Published in final edited form as:

Exp Dermatol. 2016 July ; 25(7): 563–565. doi:10.1111/exd.12991.

UCP1 in sebaceous glands correspond with increased antioxidant potential and not brown adipose tissue function

C.N. Miller^{1,2,†}, P. Yu³, S. Ambati³, E.C. McKinney³, T. Avra⁴, C.A. Baile^{1,2,5}, and R.B. Meagher³

¹Department of Animal and Dairy Science, University of Georgia, Athens, GA, USA

²Department of Foods and Nutrition, University of Georgia, Athens, GA, USA

³Department of Genetics, University of Georgia, Athens, GA, USA

⁴College of Veterinary Medicine, University of Georgia, Athens, GA, USA

Keywords

antioxidants; brown adipose tissue; sebaceous glands; skin; thermogenesis

Background

The rodent tail is a thermoregulatory organ. Further, the tail is highly sympathetically innervated and when trunk temperatures dip below thermoneutrality there is vasoconstriction in the tail (1). Heat loss occurs most frequently at the base of the tail, close to the rump (2), therefore blood temperatures circulating in the tip of the tail are the coldest in the body (3, 4). Considering increased sympathetic tone and the relatively colder environment seen in the rodent tail tip, it is reasonable to suggest that uncoupling protein 1 (UCP1), a major mediator of thermoregulation (5), would also be expressed. Regions of UCP1 expression have previously been identified in the skin of humans and include the epidermis, sweat glands, and sebaceous glands, and suggests the possibility of similar properties within the skin of the rodent tail (6).

Questions addressed

UCP1 activity in rodent sebaceous glands has yet to be identified. From the background work and conclusions of Mori *et al* (6), we hypothesized that UCP1-positive regions within the tail would have properties similar to brown adipose tissue (BAT) and contribute to thermoregulation.

[†]Corresponding Author: Dr. Colette N. Miller, cnmiller@uga.edu, Department of Animal and Dairy Science, 425 River Rd., University of Georgia, Athens, GA 30602-7223, Phone: (706) 542-0978, Fax: (706) 542-7925.

⁵Dr. C.A. Baile passed away near the completion of this study.

Conflict of interests

The authors declare no conflicting interests.

Experimental design

For the purpose of this study, 3 month old male C57BL/6J mice were used to investigate the properties of sebaceous gland UCP1. The distal 6 mm of tail was removed from euthanized mice and immediately frozen for gene expression or immunofluorescence assessment. BAT from the same mice was also excised to compare gene expression patterns of thermogenic genes, uncoupling proteins, and antioxidants to the tail by quantitative RT-PCR. Additional assessments compared the gene expression and presence of UCP1 between 3 month old and aged (>12 month old) mice. Lastly, regional sections of the tail was processed to determine if the known regional temperature differences within the tail produced variances in the quantity of UCP1 protein and RNA. Detailed information of the experimental procedures can be found within the Supporting Information section.

Results

Considering that the tail expressed well-described markers of BAT (see Fig. S1 and S2), transcript expression in tail samples was compared to that of scapular BAT. Transcript was normalized to the levels of the general lipid marker fatty acid binding protein 4 (*Fabp4*). The normalization to *Fabp4* permitted us to explore the relative gene expression within a lipid rich cell type inclusive of adipocytes and sebocytes. As UCP1 was not ubiquitously expressed within the tail section (Fig. S2), normalization to a lipid marker permits a more focused measurement of UCP1-rich cells in a mixed cell type tissue such as the tail. While the expression of *Ucp1* was not significantly different between the tail and BAT, the expression of *Ucps 2-5* were up to 1000-fold higher within the tail section relative to BAT (Fig. 1a).

UCPs have been hypothesized to play a role in many diseases including both forms of diabetes and Parkinson's Disease (7, 8). The protective effects of UCPs in these diseases are attributed to their antioxidant properties in the face of elevated mitochondrial superoxide concentrations. The increased presence of UCP1, in addition to the other UCPs, results in a reduced membrane potential thereby reducing reactive oxygen species formation as part of a negative feedback mechanism to reduce oxidative stress (8). As the UCPs were elevated within the tail section relative to BAT, we explored antioxidant gene expression between the tissues. Similarly, up to 100-fold increases in the antioxidant genes glutathione peroxidase 1 (*Gpx1*) and superoxide dismutase (*Sod 1* and *2*) were found in the tail section relative to BAT (Fig. 1b). Together with the expression patterns of the *Ucps*, our data is indicative that sebaceous glands, which express relatively high amounts of *Fabp4*, have an antioxidant potential that varies greatly from BAT.

Regional temperatures vary widely in the tail, with the temperatures decreasing dramatically from the proximal to the distal end of the tail (4). Redfern *et al* found that skin tail temperature proximal to the body started at 35°C, was reduced to 26°C towards the middle region, and dropped to 18°C at the tip. Because UCP1 is a thermogenic protein responsive to temperature fluctuations in BAT, we assayed differences in UCPs among these three distinct temperature zones of the tail. No significant differences in *Ucp1* expression or protein staining intensity were measured among the tip, middle, and proximal tail regions of 3-

month-old mice (Fig. 2a). Intriguingly, the tip did express increased amounts of the antioxidants *Ucp2*, *Gpx1*, and *Sod1* compared to the other regions. As the amount of sebocytes were controlled for by the use of *Fabp4*, the increased antioxidant expression in the tip may be due to differences within the hair cycle or for protection from external damage and renders further investigation. Despite this, the lack of a difference in the thermogenic UCP1 protein and RNA within the regions is indicative that UCP1 within the sebocyte population is unresponsive to regional temperature variations in the tail.

Lastly, because the amount of BAT and UCP1 activity are greatly reduced or even lost during aging (5) and this same aging pattern could be recapitulated in tail, *Ucp1* expression and protein localization were compared in tails among young (3 month of age) and aged (12 month of age) mice. Age had no effect on the expression of the well characterized thermogenic genes *Ucp1* or *Prdm16* between mice (Fig. S3 a). In addition, neither the immunofluorescence intensity nor the location of UCP1 protein differed notably in the terminal tail region between young or aged mice (Fig. S3 b).

Conclusions

We extended the results by Mori *et al* showing that *Ucp1* is expressed in various locations in the tail sebaceous glands (6). Our data, however, do not indicate that the role of sebaceous gland UCP1 is one of thermoregulation. Further, we provide evidence that UCPs in the sebaceous gland serves an alternative antioxidant role as described in other organs (8).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Miller performed the research, designed the study, analyzed the data, and wrote the paper. Yu, Amabti, McKinney, and Avra performed the research and analyzed associated data. Baile and Meagher participated in the study design and contributed essential reagents and equipment. All living authors participated in the drafting and revision of the manuscript and have approved of all versions of the manuscript its associated documents prior to submission. This research was supported by the Atlanta Chapter of the Achievement Awards for College Scientists (CNM), the National Institute of Diabetes and Digestive and Kidney Diseases (#DK090300) (RBM). Additional support was provided from the Georgia Research Alliance Eminent Scholar Fund and the UGA Research Foundation (CAB).

References

1. Owens NC, Ootsuka Y, Kanosue K, et al. Thermoregulatory control of sympathetic fibres supplying the rat's tail. *The Journal of physiology*. 2002; 543:849–858. [PubMed: 12231643]
2. Vanhoutte G, Verhoye M, Raman E, et al. In-vivo non-invasive study of the thermoregulatory function of the blood vessels in the rat tail using magnetic resonance angiography. *NMR in biomedicine*. 2002; 15:263–269. [PubMed: 12112608]
3. Gemmell RT, Hales JR. Cutaneous arteriovenous anastomoses present in the tail but absent from the ear of the rat. *Journal of anatomy*. 1977; 124:355–358. [PubMed: 591433]
4. Redfern WS, MacLean MR, Clague RU, et al. The role of alpha 2-adrenoceptors in the vasculature of the rat tail. *British journal of pharmacology*. 1995; 114:1724–1730. [PubMed: 7599941]
5. Seale P, Lazar MA. Brown fat in humans: turning up the heat on obesity. *Diabetes*. 2009; 58:1482–1484. [PubMed: 19564460]

6. Mori S, Yoshizuka N, Takizawa M, et al. Expression of uncoupling proteins in human skin and skin-derived cells. *The Journal of investigative dermatology*. 2008; 128:1894–1900. [PubMed: 18305572]
7. Ho PW, Ho JW, Liu HF, et al. Mitochondrial neuronal uncoupling proteins: a target for potential disease-modification in Parkinson's disease. *Transl Neurodegener*. 2012; 1:3. [PubMed: 23210978]
8. Liu J, Li J, Li WJ, et al. The role of uncoupling proteins in diabetes mellitus. *J Diabetes Res*. 2013; 2013:585897. [PubMed: 23841103]

Figure 1. Expression of uncoupling proteins and antioxidant genes in BAT and peripheral tail Peripheral tail snips (under 6 mm in length; n=3–8) and scapular brown adipose tissue (n=3) were excised from 3 month old, male mice following euthanasia. RQ values were normalized to *Fabp4* in order to target the lipid-rich cells (sebocytes and adipocytes) within each target tissue for uncoupling proteins (a) and antioxidant genes (b). Statistical significance is represented as * p<0.05, ** p<0.01, and *** p<0.001. Representative immunofluorescence images of UCP1 staining (red) counterstained with DAPI (green) within the peripheral tail sebaceous gland (c). Abbreviations: brown adipose tissue, BAT; fatty acid binding protein 4, FABP4; uncoupling protein, UCP; glutathione peroxidase 1, GPX1; superoxide dismutase, SOD. Scale bars equal 20 μm in length.

Figure 2. Expression of uncoupling proteins and antioxidant genes between tail regions
 Sections from the tip, middle, and proximal regions of the tail (n=3) were removed for qRT-PCR analysis of uncoupling proteins (a) and antioxidant genes (b). Target genes were normalized to the lipid-rich cell marker *Fabp4*. Statistical significance is represented as * p<0.05 and *** p<0.001. Representative immunofluorescence images of UCP1 staining (red) counterstained with DAPI (green) in the regional tail sections (c). Abbreviations: fatty acid binding protein 4, FABP4; uncoupling protein, UCP; glutathione peroxidase 1, GPX1; superoxide dismutase, SOD. Scale bars equal 20 μm in length.