

Improved glucose homeostasis in mice overexpressing human UCP3: a role for AMP-kinase?

Citation for published version (APA):

Schrauwen, P., Hardie, D. G., Roorda, B. D., Clapham, J. C., Abuin, A., Thomason-Hughes, M., Green, K., Frederik, P. M., & Hesselink, M. K. (2004). Improved glucose homeostasis in mice overexpressing human UCP3: a role for AMP-kinase? *International Journal of Obesity*, 28(6), 824-8. <https://doi.org/10.1038/sj.ijo.0802629>

Document status and date:

Published: 01/01/2004

DOI:

[10.1038/sj.ijo.0802629](https://doi.org/10.1038/sj.ijo.0802629)

Document Version:

Publisher's PDF, also known as Version of record

Document license:

Taverne

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

SHORT COMMUNICATION

Improved glucose homeostasis in mice overexpressing human UCP3: a role for AMP-kinase?

P Schrauwen^{1*}, DG Hardie², B Roorda¹, JC Clapham^{3,7}, A Abuin⁴, M Thomason-Hughes², K Green², PM Frederik⁵ and MKC Hesselink⁶

¹Department of Human Biology, Maastricht University, The Netherlands; ²Wellcome Trust Biocentre, University of Dundee, Dundee, Scotland, UK; ³Department of Vascular Biology, GlaxoSmithKline, Harlow, Essex, UK; ⁴Department of Comparative genetics, GlaxoSmithKline, Harlow, Essex, UK; ⁵EM-unit, Maastricht University, The Netherlands; and ⁶Department of Movement Sciences, Maastricht University, The Netherlands

OBJECTIVE: An unexplained phenotype of mice overexpressing human UCP3 is their improved glucose homeostasis. Since overexpression of UCP3 might affect the energy charge of the cell, we investigated whether these mice have an increased AMP-activated protein kinase (AMPK) activity.

METHODS: Mitochondrial localisation of UCP3 was determined by immunoelectronmicroscopy and AMPK activity was measured in medial gastrocnemius of control mice and mice overexpressing human UCP3.

RESULTS: Mice overexpressing human UCP3 had 5.8 fold higher levels of UCP3 protein, for which mitochondrial localisation was confirmed by immunoelectronmicroscopy. The ATP/AMP ratio was significantly lower in mice over-expressing UCP3 compared to the wild-type (10.9 ± 1.6 vs 20.4 ± 1.9 AU, $P=0.03$). Over-expression of UCP3 resulted in increased AMPK $\alpha 1$ activity (1.23 ± 0.05 vs 1.00 ± 0.06 normalized values, $P=0.004$) and a tendency towards increased AMPK $\alpha 2$ activity (1.18 ± 0.08 vs 1.00 ± 0.10 normalized values, $P=0.08$).

CONCLUSION: Increased AMPK activity provides a plausible explanation for the improved glucose tolerance characteristic for these mice.

International Journal of Obesity (2004) 28, 824–828. doi:10.1038/sj.ijo.0802629

Published online 16 March 2004

Keywords: AMP-activated protein kinase; glucose metabolism; UCP3; diabetes; energy metabolism

Introduction

The human uncoupling protein-3 (UCP3) has been associated with the regulation of glucose metabolism and type II diabetes mellitus. Concerted upregulation of UCP3 and GLUT4 mRNA upon cold exposure¹ and acute exercise² has been interpreted as evidence for a role of UCP3 in glucose metabolism. Furthermore, we have recently shown that type II diabetic subjects have 2-fold lower UCP3 protein levels compared to healthy controls, suggesting that here also UCP3 might be involved in the disturbances in glucose homeostasis characteristic for type II diabetes mellitus.³ In addition to these associations between UCP3 and glucose

metabolism, it has also been shown that overexpression of UCP1 in skeletal muscle (to a level of only 1% of the UCP expression naturally occurring in brown adipose tissue) increases skeletal muscle glucose transport and protects against insulin resistance and hyperglycemia induced by high-fat feeding.⁴ Also, mice overexpressing human UCP3 in skeletal muscle are characterized by reduced fasting glucose concentration, improved glucose tolerance following an oral glucose load and lower insulin levels.⁵ However, the mechanism by which UCP3 can affect glucose homeostasis is presently unknown. Nowadays, the prevailing opinion is that the primary physiological function of UCP3 is most likely in the prevention of ROS production⁶ or in fatty acid metabolism.^{7–9} It is, therefore, possible that through these putative functions in ROS production and/or fatty acid metabolism, UCP3 could indirectly interact with glucose metabolism, explaining the observed associations between UCP3 and glucose metabolism. However, it is also important to note that as a side effect of its primary role, UCP3 might still be able to uncouple mitochondria, especially when the

*Correspondence: Dr P Schrauwen, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands.
E-mail: p.schrauwen@hb.unimaas.nl

⁷Present address: Cell Biology and Biochemistry, AstraZeneca R&D, Mölndal, Sweden

Received 16 October 2003; revised 17 December 2003; accepted 2 February 2004; published online 16 March 2004

(over)expression of UCP3 is very high.¹⁰ Indeed, supraphysiological overexpressing UCP3 in yeast¹¹ and mice,⁵ reduces the proton gradient across the inner mitochondrial membrane, which is used to generate the energy for the phosphorylation of ADP to form ATP. In such conditions the adenylate kinase reaction ($2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$) may help to maintain cellular ATP levels with a concomitant increase in AMP. The latter is a well-known activator of the enzyme AMP-activated protein kinase (AMPK), an enzyme responsible for the phosphorylation of key enzymes that control metabolic flux, among which is glucose uptake (via GLUT4 translocation).¹² Therefore, the beneficial effect of (supraphysiological) UCP3 overexpression on glucose homeostasis could reflect an effect of mitochondrial uncoupling on AMPK activity. In this context, chemical compounds that uncouple mitochondrial respiration have also been shown to increase glucose uptake in muscle cells.¹³ Therefore, we used mice overexpressing human UCP3 (UCP3-Tg), which were shown to have improved glucose tolerance⁵ and their wild-type littermates (wild-type) to test the hypothesis that improved glucose homeostasis in these mice can be accounted for by increased activity of the $\alpha 1$ and/or $\alpha 2$ isoform of AMPK. In addition, we examined the localisation of UCP3 in these UCP3-Tg mice, to confirm that UCP3 indeed was located in the inner mitochondrial membrane.

Materials and methods

Animals

A total of 18 UCP3-Tg mice (aged: 21.4 ± 1.2 weeks) and 16 wild-type littermates (aged: 22.2 ± 1.4 weeks) were used. Creation of UCP3 overexpressing mice has been described previously.⁵ Animals were housed individually on a 12:12 h light–dark cycle (light from 0700 to 1900 hours), at $21\text{--}22^\circ\text{C}$, and allowed unlimited access to standard chow diet. The studies were approved by the Institutional Animal Care and Use Committee of the Maastricht University and complied with the principles of laboratory animal care. Under general anaesthesia (1.5–2.0% halothane in O_2 and N_2O (3:1, 4.01/min), medial gastrocnemius muscles were rapidly excised unilaterally and freeze-clamped within 7 s after cutting the nerve and blood vessels.

UCP3 protein

Endogenous and human UCP3 protein content was determined by Western blotting, using rabbit polyclonal antibodies against mouse (code 1338) and human UCP3 (code, 1331, kindly provided by LJ Sliker, Eli Lilly.)¹⁰ Immunogold electron microscopy was performed on an ultrathin cryosections of medial gastrocnemius essentially according to Martinez–Menarguez.¹⁴ By combining our antibody specifically detecting human UCP3 with the antibody detecting mouse UCP3, we were able to differentiate between endogenous UCP3 expression and expression of hUCP3 in the transgene.

AMPK activity and nucleotides

Immunoprecipitate kinase assays were performed as described previously, using AMPK anti- $\alpha 1$ and anti- $\alpha 2$ antibodies.¹⁵ Samples of frozen muscle of eight UCP3-Tg and wild-type mice were ground to powder under liquid nitrogen with a pestle and mortar. The powdered muscle was homogenized with an equal volume of 5% perchloric acid, and then centrifuged at 14 000 rpm and 4°C , for 3 min to remove acid-insoluble material. The supernatant was then extracted with two washes of a 10% excess (volume) of 1:1 tri-*n*-octylamine and 1,1,2-trichlorotrifluoroethane. Nucleotides were separated by capillary electrophoresis with on-column isotachopheric concentration, using run buffers consisting of 100 mM sodium phosphate, and 50 mM NaCl (pH 5.7; leading buffer); and 100 mM MES/Tris pH 5.7 (tailing buffer). To each buffer 0.2% hydroxyethyl-cellulose was added to decrease the electro-osmotic flow. Before running each sample, a 10% volume of leading buffer was added containing 8-Br-cAMP as a reference compound. Nucleotide peaks were detected by UV absorbance at 260 nm (ref. 350 nm), and integrated using System Gold software (Beckman). Nucleotide ratios were calculated from peak areas after correction for retention times. Peaks due to added ATP, ADP and AMP coincided with the main sample peaks supporting their assignment as such.

Statistical analysis

Results are presented as mean \pm s.e. Differences between groups were evaluated by unpaired *t*-tests. Pearson's correlation coefficients were calculated to determine the relationship between selected variables. Outcomes were regarded statistically significant if $P < 0.05$.

Results

UCP3-Tg mice had 5.8-fold higher levels of total UCP3 protein (human + endogenous) compared to wild types ($P < 0.0001$). Moreover, we confirmed the mitochondrial localisation of human UCP3 by immunoelectronmicroscopy (Figure 1). UCP3-Tg mice had significantly lower body weight compared to the wild-type (22.4 ± 0.9 vs 25.9 ± 0.8 g in UCP3-Tg and wild-type mice respectively, $P < 0.01$) and total UCP3 protein content correlated significantly with body weight ($r = -0.53$, $P < 0.005$, Figure 3a). ATP concentration was not affected by overexpression of UCP3 (relative fraction of the total adenine nucleotide HPLC signal: 0.78 ± 0.01 vs 0.79 ± 0.02 , in UCP3-Tg and wild-type mice respectively, NS), indicating that UCP3-Tg mice, despite the proton leak present are able to maintain levels of ATP, perhaps via the adenylate kinase reaction. In line with this, we observed a significant decrease in ADP levels (relative fraction: 0.14 ± 0.01 vs 0.20 ± 0.02 , in UCP3-Tg and wild-type mice respectively, $P = 0.03$), suggesting that ADP was converted to AMP to maintain ATP levels. Indeed, AMP levels

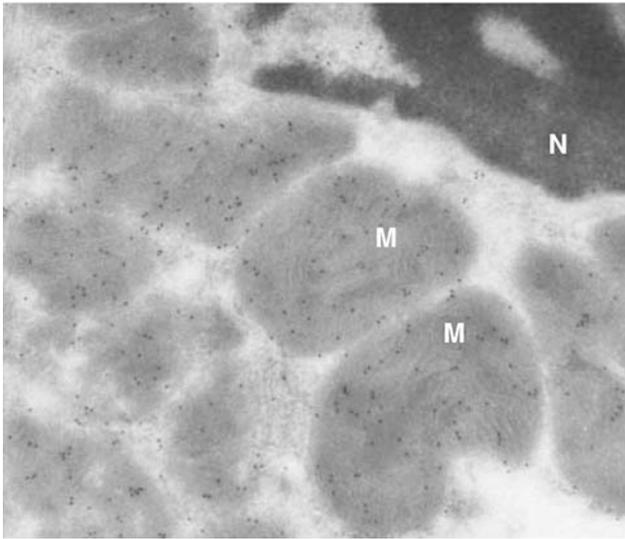


Figure 1 Immunogold electron microscopy confirming mitochondrial localisation of human UCP3 in the UCP3-Tg mice (n = nucleus, m = mitochondrion).

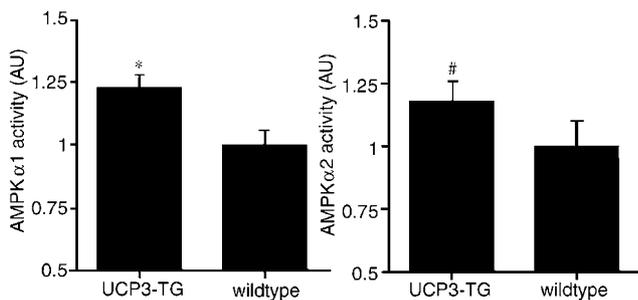


Figure 2 AMPK $\alpha 1$ and $\alpha 2$ activity in m. gastrocnemius of wild-type ($n = 16$) and UCP3-Tg mice ($n = 18$). AMPK activity was measured as described in Materials and methods. Data are means \pm s.e.m. * $P < 0.005$, # $P = 0.08$ compared to wild-type.

doubled in UCP3-Tg compared to wild-type mice (0.08 ± 0.01 vs 0.04 ± 0.00 AU, $P = 0.08$). The ATP/AMP ratio, an important regulator of AMPK activity, was significantly lower in UCP3-Tg compared to wild-type mice (10.9 ± 1.6 vs 20.4 ± 1.9 , $P = 0.03$), creating the subcellular conditions to activate AMPK.

Overexpression of UCP3 indeed resulted in increased AMPK $\alpha 1$ activity (1.23 ± 0.05 vs 1.00 ± 0.06 normalized values, $P = 0.004$, Figure 2) and a tendency towards increased AMPK $\alpha 2$ activity (1.18 ± 0.08 vs 1.00 ± 0.10 normalized values, $P = 0.08$, Figure 2). The UCP3 protein content correlated with AMPK $\alpha 1$ activity ($r = 0.43$, $P < 0.05$) and tended to correlate with AMPK $\alpha 2$ activity ($r = 0.3$, $P = 0.09$). Both AMPK $\alpha 1$ ($r = -0.52$, $P < 0.005$, Figure 3b) and $\alpha 2$ ($r = -0.69$, $P < 0.001$, Figure 3c) activity correlated with body weight. No differences in protein content of AMPK ($\alpha 1$ or $\alpha 2$) were detected between mice overexpressing human UCP3 and wild-type mice (data not shown).

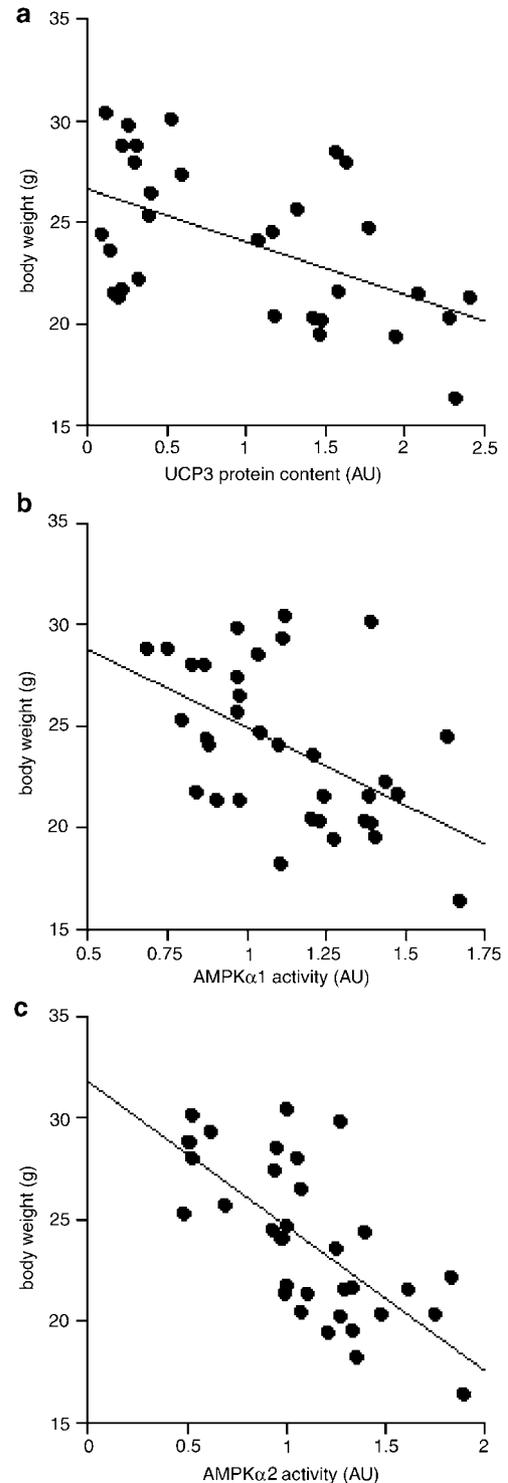


Figure 3 Relationship between m. gastrocnemius (a) UCP3 protein content ($r = -0.53$, $P < 0.005$, (a), (b) AMPK $\alpha 1$ ($r = -0.52$, $P < 0.005$) and (c) AMPK $\alpha 2$ activity and body weight ($r = -0.69$, $P < 0.001$) in wild-type ($n = 16$) and UCP3-Tg mice ($n = 18$). AMPK activity was measured as described in Materials and methods. Data are means \pm s.e.

Discussion

In recent years, AMPK has been recognized as a novel target for the pharmacological treatment of type II diabetes mellitus.¹² Chemical activation of AMPK with the compound AICAR increases glucose uptake^{16,17} and insulin sensitivity.^{18,19} Here we show that overexpressing human UCP3 in skeletal muscle of mice affects the cellular energy charge and induces activation of AMPK $\alpha 1$ and tends to induce the activation of AMPK $\alpha 2$. Why AMPK $\alpha 1$ was more affected compared to AMPK $\alpha 2$ cannot be deduced from the present study, but is remarkable considering that especially AMPK $\alpha 2$ is involved in glucose homeostasis in muscle.²⁰ In addition to the increased AMPK activity, UCP3 overexpression also affected body weight, which previously was shown to be due to an increase in metabolic rate.⁵ Interestingly, AMPK activity was tightly correlated with the body weight. This suggests that UCP3-induced AMPK activation stimulates substrate metabolism necessary to provide fuels for the increased metabolic rate, and diverts glucose towards oxidation rather than towards storage as glycogen and fat. This suggestion is also consistent with the decreased adipose tissue mass reported in UCP3-Tg mice.⁵ Most important, the increased AMPK activity observed in the present study, after supraphysiological overexpression of human UCP3 in skeletal muscle, provides a plausible explanation for improved glucose tolerance in these UCP3-Tg mice,⁵ and suggests that mitochondrial uncoupling improves glucose metabolism by activating AMPK. The latter could also explain the improved glucose handling that has been shown in muscle cells treated with chemical uncouplers¹³ and in mice overexpressing UCP1 in skeletal muscle.⁴ Therefore, our data indicate that agents able to (lifetime) activation of AMPK, for example by uncoupling mitochondria, are powerful tools in the prevention or treatment of type II diabetes mellitus. The results of the present study do not, however, indicate that the primary physiological function of UCP3 is to regulate glucose metabolism by AMPK activation. We have recently shown that upregulation of UCP3 in humans in the physiological range does not affect mitochondrial uncoupling,¹⁰ and it is now generally accepted that the primary physiological function of UCP3 is not to uncouple mitochondria.^{7,6,9} It has therefore been suggested that overexpressing UCP3 in the supraphysiological range does not represent native uncoupling, possibly due to improper incorporation of UCP3 in the mitochondrial membrane.⁶ In contrast to this suggestion, we show in the present study, using immunogold electron microscopy, that the vast majority of UCP3 was associated with the innermitochondrial membrane and the cristae and that extramitochondrial labelling was almost absent (see Figure 1). However, regardless of the nature of uncoupling (either non-native or due to the supraphysiological level), as it is the change in energy charge, which is responsible for AMPK activation, our data also provide a plausible explanation why ablation of UCP3 (that does not affect cellular energy charge) does not result in apparent disturbances in glucose homeostasis.²¹ Therefore, the previously observed

beneficial effects of UCP3 on glucose metabolism seems to be due to an effect of (supraphysiological concentrations of) UCP3, via mitochondrial uncoupling, on AMPK activity. However, whether the reduction of UCP3 protein levels in type II diabetic subjects³ also affects AMPK activity seems doubtful given that UCP3 content in the physiological range in humans does not affect mitochondrial uncoupling,¹⁰ but this should be further investigated. In this context, the reduction in UCP3 protein levels in type II diabetic patients could well be related to alteration in fatty acid metabolism in this disease, as we previously suggested.⁹

In conclusion, we have shown that mice overexpressing human UCP3 have increased AMPK activity, which provides a plausible explanation for the improved glucose tolerance characteristic for these mice. This suggests that affecting mitochondrial uncoupling, whether or not via UCPs, might be a target in the prevention or treatment of type II diabetes mellitus.

Acknowledgements

The research of Dr P Schrauwen has been made possible by a fellowship of the Royal Netherlands Academy of Arts and Sciences. Dr DG Hardie was supported by a Programme Grant from the Wellcome Trust and a Project Grant from Diabetes UK. We thank Lawrence J Sliker from Eli Lilly and Company for providing us with the UCP3 antibody. The skilful technical assistance of Hans Duimel in preparing and staining the samples for immunogold EM is gratefully acknowledged. This work was supported by a grant from the Human Nutrition Institute of the International Life Sciences Institute Research Foundation (ILSI RF). The opinions expressed herein are those of the author(s) and do not necessarily represent the views of ILSI RF.

References

- 1 Lin B, Coughlin S, Pilch PF. Bidirectional regulation of uncoupling protein-3 and GLUT-4 mRNA in skeletal muscle by cold. *Am J Physiol Endocrinol Metab* 1998; **275**: E386–E391.
- 2 Tsuboyama-Kasaoka N, Tsunoda N, Maruyama K, Takahashi M, Kim H, Ikemoto S, Ezaki O. Up-regulation of uncoupling protein 3 (UCP3) mRNA by exercise training and down-regulation of UCP3 by denervation in skeletal muscles. *Biochem Biophys Res Commun* 1998; **247**: 498–503.
- 3 Schrauwen P, Hesselink MK, Blaak EE, Borghouts LB, Schaart G, Saris WH, Keizer HA. Uncoupling protein 3 content is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* 2001; **50**: 2870–2873.
- 4 Li B, Nolte LA, Ju JS, Ho Han D, Coleman T, Holloszy JO, Semenkovich CF. Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. *Nat Med* 2000; **6**: 1115–1120.
- 5 Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 2000; **406**: 415–418.
- 6 Echtay KS, Roussel D, St Pierre J, Jekabsons MB, Cadenas S, Stuart JA, Harper JA, Roebuck SJ, Morrison A, Pickering S. Superoxide

- activates mitochondrial uncoupling proteins. *Nature* 2002; **415**: 96–99.
- 7 Himms-Hagen J, Harper ME. Physiological role of UCP3 may be export of fatty acids from mitochondria when fatty acid oxidation predominates: an hypothesis. *Exp Biol Med (Maywood)* 2001; **226**: 78–84.
 - 8 Goglia F, Skulachev VP. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. *FASEB J* 2003; **17**: 1585–1591.
 - 9 Schrauwen P, Hoeks J, Schaart G, Kornips E, Binas B, Van De Vusse GJ, Van Bilsen M, Luiken JJ, Coort SL, Glatz JF. Uncoupling protein 3 as a mitochondrial fatty acid anion exporter. *FASEB J* 2003; **17**: 2272–2274.
 - 10 Hesselink MKC, Greenhaff PL, Constantin-Teodosiu D, Hultman E, Saris WHM, Nieuwlaet R, Schaart G, Kornips E, Schrauwen P. Increased uncoupling protein 3 content does not affect mitochondrial function in human skeletal muscle *in vivo*. *J Clin Invest* 2003; **111**: 479–486.
 - 11 Gong D-W, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, β 3-adrenergic agonists, and leptin. *J Biol Chem* 1997; **272**: 24129–24132.
 - 12 Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type II diabetes. *Am J Physiol Endocrinol Metab* 1999; **277**: E1–10.
 - 13 Bashan N, Burdett E, Guma A, Sargeant R, Tumiati L, Liu Z, Klip A. Mechanisms of adaptation of glucose transporters to changes in the oxidative chain of muscle and fat cells. *Am J Physiol* 1993; **264**: C430–C440.
 - 14 Martinez-Menarguez JA, Prekeris R, Oorschot VM, Scheller R, Slot JW, Geuze HJ, Klumperman J. Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. *J Cell Biol* 2001; **155**: 1213–1224.
 - 15 Hardie DG, Salt IP, Davies SP. Analysis of the role of the AMP-activated protein kinase in the response to cellular stress. *Methods Mol Biol* 2000; **99**: 63–74.
 - 16 Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol* 1997; **273**: E1107–E1112.
 - 17 Bergeron R, Russell III RR, Young LH, Ren JM, Marcucci M, Lee A, Shulman GI. Effect of AMPK activation on muscle glucose metabolism in conscious rats. *Am J Physiol* 1999; **276**: E938–E944.
 - 18 Buhl ES, Jessen N, Schmitz O, Pedersen SB, Pedersen O, Holman GD, Lund S. Chronic treatment with 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. *Diabetes* 2001; **50**: 12–17.
 - 19 Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab* 2002; **282**: E18–E23.
 - 20 Viollet B, Andreelli F, Jorgensen SB, Perrin C, Geloan A, Flamez D, Mu J, Lenzner C, Baud O, Bennoun M. The AMP-activated protein kinase α 2 catalytic subunit controls whole-body insulin sensitivity. *J Clin Invest* 2003; **111**: 91–98.
 - 21 Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R. Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem* 2000; **275**: 16258–16266.