Journal of Advances in Medicine and Medical Research

27(3): 1-12, 2018; Article no.JAMMR.42427
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Thermal Effect on Human Skeletal Muscle-derived Cell Transcriptomes: Possibility of Prevention against Atherosclerosis

Masayo Nagai[1] and Hidesuke Kaji[1,2*]

[1]Division of Physiology and Metabolism, University of Hyogo 13-71, Kitaoji-cho, Akashi, Hyogo 673-8588, Japan.
[2]Division of Pathophysiology, Kobe Women's University 4-7-2, Minatojimanakamachi, Chuo-ku, Kobe 650-0046, Japan.

Authors' contributions

This work was carried out in collaboration between both authors. Author MN managed the literature searches, designed the study in part, performed the statistical analysis and wrote the first draft of the manuscript. Author HK principally designed the study, managed the analyses of the study and wrote the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/42427
Editor(s):
(1) Elvira Bormusov, The Lloyd Rigler Sleep Apnea Research Laboratory, Unit of Anatomy and Cell Biology, Israel.
(2) Angelo P. Giardino, Professor, Texas Children's Hospital, Houston, Texas, USA and Department of Pediatrics, Baylor College of Medicine, Houston, USA.
Reviewers:
(1) Tetsuro Tamaki, Tokai University School of Medicine, Japan.
(2) Burak Cem Soner, Necmettin Erbakan University, Konya, Turkey.
(3) Recep Kurkcu, Amasya University, Turkey.
(4) Mariana Rotta Bonfim, Universidade Municipal de São Caetano do Sul, Brazil.
(5) Carol Imbriano, University of Modena and Reggio, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history/25688

Original Research Article

Received 9th May 2018
Accepted 16th July 2018
Published 26th July 2018

ABSTRACT

Aims: Our previous study demonstrated an increase in the serum adiponectin level and decrease in soluble urokinase-type plasminogen activator receptor (suPAR) after the application of thermal sheets to femoral skeletal muscle in healthy people. Based on these results, the possibility of atherosclerotic cardiovascular disease (ASCVD) prevention effect by thermal stimulation was considered. The present study examined the effect of thermal stimulation on the skeletal muscle-derived cell (SMDC) to determine the changes of ASCVD-related molecules and the mechanism of anti-atherogenic changes in serum adiponectin level and suPAR value.

**Corresponding author: E-mail: h-kaji@yg.kobe-wu.ac.jp;*

Study Design: Experimental study comparing transcriptome between cells cultured at higher temperature and control cells.
Place and Duration of Study: From September 2015 to March 2017, Division of Physiology and Metabolism, University of Hyogo, Japan.
Methodology: SMDC were cultured at 42°C and 37°C for 20 hours, and its gene expression was analysed using microarray technology.
Results: Thermal stimulation to SMDC confirmed a significant increase in expression of 1,072 genes and significant inhibition in expression of 1,123 genes. Among these genes, multiple genes were factors affecting ASCVD. Furthermore, in gene ontology analysis and pathway analysis, factors considered to be related to ASCVD, such as lipid metabolism and glucose metabolism, were significantly included. Although thermal stimulation on SMDC induced changes in the gene expression of factors promoting ASCVD, changes in factors protecting against ASCVD were predominant. Besides, dipeptidyl peptidase IV (DPP-4) mRNA expression was decreased by thermal stimulation to SMDC. Despite the increase in serum adiponectin levels, the concentration of DPP-4 released in the medium was significantly higher in the cells exposed to thermal stimulation compared to control cells. Furthermore, any changes were not responsible for the decreased serum suPAR.
Conclusion: These results suggest that thermal stimulation might change gene expression beneficial for ASCVD prevention in SMDC. Further study is needed to verify the ASCVD preventive effect by thermal stimulation *in-vivo*.

Keywords: Tthermal stimulation; skeletal muscle-derived cells; transcriptome; anti-atherosclerosis.

1. INTRODUCTION

In recent years, the influences of skeletal muscle on metabolism have attracted a major attention. A physiologically active substance (myokine) released from skeletal muscle and its function on skeletal muscle has been reported to be related to cardiovascular diseases [1]. It has been reported that thermal stimulation of skeletal muscle may be a method to promote glucose metabolism by heat shock protein (HSP) activity [2]. Thermal stimulation of skeletal muscle is known to encourage preventive effects against atherosclerotic cardiovascular disease (ASCVD) as exercise [3]. However, the mechanism has not been clarified [4]. If it is true, thermal stimulation might be useful for prevention of ASCVD.

Our previous study [5] demonstrated an increase in the serum adiponectin level and a decrease in soluble urokinase-type plasminogen activator receptor (suPAR) after the application of thermal sheets to femoral skeletal muscle in healthy males and females. In nursing, the use of thermal sheets for various purposes has been reported as a compressing method with moderate continuous hyperthermic effects. Based on this finding, the possible effects of heat stimulation on skeletal muscle function caused by changes in serum protein levels due to changes in gene expression has been considered in the present study. The results of our previous study suggested the effects of thermal stimulation on ASCVD preventable factors. To investigate metabolic behaviour, comprehensive measurement of the activities of involved gene transcripts and proteins such as enzymes and associated metabolites are informative.

The objective of this study is to investigate the possibility whether thermal stimulation is effective to induce gene expression in preventing atherosclerosis. The second objective is to determine whether an increase in plasma adiponectin or decrease in suPAR, as reported in the previous report [5], are mediated through molecules from skeletal muscle-derived cells (SMDC).

2. MATERIALS AND METHODS

Changes in mRNA expression due to heat stimulation were compared by using microarray analysis between a heat-exposed SMDC cultured at 42°C and control cells cultured at 37°C.

SMDC (SkMC, Lonza, Japan) is embryonic human cells derived from quadriceps muscle. Routine characterisation of SMDC includes positive immunofluorescence staining for desmin (≥30% positive) following differentiation in fusion medium in first passage out of cryopreservation. SkGM-2 BulletKit (Lonza, Japan) was used as a culture medium.

The culture of SMDC was initiated at 37°C, 5% CO_2 and 100% humidity. A thermal load was initiated when growth saturation densities of 50% to 70% were confirmed in SMDC cultures. The control group was at 37°C, and the thermal load group was at 42°C. In our previous *in-vivo* study [5], we stimulated the heat with a sheet. It has been reported that the temperature of the thermal sheet application area increases by 4 to 5°C above the reference temperature [6]. Cancer cells that are more vulnerable to heat than normal cells, cell damage is not observed in Human Leukemia U937 below 42.5°C [7]. Therefore, it was considered that the culture at 42°C was available in human normal skeletal muscle cells. There is no report on *in-vivo* thermal stimulation given for consecutively 20 hrs. However, in this study, we set 20 hours to comprehensively study the effect of thermal stimulation on SMDC.

Total RNA was extracted after thermal loading for 20 hours by using Total RNA Purification Maxi Kit (Norgen Biotek Corporation, Canada). SMDC used for microarray analysis was analysed using 5 replicates. The electrophoresis pattern of total RNA was confirmed with an Agilent 2100 Bioanalyzer, and quantitative evaluation was performed by NanoDropND-1000. The microarray analysis was done by using Gene Chip Human Genome U133 Plus 20 Array (Affymetrixs). The validation of this microarray was previously performed by parallel measurement of the representative changes by real-time RT-PCR [8]. Detected signals were subjected to the scatter plot, gene ontology (GO), cluster and pathway analyses by using the Gene Spring GX software.

In this study, "Significant change in magnification" was set to 2-fold or more or 0.5-fold or less which is common in microarray analysis. Functions including the association with ASCVD of up- and down-regulated genes were confirmed using 'Pubmed' and 'NCBI gene'.

GO analysis was performed by using the gene list whose expression changed more than 2-fold and less than 0.5-fold. The probe list detected by GO analysis was classified by confirming each function of GO using the database AmiGO 2 (http://amigo.geneontology.org/amigo).

The pathway analysis was carried out by using a list where the gene expression varied more than twice or less than half by thermal stimulation at 42°C. Single Experiment Analysis was performed using WikiPathways database (http://www.Wikipathways.org / index.php / WikiPathways).

Concerning dipeptidyl peptidase IV (DPP-4), which was considered to have the possibility of acting on fat cells or blood that released from the skeletal muscle due to elevation of temperature, the concentration of DPP-4 released into the culture medium after SMDC culture under the same conditions was determined by using the enzyme-linked immunosorbent assay (ELISA) with the Human Soluble DPPIV/CD26 ELISA Kit (Aviscera Bioscience, Inc., USA). The result was statistically analyzed by Mann-Whitney U test using IBM® SPSS® Statistics Desktop version.

3. RESULTS

The number of floating cells was very low in cultures at both 37°C and 42°C for 20 hours. As shown in Fig. 1, the apparent change in cell morphology was not observed 20 hours after the start of thermal stimulation.

The extracted total RNA was appropriate for the quantitative evaluation of the total RNA amount in cells cultured at both 37°C and 42°C and was suitable as a sample for microarray analysis (Data not shown).

The effect of thermal-exposure on gene expression in SMDC has been shown by scatter plots (Fig. 2).

Also, hierarchical clustering of the top 100 regulated genes is shown by dendrogram with heatmap (Fig. 3).

The thermal-exposed cells (42°C) revealed 1,072 genes showing 2-fold higher expression and 1,123 genes showing 0.5-fold lower expression compared to the control cells (37°C) (Data not shown). Among the 1,072 genes showing >2-fold expression, 24 were considered to be associated with ASCVD (Table 1). Among the 1,123 genes showing < 0.5-fold expression, 12 were considered to be associated with ASCVD (Table 2).

GO analysis was performed for 1,072 genes with more than 2-fold expression in SMDC, and 152 were significantly ($p < 0.001$) consistent with the gene list classified by GO. According to the GO term classification, 114 of 152 genes were classified as biological processes, 15 as cellular components, and the other 23 as molecular functions. Among 1,123 genes with less than 0.5-

fold expression, 123 were significantly consistent with the gene list classified by GO, and 79 of the 123 genes were classified as biological processes, 36 as cellular components, and 8 as molecular functions. GO included genes associated with regulation of metabolism, HSP, macrophage activity, enzyme activity, cell growth, and immune system (Data not shown).

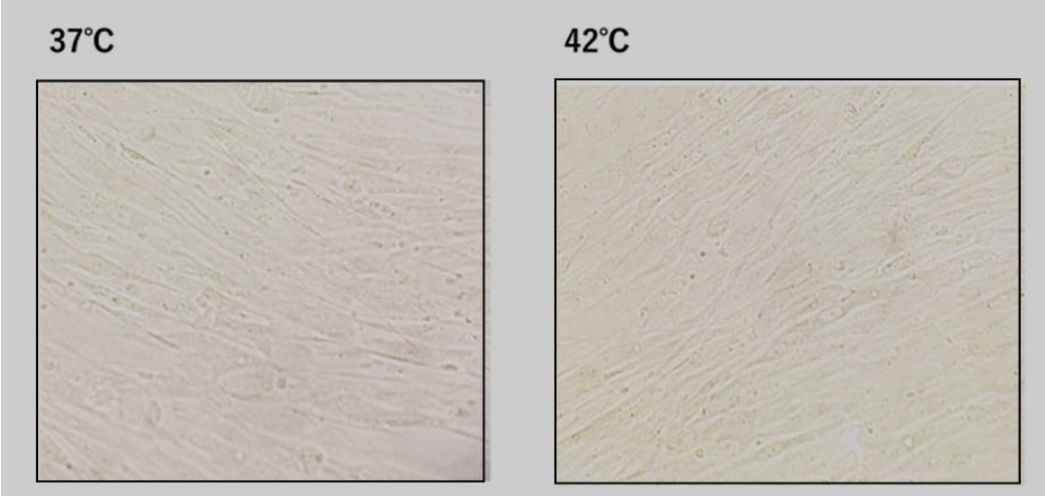


Fig. 1. SMDC appeared healthy 20 hours after the start of culture at 37°C (left panel) and 42°C (right panel) at least when observed by a phase contrast microscopy

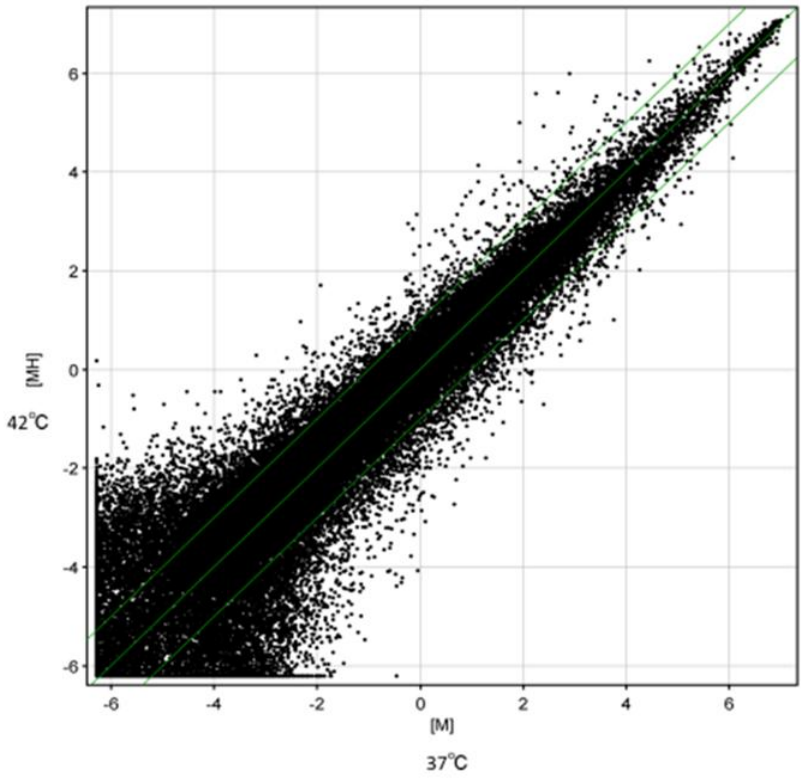


Fig. 2. Scatter plots showing the effect of thermal stimulation on gene expression in SMDC

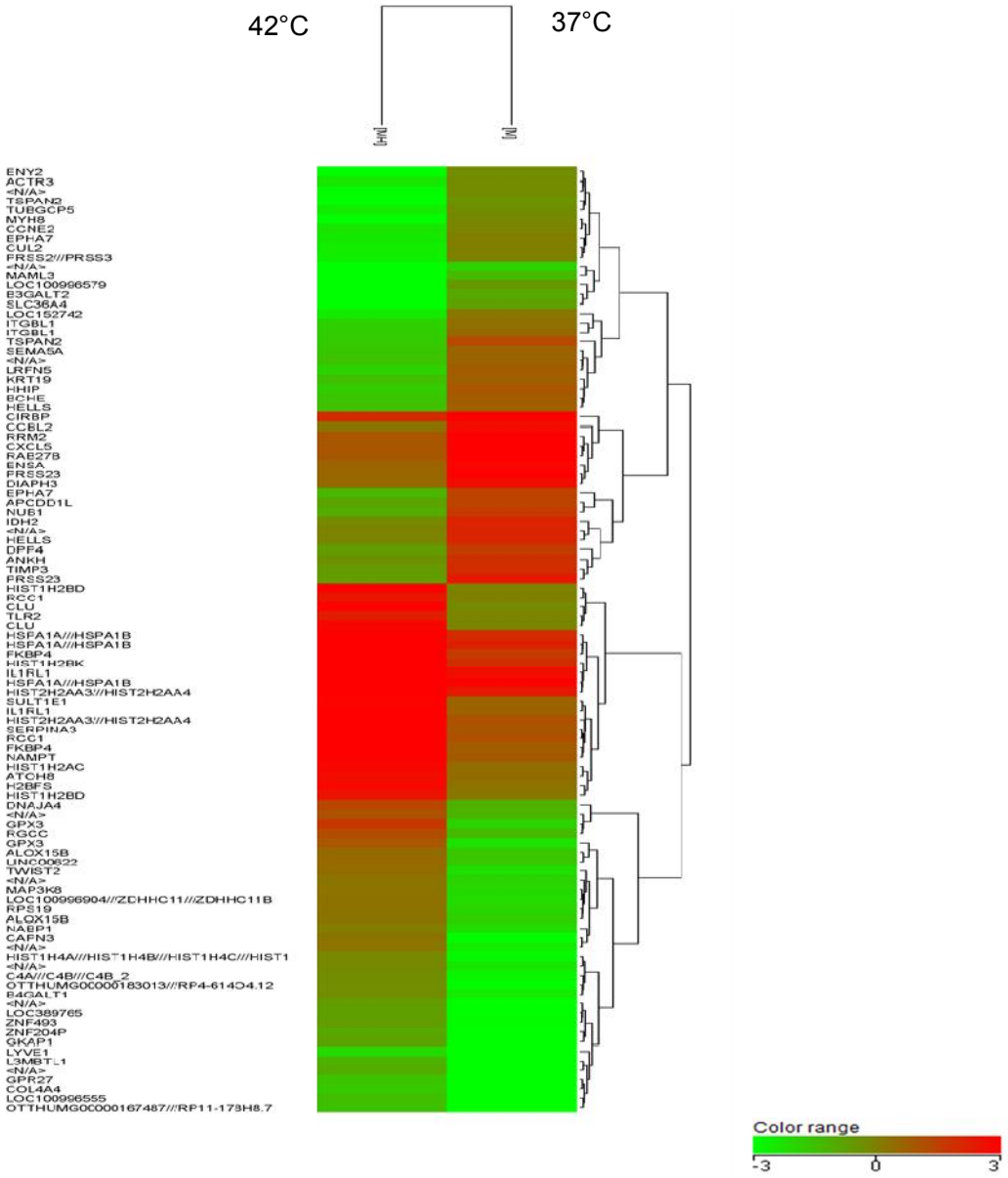


Fig. 3. Dendrogram with heatmap showing the hierarchical clustering of the top 100 regulated genes

The grade of up- and down-regulation is shown by red and green color gradation

Some of the genes showing increased expression were significantly contained in the pathways for lipid synthesis, AMP-activated protein kinase (AMPK) signal transduction, vitamin B_{12} metabolism (including high-density lipoprotein (HDL) metabolism), integrin-mediated cell adhesion, and nitric oxide metabolism. Some of the genes showing decreased expression were significantly contained in the pathways for sterol regulatory element-binding protein (SREBP) signalling, which regulates the metabolism of fatty acids and cholesterol, SREBP-associated cholesterol metabolism, homeostasis, cholesterol synthesis, glycogen metabolism, and AMPK signalling. The pathways for lipid synthesis, AMPK signal transduction, and integrin-mediated cell adhesion significantly contained some of the genes showing increased or decreased expression (Table 3).

Table 1. Genes related to ASCVD was upregulated by thermal stimulation in SMDC

	Gene symbol	Gene title	Fold change	Effect of ASCVD at skeletal muscle
○	**HSPA1A///HSPA1B**	heat shock 70kDa protein 1A///heat shock 70kDa protein 1B	11	improve insulin sensitivity
○	**CLU**	clusterin	9.086	improve insulin sensitivity, protection from cell damage
○	**SESN3**	sestrin 3	4.28	improve insulin sensitivity
○	**HSP105/110**	heat shock 105kDa/110kDa protein 1	4.12	improve insulin sensitivity
○	**GDF15**	growth differentiation factor 15	3.84	antidiabetic effect, maintenance of mitochondrial function
○	**CTRP1/C1QTNF1**	C1q and tumor necrosis factor related protein 1	3.17	improve insulin sensitivity
○	**SLN**	sarcolipin	3	promotion of glucose uptake by noninsulin-dependent
○	**SESN1**	sestrin1	2.88	maintenance of motor function of skeletal muscle
○	**LEPR**	leptin receptor	2.64	improve glucose uptake
○	**INSR**	insulin receptor	2.63	improve insulin sensitivity, antidiabetic effect,
	VEGFA	vascular endothelial growth factor A	2.63	angiogenesis
	SERPINE1 (PAI-1)	serpin peptidase inhibitor, clade E (plasminogen activator inhibitor-1)	2.57	promotion of arteriosclerosis
○	**THRA**	thyroid hormone receptor, alpha	2.55	increase in number of mitochondria, improve insulin sensitivity
○	**KLF15**	kruppel-like factor 15	2.46	improve glucose uptake
○	**IGF1R**	insulin-like growth factor 1 receptor	2.42	improve glucose tolerance
	WISP1 (CCN4)	WNT1 inducible signaling pathway protein 1	2.34	adipocytokine
○	**HRH1**	histamine receptor H1	2.31	improve insulin sensitivity
	THBS1 or TSP-1	thrombospondin 1	2.28	decrease of glucose uptake, insulin resistance, angiogenesis
○	**IRS2**	insulin receptor substrate 2	2.27	improve insulin sensitivity, antidiabetic effect
	HSPD1	heat shock 60kDa protein 1 (chaperonin)	2.2	insulin resistance
	AGT	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	2.15	insulin resistance, decrease of blood flow
	FITM2	fat storage-inducing transmembrane protein 2	2.15	enhanced skeletal muscle energy metabolism
○	**CEBPD**	CCAAT/enhancer binding protein (C/EBP), delta	2.12	enhanced skeletal muscle strength
	TLR4	toll-like receptor 4	2.11	induction of chronic inflammation

○: protective against ASCVD

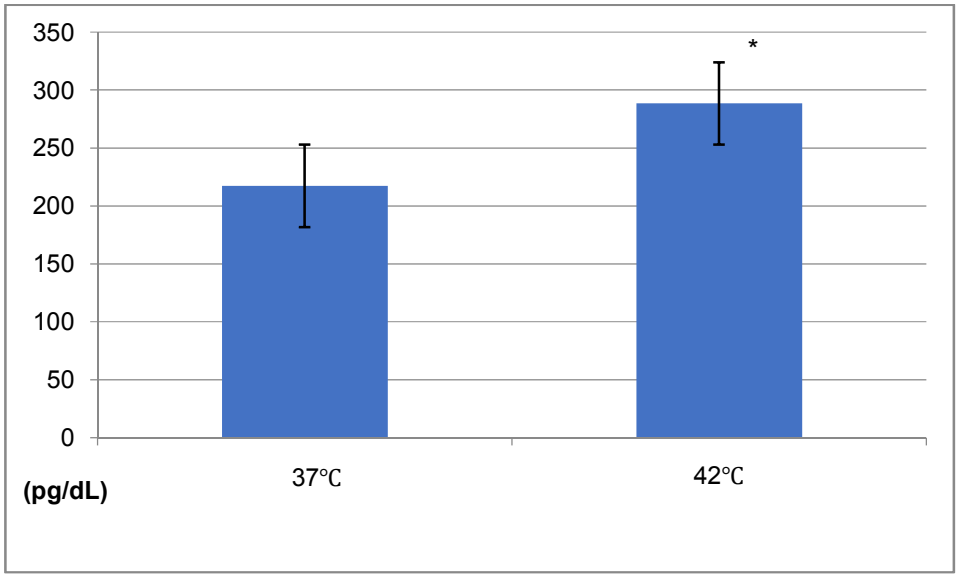
Table-2. Genes related to ASCVD was downregulated by thermal stimulation in SMDC

	Gene symbol	Gene title	Fold change	Effect of ASCVD at skeletal muscle
○	**DPP-4**	dipeptidyl-peptidase 4	0.213	suppression of adiponectin secretion, decrease of skeletal muscle mass, degradation of mitochondrial function
○	**LIPG**	lipase, endothelial	0.254	enhanced high-density lipoprotein (HDL) metabolism
	CAMK2D	calcium/calmodulin -dependent protein kinase II delta	0.29	inducible expression of GLUT4
○	**MEF2C**	myocyte enhancer factor 2C	0.321	myogenesis, control of muscle fiber type, maintenance of glucose homeostasis
○	**HSPA5**	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	0.333	glucose homeostasis
○	**IGFBP3**	insulin-like growth factor binding protein 3	0.336	promotion of arteriosclerosis
	PLAU	plasminogen activator, urokinase	0.348	fibrinolytic
○	**ADAM 12**	ADAM metallopeptidase domain 12	0.375	accumulation of fat
○	**ATF6**	activating transcription factor 6	0.465	suppressed expression of GLUT4
	NUCB2	nucleobindin 2	0.465	adipocytokine, glucose uptake at hyperglycemia
○	**IGFBP1**	insulin-like growth factor binding protein 1	0.489	promotion of arteriosclerosis, insulin resistance
○	**HB-EGF**	heparin-binding epidermal growth factor (EGF)-like growth factor	0.49	insulin resistance

○: protective against ASCVD

Table 3. Pathways related to ASCVD by thermal stimulation in SMDC

	p-value	Number of gene expression changes by thermal stimulation	Number of genes in Wikipathway
【Pathways related to up-regulated genes】			
Hs_Adipogenesis_WP236_72082	3.32E-07	14	131
Hs_TNF_alpha_Signalling_Pathway_WP231_72093	3.08E-04	8	87
Hs_Toll-like_receptor_signalling_pathway_WP75_72133	9.66E-04	8	102
Hs_AMPK_Signaling_WP1403_73193	0.002265306	6	68
Hs_Vitamin_B12_Metabolism_WP1533_70117	0.003302485	5	53
Hs_Integrin-mediated_Cell_Adhesion_WP185_71391	0.003557221	7	99
Hs_Metabolism_of_nitric_oxide_WP1850_70998	0.007666076	2	7
【Pathways related to up-regulated genes】			
Hs_SREBP_signalling_WP1982_71987	5.20E-05	8	65
Hs_Glycogen_Metabolism_WP500_63201	1.06E-04	6	36
Hs_Cholesterol_Biosynthesis_WP197_69902	3.97E-04	4	17
Hs_AMPK_Signalling_WP1403_73193	0.003353694	6	68
Hs_SREBF_and_miR33_in_cholesterol_and_lipid_homeostasis_WP2011_70089	0.004439881	3	18
Hs_Integrin-mediated_Cell_Adhesion_WP185_71391	0.005460716	7	99
Hs_Adipogenesis_WP236_72082	0.00704534	8	131



p=0.0002 (37℃ vs 42℃)

Fig. 4. Change in DPP-4 concentration in SMDC culture medium by thermal stimulation

The concentration of DPP-4 was significantly higher in the thermal stimulation cells. The concentration of DPP-4 was 214.8 pg/dL in the cell medium at 37°C and 288.5 pg/dL at 42°C (p = 0.0002)

Interestingly, DPP-4 expression was decreased by thermal stimulation to SMDC, so it is worthwhile to mention that degradation of adiponectin was reduced, thereby causing the elevation of plasma adiponectin levels. Thus, DPP-4 secretion from SMDC was measured by ELISA, and DPP-4 like protein was identified in the culture media. Unexpectedly, the concentration of DPP-4 was significantly higher in the cells exposed to thermal stimulation than in the control cells (Fig. 4).

4. DISCUSSION

SMDC exposed to heat at 42°C showed marked changes in the expression of various genes. Among the factors showing significantly increased gene expression in SMDC exposed to heat, the following molecules were expected to have antidiabetic effects. HSPA1A/HSPA1B (HSP70/HSP72) has been reported to have decreased expression when there is obesity or insulin resistance [9] or ageing [10]. HSP70 family prevention effect against obesity and insulin resistance was caused by a high-fat diet [9, 11]. Moreover, HSP72 is effective in the treatment of type 2 diabetes [12]. HSP105/110 is involved in promoting the expression of HSP70 in thermally stimulated mammalian cells [13]. Clusterin was reported to prevent high-fat-diet-induced insulin resistance through suppression of expression of relevant genes of reactive oxygen species and suppression of inflammation [14]. Clusterin also has been reported to prevent lipid accumulation [15]. Sestrin 1 [16] and Sestrin 3 [17] are thought to control oxidative stress in human skeletal muscle and control glucose and lipid metabolism. Complement C1q (CTRP1) acts in skeletal muscle and might be attributed to promote uptake of glucose by activation of p44 / 42- mitogen-activated protein kinase (MAPK) and plays a significant role of supplementing its function in case of low adiponectin [18, 19]. Additionally, factors that are thought to be involved in promoting glucose uptake in skeletal muscle were confirmed by the increased expression of insulin receptor, insulin receptor substrate 2, leptin receptor, histamine receptor H1, thyroid hormone receptor α, growth differentiation factor 15, sarcolipin, Kruppel-like factor 15, and insulin-like growth factor I receptor. Fat storage-inducing transmembrane protein 2 and CCAAT/enhancer-binding protein δ are expected to have preventive effects against ASCVD by improving the skeletal muscle function.

Among the factors showing significantly decreased gene expression in SMDC exposed to heat, the following molecules were considered to be useful for preventing ASCVD when their expressions were inhibited: DPP-4, endothelial lipase, heat shock 70 kDa protein 5, activating transcription factor 6, disintegrin and

metalloproteinase domain-containing protein 12 (ADAM 12), myocyte enhancer factor 2C, insulin-like growth factor-binding protein, and heparin-binding epidermal growth factor (EGF)-like growth factor.

Reduction of these factors has been known to improve the insulin sensitivity irrespective of dietary restrictions and promote glucose uptake from the skeletal muscle. Thus, thermal stimulation of skeletal muscle may be useful for preventing ASCVD due to their effects on the glucose and lipid metabolism. Measurement by ELISA resulted in an increase in DPP-4 concentration at 42°C in the culture medium indicating the dissociation between the mRNA expression level and released protein level. Thus, it is unlikely that the elevated plasma adiponectin levels by thigh muscle fomentation observed in the previous study [5] are due to the reduced DPP-4 from the skeletal muscle. The mRNA expression of adiponectin receptor 1 (ADIPOR1) and adiponectin receptor 2 (ADIPOR2) was not significantly elevated; an increase in serum adiponectin concentration due to a decrease in the number of receptors was unlikely. CTRP1 is known as a paralog of adiponectin. CTRP1 mRNA expression is elevated, possibly binding to ADIPOR.

Among the factors showing increased gene expression in SMDC exposed to thermal stimulation, vascular endothelial growth factor A, and thrombospondin 1 were thought to promote atherosclerosis. HSP60 family member 1 and angiotensinogen were considered to improve insulin resistance, and WNT1 inducible signalling pathway protein 1 and toll-like receptor 4 were considered to promote ASCVD. Calmodulin-dependent protein kinase and urokinase-type plasminogen activator (uPA) act to prevent diabetes mellitus and atherosclerosis, respectively, but their gene expressions were inhibited by thermal stimulation, suggesting that they exert promoting effects on each disease following thermal stimulation. In addition, urokinase-type plasminogen activator receptor (uPAR), uPA, and plasminogen activator inhibitor-1 (PAI-1) were thought as factors related to suPAR reduction by thermal stimulation. However, both cases were found to show an increasing trend of suPAR. It was unlikely that the decrease in serum suPAR concentration in our previous study was due to a change derived from skeletal muscle.

As shown in microscopic photographs, SMDC did not show severe cell damage or cell death 20 hours after the start of cultures at 42°C. However, 25% reduction of cell density was observed in cells at 42°C compared with control cells and some of the up-regulated genes at 42°C is involved in suppression of cell proliferation: nucleolar protein 3 (fold change: 4.518), THAP domain containing, apoptosis associated protein 2 (3.63), and programmed cell death 6 (3.306) (Data not shown). Furthermore, growth saturation densities at the start of the thermal stimulation to SMDC were 50% to 70%, which may have an effect. Thus, we cannot exclude the possibility of minor influences on cell growth at this experimental condition. Further verification of temperature level and time course is needed for safe application *in vivo*.

Results of GO and pathway analysis contained significant altering gene expression group related to glucose and lipid metabolism, suggested that thermal stimulation may have a favorable effect on the glucose and lipid metabolism of skeletal muscle.

Therefore, if comprehensive judgment is made from factors acting on improving insulin sensitivity and defensively on ASCVD, thermal stimulation to skeletal muscle *in vivo* are expected to have a preventive effect on ASCVD. The results demonstrated factors that work on ASCVD prevention unrelated to diets and exercise, so it is expected to utilize muscle fomentation when prevention by exercise and diets are insufficient. These findings can lead to the future research related to support for personalized prevention of ASCVD. After validation of the temperature or time conditions of thermal stimulation, further study is needed for clinical application for ASCVD prevention by using physiological or biochemical ASCVD markers as endpoint.

5. CONCLUSION

In the present study, nine of the top ten anti-atherosclerotic genes were upregulated by thermal stimulation. Six out of the top ten atherogenic genes were downregulated by thermal stimulation. These results suggest that the changes of gene expression are beneficial for ASCVD prevention, although this thermal stimulation also induced minor changes in the gene expression of factors promoting ASCVD. Further *in vivo* study is required to apply thermal stimulation for ASCVD prevention.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid for Scientific-Research from JSPS KAKENHI Grant Number JP25870930 and JK00860.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Giudice J, Taylor JM. Muscle as a paracrine and endocrine organ. Curr Opin Pharmacol. 2017;34:49–55.
2. Goto A, Egawa T, Sakon I, Oshima R, Ito K, Serizawa Y, et al. Heat stress acutely activates insulin-independent glucose transport and 5′-AMP-activated protein kinase prior to an increase in HSP72 protein in rat skeletal muscle. Physiol Rep. 2015;3(11):e12601:1-9. DOI:10.14814/phy2.12601
3. Ashley AE, Von Schulze AT, Geiger PC. Exercise, heat shock proteins and insulin resistance. Philos Trans R Soc Lond B Biol Sci. 2018;373(1738). DOI:10.1098/rstb.2016.0529.
4. Petrie MA, Kimball AL, McHenry CL, Suneja M, Yen CL, Sharma A, et al. Distinct skeletal muscle gene regulation from active contraction, passive vibration, and whole body heat stress in humans. PLoS One. 2016;11(8):e0160594. DOI:10.1371/journal.pone.0160594
5. Nagai M, Morita Y, Mori M, Sakashita R, Kaji H. Effect of thigh muscle fomentation on metabolic and inflammatory biomarkers. Integr Obes Diabetes. 2015; 1(4):98-100.
6. Oda H, Igaki M, Ugajin T, Suzuki A, Tsuchiya S, Nagashima K, et al. Effects of warming the lower back with a heat and steam generating sheet on thermoregulatory responses and sensation. Jpn J Biometeor. 2006;43(1): 43-50.
7. Tabuchi Y, Furusawa Y, Kondo T. Genes and gene networks in the apoptosis induced by heat stress in human leukemia U937 Cells. Therm Med. 2011;27(2):31-40. DOI:10.3191/thermalmed.27.31
8. Kaji H, Okada M, Mori M, Hamaue A, Nagai M. Blockade of the neuropeptide Y Y2 receptor with the potent antagonist BIIE0246 regulates gene expression levels in the lipid metabolic pathways in human hepatoma cell line HepG2. Integr Mol Med. 2016;3(2):576-582.
9. Chung J, Nguyen AK, Henstridge DC, Holmes AG, Chan MH, Mesa JL, et al. HSP72 protects against obesity-induced insulin resistance. Proc Natl Acad Sci USA. 2008;105(5):1739-1744. DOI:10.1073/pnas.0705799105
10. Chichester L, Wylie AT, Craft S, Kavanagh K. Muscle heat shock protein 70 predicts insulin resistance with aging. J Gerontol A Biol Sci Med Sci. 2015;70(2):155-162. DOI:10.1093/gerona/glu015
11. Henstridge DC, Bruce CR, Drew BG, Tory K, Kolonics A, Estevez E, et al. Activating HSP72 in rodent skeletal muscle increases mitochondrial number and oxidative capacity and decreases insulin resistance. Diabetes. 2014;63(6):1881-1894. DOI: 10.2337/db13-0967
12. Kondo T, Goto R, Ono K, Kitano S, Suico MA, Sato M, et al. Activation of heat shock response to treat obese subjects with type 2 diabetes: a prospective, frequency-escalating, randomized, open-label, triple-arm trial. Sci Rep. 2016;6:35690. DOI:10.1038/srep35690
13. Yamagishi N, Fujii H, Saito Y, Hatayama T. Hsp105beta upregulates hsp70 gene expression through signal transducer and activator of transcription-3. FEBS J. 2009; 276(20):5870-5880. DOI:10.1111/j.1742-4658.2009.07311.x
14. Kwon MJ, Ju TJ, Heo JY, Kim YW, Kim JY, Won KC, et al. Deficiency of clusterin exacerbates high-fat diet-induced insulin resistance in male mice. Endocrinology. 2014;155(6):2089-2101. DOI:10.1210/en.2013-1870
15. Matukumalli SR, Tangirala R, Rao M. Clusterin: Full-length protein and one of its chains show opposing effects on cellular lipid accumulation. Sci Rep. 2017;7: 41235. DOI: 1-13.10.1038/srep41235
16. Lee JH, Budanov AV, Park EJ, Birse R, Kim TE, Perkins G, et al. Sestrin as a

feedback inhibitor of TOR that prevents age-related pathologies. Science. 2010; 327(5970):1223-1228.
DOI:10.1126/science.1182228

17. Nascimento EB, Osler ME, Zierath JR. Sestrin 3 regulation in type 2 diabetic patients and its influence on metabolism and differentiation in skeletal muscle. Am J Physiol Endocrinol Metab. 2013;305(11):E1408-1414.
DOI:10.1152/ajpendo.00212.2013

18. Seldin MM, Tan SY, Wong GW. Metabolic function of the CTRP family of hormones. Rev Endocr Metab Disord. 2014;15(2): 111-123.
DOI: 10.1007/s11154-013-9255-7

19. Xin Y, Lyu X, Wang C, Fu Y, Zhang S, Tian C, et al. Elevated circulating levels of CTRP1, a novel adipokine, in diabetic patients. Endocr J. 2014;61(9):841-847.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25688