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BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells

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Submitted 19 September 2013; accepted in final form 22 November 2013

Elsen M, Raschke S, Tennagels N, Schwahn U, Jelenik T, Roden M, Romacho T, Eckel J. BMP4 and BMP7 induce the white-to-brown transition of primary human adipose stem cells. Am J Physiol Cell Physiol 306: C431–C440, 2014. First published November 27, 2013; doi:10.1152/ajpcell.00290.2013.—While white adipose tissue (AT) is an energy storage depot, brown AT is specialized in energy dissipation. Uncoupling protein 1 (UCP1)-expressing adipocytes with a different origin than classical brown adipocytes have been found in white AT. These “brite” (brown-in-white) adipocytes may represent a therapeutic target to counteract obesity. Bone morphogenetic proteins (BMPs) play a role in the regulation of adipogenesis. Based on studies with murine cells, BMP4 is assumed to induce stem cell commitment to the white adipocyte lineage, whereas BMP7 promotes brown adipogenesis. There is evidence for discrepancies between mouse and human AT. Therefore, we compared the effect of BMP4 and BMP7 on white-to-brown transition in primary human adipose stem cells (hASCs) from subcutaneous AT. Long-term exposure of hASCs to recombinant BMP4 or BMP7 during differentiation increased adipogenesis, as determined by lipid accumulation and peroxisome proliferator-activated receptor-α (PPARα) expression. Not only BMP7, but also BMP4, increased UCP1 expression in hASCs and decreased expression of the white-specific marker TCF21. The ability of hASCs to induce UCP1 in response to BMP4 and BMP7 markedly differed between donors and could be related to the expression of the brite marker CD137. However, mitochondrial content and oxygen consumption were not increased in hASCs challenged with BMP4 and BMP7. In conclusion, we showed for the first time that BMP4 has similar effects on white-to-brown transition as BMP7 in our human cell model. Thus the roles of BMP4 and BMP7 in adipogenesis cannot always be extrapolated from murine to human cell models.

Bone morphogenetic proteins; primary human preadipocytes; adipogenesis; brite adipocytes

Adipose tissue (AT) plays a crucial role in the regulation of energy homeostasis. Functionally, AT can be subdivided into white AT and brown AT. While white AT is the main site of energy storage and provides substrates in terms of energy needs by releasing free fatty acids and glycerol, brown AT metabolizes triglycerides to generate heat in adaptation to a cold environment (32). This unique function of brown AT is due to a high mitochondrial density and to the presence of uncoupling protein 1 (UCP1). Until recently, brown AT was believed to play a negligible role in the adult human. However, it gained substantial interest since active brown AT has been shown to be present in adults by five independent groups (4, 23, 38, 40, 43) and brown AT activity was negatively associated with increasing body mass index (BMI) (23, 38, 43). Furthermore, UCP1-expressing brown-like adipocytes have been discovered within white AT after cold exposure (31). These so-called “brite” (brown-in-white) adipocytes arise from white preadipocytes (19), whereas classical brown adipocytes are derived from the myogenic lineage (29, 35). Both increasing brown AT activity and promoting the induction of brite adipocytes in white AT represent strategies to counteract obesity. Pharmacological agents, like peroxisome proliferator-activated receptor-γ (PPARγ) agonists (8, 20), triiodothyronine (T3) (12), cardiac natriuretic peptides (CNPs) (1), and bone morphogenetic protein (BMP) 7 (27) have been reported to induce brite adipocytes in humans.

BMPs belong to the transforming growth factor-β (TGF-β) superfamily and are important regulators of developmental processes. BMP signaling is complex and dependent on the receptor composition of a cell and the presence of intra- and extracellular antagonists (3). Selected members of the BMP family play an important role in the regulation of white vs. brown adipogenesis and energy homeostasis (16). While BMP4 induces commitment of pluripotent stem cells to the white adipocyte lineage (2, 10) and promotes differentiation of white preadipocytes (9), BMP7 plays an important role in brown adipocyte lineage determination of the multipotent C3H10T1/2 cell line and promotes differentiation of murine brown preadipocytes (37). Evidence from transgenic mouse models suggests that BMP7 plays an important role in whole energy homeostasis, since BMP7 knockout mice displayed a decrease in brown AT mass (37) and systemic BMP7 administration to ob/ob mice led to increased energy expenditure and reduced food intake accompanied by weight loss (36). Therefore, it is currently assumed that BMP4 and BMP7 differentially regulate white vs. brown adipogenesis. However, BMP4 might also induce a white-to-brown switch and have positive effects on energy homeostasis (21). Qian et al. (21) showed that AT-specific overexpression of BMP4 leads to induction of...
brite adipocytes and increased energy expenditure in mice. Furthermore, murine 3T3-L1 adipocytes challenged with BMP4 during differentiation displayed a brite phenotype with increased mitochondrial content and expression of brown AT markers (21).

There is evidence that the molecular signature from human AT depots differ from mouse AT depots (42), raising the question if results from mouse studies investigating mechanisms of browning of white AT can be extrapolated to humans. Furthermore, the effects of different BMPs on white vs. brown adipogenesis seem to be dependent on the AT depot and species studied. The impact of BMP4 and BMP7 on browning of white adipocytes has not yet been explored in parallel in a human cell culture model. Therefore, we aimed to investigate for the first time the effect of BMP4 on the white-to-brown transition of primary human adipose stem cells (hASCs) compared with the known browning factor BMP7. In this study, hASCs were isolated from the subcutaneous depot of different donors and challenged with BMP4 or BMP7. In contrast to mice, in humans both BMP4 and BMP7 were found to induce a brite adipocyte phenotype, suggesting substantial differences in AT plasticity between different species.1

MATERIALS AND METHODS

Isolation and culture of primary hASCs. Subcutaneous AT (from the abdominal and mammary region) was obtained from healthy lean overweight women (n = 38; BMI: 28.8 ± 0.7 kg/m²; age: 39 ± 3 yr) undergoing plastic surgery. The procedure was approved by the ethical board of Heinrich-Heine-University, Düsseldorf, Germany. hASCs were isolated by collagenase digestion of AT as previously described by our group (6). Isolated cell pellets were resuspended in adipocyte basal medium (BM), which was DMEM/F-12 (GIBCO, Grand Island, NY) supplemented with 14 nmol/l NaHCO3, 33 mmol/l d-glucose, 66 nM insulin, 1 nM triiodo-L-thyronine, 100 nM cortisol, 10 ng/ml MTF2, 1 μM forskolin, 5 μM 9-cis-retinoic acid, 70 μg/ml of the complex III inhibitor antimycin A (A8674; Sigma-Aldrich, St. Louis, MO) to obtain maximal respiration. After observing a steady-respiratory flux, cells were titrated with the uncoupler carbonyl cyanide-4-trifluoromethoxyphenylhydrazone (FCCP; Sigma-Aldrich, St. Louis, MO) to obtain maximal respiration. Finally, respiration was inhibited with 2.5 μM of the complex III inhibitor antimycin A (Ab6764; Sigma-Aldrich). Oxygen consumption was normalized to cell number and analyzed with the Oroboros Oxygraph-2k (Oroboros Oxygraph, Innsbruck, Austria). Chambers were equilibrated with BM for 30 min before addition of freshly trypsinized cells. Measurements were done in duplicates and performed in 2 ml of adipocyte BM supplemented with 10% FCS at 37°C in an open system. After observing a steady-respiratory flux, cells were titrated with the uncoupler carbonyl cyanide-4-trifluoromethoxyphenylhydrazone (FCCP; Sigma-Aldrich, St. Louis, MO) to obtain maximal respiration. Finally, respiration was inhibited with 2.5 μM of the complex III inhibitor antimycin A (Ab6764; Sigma-Aldrich). Oxygen consumption was normalized to cell number and analyzed with the Oroboros software. Antimycin A-sensitive oxygen consumption was considered as basal respiration. Anticongenic differentiation for 14 days with or without the addition of 50 ng/ml BMP4 or BMP7 (R&D Systems, Minneapolis, MN) was added to the differentiation medium at a final concentration of 100-fold.

Generation of concentrated supernatants. BMP4 and BMP7 were used to promote browning of hASCs. In a human cell culture model. Therefore, we aimed to investigate for the first time the effect of BMP4 on the white-to-brown transition of primary human adipose stem cells (hASCs) compared with the known browning factor BMP7. In this study, hASCs were isolated from the subcutaneous depot of different donors and challenged with BMP4 or BMP7. In contrast to mice, in humans both BMP4 and BMP7 were found to induce a brite adipocyte phenotype, suggesting substantial differences in AT plasticity between different species.1

1 This article is the topic of an Editorial Focus by Maria-Jesus Obregon (17a).
UCP1 (Hs_UCP1_3_SG), TCF21 (Hs_TCF21_2_SG), ZIC1 (Hs_ZIC1_1_SG), PPARy coactivator 1 (PGC-1α; Hs_PPARC1A_1_SG), and β-actin (Hs_ACTB_2_SG) and primer pairs for CD137 (Eurofins MWG, Hamburg, Germany; forward 5′-AGCTTTACAACATAGTAGCCAC and reverse 5′-TCTGCAATGTCTTGTCTCCT) on a Step One Plus cycler (Applied Biosystems, Carlsbad, CA). Amplification was done as follows: one step at 95°C 2 min, 40 cycles at 95°C 15 s, 55–58°C 30 s, and 60°C 30 s. All samples were analyzed in triplicate. Expression of genes was normalized to β-actin mRNA according to the comparative threshold method (ΔΔCT).

Microfluidic card TaqMan gene expression assay. RNA integrity was tested on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using Agilent RNA Nano chips. Only RNAs with a RIN score of 7.5 or higher were used for analysis. Synthesis of cDNA was done from 0.5 μg of each total RNA preparation in a volume of 20 μl with the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer’s instructions. Thermal cycling of the PCR reactions was done in microfluidic cards on a ViiA7 Real Time PCR 384 well cycler and fluorescence plate reader (Applied Biosystems). Specific TaqMan Gene Expression Assays (Applied Biosystems) for PRDM16 (Hs00991677_m1), NRF1 (Hs00273372_s1), OXR1 (Hs00250562_m1), UCP2 (Hs01075227_m1), UCP3 (Hs-01106052_m1), CYCS (Hs01588974_g1), VDAC1 (Hs01631624_gH), GAPDH (Hs02758991_g1), and β-actin (Hs01060665_g1) were used. Expression levels of genes were normalized to GAPDH and β-actin as housekeeping genes and related to control samples.

Statistics. Results are expressed as means ± SE. One-way ANOVA (post hoc test: Bonferroni or Dunnett) was used to determine statistical significances. All statistical analyses were done using GraphPad Prism 5 considering P < 0.05 as statistically significant.

RESULTS

BMP4 is secreted by human adipocytes. BMP4 has been shown to induce commitment of pluripotent C3H10T1 cells to the adipocyte lineage (2, 33). Furthermore, BMP4 mRNA is induced in primary human preadipocytes undergoing adipogenic differentiation (9), suggesting that BMP4 may be a secreted factor acting on adipocyte differentiation in a paracrine manner. Thus we first examined BMP4 protein levels in primary hASCs undergoing adipogenic differentiation. BMP4 is constitutively expressed during differentiation of primary hASCs, with a trend towards a decrease at later stages of differentiation (Fig. 1A). Moreover, we show for the first time that BMP4 is secreted from differentiated hASCs, as determined by immunoblot of concentrated supernatants from seven different donors (Fig. 1C). The detected band for BMP4 has a molecular mass of 47 kDa, corresponding to the uncleaved propeptide of BMP4 (UniProt entry P12644). We also assessed BMP7 protein levels during adipogenesis of hASCs. We found that BMP7 (UniProt entry P18075) is present as a 50-kDa form, representing the precursor (data not shown), and as smaller 15.7-kDa protein, reflecting the cleaved mature protein (Fig. 1B). Levels of mature BMP7 were slightly but not significantly upregulated during adipogenesis (Fig. 1B). Interestingly, we could not show secretion of BMP7 from differentiated hASCs, since BMP7 was not detectable in the same concentrated supernatants used for detection of BMP4 (Fig. 1D).

To compare the effect of BMP4 to BMP7 on the white-to-brown shift of hASCs, we determined equipotent concentrations of BMP4 and BMP7. Therefore, undifferentiated hASCs were challenged with different concentrations of BMP4 or BMP7 for 30 min and SMAD1/5/8 phosphorylation, the main signaling pathway activated by BMPs, was measured. We observed a dose-dependent increase of BMP4- and BMP7-induced SMAD1/5/8 phosphorylation with similar maximal BMP4/7 concentrations (Fig. 2A). Furthermore, SMAD1/5/8 phosphorylation induced by BMP4 and BMP7 was time dependent (Fig. 2B). For long-term treatments we chose a concentration of 50 ng/ml for BMP4 and BMP7, which induced

A

B

C

D

Fig. 1. Bone morphogenetic protein 4 (BMP4) but not BMP7 is secreted from differentiated hASCs. A and B: human adipose stem cells (hASCs) were differentiated for the indicated time points and BMP4 (A) and BMP7 (B) protein levels were analyzed by immunoblot. All data are normalized to β-actin and expressed relative to day 0. Data are expressed as mean values ± SE; n = 5. C and D: to analyze secretion of BMP4 and BMP7 from adipocytes, supernatants of hASCs from 7 different donors at day 14 of differentiation were collected after 24 h, 100× concentrated, and subsequently analyzed by Western blot for BMP4 (C) and BMP7 (D). F9: F9 cell lysate (sc-2245; Santa Cruz Biotechnology) was used as a positive control for BMP7; ID: immunodetection.
maximal SMAD1/5/8 phosphorylation. Previous studies in preadipocytes and mesenchymal stem cells used similar BMP concentrations (9, 10, 15, 21, 27, 37).

**BMP4 and BMP7 induce a brite-like expression profile in hASCs.** It is known that BMPs play a role in preadipocyte commitment and also promote differentiation of already committed preadipocytes (28). Therefore, we first investigated the effect of chronic BMP4/7 exposure on hASC differentiation. Differentiation efficiency of hASCs was significantly increased by long-term BMP4 and BMP7 treatment, as indicated by Oil Red O Staining (Fig. 2C) and PPARγ expression (Fig. 3A).

Moreover, BMP4 and BMP7 induced a brown-like mRNA expression profile in hASCs, as determined by significantly increased UCP1 expression (Fig. 3B) and reduced expression of the white-specific marker TCF21 (Fig. 3C). ZIC1, a marker for classical brown adipocytes derived from the myogenic lineage, was not altered (Fig. 3D). The transcription factor PRDM16 is a key regulator of brown adipogenesis and activates the PPARγ coactivator 1 (PGC-1) family members PGC-1α and PGC-1β (30), which play a role in the regulation of mitochondrial function (34). The key regulator PRDM16 was slightly, but not significantly, upregulated by BMP4 and BMP7 (Fig. 3F). While PGC-1α expression was also not enhanced by BMP4 and BMP7 (Fig. 3E), PGC-1β expression was significantly increased by BMP4 and BMP7 (Fig. 3F).

Since mitochondrial content and function is enhanced in brite adipocytes (19), we studied mRNA expression levels of further genes encoding for transcriptional regulators of mitochondrial function and mitochondrial proteins. The transcription factors NRF1 and TFAM, which are involved in mitochondrial biogenesis (25), were not affected by BMP4 or BMP7. Expression of some genes like CYCS and UCP2 was significantly enhanced by BMP4 and BMP7, whereas expression of OXR1 and UCP3 was downregulated in BMP4- and BMP7-treated hASCs (Fig. 3F).

The ability to induce UCP1 in response to BMP4 and BMP7 was highly variable between donors (1.4- to 13.9-fold and 1.5- to 17.2-fold, respectively; Fig. 4A). Recently, CD137 and TMEM26 have been described as brite adipocyte-selective markers and murine precursor cells expressing high levels of CD137 showed a higher potential to induce browning in response to the newly identified myokine irisin (42). Therefore, we measured CD137 mRNA levels in undifferentiated hASCs of nine donors and separated them into CD137 low- and high-expressing group (Fig. 4B). Interestingly, the mean BMI was similar in the CD137 low- and high-expressing group (29.3 ± 1.3 and 28.9 ± 1.2 kg/m², respectively). CD137 high-expressing donors showed a significantly higher induction of UCP1 in response to BMP4 and BMP7 than CD137 low-expressing donors (Fig. 4D), while there is no significant difference in PPARγ expression between the two groups (Fig. 4C).

**Mitochondrial content and function are not increased in hASCs by BMP4 and BMP7.** Next, we aimed to assess if the observed browning effects of BMP4 and BMP7 on UCP1 and mitochondrial gene expression resulted in a higher mitochondri-
Fig. 3. BMP4 and BMP7 induce a brite gene expression pattern in hASCs. hASCs from different donors were induced for adipogenic differentiation under standard conditions or with the addition of recombinant human BMP4 or BMP7 (50 ng/ml). Medium and recombinant BMP4/7 were replaced every 2–3 days and cells were differentiated for 14 days. A–E: total mRNA was isolated and mRNA expression of the general marker for adipogenesis peroxisome proliferator-activated receptor-γ (PPARγ; A), the brite marker uncoupling protein 1 (UCP1; B), the white-specific marker transcription factor 21 (TCF21; C), the myogenic marker ZIC1 (D) and PPARγ coactivator 1 (PGC1α; E) was analyzed via quantitative real-time PCR. Data are normalized to β-actin mRNA levels and expressed relative to control on day 14. Data are mean values ± SE; n = 7–14. ***P < 0.001, **P < 0.01, *P < 0.05. F: further transcription factors involved in white-to-brown transition and mitochondria-related genes were analyzed in hASCs at day 14 of differentiation with a microfluidic card TaqMan gene expression assay. Expression levels of PRDM16, PPARα, PGC1α, NRF1, TFAM, OXR1, UCP2, UCP3, CYCS, and VDAC1 were determined in relation to GAPDH and β-actin mRNA levels and expressed related to control. Data represent mean values ± SE; n = 4–9. ***P < 0.001, **P < 0.01, *P < 0.05.

BMP4 and BMP7 promote browning of hASCs. BMP4 is a novel adipokine secreted from hASCs. BMP4 is expressed and secreted from proliferating A33 cells, which is an adipogenic subline of the pluripotent 10T1/2 cell line (2). Furthermore, BMP4 mRNA is induced in human preadipocytes undergoing differentiation. The inhibition of adipogenic differentiation by the BMP4 inhibitor noggin strongly suggests a paracrine function for BMP4 in adipogenesis (9). Our results confirm that BMP4 is secreted from differentiated hASC and promotes adipogenic differentiation of hASCs. It should be noted that we observed secretion of a 47-kDa BMP4 peptide, corresponding to the uncleaved propeptide of BMP4. BMPs are described to be fully processed to their mature form before secretion (26). However, BMP4 and other BMP members can form complexes with their prodomains after cleavage and be secreted in this form (3). Since we were not able to show BMP7 secretion, BMP7 does probably not act on adipogenesis in an auto-/paracrine manner. In line with our findings, BMP4 and BMP7 increased lipid accumulation in murine brown preadipocytes (37) and BMP7 promoted adipogenesis of human preadipocytes derived from white AT (27).

Besides the effect of BMP4 and BMP7 on adipogenic differentiation of hASCs, we observed gene expression pat-
The ability to induce UCP1 in response to BMP4/7 is variable between donors and related to CD137 expression. A: PPARγ and UCP1 expression in hASCs at day 14 of differentiation in response to BMP4 and BMP7 was assessed by quantitative RT-PCR. Some donors show a very strong induction of UCP1 in response to BMP4/7, while the effect on PPARγ is comparable between donors. Date are normalized to β-actin and expressed related to control; n = 16. B: undifferentiated hASCs from 9 different donors were analyzed for CD137 expression by quantitative RT-PCR and separated into CD137 high and low expressing donors. There was no significant difference in body mass index (BMI) between the CD137 low and CD137 high group (29.3 ± 1.3 and 28.9 ± 1.2 kg/m², respectively). C and D: comparison of PPARγ (C) and UCP1 (D) mRNA expression levels between CD137 low- and high-expressing donors on day 14 of differentiation. Donors expressing high levels of CD137 show a significant stronger induction of UCP1 in response to BMP4 and BMP7 compared with CD137 low expressing donors. Data are mean values ± SE. and expressed related to control. *P < 0.05 CD137 low vs. CD137 high.

tems that have been described for brite adipocytes (19, 20). TCF21 has been described as a white-specific marker gene, which is downregulated in white murine preadipocytes chronically challenged with rosiglitazone triggering functional features of brown adipocytes (19). We observed reduced expression of TCF21 in hASCs treated with BMP4 and BMP7. Importantly, TCF21 was a marker independent of differentiation in our model system. However, UCP1 mRNA expression was differentiation dependent and is upregulated in mature adipocytes (12, 19, 27). This raises the question whether the observed increase of UCP1 expression induced by BMP4 and BMP7 is solely related to the BMP4- and BMP7-mediated promotion of adipogenic differentiation. Nevertheless, the BMP4- and BMP7-induced increase of UCP1 mRNA (6.2- and 7.7-fold, respectively) was higher than their effect on differentiation, as determined by lipid accumulation (2.5- and 2.6-fold, respectively) and PPARγ mRNA level (4.2- and 4.1-fold, respectively). Thus increased expression levels of UCP1 together with decreased expression levels of the differentiation-independent marker TCF21 indicate a white-to-brown switch of hASCs challenged with BMP4 and BMP7. A previous study in primary human preadipocytes derived from the subcutaneous depot showed similar effects of BMP7 on UCP1 and a differentiation marker. Furthermore, this study provided evidence that the subcutaneous depot in mouse and human AT displays the highest capacity to undergo a white-to-brown transition (27). All hASCs used for this study were isolated from the subcutaneous AT.

An interesting observation of this study was the variable ability of different donors to induce UCP1 expression in response to BMP4/7. Recently, the cell surface protein CD137 has been described as a new brite-selective marker gene in mice. CD137-selected murine preadipocytes displayed elevated expression levels of UCP1 and a higher ability to induce a brite phenotype in response to the recently described hormone irisin (42). We detected a stronger potential to induce UCP1 expression in response to BMP4/7 in those donors highly expressing CD137. Thus CD137 might also be a marker for brite precursor cells in humans. However, the comparability between mouse and human AT depots is currently puzzling. Wu et al. (42) suggested, that brown AT from the adult human consists of brite adipocytes rather than of classical brown adipocytes derived from a myogenic origin. However, recent studies provided evidence for the existence of classical brown AT in humans (5, 11, 13), indicating that human brown AT is composed of brite and classical brown adipocytes. It should be noted that active brown AT cannot be found in all humans and decreases with increasing BMI (23, 38, 43). Vice versa, brown AT can be recruited in humans and morbidly obese patients negative for brown AT became brown AT-positive after weight loss. Interestingly, not all patients were able to recruit brown AT (39). In line with that, our results indicate that not all individuals might have the ability to induce thermogenically active adipocytes. To counteract human obesity by targeting brown/brite AT, it is of importance to identify the developmental origins of these tissues in humans and to identify markers that label cells capable to induce thermogenically active adipocytes.

Assessment of white-to-brown transition solely based on UCP1 expression without any functional readout is critical,
since expression of UCP1 mRNA does not necessarily lead to increased energy dissipation (17). To strengthen this study we analyzed mitochondrial biogenesis and function, which are enhanced in brite adipocytes. Increased mitochondrial biogenesis can be induced in human adipocytes by certain stimuli, such as CNPs (1), T3 (12), and PPARγ agonists (8, 20). Here, we found that BMP4 and BMP7 upregulate OXPHOS complexes in hASCs. This effect probably reflects the increased differentiation induced by BMP4 and BMP7, since mitochondrial content is upregulated during differentiation of 3T3-L1 adipocytes (41), rat adipocytes (14), and also in our human cell model. Immunofluorescence staining for MCT02 confirmed that there is no difference in MCT02 abundance between the differentiated lipid-loaden hASCs treated with and without BMP4/7. The increased oxygen consumption in BMP4/7 challenged hASCs can therefore also be related to the differentia-

Fig. 5. Mitochondrial content and function in differentiated hASCs is not increased by BMP4 and BMP7. hASCs from different donors were induced for adipogenic differentiation under standard conditions or with the addition of recombinant human BMP4 or BMP7 (50 ng/ml). Medium and recombinant BMP4 and BMP7 were replaced every 2–3 days. A: on day 14 of differentiation cells were lysed and analyzed for OXPHOS complexes by immunoblot with a commercially available OXPHOS antibody cocktail. BMP4 and BMP7 increased protein levels of the single complexes and of OXPHOS complexes in total. Results are normalized to β-actin protein levels and expressed relative to control. Data are mean values ± SE; n = 9. ***P < 0.001, **P < 0.01, *P < 0.05. Representative blots are shown. B: OXPHOS complexes in differentiated hASCs (day 14) compared with undifferentiated hASCs (day 0). Results are normalized to β-actin and expressed relative to day 0. Data are mean values ± SE; n = 6–7. *P < 0.05. Representative blots are shown. C: basal and uncoupled respiration was measured in differentiated hASC at day 14. Data are mean values ± SE; n = 5–6. *P < 0.05 vs. control. D: immunofluorescence staining against mitochondrial marker MTC02 (green) and staining of nuclei with DAPI (blue) in hASCs differentiated under control conditions and challenged with BMP4 or BMP7. MTC02 abundance is higher in differentiated hASCs (open arrowhead) compared with nondifferentiated hASCs (closed arrowhead).
tion effect of the BMPs. Mitochondrial biogenesis in AT is mainly controlled by members of the PGC-1 family (24). Of note, PGC-1β, but not PGC-1α was upregulated in differentiated hASCs by BMP4/7 treatment. In line with that, PGC-1β has been proposed to be involved in general mitochondrial biogenesis in white AT, while PGC-1α plays a role in brown AT mitochondrial function (18). Nevertheless, BMP7 is able to enhance mitochondrial biogenesis in murine brown preadipocytes accompanied by increased PGC-1α mRNA expression (37) and overexpression of BMP4 in murine white AT increased PGC-1α mRNA levels and mitochondrial biogenesis (21). Thus BMP4 and BMP7 can affect mitochondrial biogenesis in murine models but were not able to increase brown-related mitochondrial biogenesis in our human cell model. To our knowledge, there are no reports on the role of BMP4 and BMP7 on mitochondria in primary human adipocytes. Taken together, we showed here that BMP4 and BMP7 induce a brite gene expression pattern in hASCs but do not induce a full white-to-brown transition with increased mitochondrial function. AT plasticity is a complex scenario, and other AT-derived factors may be necessary to synergize with BMP4 and BMP7 to induce complete browning in human white AT.

Our findings differ from the current view of BMP4 as a player in white adipogenesis and BMP7 as promoter of brown adipogenesis (16, 28, 37). Importantly, this view is based on different studies using different cell model systems (murine vs. human; brown AT vs. white AT) and the effects of BMP4 and BMP7 have been compared only in a few cases. In the study by Tseng et al. (37), BMP4 and BMP7 had the same effect on lipid accumulation in murine brown preadipocytes, but only BMP7 was able to significantly induce UCP1 expression whereas BMP4 suppressed expression of UCP1 in these brown preadipocytes. The induction of UCP1 mRNA expression by BMP7 has also been shown in primary human preadipocytes derived from subcutaneous AT. But a longer treatment with BMP7 was necessary to induce browning in human preadipocytes than in murine preadipocytes and the effect on UCP1 expression was less strong (27). Furthermore, only a slight effect of BMP7 on UCP1 expression has been observed in human multipotent adipose-derived stem cells (20). We also show a slight effect of BMP7 on UCP1 expression and no enhancement of mitochondrial biogenesis, suggesting a lower potency of BMP7 to induce browning in human preadipocytes compared with murine cells. Moreover, BMP4 did not suppress UCP1 expression in hASCs but exerted similar effects as BMP7 on all aspects of white-to-brown transition in primary hASCs. The inhibitory effect of BMP4 on brown adipogenesis, as shown in murine brown preadipocytes (37), has never been investigated in human white preadipocytes. Furthermore, there was recent evidence that BMP4 plays a different role in white and brown

![Diagram showing the effect of BMP4 and BMP7 on AT precursor cells in mouse and human models.](http://ajpcell.physiology.org/)

**Fig. 6.** Effect of BMP4 and BMP7 on AT precursor cells is dependent on the AT depot and species. BMP4 and BMP7 are able to induce UCP1 in murine and human white preadipocytes. In brown preadipocytes/AT, exclusively BMP7 promotes brown adipogenesis, while BMP4 suppresses UCP1 expression. Comparing murine and human models, BMP4 and BMP7 show a stronger effect on browning in murine model systems. MSC, mesenchymal stem cells. ↑ Slight UCP1 upregulation; ↑ strong UCP1 upregulation; ↓ UCP1 downregulation.

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AT. In transgenic mice with AT-specific overexpression of BMP4, mRNA levels of UCP1 and the key factors of brown adipogenesis PGC-1α and PRDM16 were strongly increased in white AT, while the expression of these genes were decreased in brown AT (21). The myokine irisin is another example uncovering differences between mice and humans. While irisin induced browning in murine preadipocytes derived from white AT (42), we recently showed that irisin has no effect on white-to-brown transition in human white preadipocytes (22).

Comparing our results with these previous studies supports the idea that there are discrepancies of the action of BMPs between human vs. murine models and white vs. brown AT precursor cell origin (summarized in Fig. 6). Additionally, AT plasticity is a complex scenario and rather a cocktail of factors than BMP4 and BMP7 alone may be necessary to induce a complete white-to-brown transition in human white AT.

Given this and taking into account, that BMP signaling is dependent on the receptor composition, the presence of ligands, and the extracellular antagonists (3), it is likely that BMP4 and BMP7 might have a similar effect on precursor cells derived from white AT but play a differential role in brown precursor cells. In addition, BMP4 and BMP7 may be more potent inducers of browning in mice than in humans. Furthermore, we here show that BMP4 is a new adipokine and acts on adipogenesis and white-to-brown transition in an auto-/paracrine manner. It has been shown recently that BMP4 expression is higher in white AT from lean compared with obese humans (21). Therefore, BMP4 represents a potential target in the fight against obesity and its regulation in states of AT inflammation should be addressed in further studies. Finally, our results indicate that not all donors have the ability to strongly induce UCP1 in response to BMP4/7 and this feature was related to CD137 expression. To promote white-to-brown transition of human white AT as a therapeutic approach, it is essential to know which cells can be targeted. Thus markers labeling human cells that possess a high potential to undergo browning should be investigated.

ACKNOWLEDGMENTS

We thank J. Liebau (Department of Plastic Surgery, Florence-Nightingale-Hospital, Düsseldorf, Germany) and C. Andree (Department of Plastic Surgery, Sana-Hospital, Düsseldorf-Gerresheim, Germany) for support in obtaining AT samples. The secretarial assistance of B. Hurow and the technical help of A. Cramer, M. Esser, I. Rokitta, S. Kauffelt, and A. Schlüter are gratefully acknowledged.

GRANTS

This work was supported by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen (Ministry of Science and Research of the State of North Rhine-Westphalia) and the Bundesministerium für Gesundheit (Federal Ministry of Health). T. Romacho is the recipient of a FP7 Marie Curie Intra-European Fellowship. This study was supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e.V.) and the European Foundation for the Study of Diabetes (EFSID).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


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