

OPEN
MATTERS ARISING

Browning capabilities of human primary adipose-derived stromal cells compared to SGBS cells

D. Halbgebauer, M. Dahlhaus, M. Wabitsch, P. Fischer-Posovszky & D. Tews

ARISING FROM: C. R. Yeo *et al.*; *Scientific Reports* <https://doi.org/10.1038/s41598-017-04369-2> (2017).

Induction of a brown adipocyte differentiation program in white adipocytes (so-called “browning”) by pharmacological agents leads to improved energy metabolism thus providing a therapeutic option to treat obesity. In a recent study, Yeo *et al.* claimed that the Simpson-Golabi-Behmel syndrome (SGBS) cell strain is superior in browning compared to primary adipose-derived stromal cells (hASCs); however, different media compositions were used for the different cell types potentially hampering interpretation of results. Comparing both cell types under equal conditions we demonstrate that both, SGBS and hASCs, differentiate into brown-like adipocytes, but with differences regarding UCP1 expression and mitochondrial content underlining that media conditions strongly influence the adipocyte phenotype. This should be considered in future studies.

In contrast to white adipose tissue (WAT), the primary energy storage organ of the body, brown adipose tissue (BAT) is able to metabolize lipids and glucose to produce heat in order to defend body temperature against cold¹.

Upon chronic cold exposure, white adipose tissue (WAT) can partially convert into thermogenic, beige adipose tissue, which shares main similarities with classical brown adipose tissue (BAT). This conversion of WAT to beige fat, often called “browning” or “beigeing” is associated with improved glucose and lipid metabolism in mice². In humans, data on WAT browning is scarce³ but long-term cold exposure of human subjects is associated with increased BAT activity and a decrease in body fat mass^{4,5}. Thus, activation of BAT has frequently been suggested as a therapeutic option to prevent or to treat obesity⁶. While BAT and beige fat develops in clear distinct depots in mice, the discrimination between brown and beige adipocytes is less defined in humans⁷.

To better understand the molecular mechanism of WAT browning, *in vitro* cell models of murine and human origin have been frequently used, including primary adipose-derived stem cells, iPS cells, hMADS and SGBS cells^{8–10}. In a recent paper published by Yeo *et al.* in this journal, SGBS cells were compared to human primary adipose-derived stromal cells (hASCs) in terms of adipocyte browning *in vitro*¹¹. The authors showed that SGBS adipocytes had more UCP1 mRNA expression compared to primary cells. Additionally, SGBS adipocytes showed higher respiration rates compared to primary hASCs. Based on these findings, they claimed that SGBS cells have a higher capacity to differentiate into brown-like adipocytes and concluded that SGBS cells represent a model for human brown adipocytes. Obviously however, SGBS cells in their study were apparently better differentiated than primary cells as judged by differences in lipid content and expression of adipogenic marker genes. This is most likely based on differences in media conditions used in this study.

SGBS cells were derived from subcutaneous white adipose tissue of an infant suffering from the Simpson-Golabi-Behmel syndrome (SGBS) and were established as cell strain by our lab¹². Although the cells are not transformed or immortalized, they retain their capacity to differentiate into adipocytes *in vitro* for more than 50 generations¹³. The molecular reason for this is currently unknown. With more than 150 published articles, SGBS cells represent a valuable cell model for human adipogenesis and adipocyte biology. Yeo *et al.* recently claimed that SGBS cells have a particularly high capacity to undergo browning, whereas hASCs derived from subcutaneous white adipose tissue represent a model system to study white adipocytes¹¹. Indeed, SGBS cells have been used to study *in vitro* browning of adipocytes^{10,14}. In comparative studies performed so far SGBS behaved very similar to primary *in vitro* differentiated adipocytes¹³.

Therefore, we hypothesize that both SGBS as well as hASCs behave similarly regarding differentiation into brown-like adipocytes. To address this, we investigated marker gene expression of brown adipogenesis in SGBS and hASCs upon differentiation. In order to account for differences in media composition, we also compared rosiglitazone and indomethacin towards their ability to induce adipogenic differentiation, BAT marker expression and mitochondrial metabolism.

Division of Pediatric Endocrinology and Diabetes, Department of Pediatric and Adolescent Medicine, Ulm University Medical Center, Ulm, Germany. ✉e-mail: Daniel.Tews@uniklinik-ulm.de

Materials and Methods

Ethical note. All procedures involving human subjects were approved by the ethics committee of the University of Ulm (entry number 300/16). Written informed consent was obtained from all subjects and all associated methods were conducted in accordance with approved guidelines for human experimental research.

Cell culture. Human Simpson-Golabi-Behmel (SGBS) preadipocytes were cultured as described in the original publication¹². Human adipose-derived stromal cells were isolated from mammary adipose tissue from $n = 7$ women (mean age 48 ± 16 years, mean BMI 27.4 ± 5.2 kg/m²) undergoing elective surgery using collagenase digestion (type II, Sigma-Aldrich, Munich, Germany) according to established protocols¹⁵.

SGBS preadipocytes and hASCs were seeded into cell culture vessels and were differentiated for 14 days into mature adipocytes using differentiation media (DMEM:F12 supplemented with 20 nM insulin, 100 nM cortisol, 25 nM dexamethasone, 250 μ M IBMX, 10 μ g/ml apo-transferrin, 3.3 mM biotin, 1.7 mM panthotenate) supplemented with either rosiglitazone (2 μ M) or indomethacin (100 μ M). After 4 days, rosiglitazone/indomethacin, IBMX and dexamethasone were omitted. Differentiation rates were determined by microscopic cell counting using a net micrometer and dividing differentiated adipocytes by total cell number.

In a different study, ASCs were isolated from paired deep neck and subcutaneous neck adipose tissue from $n = 12$ patients undergoing neck surgery¹⁶ (age 47.4 ± 18.0 years; BMI 27.3 ± 5.3 kg/m²), and were differentiated as described before¹⁶.

Triglycerides were extracted from adipocytes using hexane:isopropanol (3:1) and were dissolved in isopropanol after evaporation. Triglyceride content was determined using the Triglyceride determination kit (Merck, Darmstadt, Germany).

Expression analysis. Extraction of total RNA, synthesis of cDNA and analysis of mRNA expression by qPCR was done as described previously¹⁷. Relative mRNA levels were determined by comparison to a reference gene (TF2B, SDHA) using the ddCT method. Primer sequences are available on request.

Protein quantification and Western blot. Extraction of cellular proteins, determination of protein content and immunodetection was described before¹⁷. Expression of target proteins was analyzed by incubating membranes with primary antibodies (anti-UCP1 MAP6158, R&D; anti-OXPHOS ab110411, Abcam; anti-PGC1a ab54481, Abcam; anti-PLIN ab3525, Abcam; anti-TIMM23 ab116329, Abcam; hFAB rhodamine anti-GAPDH 12004168, BioRad) and HRP-conjugated secondary antibodies. ECL signals were detected using a ChemiDoc MP Imaging system (BioRad Laboratories GmbH, Munich, Germany).

Citrate synthase assay. Citric acid synthase activity was assayed as a measure for mitochondrial content as described previously¹⁷.

Functional extracellular flux analysis. Oxygen consumption was determined using a plate-based respirometer (Seahorse XFe96 Flux Analyzer, Agilent Technologies). Preparation of cells and measurement of the cellular respiration was described before¹⁷. Data was normalized to cell number by quantification of Janus Green incorporation¹⁸.

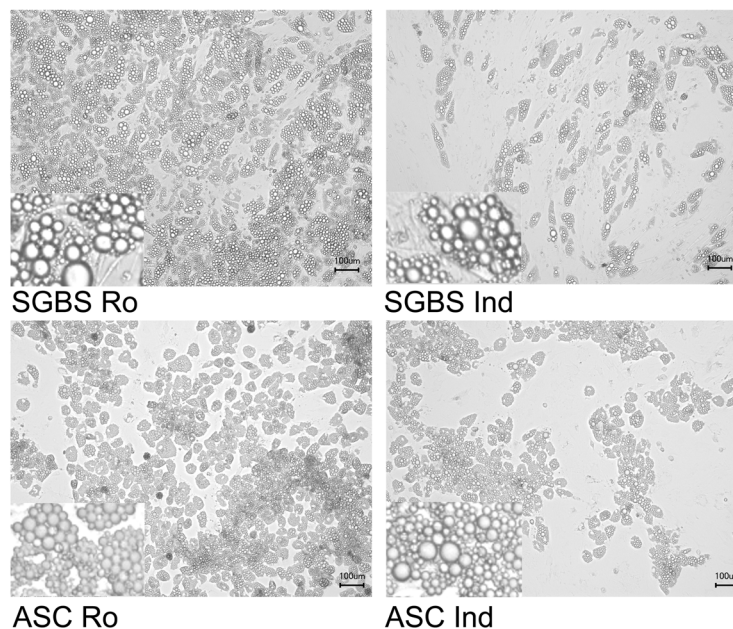
Statistics. GraphPad Prism version 7.03 (GraphPad Software Inc., San Diego, USA) was used for statistical analysis. If not otherwise stated, data from three independent triplicate experiments were expressed as mean \pm standard error of means (SEM). For statistical comparison, Analysis of variants test (ANOVA) or t-test was used as indicated in the figure legends. A p value $p < 0.05$ was considered statistically significant.

Results

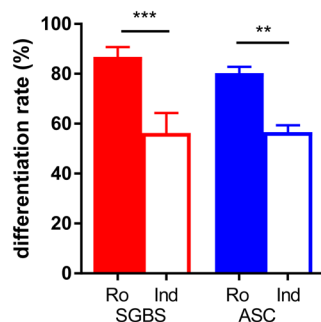
Adipogenic differentiation depends on media conditions. SGBS and hASCs derived from mammary subcutaneous adipose tissue were subjected to adipogenic differentiation in media supplemented with either rosiglitazone or indomethacin. Within 14 days, cells accumulated lipids and turned from fibroblastic into the characteristic shape of *in vitro* differentiated adipocytes. Obviously, fewer cells were differentiated when using indomethacin in both SGBS and hASCs (Fig. 1A). This was also evident when analyzing differentiation rates (Fig. 1B). Under rosiglitazone conditions, both SGBS and hASC differentiated to a comparable extent (86.8 ± 8.7 and $80.3 \pm 6.6\%$). Using indomethacin, differentiation rates were much lower in general, but also comparable between both cell types (56.3 ± 17.9 and $56.6 \pm 7.1\%$). These results were also reflected by comparable expression rates of the adipogenic marker genes PPAR γ (peroxisome proliferator-activated receptor gamma), adiponectin, and GLUT4 (glucose transporter 4, Fig. 1D). Interestingly, SGBS cells seemed to have larger lipid droplets compared to hASCs (Fig. 1A), which was also reflected by increased triglyceride content (Fig. 1C).

Adipocyte browning capacity is different in SGBS cells and hASCs. Subsequently, we assessed the expression of BAT-related genes in SGBS and hASCs under the chosen conditions. UCP1 was higher expressed in adipocytes differentiated with rosi compared to indomethacin, where UCP1 expression was hardly detectable (Fig. 2A). Interestingly, SGBS cells showed stronger UCP1 expression compared to hASCs when differentiated with rosiglitazone. CIDEA was equally expressed between SGBS and hASCs, being approximately 3-fold higher expressed in rosi compared to indo conditions. DIO2 (deiodinase 2) was induced by rosiglitazone compared to indo in hASCs only. Surprisingly, PRDM16 was not induced by rosiglitazone in both cell types (Fig. 2A).

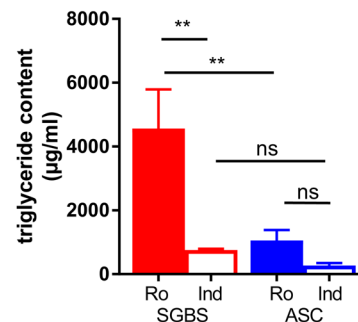
A



B



C



D

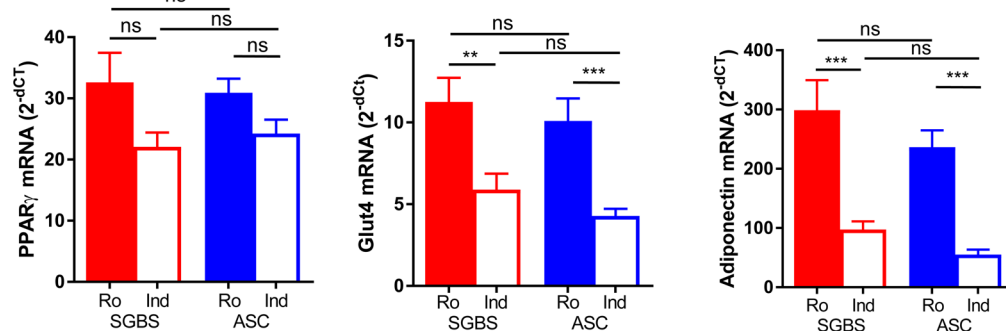


Figure 1. Differentiation of SGBS preadipocytes or hASCs with indomethacin or rosiglitazone. SGBS preadipocytes ($n=5$ independently performed experiments) or human adipose stromal cells (hASC, $n=7$ different donors) were differentiated *in vitro* using either 100 μM indomethacin (Ind) or 2 μM rosiglitazone (Ro) for 14 days, representative photomicrographs shown in (A) insets 5-fold enlarged. The adipogenic differentiation rate (B) was determined microscopically. Triglyceride content was enzymatically determined ($n=3-4$) (C). The expression of key adipogenic marker genes was assessed by qRT-PCR using the dCt method, TF2B was used as reference gene (D). Mean \pm SEM is shown, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

To account for differences in differentiation rates between cell types and individual samples, expression data of BAT-associated genes were normalized to the geometric mean of the CT values of PPAR γ , adiponectin, and GLUT-4. Interestingly, UCP1 mRNA as well as protein expression was higher in SGBS compared to hASCs even after correction for differentiation, suggesting higher propensity to browning in SGBS cells. (Fig. 2B).

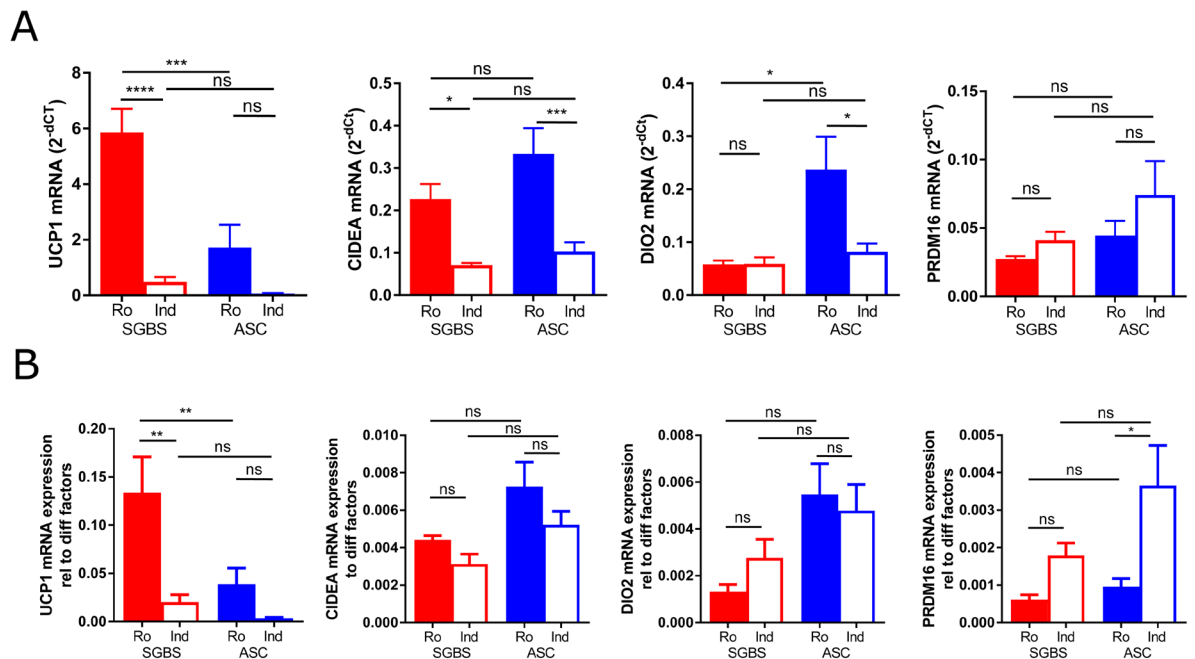


Figure 2. Expression of BAT-associated genes in SGBS or hASCs differentiated with indomethacin or rosiglitazone. SGBS preadipocytes ($n=5$ independently performed experiments) or human adipose stromal cells (hASC, $n=7$ different donors) were differentiated *in vitro* using either $100\mu\text{M}$ indomethacin (indo) or $2\mu\text{M}$ rosiglitazone (rosi) for 14 days. Total RNA was isolated and the expression of UCP1, CPT1B, PGC1a and DIO2 was determined by qRT-PCR using TF2B as reference (A). Data was also normalized using the geometric mean of adipogenic marker genes in Fig. 1D as reference (B). Mean +SEM is shown, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

To identify differences in mitochondrial metabolism, we performed respiration analyses using a plate-based respirometer (Fig. 3A). Upon differentiation with rosiglitazone, basal, proton leak, and maximal respiration was significantly higher in hASCs compared to SGBS adipocytes (Fig. 3C). This effect was also observed, although not significantly different, in indomethacin-differentiated cells (Fig. 3D). Of note, cAMP-driven increase in respiration was higher in cells differentiated with rosiglitazone, but there was no difference between hASCs and SGBS (Fig. 3B), suggesting that UCP1 activation by the release of free fatty acids was not different between cell types. Respiration according to ATP production was not different between SGBS and hASC adipocytes, indicating that differences in basal respiration are based on proton leak only.

Mitochondrial density is increased in hASC compared to SGBS cells. The overall higher mitochondrial metabolism in hASC suggests that the amount of mitochondria is higher in these cells compared to SGBS cells. Indeed, mRNA expression of the mitochondrial marker genes CPT1B, COX8A, and CYC1 was significantly higher in hASC compared to SGBS adipocytes when differentiated with rosiglitazone (Fig. 4A). Under indomethacin conditions, COX8A and CYC1 expression was elevated as well. In both conditions, the transcription factor essential for mitochondrial biogenesis, PGC1a, was highly increased in hASCs compared to SGBS, indicating enlarged mitochondrial content in hASC adipocytes. Protein markers of the mitochondrial electron transport chain (ETC) and the marker of the inner mitochondrial membrane TIMM23 were elevated in hASCs compared to SGBS when differentiated with rosi (Fig. 4B). Interestingly, PGC1a, the key factor for mitochondrial biogenesis, was also higher expressed in hASCs upon rosi treatment compared to SGBS. In concordance with this, activity of citrate acid synthase was also increased in rosi-differentiated hASC adipocytes compared to SGBS (Fig. 4C).

Browning capacity is dependent on adipose depot/microenvironment. Both SGBS cells as well as hASCs derive from subcutaneous white adipose tissue. In order to appreciate their browning capacity, we compared SGBS and hASCs to progenitor cells isolated from subcutaneous and deep neck adipose tissue obtained from neck surgeries¹⁶, all differentiated in the presence of rosiglitazone. Clearly, cells derived from the deep neck depot displayed the strongest UCP1 expression (approx. 6-fold higher compared to subcutaneous cells), indicating highest ability to differentiate into brown adipocytes (Fig. 5). All the other cells derived from different subcutaneous adipose tissue had comparable UCP1 expression. Of note, we could not detect differences in UCP1 expression between males and females (Supplemental Fig. 2).

Discussion

Cell culture models of human adipocyte progenitor cells are a valuable tool to evaluate the impact of genetic modulation or pharmacological treatment on induction of a white-to-brown shift in adipogenesis. Intrinsic differences in cell models (e.g. genetic background, immortalization etc.) as well as differences in adipogenic inducers might have strong effects on the expected outcome of the experiments. Thus, comparison of cell models and differentiation cocktails is needed to understand their impact on the regulation of adipocyte browning.

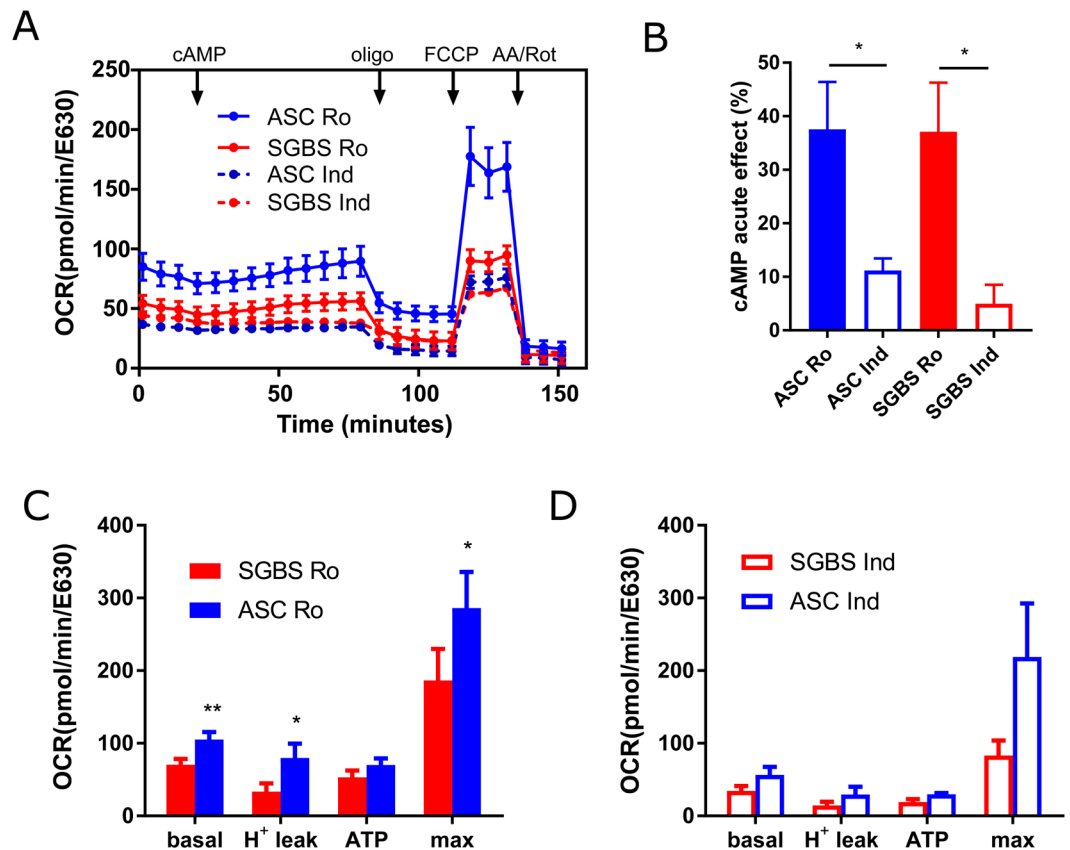


Figure 3. Mitochondrial activity of SGBS and hASC adipocytes. SGBS and hASCs ($n = 4$ each) were differentiated *in vitro* using either 100 μM indomethacin (Ind) or 2 μM rosiglitazone (Ro) for 14 days and subjected to respiration measurements using a plate-based respirometer (Seahorse XFe96, Agilent, **A**). Cells were measured in a 3 min mix – 3 min measure scheme. After 3 basal measurements, 0.5 mM dibutyryl-cAMP was injected to induce UCP1 activity by lipolysis. Oligomycin (2 μM) was added to determine ATP and proton leak-dependent respiration. Full uncoupling of mitochondrial ETC was achieved by addition of 4 μM FCCP. Finally, ETC was completely blocked by antimycin A and rotenone (1.5 μM each). Assay was performed in medium containing 1% BSA. Relative induction of respiration by cAMP was calculated as difference between first and second injection (**B**). Basal and proton leak respiration in cells differentiated with rosiglitazone (**C**) or indomethacin (**D**) was calculated from the oxygen consumption rate (OCR) plots. Data are shown as mean + SEM is shown, * $p < 0.05$, ** $p < 0.01$.

The most commonly used agents to differentiate adipocyte progenitor cells into adipocytes are thiazolidinediones (TZDs) such as troglitazone or rosiglitazone, which directly target PPAR γ , the key mediator of adipogenesis. As a „side-effect“ of strong PPAR γ induction however, thiazolidinediones are able to induce a white-to-brown phenotype switch (e.g. increased UCP1 expression) in adipocytes¹⁹. Mechanistically, TZDs act by binding to and activating PPAR γ and PPAR-response elements (PPREs) on the promoter and/or enhancer of brown fat-selective genes²⁰. This mechanism of action, however, cannot be solely responsible for white-to-brown transitions because PPAR γ is highly and equally expressed in both brown and white adipocytes and is essential for adipogenesis in both cell types^{21,22}. Of note, ectopic expression of PPAR γ does not induce white-to-brown transition²³.

In earlier differentiation protocols, indomethacin, a non-steroidal anti-inflammatory drug, was frequently used as an inducer of adipogenesis²⁴. It acts by inhibition of cyclooxygenases 1 (COX1) and -2 (COX2), and also induces PPAR γ , although activation is far lower compared to rosiglitazone²⁵. As a consequence, indomethacin does not induce UCP1 expression in white adipose tissue²⁶.

Interestingly, also other specific PPAR γ agonists fail to induce UCP1 upon adipogenic differentiation¹⁹, suggesting that UCP1 induction by rosiglitazone is not mediated by PPAR γ activation. It has been previously shown that rosiglitazone induces a white-to-brown transition in murine preadipocytes via stabilization of PRDM16, a coactivator of PPAR γ ¹⁹. Whether or not PRDM16 stabilization plays a major role in human cells is not well understood. In our study, PRDM16 expression levels were low and we did not observe differences between SGBS and hASCs, which however does not exclude a role of PRDM16 in this context. Our data are in line with this literature as adipogenic differentiation was lower after indomethacin treatment in both cell models in comparison with rosiglitazone treatment. Moreover, UCP1 expression was far less induced, indicating rosiglitazone as the more potent browning inducer in this setting. Here we want to point out the importance in choosing the right differentiation condition in experiments regarding adipose browning. One should consider that rosiglitazone is

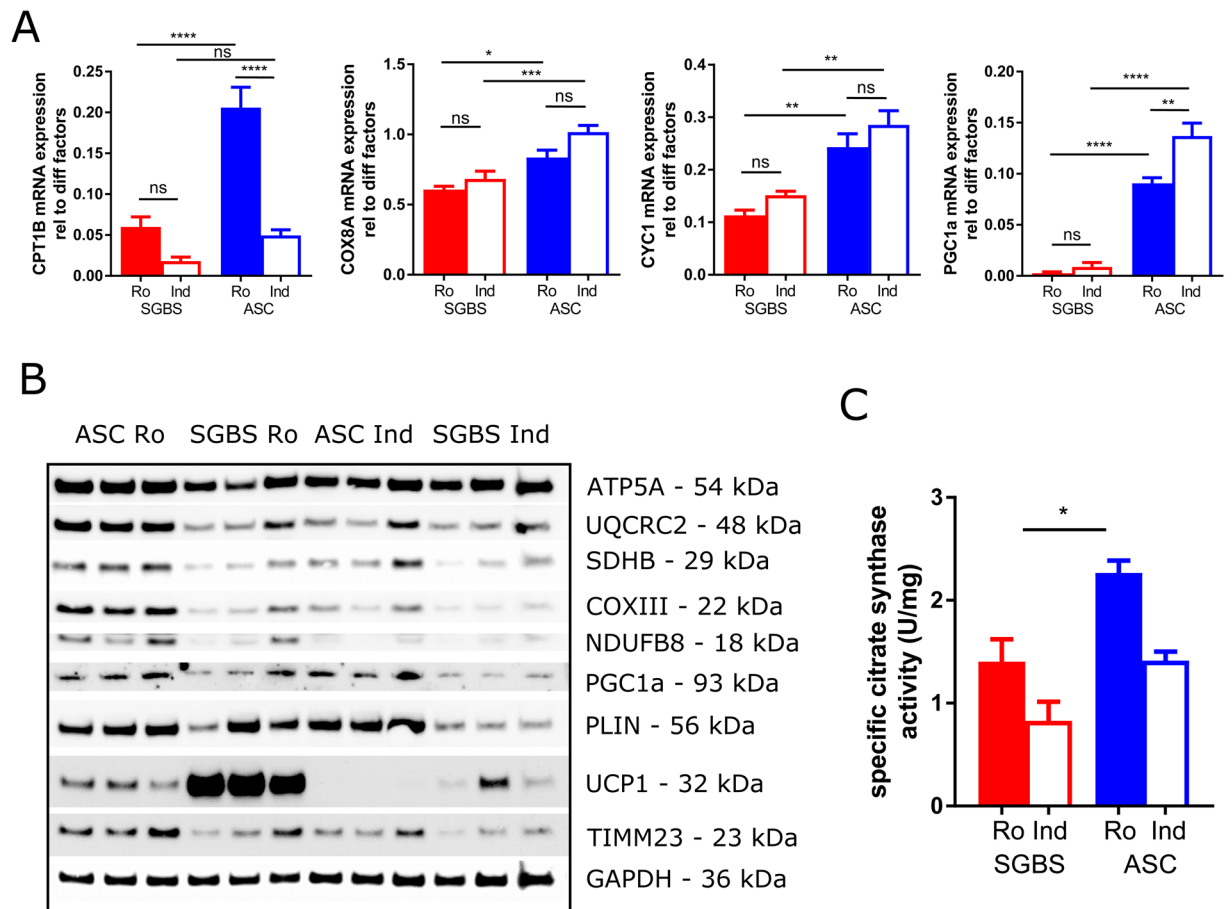


Figure 4. Mitochondrial content of differentiated SGSB and hASC adipocytes. SGSB preadipocytes (n = 5) or human adipose stromal cells (hASC, n = 7) were differentiated *in vitro* using either indomethacin (indo) or rosiglitazone (rosi) for 14 days. Expression of mitochondrial genes were analyzed using qRT-PCR (A) and on protein level (B). Activity of citrate synthase in cell lysates (n = 3 each) was assayed as a marker for mitochondrial content (C). Mean +SEM is shown, **p < 0.01, ***p < 0.001, ****p < 0.0001.

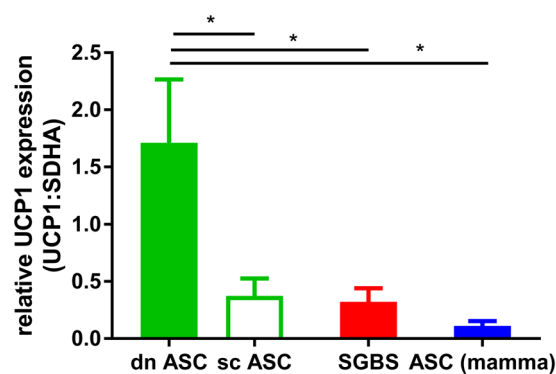


Figure 5. UCP1 expression in SGSB adipocytes compared to differentiated adipocytes derived from deep neck tissue. SGSB preadipocytes (n = 11) and hASCs from human deep neck (dn, n = 12) and subcutaneous neck (sc, n = 12) and mammary adipose tissue (n = 7) were differentiated into adipocytes as outlined in Fig. 1. UCP1 expression was analyzed by qRT-PCR using SDHA as reference gene. Mean +SEM is shown, *p < 0.05.

a potent inducer of browning¹⁹ – thus, expected targeted effects might be covered by rosiglitazone action. Even more importantly, different cell types should be differentiated under the exact same conditions as they interfere with both differentiation itself, but also with the process of browning. In contrast to the earlier publication by Yeo *et al.* we could demonstrate that – if differentiated under equal conditions – SGSB and hASCs do not differ

in expression of key adipogenic differentiation marker genes (e.g. PPAR γ , adiponectin, and GLUT-4). Thus, the absence of UCP1 mRNA expression as well as low respiration rates in hASCs in the previous paper were most likely caused by indomethacin used as an inducing agent leading to a low differentiation rate.

Although there was no significant difference in adipogenic differentiation, our data indicate that hASC and SGBS cells differ in terms of UCP1 inducibility and mitochondrial content. UCP1 expression was higher in SGBS cells compared to hASCs, however other BAT markers such as CIDEA, DIO2 and PRDM16 were either higher in hASCs or equal between the two cell types. This indicates that SGBS and primary hASCs behave differently in response to rosiglitazone treatment. Moreover, mitochondrial biogenesis is apparently higher in primary hASCs as given by the elevated mRNA levels of PGC1 α and protein expression of OXPHOS components. Higher mitochondrial content in hASCs might also explain elevated basal and maximal respiration levels compared to SGBS cells.

SGBS cells were isolated from a 3-month-old infant, this might explain their capacity to stronger UCP1 induction. During ageing there is a gradually loss of BAT in humans²⁷, and human studies also suggest that BAT activity is reduced in older subjects²⁸. Additionally, mouse studies suggest that browning capacity decreases with ageing²⁹. Variances in mitochondrial biogenesis might be caused by either to different origin of adipose tissue (subcutaneous versus mammary) or the underlying disease of the donor (SGBS). In-depth sequencing of the SGBS cells might identify the cause for reduced mitochondrial content.

Both SGBS cells and hASCs showed the capacity to differentiate into UCP1 expressing adipocytes and might reflect the situation occurring in white adipose tissue *in vivo*. Compared to progenitor cells from the deep neck depot¹⁶, a site where brown adipocytes can be found in humans, the induction of UCP1 expression is lower in SGBS and hASCs and is comparable to subcutaneous-derived cells from the neck. It has been shown by us and others that cells from the deep neck depot have stronger ability to differentiate into brown adipocytes *in vitro* compared to those isolated from subcutaneous tissues^{16,30,31}. This suggests that browning capacity is dependent on the given adipose tissue niche or that a certain progenitor cell exists which has the machinery to differentiate into brown-like adipocytes.

In summary, we provide evidence that both SGBS cells and hASCs are both able to differentiate into UCP1-positive adipocytes, using rosiglitazone as adipogenic inducer. Comparing these cells to cells originating from a brown adipose location it is obvious that the respective depot is an important driver of brown adipogenesis.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 27 November 2018; Accepted: 16 April 2020;

Published online: 15 June 2020

References

- Cannon, B. & Nedergaard, J. Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277–359 (2004).
- Seale, P. *et al.* Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J. Clin. Invest.* **121**, 96–105 (2010).
- Finlin, B. S. *et al.* Human adipose beiging in response to cold and mirabegron. *JCI Insight* **3**, e121510 (2018).
- Van Der Lans, Aa. J. J. *et al.* Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J. Clin. Invest.* **123**, 3395–3403 (2013).
- Yoneshiro, T. *et al.* Recruited brown adipose tissue as an antiobesity agent in humans. *J. Clin. Invest.* **123**, 3404–3408 (2013).
- Bartelt, A. & Heeren, J. Adipose tissue browning and metabolic health. *Nat. Rev. Endocrinol.* **10**, 24–36 (2014).
- Cypess, A. M. *et al.* Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat. Med.* **19**, 635–9 (2013).
- Elabd, C. *et al.* Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* **27**, 2753–2760 (2009).
- Mohsen-Kanson, T. *et al.* Differentiation of human induced pluripotent stem cells into brown and white adipocytes: Role of Pax3. *Stem Cells* **32**, 1459–1467 (2014).
- Tews, D. *et al.* FTO Deficiency Induces UCP-1 Expression and Mitochondrial Uncoupling in Adipocytes. *Endocrinology* **154**, 3141–3151 (2013).
- Yeo, C. R. *et al.* SGBS cells as a model of human adipocyte browning: A comprehensive comparative study with primary human white subcutaneous adipocytes. *Sci. Rep.* **7**, 4031 (2017).
- Wabitsch, M. *et al.* Characterization of a human preadipocyte cell strain with high capacity for adipose differentiation. *Int. J. Obes. Relat. Metab. Disord.* **25**, 8–15 (2001).
- Fischer-Posovszky, P., Newell, F. S., Wabitsch, M. & Tornqvist, H. E. Human {SGBS} Cells - a Unique Tool for Studies of Human Fat Cell Biology. *Obes. Facts Eur. J. Obes.* **1**, 184–189 (2008).
- Tews, D. *et al.* Teneurin-2 (TENM2) deficiency induces UCP1 expression in differentiating human fat cells. *Mol. Cell. Endocrinol.* **443**, 106–113 (2017).
- Hauner, H., Skurk, T. & Wabitsch, M. Cultures of human adipose precursor cells. *Methods Mol. Biol.* **155**, 239–247 (2001).
- Tews, D. *et al.* Comparative gene array analysis of progenitor cells from human paired deep neck and subcutaneous adipose tissue. *Mol. Cell. Endocrinol.* **395**, 41–50 (2014).
- Tews, D. *et al.* Elevated UCP1 levels are sufficient to improve glucose uptake in human white adipocytes. *Redox Biol.* **26**, 101286 (2019).
- Raspotnig, G. *et al.* Colorimetric Determination of Cell Numbers by Janus Green Staining. *Anal. Biochem.* **275**, 74–83 (1999).
- Ohno, H., Shinoda, K., Spiegelman, B. M. & Kajimura, S. {PPAR γ } agonists Induce a White-to-Brown Fat Conversion through Stabilization of {PRDM}16 Protein. *Cell Metab.* **15**, 395–404 (2012).
- Sears, I. B., MacGinnitie, M. A., Kovacs, L. G. & Graves, R. A. Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. *Mol. Cell. Biol.* **16**, 3410–3419 (1996).
- He, W. *et al.* Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl. Acad. Sci. USA* **100**, 15712–15717 (2003).

22. Imai, T. *et al.* Peroxisome proliferator-activated receptor gamma is required in mature white and brown adipocytes for their survival in the mouse. *Proc Natl Acad Sci USA* **101**, 4543–4547 (2004).
23. Sugii, S. *et al.* PPAR γ activation in adipocytes is sufficient for systemic insulin sensitization. *Proc. Natl. Acad. Sci.* **106**, 22504–22509 (2009).
24. Scott, M. A., Nguyen, V. T., Levi, B. & James, A. W. Current Methods of Adipogenic Differentiation of Mesenchymal Stem Cells. *Stem Cells Dev.* **20**, 1793–1804 (2011).
25. Puhl, A. C. *et al.* Mechanisms of peroxisome proliferator activated receptor γ regulation by non-steroidal anti-inflammatory drugs. *Nucl. Recept. Signal.* **13**, 1–17 (2015).
26. Madsen, L. *et al.* UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. *PLoS One* **5** (2010).
27. Heaton, J. M. The distribution of brown adipose tissue in the human. *J. Anat.* **112**, 35–39 (1972).
28. Saito, M. *et al.* High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* **58**, 1526–1531 (2009).
29. Gonçalves, L. F. *et al.* Ageing is associated with brown adipose tissue remodelling and loss of white fat browning in female C57BL/6 mice. *Int. J. Exp. Pathol.* **98**, 100–108 (2017).
30. Lee, P., Werner, C. D., Kebebew, E. & Celi, F. S. Functional thermogenic beige adipogenesis is inducible in human neck fat. *Int. J. Obes. (Lond)*. **38**, 170–176 (2014).
31. Zingaretti, M. C. *et al.* The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* **23**, 3113–3120 (2009).

Acknowledgements

This work has been supported by the German Research Association (TE912/2-2, FI1700/5-1) and by the Boehringer Ingelheim Ulm University BioCenter (BIU). PFP is supported by the Heisenberg program of the German Research Association (FI1700/7-1). DH is supported by the International Graduate School of Molecular Medicine Ulm (IGradU). The authors wish to thank Alexandra Killian for her excellent technical assistance.

Author contributions

D.H., D.T., M.W. and P.F.P. designed the study, analyzed and interpreted the data and wrote the manuscript. D.H. and M.D. collected and assembled data. All authors revised the manuscript and approved the final version of the paper.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41598-020-64369-7>.

Correspondence and requests for materials should be addressed to D.T.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2020