measured in this study have been assessed at the protein and phenotype levels in other studies [2,3]. Also, additional studies to determine thermogenic effects of short-chain fatty acids (SCFA) on obese adipocytes would be advantageous since multiple studies reported the beneficial effects of SCFA on body composition and metabolic health [43,44].

SUMMARY

Taken together, HFD feeding suppressed *Ucp1* expression in WAT, *Pparg* expression in SVC isolated from BAT, and *Pgc1a* upregulation under NE treatment in white primary adipocytes. HFD challenge was also shown to upregulate the basal level of *Ucp1* in white primary adipocytes and that of *Pgc1a* in brown primary adipocytes. These data indicate that diet-induced obesity suppresses adipose thermogenesis; however, increased fatty acid oxidation caused by HFD feeding may promote thermogenic capacity of adipose progenitor cells. *Ucp1* induction by OLA and GLA in white primary adipocytes derived from HFD-fed mice suggests that dietary consumption or supplementation of these fatty acids may lead to the activation of adipose thermogenesis in obese animals and humans.

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