HNUE JOURNAL OF SCIENCE DOI: 10.18173/2354-1059.2022-0029
Natural Sciences 2022, Volume 67, Issue 2, pp. 120-128
This paper is available online at http://stdb.hnue.edu.vn

COLD INDUCES EXPRESSION OF GENES SPECIFIC TO THERMOGENESIS (*PGC-1α, UCP1*) AND BEIGE CELLS (*TMEM26, SLC27A1*) IN MOUSE INGUINAL WHITE ADIPOSE TISSUE

Le Ngoc Hoan
Faculty of Biology, Hanoi National University of Education

Abstract. Studying adaptive thermogenic responses as being a strategy to protect against several metabolic disorders. The browning process is a crucial point of adaptive thermogenic responses. The present study was carried out to examine whether inguinal white adipose tissue (iWAT) is a candidate for the browning process. Mice were caged in a 4 °C cold chamber (Cold group) for 24 hours and 23 °C room temperature condition (RT group). The result showed that mRNA expression of genes specific to thermogenesis, including *peroxisome proliferator-activated receptor gamma coactivator 1-alpha* (*PGC-1α*) and *uncoupling protein 1* (*UCP1*), was markedly upregulated in iWAT of the mice in the Cold group compared to the mice in the RT group. Consistent with this, mRNA expression of the *Transmembrane protein 26* (*TMEM26*) gene, specific to beige cells, was also significantly higher in the iWAT of the cold exposed mice than that in the iWAT of the mice housed at room temperature. Expression of *solute carrier family 27member 1* (*SLC27A1*) gene, another gene coding for a protein of beige cells, was tendency to increase in iWAT of the cold exposed mice compared with that in iWAT of the control mice. However, this change was not significantly differed between the two groups. These data demonstrate that iWAT is an important tissue responding to cold-induced thermogenesis and browning process. Thus, iWAT is a promising candidate for further studies on the mechanism of cold-induced thermogenic responses in white adipose tissues.
***Keywords*:** cold, inguinal adipose tissue, thermogenesis, browning.

1. Introduction

Metabolic disorders, such as cardiovascular diseases, type 2 diabetes and fatty liver diseases, are rapidly increasing which burden medical and health care systems in the World [1]. These metabolic disorders are closely associated with obesity that is caused by the misbalancing between food intake and energy expenditure [2]. Adaptive nonshivering thermogenesis responses such as thermogenesis and the browning process are being critical parts of whole body energy expenditure [3]. Generally, those responses are studied in brown adipose tissue which expresses the highest level of specific markers for

Received May 11, 2022. Revised June 20, 2022. Accepted June 27, 2022.
Contact Le Ngoc Hoan, e-mail address: lengochoan@gmail.com

thermogenesis and brown fat cells such as peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) and uncoupling protein 1 (UCP1) [4]. Since, increases in these molecules (PGC-1α, UCP1) are accompanied by enhanced energy expenditure, thus brown adipose tissue plays an important role in body energy consumption. Therefore, there have been many studies on the manipulation of the development and activity of brown adipose tissue to treat obesity and its related metabolic dysfunctions [5, 6]. Notably, white adipose tissue (WAT), another type of adipose tissue, is bigger than brown adipose tissue in the human body and white adipose tissue hypertrophy leads to overweight, obesity, and metabolic disorders [7, 8]. In contrast, factors induce WAT to become brown and increase system thermogenesis leading to lowering the risk of obesity [9].

Latest studies have demonstrated that white adipose tissue can be induced browning in which white adipocytes are switched to beige cells whose characteristics are like brown adipocytes. For example, mice caged in an enriched environment with plenty of physical and social activities show the upregulation of molecules involved in browning and thermogenesis in WAT such as *PGC-1α*, *UCP1* [10]. Resveratrol supplementation showed increased expression of mRNA levels of *transmembrane protein 26* (*TMEM26)* and *solute carrier family 27 member 1* (*SLC27A1)*, specific markers of beige adipocytes, in WAT of mice. This change was accompanied by an increase in expression of *PGC-1α*, and *UCP1,* increased energy expenditure, as well as inhibition of high-fat diet-induced obesity [11]. White adipose tissues in the body are divided into several types following their distribution, including mesenteric white adipose tissue (mWAT), epididymal white adipose tissue (eWAT), abdominal subcutaneous white adipose tissue (asWAT), inguinal white adipose tissue (iWAT) [12]. Among those WATs, the iWAT distributes in the area next to the brown adipose tissue, making it a promising candidate for induction of browning and thermogenesis [13]. Therefore, in the current study, iWAT was removed from the cold-exposed mice to test for its thermogenic responses by detecting mRNA expression of genes specific to thermogenesis (*PGC-1α*, *UCP1*) as well as genes specific to beige cells (*TMEM26*, *SLC27A1*). As a result, the data of the study showed that almost those molecules were significantly upregulated in iWAT of the cold exposed mice compared with that of the room temperature housed control mice.

2. Content

2.1. Material and method

2.1.1. Mice setting

C57BL/6 male mice at eight weeks of age were individually housed in plastic cages for a week at room temperature (23 °C) for acclimatization. Then, the experimental mice were also individually housed in plastic cages in a cold refrigerator (4 °C) for 24 h. The control mice were housed at room temperature. The mice were fed a regular diet and given free access to food and water. Each experimental or control group was consisting of 4 animals. Mice were purchased from Animal House of the University of Ulsan. Animal experiments were approved by the animal ethics committee of the University of Ulsan and conformed to National Institutes of Health guidelines. The mice were killed by

CO_2 asphyxiation and inguinal white adipose tissues were dissected. All experiments were carried out in the Laboratory of Food Science and Nutrition of The University of Ulsan, South Korea in 2013.

2.1.2. Quantitative Real-Time PCR

Two microgram aliquots of total RNA extracted from the homogenated inguinal adipose tissue of each mouse in each group were reverse transcribed to cDNA using M-MLV reverse transcriptase (Promega, USA). The quantitative real-time-PCR (qRT-PCR) amplification of the cDNA was performed in duplicate with an SYBR premix ExTaq kit (TaKaRa Bio Inc., USA) using a Thermal Cycler Dice (TaKaRa Bio Inc., Japan). In standard PCR, DNA is amplified by 3 repeating steps: denaturation, annealing, and elongation. However, in qRT-PCR, fluorescent labeling enables the collection of data as PCR progresses. The fluorescent labeling allows the quantification of the amplified DNA molecules by borrowing the use of a double strain DNA binding dye. During each cycle, the fluorescence is measured. The fluorescence signal shows the amount of replicated DNA and thus, the DNA is quantified in "real-time". Reactions of qRT-PCR were performed with the same schedule: pre-denaturation at 95°C for 10 s and 40 cycles of denaturation at 95°C for 5 s and annealing at 60°C for 30 s. Results were analyzed with Real-Time System TP800 software (Takara Bio Inc., Japan) and the values were normalized to the levels of the housekeeping gene *β-actin*. The primers are shown in Table 1.

Table 1. Mouse primers were used for qRT-PCR analysis.

Gene	Forward primer (5' → 3')	Reverse primer (5' → 3')
PGC-1α	CCGTAAATCTGCGGGATGATG	CAGTTTCGTTCGACCTGCGTAA
UCP1	TACCAAGCTGTGCGATGTCCA	GCACACAAACATGATGACGTTCC
TMEM26	GGCCGTGAAGCCATAAAGCTA	AAAGGCTCCTGTTGAACCAAGAC
SLC27A1	GCAGCATTGCCAACATGGAC	GTGTCCTCATTGACCTTGACCAGA
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA

2.1.3. Statistical Analysis

The results were presented as means ± standard error of the mean (*SE*). Comparisons of variables were performed by using Student's *t*-test. The *P values* < 0.05 were considered significant differences in comparisons.

2.2. Results and discussions

2.2.1. Cold markedly induces expression of PGC-1α mRNA in inguinal adipose tissue

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is well known as a key molecule in the regulation of thermogenesis [14]. PGC-1α is highly expressed in metabolic tissues such as skeletal muscle, liver, brown adipose tissue, and white adipose tissues [15]. Here, the first aim of this study was to test whether cold induces the expression of *PGC-1α* in iWAT. As a result, the expression of *PGC-1α*

mRNA in iWAT of the cold-exposed mice was significantly higher than that in iWAT of the control mice (Figures 1A and 1B).

A recent study has revealed that increased mRNA level of *PGC-1α* in metabolic tissues including white adipose tissues is associated with increases in body oxygen consumption as well as total body energy expenditure which are accompanied by enhanced body temperature [16]. Thus, upregulation of *PGC-1α* mRNA levels in iWAT of the cold-exposed mice indicates that iWAT is an important tissue responding to cold-induced thermogenesis. Moreover, increased *PGC-1α* mRNA levels in adipose tissues are also related to decreases in high fat diet-induced obesity and its metabolic disorders (such as insulin resistance and type 2 diabetes) [17]. Strategies for upregulation of the *PGC-1α* mRNA level in iWAT will be contributing to the battle against obesity and its related metabolic dysfunction.

(A)

Mouse	RT	Cold
1	1.82	11.71
2	2.67	7.23
3	1.45	15.81
4	0.75	9.85
Mean	***1.67***	***11.15***
SE	***0.40***	***1.80***
P	***0.0022***	

(B)

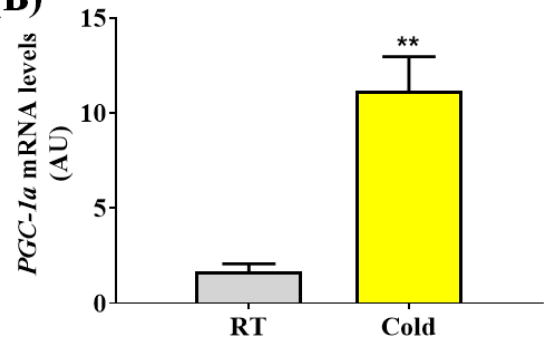

Figure 1. Expression of PGC-1α mRNA in iWAT

*Inguinal adipose tissues (iWAT) were isolated from the mice housed at room temperature (RT) or in a cold refrigerator for 24 hours (Cold) conditions. Real-time RT-PCT analysis for expression of PGC-1α mRNA. Levels of mRNA were normalized to levels of β-actin mRNA. (A) data analysis of PGC-1α mRNA levels. (B) comparison of PGC-1α mRNA levels. Data represent the results of four mice per group. Values are means (X) ± standard error (SE). **P < 0.01 compared between the Cold group and the RT group. AU is an arbitrary unit.*

2.2.2. Cold strongly enhances expression of UCP1 mRNA in inguinal adipose tissue

It is worth noting that PGC-1α is a regulator of uncoupling protein 1 (UCP1) which is highly expressed in brown adipose tissue [18]. Upregulated *PGC-1α* mRNA level is associated with increased *UCP1* gene and protein in brown adipose tissue of mammals. In contrast, *PGC-1α* knock-out (KO) mice show low levels of *UCP1* molecules in brown adipose tissue [14, 18]. Therefore, the current study tested whether cold-induced *PGC-1α* mRNA upregulation also affected the expression of *UCP1* mRNA in iWAT of the cold-exposed mice. Here, the data revealed that the expression mRNA level of *UCP1* was significantly increased in iWAT of the cold-exposed mice compared to that of the room temperature housed control mice (Figures 2A and 2B). Since UCP1 is an important protein that regulates oxidative phosphorylation in mitochondria leading to enhanced

mitochondrial heat production [19], thus, increased *UCP1* mRNA level in iWAT of the cold exposed mice show a thermogenic response in this tissue.

(A)

Mouse	RT	Cold
1	0.74	16.55
2	1.36	8.46
3	0.83	12.17
4	1.98	7.76
Mean	***1.23***	***11.24***
SE	***0.29***	***2.02***
P	***0.0027***	

(B)

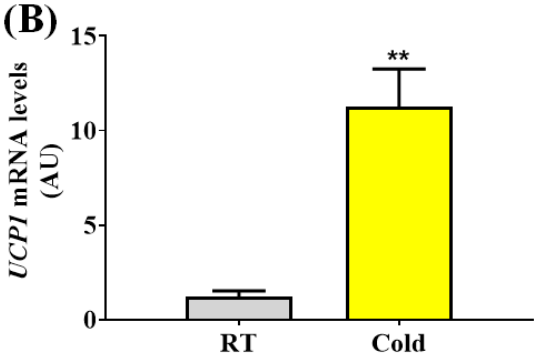

Figure 2. Expression of UCP1 mRNA in iWAT

*Inguinal adipose tissues (iWAT) were isolated from the mice housed at room temperature (RT) or in a cold refrigerator for 24 hours (Cold) conditions. Real-time RT-PCT analysis for expression of UCP1 mRNA. Levels of mRNA were normalized to levels of β-actin mRNA. (A) data analysis of UCP1 mRNA levels. (B) comparison of UCP1 mRNA levels. Data represent the results of four mice per group. Values are means (X) ± standard error (SE). **P < 0.01 compared between the Cold group and the RT group. AU is an arbitrary unit.*

2.2.3. Cold significantly increases expression of TMEM26 mRNA in inguinal adipose tissue

Notably, the UCP1 molecule is very well known as a specific marker of brown adipocytes [20]. Thus, increased expression of *UCP1* in iWAT of the cold exposed mice is not only showing the thermogenic response in the tissue but also demonstrates a possibility of switching from white adipocytes to brown adipocytes – like cells, naming beige cells, in the iWAT. Consistent with this, the result of this study showed that expression of mRNA level of *Transmembrane protein 26* (*TMEM26)* was significantly increased in iWAT of the cold exposed mice compared with that of the room temperature housed control mice (Figures 3A and 3B).

A recent study has suggested that TMEM26 is highly expressed in white adipose tissues in response to factors induced by thermogenesis and the browning process [21]. Beige cells possess characteristics like brown adipocytes which contain plenty number of mitochondria and small lipid droplets, and express high levels of molecules specific to thermogenesis (e.g., PGC-1α, UCP1) [22]. Moreover, the previous study has discovered that TMEM26 is a marker specific to beige cells which are more highly expressed in these cells than in any other cells in the body [22]. Thus, the upregulation of mRNA levels of *TMEM26* as well as *PGC-1α* and *UCP1* in iWAT of the cold exposed mice proved the induction of beige cell formation in this tissue.

(A)

Mouse	RT	Cold
1	0.65	2.95
2	0.98	1.48
3	1.12	1.93
4	1.36	1.54
Mean	***1.03***	***1.98***
SE	***0.15***	***0.34***
P	***0.043***	

(B)

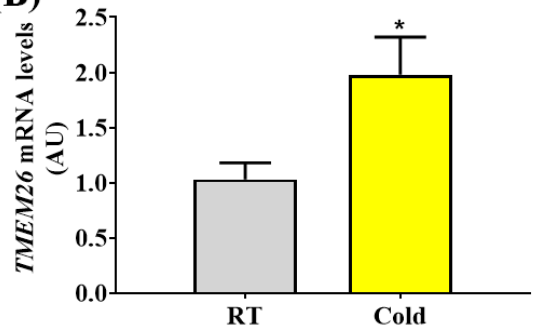

Figure 3. Expression of TMEM26 mRNA in iWAT

*Inguinal adipose tissues (iWAT) were isolated from the mice housed at room temperature (RT) or in a cold refrigerator for 24 hours (Cold) conditions. Real-time RT-PCT analysis for expression of TMEM26 mRNA. Levels of mRNA were normalized to levels of β-actin mRNA. (A) data analysis of TMEM26 mRNA levels. (B) comparison of TMEM26 mRNA levels. Data represent the results of four mice per group. Values are means (X) ± standard error (SE). *P < 0.05 compared between the Cold group and the RT group. AU is an arbitrary unit.*

2.2.4. Cold mildly induces expression of SLC27A1 mRNA in inguinal adipose tissue

To confirm the induction of beige cell formation in iWAT of the cold exposed mice, the final test was examining the expression of mRNA level of *Solute carrier family 27 member 1* (*SLC27A1)*, another specific marker of beige adipocytes [22]. Unfortunately, the present data showed that the expression of mRNA level of *SLC27A1* in iWAT of the cold-exposed mice tendency higher than that of the control mice but there was no significant difference between both groups (Figures 4A and 4B).

SLC27A1 has been recognized as a marker of beige cells that is highly expressed in white adipose tissues in several conditions induced browning in these tissues [22]. Some factors can cause beige cell formation in white adipose tissues but do not enhance the expression of the SCL27A1 molecule. For example, primary white retinoblastoma haploinsufficient (Rb(+/-)) adipocytes express brown adipocyte-related genes (*PGC-1α*, *PR domain containing 16/PRDM16*) but do not enhance the expression of beige cell marker *SCL27A1* [23]. Therefore, the data of the present study suggest that cold-induced high expression of beige cell gene *TMEM26* did not strongly affect the expression of *SCL27A1*, another gene-specific to beige cells, in iWAT of the mice. However, the mechanism involved in the deference in the expression of *TMEM26* and *SCL27A1* in iWAT of the cold exposed mice are unknown and it needs to be elucidated in further studies.

(A)

Mouse	RT	Cold
1	0.92	1.48
2	1.05	1.21
3	1.21	1.39
4	0.93	1.09
Mean	***1.03***	***1.29***
SE	***0.07***	***0.09***
P	***0.054***	

(B)

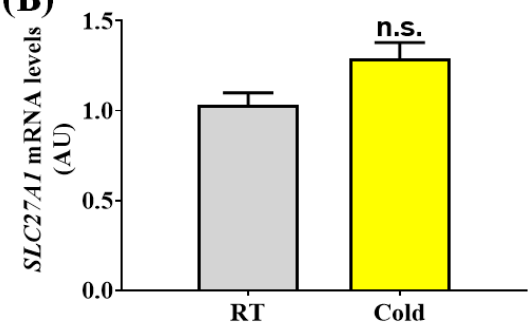

Figure 4. Expression of SLC27A1 mRNA in iWAT

Inguinal adipose tissues (iWAT) were isolated from the mice housed at room temperature (RT) or in a cold refrigerator for 24 hours (Cold) conditions. Real-time RT-PCT analysis for expression of SLC27A1 mRNA. Levels of mRNA were normalized to levels of β-actin mRNA. (A) data analysis of SLC27A1 mRNA levels. (B) comparison of SLC27A1 mRNA levels. Data represent the results of four mice per group. Values are means (X) ± standard error (SE). n.s. is not significant between the Cold group and the RT group. AU is an arbitrary unit.

3. Conclusions

As a consequence, the current research demonstrates the potential of inguinal white adipose tissue (iWAT) in responding to cold-induced adaptive thermogenesis and browning process in mice. Cold-induced strong expression of mRNA levels of genes specific to thermogenesis (*PGC-1α*, *UCP1*) and beige cell gene (*TMEM26*). Expression of mRNA level of *SCL27A1*, another beige cell marker, was slightly increased in iWAT of the cold exposed mice compared to the room temperature housed control mice. Therefore, PGC-1α, UCP1, and TMEM26 are selective markers for studying cold-induced adaptive thermogenic responses in iWAT, whereas, SCL27A1 should not be used as a specific molecule of cold-induced thermogenesis in iWAT. The iWAT will be a promising site for the induction of beige cell formation and thermogenesis that enhance energy expenditure and lower body weight gain as well as fat accumulation and thus, contributing to the combat against high-fat diet induced obesity and obesity-related metabolic disorders.

REFERENCES

[1] Zhao Y, Zhang P, Lee J.T, and Oldenburg B *et al.*, 2021. The Prevalence of Metabolic Disease Multimorbidity and Its Associations With Spending and Health Outcomes in Middle-Aged and Elderly Chinese Adults. *Frontiers in Public Health,* May 3; 9:658706. doi: 10.3389/fpubh.2021.658706

[2] Lutsey P.L, Steffen L.M, and Stevens J, 2008. Dietary Intake and the Development of the Metabolic Syndrome. *Circulation,* Vol. 117, pp. 754-761.

[3] Himms-Hagen J, 1984. Nonshivering thermogenesis. *Brain research bulletin,* Vol. 12, pp. 151-160.

[4] Gill J.A and La Merrill M.A, 2017. An emerging role for epigenetic regulation of Pgc-1α expression in environmentally stimulated brown adipose thermogenesis. Environmental epigenetics, 30; 3(2):dvx009. doi: 10.1093/eep/dvx009.

[5] Cypess A.M and Kahn C.R, 2010. Brown fat as a therapy for obesity and diabetes. *Current Opinion in Endocrinology. Diabetes, and Obesity,* Vol. 17, pp. 143-149.

[6] Wu L, Xia M, Duan Y, Zhang L, and Li J *et al.*, 2019. Berberine promotes the recruitment and activation of brown adipose tissue in mice and humans. *Cell Death & Disease,* Vol. 10, pp. 468.

[7] Longo M, Zatterale F, Naderi J, and Miele C *et al.*, 2019. Adipose Tissue Dysfunction as Determinant of Obesity-Associated Metabolic Complications. *International Journal of Molecular Sciences,* Vol. 20, pp. 2358.

[8] Le Ngoc Hoan, Nguyen Phuc Hung, Ho Thi Hong Van, and Chu Dinh Toi, 2018. Characteristics of white adipose tissue shape and weight in the restricted high-fat diet-fed mice. *HNUE Journal of Science, Natural Sciences,* Vol. 63, pp. 142-146.

[9] El Hadi H, Di Vincenzo A, Vettor R, and Rossato M, 2019. Food Ingredients Involved in White-to-Brown Adipose Tissue Conversion and Calorie Burning. *Frontiers in Physiology,* Vol. 9, pp. 1954.

[10] Cao L, Choi E.Y, Liu X, and During M.J *et al.*, 2011. White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab.,* Vol. 14, pp. 324-38.

[11] Asnani-Kishnani M, Rodríguez A.M, Serrano A, and Palou A *el al*., 2019. Neonatal Resveratrol and Nicotinamide Riboside Supplementations Sex-Dependently Affect Beige Transcriptional Programming of Preadipocytes in Mouse Adipose Tissue. *Frontiers in Physiology,* doi.org/10.3389/fphys.2019.00083

[12] Magri T.P, Fernades F.S, Souza A.S, and Mello Coelho V.D *et al.*, 2015. Interesterified fat or palm oil as substitutes for partially hydrogenated fat in maternal diet can predispose obesity in adult male offspring. *Clinical Nutrition,* Vol. 34, pp. 904-910.

[13] Peppler W.T, Anderson Z.G, MacRae L.M, MacPherson R.E, and Wright D.C, 2017. Habitual physical activity protects against lipopolysaccharide-induced inflammation in mouse adipose tissue. *Adipocyte,* Vol. 6, pp. 1-11.

[14] H. Liang and W. F. Ward, 2006. PGC-1alpha: a key regulator of energy metabolism. *Adv Physiol Educ,* Vol. 30, pp. 145-51.

[15] Cheng C.F, Ku H.C, and Lin H, 2018. PGC-1α as a Pivotal Factor in Lipid and Metabolic Regulation. *International Journal of Molecular Sciences,* Vol. 19, 3447.

[16] Kleiner S, Mepani R.J, Laznik D, and Spiegelman B.M *et al.*, 2012. Development of insulin resistance in mice lacking PGC-1α in adipose tissues. *Proceedings of the National Academy of Sciences,* Vol. 109, pp. 9635-9640.

[17] Boström P, Wu J, Korde A, Ye L, and Spiegelman B.M *et al.*, 2012. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature,* Vol. 481, pp. 463-468.

[18] Uldry M, Yang W, St-Pierre J, Lin J, Seale P, and Spiegelman B.M, 2006. Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab.,* Vol. 3, pp. 333-41.

[19] Ricquier D and Bouillaud F, 2000. Mitochondrial uncoupling proteins: from mitochondria to the regulation of energy balance. *The Journal of Physiology,* Vol. 529, pp. 3-10.

[20] Perdikari A, Leparc G.G, Balaz M, and Wolfrum C *et al.*, 2018. BATLAS: Deconvoluting Brown Adipose Tissue. *Cell Rep.,* Vol. 25, pp. 784-797.

[21] Pilkington A.C, Paz H.A, and Wankhade U.D, 2021. Beige Adipose Tissue Identification and Marker Specificity-Overview. *Frontiers in Endocrinology,* Vol. 12, 599134.

[22] Wu J, Sparks L.M, Ye L, and Spiegelman B.M *et al.*, 2012. Beige adipocytes are a distinct type of thermogenic fat cell in mice and humans. *Cell,* Vol. 150, pp. 366-76.

[23] Petrov P.D, Palou A, Bonet M.L, and Ribot J, 2016. Cell-Autonomous Brown-Like Adipogenesis of Preadipocytes From Retinoblastoma Haploinsufficient Mice. *J. Cell Physiol.,* Vol. 231, pp. 1941-1952.