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Letter to the Editor

PPARγ-mediated signalling regulates mitochondrial energy metabolism in human hair follicle epithelium

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To the editor,

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the family of nuclear hormone receptors. They include three different isoforms; namely PPAR-α, PPAR-β/δ and PPAR-γ, and have many important roles in the regulation of a large number of physiological processes, including cell proliferation, differentiation and inflammatory responses (Sertznig *et al.*, 2008). Due to their prominent expression in human skin and its appendages, there is a growing interest in PPARs in human skin biology and pathology (Dozsa *et al.*, 2016; Ramot *et al.*, 2015; Ruzehaji *et al.*, 2016; Wallmeyer *et al.*, 2015; Yin and Smith, 2016). PPARγ, the most widely investigated subtype, is expressed in the epidermis and the hair follicles (HFs), and controls skin barrier permeability, inhibits keratinocyte proliferation and promotes epidermal terminal differentiation (Ramot *et al.*, 2015). We have previously shown that agonistic PPARγ modulators may exert protective functions on keratin 15+ epithelial progenitor function in human HFs, while they inhibit hair growth by inducing catagen and inhibiting the proliferation of hair matrix keratinocytes (MKs) (Ramot *et al.*, 2014).

Recently, PPARγ-mediated signalling has also been implicated in the regulation of mitochondrial energy metabolism, e.g. in adipose tissue and the brain (Chaturvedi and Flint Beal, 2013; Hock and Kralli, 2009). However, it remains to be studied whether PPARγ-stimulation impacts on the mitochondrial biology of human HF keratinocytes *in situ*, which are known to be key players in HF energy metabolism (Kloepper *et al.*, 2015). Therefore, we have asked whether the selective (agonistic) PPARγ modulator, N-Acetyl-GED-0507-34-Levo (N-Acetyl-GED) (Ramot *et al.*, 2014), can modulate mitochondrial properties, using microdissected, organ-cultured human scalp HFs as a physiologically and clinically relevant assay system (Langan *et al.*, 2015).

In order to screen whether PPARγ-stimulation can lead to changes in mitochondria-related genes, we have analysed our previously executed genome-wide microarrays (data are accessible through GEO series accession number GSE109009), performed on two independent sets of organ-cultured HFs from a female patient's scalp, treated with 0.01 mM N-Acetyl-GED for 6 hrs (Ramot *et al.*, 2014). Using more permissive selection criteria ($p<0.05$, >2-fold, equidirectional changes in both patients), we have identified four genes involved in the control of mitochondrial function to be upregulated (**Suppl. Table 1**), thereby suggesting a role for PPARγ-stimulation in mitochondrial function.

To further test the possible role of PPARγ in mitochondrial function, human anagen VI HFs were microdissected from normal scalp skin that was obtained after written informed consent from two healthy patients, as previously described, adhering to Helsinki guidelines and under a licence from the ethics committee of the University of Münster (Reference No.: 2015-602-f-S). The HFs were organ cultured for 6 days with vehicle or 0.01-1 mM N-Acetyl-GED (concentrations were chosen based on the previously identified optimal dose; (Ramot *et al.*, 2014), with change of culture media every 48 hrs.

To confirm a possible role for N-Acetyl-GED in mitochondrial function, we investigated expression of four genes that are known to be key players in mitochondrial biology, namely Mitochondrially Encoded Cytochrome C Oxidase I (MTCO1), PPARγ coactivator 1-α (PGC1α), Mitochondrial Transcription Factor A (TFAM) and Solute Carrier Family 25 Member 3 (SLC25A3) (Ramot *et al.*, 2011; Vidali *et al.*, 2014). q-PCR analyses revealed that there was a significant and concentration-dependent stimulation of PGC1α transcription, while for the other genes there was a strong trend towards upregulation of mRNA levels following the treatment (**Fig. 1A**).

To further dissect the stimulating effect of N-Acetyl-GED on key mitochondrial elements, we also analysed their expression at the protein level. N-Acetyl-GED significantly increased immunoreactivity for MTCO1, a key enzyme of the respiratory chain (Knuever *et al.*, 2012) (**Fig. 1B**). This increase was observed in both the outer root sheath (ORS) and hair MKs. A very similar effect was also evidenced for the protein expression of TFAM, a key transcription factor for mitochondrial DNA synthesis (Knuever *et al.*, 2012) (**Fig. 1C**). A slightly different effect was observed when we checked the protein expression of voltage-dependent anion channel 1 (VDAC1), a reliable marker for mitochondrial mass in general (Vidali *et al.*, 2014). In the lower concentration (0.01 mM), there was a slight decrease in the protein expression. However, like the other mitochondrial markers, the two higher concentrations led to significant upregulation of immunoreactivity in both the ORS and MKs (**Fig. 1D**).

As complementary evidence, we also tested the effects of N-Acetyl-GED on isolated human ORS keratinocytes. Interestingly, we found that 6-hr treatments by using the same, non-cytotoxic (MTT- and CyQUANT assays, **Suppl. Fig. S1a-d**) concentrations (i.e. 0.01, 0.1. and 1 mM) of N-Acetyl-GED were able to significantly upregulate expressions of all the tested "mitochondrion-relevant" genes (i.e. MTCO1, TFAM, PGC1α, VDAC1 and SLC25A3) in the cells of the investigated donor as compared to the vehicle treated group (**Fig. 2A**). Thus, results of this pilot experiment revealing more prominent actions on "pure" ORS keratinocyte cultures than in intact HFs (**Fig. 1A**) invite the hypothesis that, within the HFs, ORS keratinocytes may be the primary targets of N-Acetyl-GED-mediated PPARγ activation.

Furthermore, to get an independent, indirect proof of the influence of PPARγ stimulation on the mitochondrial actions, we also measured ATP release of ORS keratinocytes upon the above 6-hr N-Acetyl-GED treatments (for details, see the **Supplementary Methods** section). Of great importance, we found that N-Acetyl-GED could concentration-dependently increase the amount of the released ATP of the ORS keratinocytes, highlighting again that it is indeed likely to positively regulate mitochondrial activity in these cells (**Fig. 2B**).

N-Acetyl-GED has been shown before to induce catagen and decrease hair matrix keratinocyte proliferation (Ramot *et al.*, 2014), while our current study shows that N-Acetyl-GED induce mitochondrial energy metabolism. These finding are in agreement with the concept that the hair follicle mainly engages in aerobic glycolysis and not rely predominantly on mitochondria-dependent glucose metabolism (Philpott and Kealey, 1990; Williams *et al.*, 1993). These findings also suggest that the catagen-promoting impact of N-Acetyl-GED may be dependent on the promotion of catagen-/terminal differentiation-associated processes in the hair bulb that are more reliant on oxidative phosphorylation.

These preliminary results suggest that, similar to its effects in other tissues, PPARγ-mediated signalling is a player in regulating the energy metabolism of human scalp HFs by enhancing mitochondrial function, most probably primarily in the ORS keratinocytes. Next, it deserves to be evaluated whether this modulation of mitochondrial biology read-out parameters by PPARγ modulators in human skin shown here can be translated into clinically beneficial effects (e.g. anti-HF aging).

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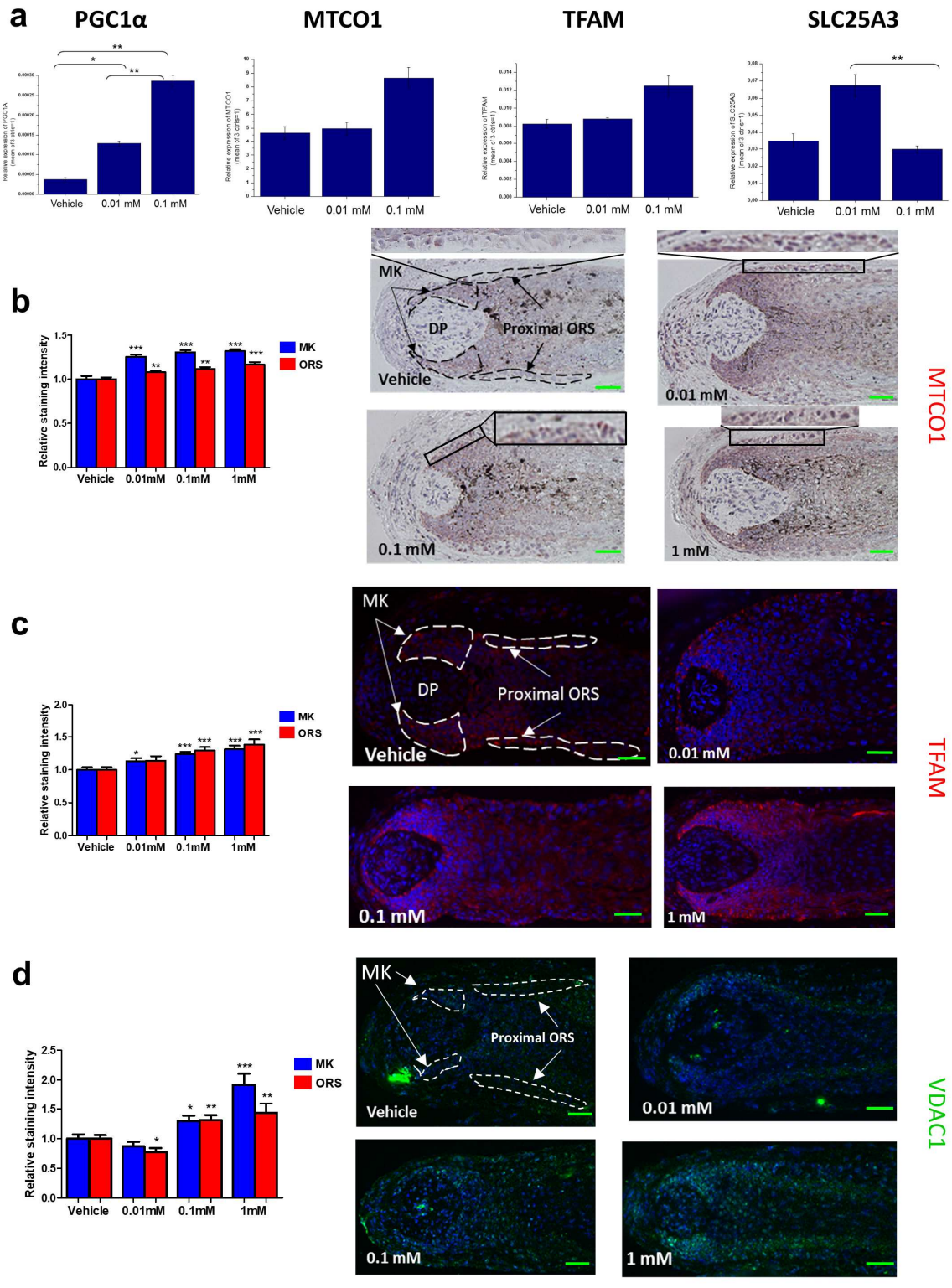
Figure legends

Figure 1. N-Acetyl-GED modulates mitochondrial activity in microdissected human HFs. A. mRNA levels of PGC1α, MTCO1, TFAM and SLC25A3, in human HFs, treated with 0.01 or 0.1mM N-Acetyl-GED for 6 hrs. Mean ± SEM. B. N-Acetyl-GED stimulated MTCO1 immunoreactivity after 6 days in culture. Mean ± SEM, n=11-20 HFs per group. Insets: higher magnification of the MTCO1 staining in the outer root sheath keratinocytes. C. N-Acetyl-GED stimulated TFAM immunoreactivity after 6 days in culture. Mean ± SEM, n=15-19 HFs per group. D. While decreasing VDAC1 immunoreactivity in the lower concentration, N-Acetyl-GED stimulated VDAC1 immunoreactivity in the higher concentrations after 6 days in culture. Mean ± SEM, n= 7-12 HFs per group. A-D: *p≤0.05, **p≤0.01, ***p≤0.001 as indicated, using two-tailed, unpaired Student's t-test. MK: matrix keratinocytes, ORS: outer root sheath, DP: dermal papilla. Scale bars = 50 μm.

Figure 2. N-Acetyl-GED acts on outer root sheath keratinocytes of human HFs. A. mRNA levels of PGC1α, MTCO1, TFAM, VDAC1 and SLC25A3, in primary, human ORS keratinocytes treated with 0.01, 0.1 or 1 mM N-Acetyl-GED for 6 hrs, as indicated. Mean ± SEM of 2-3 determinations. See also Supplementary Text. B. G6PD-corrected ATP release following the indicated 6-hr N-Acetyl-GED treatments of primary, human ORS keratinocytes. Mean ± SEM of 3 determinations. A-B: *p≤0.05, **p≤0.01, ***p≤0.001 as indicated, using two-tailed, unpaired Student's t-test.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