Acute exercise regulates adipogenic gene expression in white adipose tissue

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ABSTRACT: White adipose tissue expansion is associated with both hypertrophy and hyperplasia of adipocytes. Exercise training results in adipocyte hypertrophy by activating lipolysis, but it is poorly understood whether exercise regulates adipogenesis by altering adipogenic gene expression. The purpose of this study was to evaluate the effect of a single bout of swimming exercise on adipogenic gene expression in white adipose tissue (WAT). Male C57BL/6J mice were divided into two groups: a sedentary control group and a 120-minute swimming exercise group. Immediately after acute exercise, adipogenic gene expression in WAT was analysed by RT-PCR, and tdTomato positive cells in WAT from UCP1-cre-tdTomato mice were observed under a confocal microscope. In epididymal white adipose tissue (eWAT), PPARγ2 and C/EBPα expression at the mRNA level was significantly decreased with high induction of Wnt10b and KLFs (KLF2, KLF3, KLF7, KLF6, KLF9 and KLF15), whereas PPARγ2, not C/EBPα, was decreased with high induction of Wnt6 and KLFs (KLF2, KLF3, KLF7, KLF6 and KLF9) in inguinal white adipose tissue (iWAT) after acute exercise. The expression of C/EBPβ and C/EBPδ was upregulated in both WATs with a high level of PGC-1α expression. Expression level of UCP1 was increased only in adipocytes of eWAT, while beige cell specific gene expression was comparable between groups and tdTomato positive cells were not found in WAT of UCP1-cre-tdTomato reporter mouse immediately after acute exercise. These results suggest that acute exercise suppresses adipogenic gene expression and may regulate thermogenesis by activating C/EBPβ, PGC-1α and UCP1 in WAT.

INTRODUCTION

White adipose tissue (WAT) is a specialized organ for lipid storage and regulates whole body metabolism as a major endocrine organ secreting adipokines [1]. WAT has high plasticity since it expands its mass by increasing the size of existing adipocytes (hypertrophy) and/or forming new adipocytes (hyperplasia) in response to the demand for additional lipid storage [2, 3]. In mouse, epididymal white adipose tissue (eWAT) expansion under a high fat diet condition, which contributes to the development of metabolic syndromes such as obesity, insulin resistance, type 2 diabetes and cardiovascular disease [1], is preferentially associated with adipocyte hyperplasia, whereas inguinal white adipose tissue (iWAT) expansion mainly depends on adipocyte hypertrophy [4], supporting that inhibition of adipogenesis is critical for the treatment of metabolic syndromes. Peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding proteins (C/EBPs) have received much attention as master regulators for adipogenesis [5]. PPARγ is a member of the nuclear receptor superfamily and is expressed as two isoforms, PPARγ1 and PPARγ2, depending on different promoter usage and alternative splicing [6, 7]. While PPARγ1 is expressed in multiple tissues, PPARγ2 expression is restricted in adipose tissue [8, 9]. PPAR2 deficient mouse shows impairment of adipose tissue development with adipocyte death and deterioration of insulin sensitivity [10, 11], whereas treatment with PPARγ activators results in the development of a large number of small adipocytes in obese rat WAT with increased fat mass [12, 13]. C/EBPs belong to the large family of basic leucine zipper transcription factors and have six members (C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPε and CHOP) that form hetero- or homo-dimers to bind the same C/EBP consensus sequence. Three of them (C/EBPα, C/EBPβ and C/EBPδ) are expressed in both WAT and brown adipose tissue (BAT) [5, 14]. C/EBPα, either alone or in combination with PPARγ, promotes adipogenesis in mouse fibroblastic cells since they regulate each other’s expression [15, 16]. Additionally, C/EBP null mice have a perinatal lethal phenotype due to defective liver gluconeogenesis and subsequent hypoglycaemia [17]. Restoration of hepatic C/EBPα level in this mouse causes an absence of WAT except
EBPs, Wnts and KLFs in WAT. Show that acute exercise regulates expression levels of PPARα and PPARγ, which in turn regulate adipogenesis. In this study, we evaluated the effect of acute exercise on the expression of adipogenic genes. Therefore, we show that acute exercise regulates expression levels of PPARγ2, C/EBPα, Wnts and KLFs in WAT.

Adipogenesis is also regulated by Wnt signalling. Wnt signalling has been reported to inhibit adipogenesis by suppressing PPARγ and C/EBPα expression. Wnts are secreted glycoproteins that act through autocrine and paracrine mechanisms to regulate the development of many cell types. Wnt signalling has been reported to inhibit adipogenesis by suppressing PPARγ and C/EBPα expression. In particular, KLF6, KLF9 and KLF15 promote adipogenesis by regulating PPARγ expression. In contrast, KLF12, KLF13 and KLF16 suppress adipogenesis by regulating PPARγ expression. KLFs are a large family of C2H2 zinc-finger proteins and play an important role in adipogenesis and obesity as positive or negative regulators. SREBP1c is a pro-adipogenic factor that is indispensable for adipogenesis, but C/EBPδ and/or C/EBPγ are not indispensable for adipogenesis, but C/EBPδ and C/EBPγ act synergistically to induce adipogenesis.

Adipogenesis is also regulated by Wingless-type MMTV integration site family members (Wnts), Krüppel-like factors (KLFs), GATA binding protein (GATA) 2, GATA3 and sterol regulatory element binding transcription factor 1 (SREBP1c). WNTs are secreted glycoproteins that act through autocrine and paracrine mechanisms to regulate the development of many cell types. Wnt signalling has been reported to inhibit adipogenesis by suppressing PPARγ and C/EBPα expression. In particular, KLF6, KLF9 and KLF15 promote adipogenesis by regulating PPARγ expression. In contrast, KLF12, KLF13 and KLF16 suppress adipogenesis by regulating PPARγ expression. KLFs are a large family of C2H2 zinc-finger proteins and play an important role in adipogenesis and obesity as positive or negative regulators. SREBP1c is a pro-adipogenic factor that is indispensable for adipogenesis, but C/EBPδ and/or C/EBPγ are not indispensable for adipogenesis, but C/EBPδ and C/EBPγ act synergistically to induce adipogenesis.

Acute exercise protocol

At 10 weeks of age, C57BL/6J male mice were randomly divided into two groups: a sedentary control group (Sed) and a swimming exercise group (Exe). Mice in Exe (N=8) were adapted to swimming for 10 min for 2 days in a row to avoid water stress and then 1 week later were subjected to swimming exercise with lead fish sinks (5% of body weight) on the tail for 2 h in plastic barrels filled with water (32±1°C) (42, 43). Mice in Sed (N=8) were kept in barrels without water for 2 h. After exercise, mice were sacrificed immediately.

Adipocytes and stromal vascular fraction isolation

Adipocytes and the stromal vascular fraction (SVF) were isolated from WATs as described previously (44). WATs in DMEM/F-12 media (Invitrogen) containing 1.0% BSA were chopped with surgical scissors then digested with 0.2% collagenase type 2 (Invitrogen) for 25 min at 37°C. After filtering the mixture through 100 μM mesh to remove undigested fragments, the filtrate was centrifuged at 300 x g rpm for 5 min at 4°C. Separated adipocytes were collected with a disposable transfer pipette, and washed 3 times with DMEM/F-12 media. The SVF pellet was resuspended and washed 3 times with DMEM/F-12 media. After isolation, samples were snap frozen in liquid nitrogen for RNA isolation.

Cold exposure

C57BL/6J male mice (10 weeks old) were placed in a cold room (4°C) for 2 h without food to analyse thermogenic gene expression in WATs (N=8). Control mice were placed at room temperature (RT) and fasted for 2 h (N=8). After sacrifice, eWAT and iWAT were removed and snap frozen in liquid nitrogen for RNA isolation. For identifying UCP1 expressing cells in WAT, 10-week old UCP1-cre tdTomato reporter mice were randomly divided into three groups; Sed, Exe, and a cold exposure group (8°C for 72 h) as a positive control (N=3 in each group). After sacrifice, eWAT and iWAT were removed then formalin fixed for histological analyses.

RT-PCR

Total RNA was extracted from eWAT, iWAT, adipocytes and SVF
samples collected from Con and Exe using TRIzol isolation reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. RNA concentration was spectrophotometrically determined using NanoDrop (Thermo Scientific). Two micrograms of RNA from WATs or SVF and 150 ng of RNA from adipocytes were reverse transcribed using murine leukaemia virus reverse transcriptase and oligo (dT)16 primer. The resulting cDNAs from tissue samples were assayed in duplicate. qRT-PCR was conducted using 2X SYBR green PCR master mix on a real-time PCR system (Applied Biosystems). Gene expression data were normalized to the housekeeping gene cyclophilin A and analysed using the delta delta cycle threshold method ($\Delta\Delta ^{C_t}$) [45]. Primer sets are described in Table 1.

**Immunohistochemistry**

Both eWAT and iWAT were removed from UCP1-cre-tdTomato reporter mouse, fixed with 1% formalin for 12 h at 4°C, and then washed three times. WATs were minced into pieces (~50 mm) using a scalpel, permeabilized with 1% Triton-X 100 for 6 h at 4°C, blocked with 5% goat serum for 1 h at RT and then incubated with 2 μg of Bodipy (Invitrogen, Carlsbad, CA) for 2 h at RT . After three washes with PBS, images were acquired with a Zeiss LSM780 laser scanning confocal microscope (Carl Zeiss).

**Statistical analysis**

All results are expressed as means ± standard deviation (SD). To test normality, the Shapiro-Wilk test was performed and then, depending its outcome, data were analysed using Student’s t test or the Mann-Whitney U test. Statistical significance was set at P<0.05.

**RESULTS**

**Acute exercise modulates PPARγ2 and C/EBPs expression in WAT.**

To determine whether acute exercise regulates adipogenic gene expression, we analysed expression levels of PPARγ2 and C/EBPs in WATs of Sed and Exe mice. Acute exercise markedly reduced PPARγ2 expression in eWAT (P<0.0001) and iWAT (P<0.05) (Fig. 1A). Additionally, C/EBPα expression was significantly attenuated in eWAT (P<0.05) but was unchanged in iWAT after acute exercise (Fig. 1B). Conversely, the expression levels of C/EBPβ and C/EBPδ, known to stimulate PPARγ and C/EBPα expression, were highly upregulated in both WATs after acute exercise (P<0.0001) (Fig. 1C-D). Our results indicate that acute exercise contributes to regulating adipogenic gene expression in WAT.

**Acute exercise regulates Wnt6 and Wnt10b expression in a fat depot-specific manner**

Since PPARγ2 expression was downregulated in WAT after acute exercise, we evaluated whether acute exercise leads to increased expression levels of Wnts. After acute exercise, Wnt6 expression was higher in iWAT (P<0.05) (Fig. 2A), whereas Wnt10b expression was significantly increased in eWAT (P<0.01) (Fig. 2C). However, Wnt10a expression was comparable in both WATs between groups (Fig. 2B).

**TABLE 1. Sequences of primers for RT-PCR.**

| Transcript | Primer sequence (5’-3’) |
|------------|------------------------|
| PPARγ2     | F: TCGCTGATGCACTGCTATTG |
|            | R: GAGAGTTCCACAGACGATT |
| C/EBPα     | F: GCGGGAGGCAACAAACACTC |
|            | R: GTCTCTGAGCACTTCCAGAC |
| C/EBPβ     | F: TGAAGCAACAGGCTGCTAAGT |
|            | R: ACCAAACCCCGCAGGAACAT |
| C/EBPδ     | F: GTTGGCTCTAATTCTGCAAGA |
|            | R: GTGAAAGCCCGCAACATTAC |
| Wnt6       | F: CGCCGAGACGTGATGACCTTC |
|            | R: ATGCAACAGATATCCTACAGG |
| Wnt10a     | F: ACCCTCGTGCTCTTATTTG |
|            | R: ACCCTGACGGTCTTACATT |
| Wnt10b     | F: GGCTGTAACCCAGCACAT |
|            | R: GGCCTGAAACACAGACAT |
| KLF2       | F: GAGCTGACGCAACAGGTCTT |
|            | R: CACACGAGGGAACAGCAT |
| KLF3       | F: GCTCAGACGACAGCCTTAC |
|            | R: GAGGAGGAGGAAAGGAAGGAG |
| KLF4       | F: CTCAACAGGACCACTCCTAATC |
|            | R: AAGGAGATACCCCTTAAAGC |
| KLF5       | F: CTGCCATCTGCGCAATGATAAT |
|            | R: GAAGTGAGTACGCTCGCTTCTC |
| KLF6       | F: GAGGAGAAGGGAGGATCAGAC |
|            | R: CAGAGTCTGAGGGCTGTTTTC |
| KLF7       | F: CACAGGGTGAAGCGTCAAA |
|            | R: ACCCTGCTGTCTTCTGAGT |
| KLF9       | F: GCTGACCTGACAGCTTCAATAC |
|            | R: GCTGCCAGTCTCTTAAATGAA |
| KLF15      | F: GAAGTGAGTATGGAGGATAG |
|            | R: AGAAGTCAACCCAGAGAAAAG |
| GATA2      | F: GGAGAAAGGAGTAGGACAGAA |
|            | R: CCAAAAGACAAACAATGACAC |
| GATA3      | F: AGCTGCGACATGACATGAAAG |
|            | R: TAGGGCGAGATGGGTGTAAG |
| SREBP1c    | F: CCTGGCTGGGTCTCTTTTTC |
|            | R: TGCACTGTTCTTGGAGATGTC |
| PRDM16     | F: CAGGAGCAGGACAGCCATTC |
|            | R: GGCTGCACTCGGTCTTGGT |
| PGC-1α     | F: TCTGGGCTCAGAGGAAGAGA |
|            | R: TCTGGGCTCAGAGGAAGAGA |
| UCP1       | F: GAGGTGCTGAGGAGGTCAAGT |
|            | R: AAGCTTCTGCTGGTGCTTATAA |
| TMEM26     | F: ACCCTGCTGACGCAGAGAG |
|            | R: GTGGTGTGGGCTGCAAGGTC |
| TH         | F: AGGGGCTCTTCCAAAGGTC |
|            | R: ATCAAAGGCTTCAGCCACAC |
| Dio2       | F: CCCATCGACATGAGATGAAG |
|            | R: TGGGAAATCATCGGGCTCAGA |
| CD137      | F: CGTCGCAAACTCTCTGTGTAAC |
|            | R: GTCCACTTCTGCTGGAGAAAG |
| HOX8c      | F: CTGTGCTGAACCCCCCTTGGATC |
|            | R: GTGGTGTGGGCTGCAAGGTC |
| TBX1       | F: GGAGGGAAGGGAGTGGTCG |
|            | R: TGGTCTACTACGGGCACAAAG |
| Cyclophilin A | F: TCAAGAAGCAAGAGAAACCTTTCG |
|            | R: TCATTCTGCTGGTCTTGGCATTCC |

*Note: All primers were designed using Primer3 software.*
These results suggest that Wnt6 and Wnt10b expression is regulated by acute exercise in a fat depot-specific manner.

**Acute exercise leads to alteration of KLF expression**

Other factors regulated during adipogenesis include KLFs, GATA2, GATA3 and SREBP1c. Immediately after swimming exercise, the expression levels of KLF2, KLF3 and KLF7, known as anti-adipogenic factors, were significantly higher in both WATs (Fig. 3A-C). Interestingly, the expression levels of pro-adipogenic KLFs such as KLF6, KLF9 and KLF15 were also significantly upregulated in WAT from Exe (Fig. 3D-F), whereas KLF4 and KLF6 expression levels were not altered after acute exercise (Fig. 3G-H). Also, the expression levels of GATA2, GATA3 and SREBP1c in WATs were not altered after acute exercise (Fig. 3I-K). These results suggest that acute exercise regulates both pro- and anti-adipogenic KLF expression.

**The expression level of UCP1 is increased in eWAT after acute exercise**

Acute exercise increased the expression level of C/EBPβ, which forms complexes with PRD1-BF-1-RIZ1 homologous domain 16 (PRDM16) and then regulates peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and uncoupling protein 1 (UCP1) expression [46]. Thus, we next examined whether acute exercise induces PGC-1α and UCP1 expression in WAT. Although there was no difference in PRDM16 expression (Fig. 4A), the expression level of PGC-1α in WATs was not altered after acute exercise (Fig. 4B-C). These results suggest that acute exercise regulates both pro- and anti-adipogenic KLF expression.

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**FIG. 1.** Expression of PPARγ2 and C/EBPs after acute exercise.
Note: The expression of PPARγ2 (A), C/EBPα (B), C/EBPβ (C) and C/EBPδ (D) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, #P<0.001 vs. Sed.

**FIG. 2.** Wnts expression after acute exercise.
The expression of Wnt6 (A), Wnt10a (B) and Wnt10b (C) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, **P<0.01 vs. Sed.
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FIG. 3. KLFs expression after acute exercise.

The expression of KLFs (A-H), GATA2 (I) GATA3 (J) and SREBP1c (K) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, **P<0.01, #P<0.001 vs. Sed.
(P<0.01) after acute exercise (Fig. 4B). Interestingly, acute exercise induced expression levels of C/EBPβ and PGC-1α to a higher extent in iWAT than in eWAT; however, UCP1 expression was upregulated only in eWAT (P<0.001), not in iWAT (Fig. 4C). Next, we evaluated UCP1 expression in adipocytes and SVF from eWAT of both groups. The expression level of perilipin, a mature adipocyte marker, was significantly higher in adipocytes (Fig. 4D), indicating that adipocytes and SVF were successfully isolated. Interestingly, UCP1 upregulation was found in adipocytes (Fig. 4E), indicating that acute swimming exercise increased UCP1 expression in adipocytes of eWAT. It is suggested that swimming exercise even in warm water could activate non-shivering thermogenesis (NST) to counteract the heat loss due to high conductivity of water [47]. NST is mainly regulated by the sympathetic nervous system and thyroid hormone [48, 49]. Therefore, mice were placed in a cold room (4°C) for 2 h and then we analysed the expression levels of tyrosine hydroxylase (TH), which converts phenylalanine into dopamine and iodothyronine deiodinase type 2 (dio2), which is the enzyme responsible for the conversion of T4 to T3. After cold exposure, TH expression was significantly downregulated in eWAT; however, there was no alteration in eWAT after swimming exercise (Fig. 4F-G). The induction of dio2 was observed in both WATs after cold exposure, whereas it was upregulated only in iWAT after swimming exercise to a greater extent than after cold exposure (Fig. 4H-I). Although higher induction of dio2 after swimming exercise than cold exposure could result from combined effects of swimming exercise and NST, more than a 20-fold increase of dio2 expression after swimming exercise may suggest that its induction could be caused more by exercise than NST, since acute exercise also increases serum T3 immediately after exercise [50]. In addition, the expression level of PRDM16 and PGC-1α was not altered in WATs (Fig. 4J-K), and UCP1 expression was highly upregulated only in iWAT (Fig. 4L) after 2 h of cold exposure. These different gene expression patterns in TH, dio2, PGC-1α and UCP1 between swimming exercise and cold exposure suggest that activation of C/EBPβ mediates PGC-1α and UCP1 in eWAT after swimming exercise could be mainly the result of exercise rather than NST.

**Beige cells are not found in eWAT immediately after acute exercise**

UCP1 expressing cells in WAT, known as beige cells, are identified by specific markers such as transmembrane protein 26 (TMEM26), CD137, homeobox C8 (HOXC8) and t-box1 transcription factor c (TBX1) [51]. Therefore, we measured the expression levels of beige cell specific markers via qPCR to investigate whether UCP1 upregulation accompanies the recruitment of beige cells in eWAT after acute exercise. However, expression levels of TMEM26, CD137, HOXC8 and TBX1 were not altered in either WAT after acute exercise (Fig. 5A-D). For further investigation, we analysed UCP1 expression in WAT from UCP1-cre-tdTomato reporter mice after acute exercise. WATs from cold-exposed UCP1-cre-tdTomato reporter mice were used as a positive control. Consistent with mRNA expression levels of beige cell specific markers, tdTomato positive cells were not found in either WAT from UCP1-cre-tdTomato mice after acute exercise. However, tdTomato positive cells were dramatically increased in iWAT from UCP1-cre-tdTomato reporter mice at 3 days after cold exposure (Fig. 5E-F). These results indicate that beige cells are not detected in eWAT at least immediately after 2 h of single swimming exercise.

**DISCUSSION**

Exercise training is known to cause numerous beneficial effects on adipose tissue biology; however, it is not fully understood whether acute exercise contributes to regulating adipogenic gene expression. To address this, we analysed adipogenic gene expression in mouse eWAT and iWAT after a single bout of swimming exercise. The findings of the present study demonstrate that acute exercise regulated the expression levels of PPARγ2, C/EBPs, Wnts and KLFs in WAT.

The intensity, mode and duration of exercise may play a role in regulating PPARγ expression at the mRNA level. Previous studies have shown that exercise training does not increase PPARγ protein expression in WAT [52, 53]. Since exercise increases plasma FA that could function as a ligand for PPARα and PPARγ [54], the lack of changes in the PPAR expression may suggest that exercise could activate rather than express PPARs, as noted by Petridou et al [53]. However, exercise training downregulates PPARγ mRNA expression in the stromal-vascular fraction from adipose tissue [55]. We found that acute exercise also attenuated the expression level of PPARγ2 in both WATs in this study. On the other hand, C/EBPα expression was markedly decreased only in eWAT, not iWAT, after acute exercise in our study. C/EBPα may not be a critical factor for adipogenesis at least in iWAT since restoration of hepatic C/EBPα level in C/EBPα null mice does not effect the development of iWAT [18].

Wnt6, Wnt10a and Wnt10b act as negative regulators of adipogenesis [22, 23, 56, 57]. Although resistance training has been observed to increase Wnt expression in muscle and serum [58, 59], it has not been studied whether acute exercise regulates Wnt expression in WAT. Interestingly, acute exercise increased Wnt10b expression in eWAT and Wnt6 expression in iWAT, indicating that acute exercise regulates Wnts expression in a fat-depot specific manner. Future studies with genetically modified mice models are necessary to elucidate fat-depot specific Wnt function in adipogenesis. KLFs regulate adipogenesis and obesity as positive or negative regulators [24], and it is not clear whether acute exercise regulates expression of KLFs in WAT. In our study, acute exercise significantly increased the expression levels of anti-adipogenic KLFs (KLF2, KLF3 and KLF7) in both WATs. However, the expression levels of three pro-adipogenic KLFs (KLF6, KLF9 and KLF15) were also upregulated in WAT after acute exercise. Since these three KLFs regulate adipogenesis by activating PPAR and/or C/EBPs, induction of these three genes may result from the downregulation of PPARγ and C/EBPα mRNA expression as a compensatory response.

Collectively, our results suggest that acute exercise may contribute to inhibiting adipogenesis by regulating the induction of adipogenic...
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**FIG. 4.** PRDM16, PGC-1α and UCP1 expression after acute exercise.
Total RNA from eWAT and iWAT was isolated and the expression of PRDM16 (A), PGC-1α (B), UCP1 (C), TH (F) and Dio2 (H) was assessed by RT-PCR. Total RNA from SVF and adipocytes from eWAT were isolated and the expression of perilipin (D) and UCP1 (E) was assessed by RT-PCR. Total RNA from eWAT and iWAT was isolated and the expression of TH (G), Dio2 (I), PRDM16 (J), PGC-1α (K) and UCP1 (L) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, **P<0.01, #P<0.001 vs. Sed. After 2 h of cold exposure (4°C), total RNA from eWAT and iWAT was isolated and the expression of TH (G), Dio2 (I), PRDM16 (J), PGC-1α (K) and UCP1 (L) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, **P<0.01 vs. RT.
FIG. 5. Beige cell recruitment in WATs after acute exercise.
The expression of TMEM26 (A), CD137 (B), HOXC8 (C) and TBX1 (D) was assessed by RT-PCR. Each value was normalized to cyclophilin A. Bars represent the means and error bars represent SD (N=8). *P<0.05, **P<0.01, #P<0.001 vs. Sed. tdTomato expression was detected from eWAT and iWAT of UCP1-cre-tdTomato mouse after acute exercise or cold exposure (E-F).

genes such as PPARγ2, C/EBPα, Wnts and anti-adipogenic KLFs (KLF2, KLF3 and KLF7) in WAT.

In contrast with PPARγ and C/EBPα, acute exercise led to higher expression levels of C/EBPβ and C/EBPδ in both WATs. Induction of these genes in response to acute exercise may result from an acute compensatory mechanism for the reduction of PPARγ and/or C/EBPα expression, since C/EBPβ and C/EBPδ are known to induce the C/EBPα and PPARγ2 genes in preadipocytes for the development of adipogenesis [19, 20]. Another possible explanation is their various biological functions other than adipogenesis in WAT. In particular, it was recently found that PRDM16-C/EBPβ complex synergistically enhances the promoter activity of PGC-1α [46, 60]. PGC-1α plays an important role in adipogenesis and adaptive thermogenesis by regulating mitochondrial biogenesis via activating UCP1 expression and fatty acid oxidation enzymes [46, 61-64]. Our finding that acute exercise markedly increased PGC-1α expression in both WATs is consistent with a previous study showing that 2 h swimming exercise increases PGC-1 mRNA expression in visceral WAT in rat [65]. It is noteworthy that UCP1 expression in WAT is mediated by PGC-1α after acute exercise or exercise training [66]. Ringholm et al. found that UCP1 mRNA expression in eWAT peaked immediately after acute exercise and then decreased gradually to the rest level at 10
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hours after exercise, whereas mRNA and protein levels of UCP1 in iWAT were upregulated only at 6 hours after acute exercise [66]. In this study, we also observed that acute exercise increased UCP1 mRNA expression in eWAT, not iWAT, immediately after acute exercise. Nonetheless, expression levels of beige cell markers, such as TMEM26, CD137, HOXC8 and TBX1, were not changed and UCP1 positive cells were not detected in eWAT of UCP1-cre-tdTomato mouse at least after acute exercise. It seems that long-term adaptation to exercise may be required to recruit UCP1 positive cells in eWAT, since beige cells in WAT are found after a week of endurance exercise training [67]. Also it may require a long time between acute exercise and sacrifice to identify UCP1 positive cells after acute exercise, as it is well established that UCP1 positive cells are recruited in visceral fat in animals subjected to exercise training. Taken together, our results show that C/EBPβ expression in response to acute exercise may be associated with PGC-1α and UCP1 expression in eWAT.

Swimming exercise even in warm water could activate non-shivering thermogenesis to counteract the heat loss due to the high conductivity of water [47]. In our observations, the expression patterns of TH, dio2, PGC-1α and UCP1 in eWATs after 2 h of swimming exercise were not identical to those in eWAT after 2 h of cold exposure. Also, induction of dio2 in iWAT was dramatically higher after acute exercise than after cold exposure, and this is consistent with a previous study showing that a significant increase in serum T3 may regulate thermogenesis by activating PGC-1α and UCP1 in eWAT.

Since changes in gene expression at the mRNA level do not reflect changes at the protein level, the protein levels of adipogenic genes after acute exercise need to be analysed in future studies. Also, mice in Sed that were staying in air at RT rather than at 32±1°C for 2 h may be considered to be a weakness of this study, as the difference in temperature between groups could affect gene expression. Therefore, future study with treadmill exercise is required to confirm the effects of acute exercise on adipogenic gene expression at mRNA and protein levels.

CONCLUSIONS

In summary, we demonstrated for the first time that acute exercise could regulate adipogenic gene expression in WAT. We made a novel observation that even a single swimming exercise resulted in attenuations of PPARγ2 and C/EBPα expression at the mRNA level in eWAT, with high induction of Wnt10b and anti-adipogenic KLFs (KLF2, KLF3 and KLF7). In addition, C/EBPβ and C/EBPγ expression was upregulated, in parallel with expression levels of PGC-1α and UCP1. In iWAT, acute exercise attenuated the expression of PPARγ2, with high induction of Wnt6 and anti-adipogenic KLFs (KLF2, KLF3 and KLF7). While expression of C/EBPβ and PGC-1γ was also increased in iWAT, UCP1 expression was not changed. These results suggest that acute exercise inhibits adipogenic gene expression and may regulate thermogenesis by activating PGC-1α and UCP1 in WATs.

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REFERENCES

1. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol. 2008;9:367-377.
2. Garault M, Hernandez-Morante JJ, Lujan J, Tebar FJ, Zamora S. Relationship between fat cell size and number and fatty acid composition in adipose tissue from different fat depots in overweight/obese humans. Int J Obes (Lond). 2006;30:899-905.
3. Marques BG, Hausman DB, Martin RJ. Association of fat cell size and paracrine growth factors in development of hyperplastic obesity. Am J Physiol 1998;275:R1898-1908.
4. Wang Q, Tao C, Gupta RK, Scherer PE, Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med. 2013;19:1338-1344.
5. Rosen ED, MacDougall OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 2006;7:885-896.
6. Zhu Y, Qi C, Kerenberg JR, Chen XN, Noya D, Rao MS, Reddy JK. Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. Proc Natl Acad Sci U S A. 1995;92:7921-7925.
7. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem. 1997;272:18779-18789.
8. Braissant O, Foufelle F, Scotto C, Daucu M, Wahl W. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. Endocrinology. 1996;137:354-366.
9. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. 1994;8:1224-1234.
10. Zhang J, Fu M, Cui T, Xiong C, Xu K, Zhong W, Xiao Y, Floyd D, Liang J, Li E, Song Q, Chen YE. Selective disruption of PPARgamma 2 impairs the development of adipose tissue and insulin sensitivity. Proc Natl Acad Sci U S A. 2004;101:10703-10708.
11. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, Perrin C, Jimenez-Linan M, Blount M, Dixon J, Zahn D, Thresher RR, Aparicio S, Martin M, Collodette WH, Kettunen MI, Seppanen-Laakso T, Sethi JK, O'Rahilly S, Brindle K, Cinti S, Oresic M, Burcelin R, Vidal-Puig A. The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor gamma2isoform. Diabetes. 2005;54:1706-1716.
12. Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y, Kadowaki T. TregilTazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest. 1998;101:1354-1361.
13. de Souza CJ, Eckhardt M, Gagen K,
Dong M, Chen W, Laurent D, Burkey BF. Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. Diabetes. 2001;50:1863-1871.

14. Landschulz WH, Johnson PF, McKnight SL. The DNA binding domain of the rat liver nuclear protein C/EBP is bipartite. Science. 1989;243:1681-1688.

15. Freytag SO, Pailelli DL, Gilbert JD. Ectopic expression of the CCAAT-enhancer-binding protein alpha promotes the adipogenic program in a variety of mouse fibroblastic cells. Genes Dev. 1994;8:1654-1663.

16. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Spiegelman BM. C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev. 2002;16:22-26.

17. Wang ND, Finegold MJ, Bradley A, Ou CN, Abdelsayed SV, Wilde MD, Taylor LR, Wilson DR, Darlington GJ. Impaired energy homeostasis in C/EBP alpha knockout mice. Science. 1995;269:1108-1112.

18. Linhart HG, Ishimura-Oka K, DeMayo F, Kibe T, Repka D, Poindexter B, Bick RJ, Darlington GJ. C/EBPalpha is required for differentiation of white, but not brown, adipose tissue. Proc Natl Acad Sci U S A. 2001;98:12532-12537.

19. Yeh WC, Cao Z, Classon M, McKnight SL. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. Genes Dev. 1995;9:168-181.

20. Wu Z, Bucker NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBPdelta, and glucocorticoids. Mol Cell Biol. 1996;16:4128-4136.

21. Tanaka T, Yoshida N, Kishimoto T, Akira S. Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. EMBO J. 1997;16:7432-7443.

22. Ross SE, Hemiati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougall OA. Inhibition of adipogenesis by Wnt signaling. Science. 2000;289:950-953.

23. Cawthorn WP, Bree AJ, Yao Y, Du B, Hemiati N, Martinez-Santibanez G, MacDougall OA. Wnt6, Wnt10a and Wnt10b inhibit adipogenesis and stimulate osteoblastogenesis through a beta-catenin-dependent mechanism. Bone. 2012;50:477-489.

24. Wu Z, Wang S. Role of kruppel-like transcription factors in adipogenesis. Dev Biol. 2013;373:235-243.

25. Mori T, Sakaue H, Igiuchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsu Y, Ogawa W, Hiramatsu R, Kasuga M. Role of Kruppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J Biol Chem. 2005;280:12867-12875.

26. Pei H, Yao Y, Yang Y, Liao K, Wu JR. Kruppel-like factor KLF9 regulates PPARgamma transactivation at the middle stage of adipogenesis. Cell Death Differ. 2011;18:315-327.

27. Li D, Yea S, Li S, Chen Z, Narla G, Banck M, Laborda J, Tan S, Friedman JM, Friedman SL, Walsh MJ. Kruppel-like factor-6 promotes preadipocyte differentiation through histone deacetylase 3-dependent repression of DLK1. J Biol Chem. 2005;280:26941-26952.

28. Sue N, Jack BH, Eaton SA, Pearson RC, Funnell AP, Tumer J, Czolij R, Denyer G, Bao S, Molero-Navajas JC, Perkins A, Fujivara Y, Orkin SH, Bell-Anderson K, Crossley M. Targeted disruption of the basic Kruppel-like factor gene (Klf3) reveals a role in adipogenesis. Mol Cell Biol. 2008;28:3967-3978.

29. Kawamura Y, Tanaka Y, Kawamori R, Maeda S. Overexpression of Kruppel-like factor-7 regulates adipocytokine gene expressions in human adipocytes and inhibits glucose-induced insulin secretion in pancreatic beta-cell line. Mol Endocrinol. 2006;20:844-856.

30. Banerjee SS, Feinberg MW, Watanabe M, Gray S, Haspel RL, Denkinger DJ, Kawahara R, Hauner H, Jain MK. The Kruppel-like factor-like gene (Klf3) regulates peroxisome proliferator-activated receptor gamma expression and adipogenesis. J Biol Chem. 2003;278:2581-2584.

31. Tong Q, Dalgin G, Xu H, Ting CN, Leiden JM, Hotamisligil GS. Function of GATA transcription factors in preadipocyte-adipocyte transition. Science. 2000;290:134-138.

32. Tong Q, Tsai J, Tan G, Dalgin G, Hotamisligil GS. Interaction between GATA and the C/EBP family of transcription factors is critical in GATA-mediated suppression of adipocyte differentiation. Mol Cell Biol. 2005;25:706-715.

33. Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 1996;10:1096-1107.

34. Kim JB, Sarraf P, Wright M, Yao KM, Mueller E, Solanes G, Lowell BB, Spiegelman BM. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J Clin Invest. 1998;101:1-9.

35. Shimano H, Shimomura I, Hammer RE, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev. 1998;12:3182-3194.

36. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev. 1998;12:3182-3194.

37. Sakurai T, Ogasawara J, Kizaki T, Sato S, Ishibashi Y, Takahashi M, Kobayashi O, Oh-Ishi S, Nagasawa J, Takahashi K, Ishida H, Izawa T, Ohno H. The effects of exercise training on obesity-induced dysregulated expression of adipokines in white adipose tissue. Int J Endocrinol. 2013;2013:801743.

38. Horowitz JF. Fatty acid mobilization from adipose tissue during exercise. Trends Endocrinol Metab. 2003;14:386-392.

39. Zechner R, Kienesberger PC, Haemmerle G, Zimmermann R, Lass A. Adipose triglyceride lipase and the lipolytic catalobolism of cellular fat stores. J Lipid Res. 2009;50:3-21.

40. Ogasawara J, Nomura S, Rahman N, Sakurai T, Kizaki T, Izawa T, Ishida H, Haga S, Ohno H. Hormone-sensitive lipase is critical mediators of acute exercise-induced regulation of lipolysis in rat adipocytes. Biochem Biophys Res Commun. 2010;400:134-139.

41. Ogasawara J, Sakurai T, Kizaki T, Ishibashi Y, Izawa T, Sumitani Y, Ishida H, Radak Z, Haga S, Ohno H. Higher levels of ATGL are associated with exercise-induced enhancement of lipolysis in rat epidymal adipocytes. PLoS One. 2012;7:e40876.

42. Lukaszuk B, BlaIou I, Gorski J, Zajaczkiewicz M, Winnicka MM, Chabowski A. A single bout of exercise increases the expression of glucose but not fatty acid transporters in skeletal muscle of IL-6 KO mice. Lipids. 2012;47:763-772.

43. Borghi SM, Pinho-Ribeiro FA, Zarpelon AC, Cunha TM, Alves-Filho JC, Ferreira SH, Cunha FQ, Casagrande R, Verri WA, Jr. Interleukin-10 limits intense acute swimming-induced muscle injury. J Physiol. 2015;100:531-544.

44. Matsushita H, Wux, Sinha MK, Hardy VE, Rosato EL, Barbot DJ, Rosato FE, Goldstein BJ. Differential regulation of adiponectin secretion from cultured epididymal adipocytes. PLoS One. 2011;6:e22483.

45. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta CT) Method. Methods. 2001;25:402-408.
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46. Kajimura S, Seale P, Kubota K, Lunsford E, Frangioni JV, Gygi SP, Spiegelman BM. Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-beta transcriptional complex. Nature. 2009;460:1154-1158.

47. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84:277-359.

48. Hims-Hagen J. Nonshivering thermogenesis. Brain Res Bull. 1984;12:151-160.

49. Ribeiro MO, Carvalho SD, Schultz JJ, Chielilini G, Scanlan TS, Bianco AC, Brent GA. Thyroid hormone--sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform--specific. J Clin Invest. 2001;108:97-105.

50. Fortunato RS, Ignacio DL, Padron AS, Pecanha R, Marassi MR, Rosenthal D, Wernick-de-Castro JP, Carvalho DP. The effect of acute exercise session on thyroid hormone economy in rats. J Endocrinol. 2008;198:347-353.

51. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Jiang AH, Khandekar M, Virtanen KA, Nuuttila P, Schara G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Carvalho DP. Upper-body resistance exercise augments upper-body resistance exercise augments vastus lateralis androgen receptor-DNA binding and canonical Wnt/beta-catenin signaling compared to lower-body exercise training on adipogenesis of stromal-vascular fraction cells in rat epididymal white adipose tissue. Acta Physiol (Oxf). 2011;200:325-338.

52. Kawamura T, Yoshida K, Sugawara A, Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Jiang AH, Khandekar M, Virtanen KA, Nuuttila P, Schara G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Carvalho DP. The effect of acute exercise session on thyroid hormone economy in rats. J Endocrinol. 2008;198:347-353.

53. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Jiang AH, Khandekar M, Virtanen KA, Nuuttila P, Schara G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Carvalho DP. Upper-body resistance exercise augments upper-body resistance exercise augments vastus lateralis androgen receptor-DNA binding and canonical Wnt/beta-catenin signaling compared to lower-body exercise training on adipogenesis of stromal-vascular fraction cells in rat epididymal white adipose tissue. Acta Physiol (Oxf). 2011;200:325-338.

54. Kliewer SA, Sundsøth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahl W, Wilson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. Proc Natl Acad Sci U S A. 1997;94:4318-4323.

55. Sakurai T, Endo S, Hatano D, Ogasa-wara J, Kizaki T, Ohishi S, Izawa T, Ishida H, Ohno H. Effects of exercise training on adipogenesis of stromal-vascular fraction cells in rat epididymal white adipose tissue. Acta Physiol (Oxf). 2010;200:325-338.

56. Longo KA, Wright WS, Kang S, Gerin I, Chiang SH, Lucas PC, Qpp MR, MacDougald OA. Wnt10b inhibits development of white and brown adipose tissues. J Biol Chem. 2004;279:35503-35509.

57. Wright WS, Longo KA, Dolinsky VW, Gerin I, Kang S, Bennett CN, Chiang SH, Prestwich TC, Gress C, Burant CF, Susulic VS, MacDougald OA. Wnt10b inhibits obesity in ob/ob and agouti mice. Diabetes. 2007;56:295-303.

58. Leal ML, Lamas L, Aoki MS, Wright WS, Kang S, Bennett CN, Chiang SH, Prestwich TC, Gress C, Burant CF, Susulic VS, MacDougald OA. Wnt10b inhibits obesity in ob/ob and agouti mice. Diabetes. 2007;56:295-303.

59. Pilegaard H. PGC-1alpha is required for mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1alpha. Cell. 1999;98:115-124.

60. Sutherland LN, Bomhof MR, Capozzi LC, Basaraba SA, Wright DC. Exercise and adrenaline increase PGC-1 (alpha) mRNA expression in rat adipose tissue. J Physiol. 2009;587:1607-1617.

61. Ringholm S, Grunnet Knudsen J, Leick L, Lundgaard A, Munk Nielsen M, Pilegaard H. PGC-1alpha is required for exercise- and exercise training-induced UCP1 up-regulation in mouse white adipose tissue. PLoS One. 2013;8:e64123.

62. De Matteis R, Lucertini F, Guescini M, Polidori E, Zeppa S, Stocchi V, Cinti S, Cuppini R. Exercise as a new physiological stimulus for brown adipose tissue activity. Nutr Metab Cardiovasc Dis. 2013;23:582-590.