A Themed Meeting of The Physiological Society

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Metabolism & Endocrinology

The Royal Society, London, UK

Including focused symposia on 'Brown adipose tissue: a new human organ?'

11–13 December 2012

The Physiological Society

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Including focused symposia on: 'Brown adipose tissue: a new human organ?'

Tuesday 11 December 2012 to Thursday 13 December 2012

The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG, UK

Organised by Jan Nedergaard (The Wenner-Gren Institute, Sweden), Michael Symonds (University of Nottingham, UK) and Antonio Vidal-Puig (University of Cambridge, UK)

The Physiological Society

Welcome to this Metabolism & Endocrinology Themed Meeting of The Physiological Society. We are delighted to be here at The Royal Society, in London.

The Physiological Society has a strong tradition in enabling all physiologists in all Themes to come together to share the latest knowledge at its Meetings.

With this in mind, the scientific organisers, Jan Nedergaard (The Wenner-Gren Institute, Sweden), Michael Symonds (University of Nottingham, UK) and Antonio Vidal-Puig (University of Cambridge, UK) have put together an excellent programme including focused symposia on 'Brown adipose tissue: a new human organ?'.

The aim of these Meetings is to bring together those working in Metabolism & Endocrinology Physiology to hear the latest advances across a broad range of areas, and generate new research ideas. Over the next three days the international speaker panel will host talks on Brown adipose tissue and its effects and developments. There will be 12 oral communications and 38 poster communications.

As some of you may know, a key feature of any Society Meeting is the Society Dinner and we look forward to seeing you on Wednesday at The Churchill War Rooms for an evening of good food and drink and the chance to experience a piece of history in this unique venue.

Tuesday 11 December 2012

13.00 **Welcome and introduction to the Themed Meeting**
Jan Nedergaard (The Wenner-Gren Institute, Sweden), Michael Symonds (University of Nottingham, UK) and Antonio Vidal-Puig (Universityof Cambridge, UK)

Chair:
Antonio Vidal-Puig (University of Cambridge, UK)

13.05 **Basic concepts in brown adipose tissue biology**
Jan Nedergaard (The Wenner-Gren Institute, Sweden)

13.15 SA01 **Diet-induced thermogenesis and brown adipose tissue**
Jan Nedergaard (The Wenner-Gren Institute, Sweden)

13.45 SA02 **Brown adipose tissue (BAT) development and new insights into its role in energy balance in early life**
Michael Symonds (University of Nottingham, UK)

14.15 Refreshments

Chair:
Malcolm Parker (Imperial College London, UK)

14.45 SA03 **Transcriptional control of brown adipose tissue development**
Patrick Seale (University of Pennsylvania, USA)

15.15 C01 **Cholesteryl Ester Transfer Protein (CETP) enhances beta-3 adrenergic receptor and UCP1 expression, inducing energy expenditure and reducing adiposity in transgenic mice**
Helena Raposo (Institute of Biology, Campinas, Brazil)

15.30 C02 **Expression of Zinc finger of the cerebellum 1 suggests a possible role in development and/or function of brown adipose tissue**
Jasper de Jong (The Wenner-Gren Institute, Sweden)

15.45 Refreshments

Chair:
Martin Klingenspor (Technische Universität München, Germany)

16.15 SA04 **A role for brown fat in energy balance?**
Leslie Kozak (Polish Academy of Sciences, Poland)

16.45 C03 **Imaging brown adipose tissue at work**
Alexander Bartelt (University Medical Center Hamburg-Eppendorf, Germany)**17.00**

17.15 C04 **Quantification of de novo lipogenesis by room calorimetry**
Gustavo Abreu-Vieira (The Wenner-Gren Institute, Sweden)

17.15 Posters with wine and beer

18.45 End of day one

Wednesday 12 December 2012

Chair:
Michael Symonds (University of Nottingham, UK)

09.15 SA05 **Insights into brown adipose tissue function in cold**
Martin Klingenspor (Technische Universität München, Germany)

09.45 SA06 **Brown adipose tissue as an endocrine organ**
Francesc Villarroya (University of Barcelona, Spain)

10.15 C05 **Central prolactin-releasing peptide is required for cold- and leptin-induced thermogenesis in mice**
Simon Luckman (University of Manchester, UK)

10.30 C06 **LR11 negatively regulates thermogenesis**
Andrew Whittle (University of Cambridge, UK)

10.45 Refreshments

Chair:
Leslie Kozak (Polish Academy of Sciences, Poland)

11.30 SA07	**BAT sensitisers: Role of BMP8b** Antonio Vidal-Puig (University of Cambridge, UK)
12.00 C07	**Effect of novel thiazolidinadiones on expression of UCP1 and mitochondrial respiratory chain proteins in brown and white adipose tissues** Anastacia Kalinovich (The Wenner-Gren Institute, Sweden)
12.15 C08	**Pyruvate kinase M2 deficiency promotes a brown fat-like program in white adipocytes** Fawaz Haj (University of California Davis, USA)
12.30	Lunch
	Chair: Francesc Villarroya (University of Barcelona, Spain)
14.00 SA08	**RIP140 and the relationship between brown and white adipocytes** Mark Christian (Imperial College London, UK)
14.30 C09	**Loss of neuronatin promotes 'browning' of primary mouse adipocytes** Valentina Gburcik (Royal Veterinary College, UK)
14.45 C10	**Brown adipose tissue in epicardial adipose tissue of adult humans** Shalini Ojha (University of Nottingham, UK)
15.00	Refreshments
15.30	Posters with wine and beer
17.00	End of day two
19.00	Society Dinner at The Churchill War Rooms

Thursday 13 December 2012

	Chair Jan Nedergaard (The Wenner-Gren Institute, Sweden)
09.15 SA09	**Lessons from PET-CT imaging of brown adipose tissue** Wouter van Marken Lichtenbelt (Maastricht University, The Netherlands)
09.45 SA10	**Brown adipose tissue and longevity** Susanne Klaus (German Institute of Human Nutrition in Postdam, Germany)

10.15 C11	**Unequivocal identification of brown adipose tissue in an adult human using Magnetic Resonance Imaging** Narendra Reddy (University of Warwick, UK)
10.30 C12	**Brown adipose tissue function and premature ageing: Impaired thermogenesis in mice expressing defective mitochondrial DNA polymerase** Irina Shabalina (The Wenner-Gren Institute, Sweden)
10.45	Refreshments
	Chair: Michael Symonds (University of Nottingham, UK)
11.30 SA11	**UCP1-independent unmasking of mitochondrial oxidative capacity in adipocytes: Amelioration of obesity** Jan Kopecky (Academy of Sciences of the Czech Republic, Czech Republic)
12.00 SA12	**Systemic metabolic effects of brown adipose tissue** Jörg Heeren (University Medical Center Hamburg-Eppendorf, Germany)
12.30	Closing remarks
12.45	Theme Business Meeting
13.15	Close of meeting

Speaker Biographies

Mark Christian
Imperial College London, UK

Research interests
Mark Christian has demonstrated a role for the nuclear receptor coregulator RIP140 in repressing the expression of brown fat genes in white adipose tissue. Current research programs are determining the regulation, function & molecular characterization of lipid droplet-associated proteins and defining the genes that are regulated in adipose tissues in response stimuli that promote brown adipocytes.

Publications

1. Hallberg M, Morganstein DL, Kiskinis E, Shah K, Kralli A, Dilworth SM, White R, Parker MG, Christian M. A functional interaction between RIP140 and PGC-1a regulates the expression of the lipid droplet protein CIDEA. Mol Cell Biol. 2008. 28(22):6785-6798.
2. Christian M, Kiskinis E, Debevec D, Leonardsson G, White R, Parker MG. RIP140-targeted repression of gene expression in adipocytes. Mol Cell Biol. 2005. 25(21):9383-9391.

Jörg Heeren
University Medical Center Hamburg-Eppendorf, Germany

Research interests
The main research interest of Prof. Dr. Jörg Heeren is to understand molecular mechanisms causing abnormalities in lipid and lipoprotein metabolism, which are associated with the development of chronic inflammatory disorders such as atherosclerosis and type 2 diabetes. He is recognized for his work on the intravascular processing and hepatic uptake of triglyceride-rich lipoproteins. His research on the intracellular sorting of lipoprotein components provided a new intracellular link between the metabolism of triglyceride-rich lipoproteins and the 'good', atheroprotective cholesterol, HDL. More recently, Prof. Heeren has utilized state of the art nanoparticle-based metabolic imaging1, to establish a key role for the brown adipose tissue in the regulation of plasma triglyceride levels.

Publications

1. Bruns OT, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Lauterwasser J, Nikolic MS, Mollwitz B, Merkel M, Bigall NC, Sapra S, Reimer R, Hohenberg H, Weller H, Eychmüller A, Adam G, Beisiegel U, Heeren J. Real-time magnetic resonance imaging and quantification of lipoprotein metabolism *in vivo* using nanocrystals. Nat Nanotechnol. 2009 Mar;4(3):193-201.
2. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, Kaul MG, Tromsdorf UI, Weller H, Waurisch C, Eychmüller A, Gordts PL, Rinninger F, Bruegelmann K, Freund B, Nielsen P, Merkel M, Heeren J. Brown adipose tissue activity controls triglyceride clearance. Nat Med. 2011 Feb;17(2):200-5.

Susanne Klaus
German Institute of Human Nutrition in Postdam, Germany

Research interests
Susanne Klaus is a zoologist by training and obtained a PhD in animal physiology at the University of Marburg (Germany) in 1988. Since 1997 she has been group leader at the German Institute of Human Nutrition in Potsdam and a Professor for Energy Metabolism at the Department of Nutrition at the University of Potsdam.
Her research is focused on the interaction of diet components, mainly macronutrients with genetic factors in the development of obesity and associated health problems. A particular long lasting focus has been on the role of brown adipose tissue and mitochondrial uncoupling in energy homeostasis.

Publications

1. Freudenberg A, Petzke KJ, Klaus S. (2012) Comparison of high-protein diets and leucine supplementation in the prevention of metabolic syndrome and related disorders in mice. J Nutr Biochem. 23(11): 1524-30.
2. Keipert S, Voigt A, Klaus S. (2011) Dietary effects on body composition, glucose metabolism, and longevity are modulated by skeletal muscle mitochondrial uncoupling in mice. Aging Cell. 10: 122-136.

Martin Klingenspor
Technische Universität München, Germany

Research interests
Physiological and genetic adaptations of metabolism tune our body to adequately respond to food components and nutritional challenges. Our group investigates the impact of genes, nutrition and environmental temperature on the balance between energy intake and energy expenditure with a particular focus on brown adipose tissue function. In our previous work we investigated the recruitment of heating power in mice with non-functional brown fat and identified genomic regions required for brown adipocyte specific gene expression.

Publications

1. Meyer CW, Willershäuser M, Jastroch M, Rourke B, Fromme T, Oelkrug R, Heldmaier G, Klingenspor M. Adaptive thermogenesis and thermal conductance in wildtype and UCP1-KO mice. Am J Physiol Regul Integr Comp Physiol 299(5):R1396-406, 2010.
2. Fromme T, Hoffmann C, Nau K, Rozman J, Reichwald K, Utting M, Platzer M, Klingenspor M. An intronic single base exchange leads to a brown adipose tissue specific loss of Ucp3 expression and an altered body mass trajectory. Physiol Genomics 38(1):54-62, 2009.

Jan Kopecky
Academy of Sciences of the Czech Republic

Research interests

Over 30 years of research experience, starting from studies on the mechanism of mitochondrial energy conversion, brown adipose tissue thermogenesis, and perinatal recruitment of brown fat. Since 1992, JK serves as a Head of the Department of Adipose Tissue Biology at the Institute of Physiology (Prague). JK was an International Research Scholar of the Howard Hughes Medical Institute (1995-2000), served as a co-president of the 13th European Congress on Obesity (2004), and participated at several internationally funded projects, including the grants from the March of Dimes Birth Defects Foundation, the Wellcome Trust, and EU FP7 projects (EXGENESIS, EARNEST, BIOCLAIM, DIABAT). Current projects are focused on characterization of the mechanisms enabling beneficial effects of lipids of marine origin on obesity and associated disorders, and on the induction of energy expenditure in white fat.

Publications

1. Jelenik T, Rossmeisl M, Kuda O, Macek Jilkova Z, Medrikova D, Kus V, Hensler M, Janovska P, Miksik I, Baranowski M, Gorski J, Hébrard S, Jensen TE, Flachs P, Hawley S, Viollet B, Kopecky J. 2010. AMPK-activated protein kinase alpha 2 subunit is required for the preservation of hepatic insulin sensitivity by *n*-3 polyunsaturated fatty acids. *Diabetes* 59:2737-2746
2. Flachs P, Rühl R, Hensler M, Janovska P, Zouhar P, Kus V, Macek Jilkova Z, Papp E, Kuda O, Svobodova M, Rossmeisl M, Tsenov G, Mohamed-Ali V, Kopecky J. 2011. Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and *n*-3 fatty acids. *Diabetologia* 54:2626-2683

Leslie Kozak
Polish Academy of Sciences, Poland

Research interests
In 1970 I began investigating how the cytoplasmic glycerol-3-P dehydrogenase in Bergmann glial cells of the cerebellum was regulated by interactions with Purkinje cell neurons. By the end of the 70's it became apparent that this problem could be analyzed more effectively with cloned genes, but not in the cerebellum, because of the very low levels of gene expression. We looked for another tissue with more abundant expression and possible regulation by neural interactions. The winner was brown fat. We cloned the glycerol -3-P dehydrogenase cDNA from brown fat and in the process the Ucp1 cDNA fell out. But in time *UCP1* became interesting enough to study in its own right.

Publications

1. Rea P. Anunciado-Koza, Jingying Zhang, Jozef Ukropec, Sudip Bajpeyi, Robert A. Koza, Richard C. Rogers, William T. Cefalu, Randall L. Mynatt, and Leslie P. Kozak. Inactivation of the Mitochondrial Carrier SLC25A25 (ATP-Mg2_/Pi Transporter) Reduces Physical Endurance and Metabolic Efficiency in Mice. JBC Papers in Press, February 4, 2011, DOI 10.1074/jbc M110.203000
2. Leslie P. Kozak*, Robert A. Koza, Rea Anunciado-Koza, Tamra Mendoza, Susan Newman Inherent Plasticity of Brown Adipogenesis in White Fat of Mice Allows for Recovery from Effects of Post-Natal Malnutrition. PLoS ONE 7(2): e30392. doi:10.1371/journal.pone.0030392

Wouter van Marken Lichtenbelt
Maastricht University, The Netherlands

Research interests
Energy metabolism and thermoregulation in health and disease.
The focus is on the effects of environmental conditions, such as (indoor) temperature and nutrition on physiological processes. The studies relate to whole body physiology and to the underlying mechanisms on molecular, cellular, and tissue (skeletal muscle and brown adipose tissue) level.
A key achievement is the discovery of functional brown adipose tissue in adult humans.

Publications

1. Maarten J. Vosselman, Anouk A.J.J. van der Lans, Boudewijn Brans, Roel Wierts, Marleen A. van Baak, Patrick Schrauwen, and Wouter D. van Marken Lichtenbelt. Systemic ^{y}-Adrenergic stimulation of thermogenesis is not accompanied by brown adipose tissue activity in humans. diabetes.diabetesjournals.org (2012)
2. G. H. E. J. Vijgen, N. D. Bouvy, G. J. J. Teule, B. Brans, J. Hoeks, P. Schrauwen, and W. D. van Marken Lichtenbelt. Increase in brown adipose tissue activity after weight loss in morbidly obese subjects. The Endocrine Society doi: 10.1210/jc.2012-1289 (2012)

Jan Nedergaard
The Wenner-Gren Institute, Sweden

Research interests
Jan Nedergaard is professor of physiology at The Wenner-Gren Institute, Stockholm University. His scientific efforts have concentrated on the understanding of the function and physiological significance of brown adipose tissue. In recent years, he has been significantly involved in the establishment of new concepts in brown adipose tissue research: - that brown adipocyte precursors are principally different from white adipocytes in that they display a myogenic gene expression phenotype (2007); - that the absence of brown adipose tissue is sufficient to cause or aggravate obesity (2009); - that existing radiological data implied that brown adipose tissue is present and active in adult humans (2007), – and that the gene expression profile observed in UCP1-expressing cells in white adipose depots is so distinct from that of classical brown adipocytes that these cells/depots should be considered to be of a different nature ("brite adipocytes") (2010).

Publications

1. Waldén TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am J Physiol Endocrinol Metab. 2012 302:E19-31
2. Cannon B, Nedergaard J. Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). Int J Obes (Lond). 2010 34 Suppl 1:S7-16.

Patrick Seale
University of Pennsylvania, USA

Research interests
My laboratory studies the transcriptional machinery that controls the fate of adipocytes. We are particularly focused on the pathways responsible for directing the differentiation and function of thermogenic adipocytes, both in the brown adipose and white adipose depots. We previously identified a large zinc finger co-activator protein, Prdm16, as an important regulator of brown adipocyte differentiation.

Publications

1. Seale, P., Conroe, H.M., Estall, J.E., Kajimura, S., Frontini, A., Cinti, S., Ishibashi, J., Cohen, P., Spiegelman, B.M. (2011). Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. J. Clin Invest. 121: 96-105.
2. Ishibashi J, Firtina Z, Rajakumari S, Wood KH, Conroe HM, Steger DJ, Seale P. (2012). An Evi1-C/EBPβ complex controls Pparγ2 expression to initiate white fat cell differentiation. Mol Cell Biol. 32:2289-99.

Michael Symonds
University of Nottingham, UK

Research interests
The developmental regulation of adipose tissue and the impact of early life nutrition. Assessment, quantification and comparative physiology of brown adipose tissue.

Publications
1. Symonds, M. E., Henderson, K., Elvidge, L., Bosman, C., Sharkey, D., Perkins, A. C., and Budge, H. (2012) Thermal imaging to assess age-related changes of skin temperature within the supraclavicular region co-locating with brown adipose tissue in healthy children. J Pediatr 161, 892-898
2. Symonds, M. E., Pope, M., Sharkey, D., and Budge, H. (2012) Adipose tissue and fetal programming. Diabetologia 55, 1597-1606

Antonio Vidal-Puig
Institute of Metabolic Science, University of Cambridge, UK

Research interests
My laboratory focuses on the link between obesity and its metabolic complications and aims to develop novel therapeutic approaches for these conditions. We have contributed to establish the concept of lipotoxicity, as a valid intellectual framework to study the Metabolic Syndrome and have proposed the use of energy dissipating strategies to reverse lipotoxicity by promoting mitochondrial biogenesis, mitochondrial uncoupling and brown fat differentiation and activation..

Publications
1. Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vázquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, Dale M, Virtue S, Villarroya F, Cannon B, Rahmouni K, López M, Vidal-Puig A. BMP8B Increases Brown Adipose Tissue Thermogenesis through Both Central and Peripheral Actions. Cell. 2012 May 11;149(4):871-85.
2. Virtue S, Feldmann H, Christian M, Tan CY, Masoodi M, Dale M, Lelliott C, Burling K, Campbell M, Eguchi N, Voshol P, Sethi JK, Parker M, Urade Y, Griffin JL, Cannon B, Vidal-Puig . A new role for Lipocalin Prostaglandin D Synthase in the regulation of brown adipose tissue substrate utilisation. Diabetes 2012. 24 August.

Francesc Villarroya
University of Barcelona, Spain

Research interests

Francesc Villarroya is the head of a laboratory in the University of Barcelona focused to the study of the control of gene expression in relation to energy metabolism. The laboratory has been studying in the last decades multiple aspects of the function of brown adipose tissue in response to physio-pathological conditions using animal and cellular models. Transcriptional regulation of gene expression in brown adipose tissue is a particular focus of the laboratory. At present, Francesc Villarroya is director of the Institute of Biomedicine of the University of Barcelona, and member of the directory board of the Spanish Society for Studies on Obesity and of the Spanish research network CIBER Fisiopatologia de la Obesidas y Nutrición.

Publications

1. Giralt A, Hondares E, Villena JA, Ribas F, Díaz-Delfín J, Giralt M, Iglesias R, Villarroya F. Peroxisome proliferator-activated receptor-gamma coactivator-1alpha controls transcription of the Sirt3 gene, an essential component of the thermogenic brown adipocyte phenotype. J Biol Chem. 2011 May 13;286(19):16958-66. Epub 2011 Mar 27.
2. Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F. Hepatic FGF21 expression is induced at birth via PPARalpha in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metab. 2010 Mar 3;11(3):206-12.

Communications

Denotes presenting author

C01 & PC01	Cholesteryl Ester Transfer Protein (CETP) enhances beta-3 adrenergic receptor and UCP1 expression, inducing energy expenditure and reducing adiposity in transgenic mice H. F. Raposo*, A. A. Paiva, J. C. Christovam, H. C. F Oliveira
C02 & PC02	Expression of Zinc finger of the cerebellum 1 suggests a possible role in development and/or function of brown adipose tissue J. de Jong*, T. B. Waldén, J. A. Timmons, N. Petrovic, I. R. Hansen, B. Cannon, J. Nedergaard
C03 & PC03	Imaging brown adipose tissue at work A. Bartelt*, B. Freund, H. Ittrich, R. Reimer, J. Schmidt, N. Schaltenberg, O. T. Bruns, J. Heeren
C04 & PC04	Quantification of de novo lipogenesis by room calorimetry G. Abreu-Vieira*, B. Cannon, J. Nedergaard
C05 & PC05	Central prolactin-releasing peptide is required for cold- and leptin-induced thermogenesis in mice S. M. Luckman*
C06 & PC06	LR11 negatively regulates thermogenesis A. Whittle*, V. Peirce, M. Jiang, H. Bujo, A. Vidal-Puig
C07 & PC07	Effect of novel thiazolidinadiones on expression of UCP1 and mitochondrial respiratory chain proteins in brown and white adipose tissues A. Kalinovich*, G. Abreu-Vieira, I. G. Shabalina, N. Petrovic, B. Cannon, J. Nedergaard
C08 & PC08	Pyruvate kinase M2 deficiency promotes a brown fat-like program in white adipocytes A. Bettaieb, J. Bakke, N. Nagata, A. Tomilov, C. Lyssiotis, J. Asara, G. Cortopassi, L. Cantley, F. G. Haj*
C09 & PC09	Loss of neuronatin promotes 'browning' of primary mouse adipocytes V. Gburcik*, B. Cannon, M. E. Cleasby, J. A. Timmons
C10 & PC10	Brown adipose tissue in epicardial adipose tissue of adult humans S. Ojha*, H. S. Sacks, H. Budge, M. E. Symonds
C11 & PC11	Unequivocal identification of brown adipose tissue in an adult human using Magnetic Resonance Imaging N. L. Reddy*, T. A. Jones, S. C. Wayte, O. Adesanya, Y. Yeo, H. Randeva, S. Kumar, C. E. Hutchinson, T. M. Barber
C12 & PC12	Brown adipose tissue function and premature ageing: Impaired thermogenesis in mice expressing defective mitochondrial DNA polymerase I. G. Shabalina*, R. Csikasz, N. Petrovic, A. Trifunovic, N. Larsson, B. Cannon, J. Nedergaard

PC13 Actions of L-citrulline on gastric mucus secretion and gastric mucous cell counts in albino rats G. I. Grace*, O. S. Francis, F. S. Ayodele

PC14 Application of high content image analysis to identify factors that encourage the brown-like phenotype in human adipose-derived stem cells B. Kihlberg, A. Forslöw, B. Magnusson, R. Westergren, L. Drowley, S. Bartesaghi, S. Hallén, P. Seale, X. Peng*

PC15 Association of circulating peroxiredoxins levels with indicators of glycemic control in patients with type II diabetes mellitus E. A. El Eter*, H. AlZamil, S. Tayel, A. Almasri

PC16 Candidate Genes involved in cAMP-stimulated cell proliferation of brown preadipocytes Y. Wang*, J. Nedergaard

PC17 Coffee attenuates induction of insulin resistance by high sucrose-diet in rats A. O. Morakinyo*, D. A. Adekunbi, O. A. Adegoke

PC18 Decreased fatty acid uptake and metabolism during acute and chronic cardiac ischemia are mediated by different mechanisms K. Pates, J. Griffin, J. Luiken, K. Clarke, L. Heather*

PC19 Defective regulation of energy expenditure in IL10 knockout mice G. F. Souza*, E. P. de Araujo, J. Morari, C. Solon, L. F. do Nascimento, R. F. Moura, L. A. Velloso

PC20 Effect of (+)-snic acid as a fat burner on the rat hepatocyte; correlated histological and biochemical *in vivo* study A. A. Al-Ahmadi*, N. Ayuob, S. Ali, A. Al-Robai, N. Abo-Khatwa

PC21 Fetal macrosomia of diabetic rats: histological and histochemical studies of skin and brown fat S. S. Ali*, N. Ben Zakar, F. Al Qudsi, S. Karim

PC22 Hypothalamic BMP8B induces thermogenesis in BAT in a manner dependent of AMPK activation L. Martins*, A. J. Whittle, R. Nogueiras, C. Diéguez, A. Vidal-Puig, M. López

PC23 Identification of a clavicular brown adipose tissue depot in the sheep M. Pope*, M. Birtwistle, H. Budge, M. Symonds

PC24 Inhibitory effect of visfatin and leptin on human and rat myometrial contractility S. Mumtaz*, S. Alsaif, S. Wray, K. Noble

PC25 Late gestation maternal nutrient restriction reprogrammes the transcriptional control of uncoupling protein 1 in neonatal brown adipose tissue L.Elvidge*, M Pope, M.E.Symonds, H.Budge

PC26 Mechanisms of carbamoylcholine action on respiration and oxidative phosphorylation of isolated pancreatic acini B. O. Manko*, M. Y. Klevets, V. V. Manko

PC27 Mercury chloride-induced glucose intolerance in rats: role of oxidative stress A. O. Morakinyo, B. O. Iranloye, G. O. Oludare*, J. O. Oyedele, O. O. Ayeni

PC28 Modification of PPARα activity is detrimental to cardiac function during chronic hypoxia – critical role of PPARα on cardiac substrate metabolism A. Abd Jamil*, M. Cole, L. Heather, R. Evans, E. Sutton, K. Clarke

PC29 Role of retinoic acid, rosiglitazone and forskolin in the expression of human and mouse UCP1 reporter constructs in differentiated and undifferentiated HIB1B brown adipocytes M. Malibary*, T. M Perehinic, P. J Hill, M. E. Symonds, H. Budge, M. A. Lomax

PC30 Severe respiratory chain defects cause increased recruitment of brown adipose tissue A. Misra*, R. W. Taylor, D. M. Turnbull

PC31 Short-chain 3-L-hydroxyacyl-CoA Dehydrogenase (SCHAD) and its role in the regulation of thermogenesis N. Schulz*, A. Helms, N. Perwitz, J. Klein, T. Kanzleiter, H. Joost, A. Schurmann

PC32 Transcriptomic profiles in epididymal, inguinal, and brown adipose depots S. Naknukool*, T. Goto, N. Takahashi, T. Kawada, S. Seno, H. Daiyasu, H. Matsuda

PC33 Dead in bed syndrome: a misnomer? E. J. Kealaher*

PC34 Enhanced spermiogram in Cinnamon verum treated male Alloxan-induced diabetic Wistar albino rats O. E. Adeleye*, O. B. Akinlabi, A. I. Adeleye

PC35 Sweating and thirst perception in premenopausal, perimenopausal and postmenopausal women during moderate exercise E. Amabebe*, S. I. Omorodion, J. O. Ozoene

PC36 The effect of dietary fat and glycaemic index on metabolic profiles of human urine N. A. Ismail*, I. Garcia-Perez, J. M. Posma, L. Goff, E. Holmes, G. Frost

PC37 The effects of constituents of garlic on uterine contractility J. McNamee*, H. Robinson, S. Wray

PC38 The histochemistry of enteroendocrine cells in streptozotocin induced diabetic albino wister rats T. M. Otobo*, R. Tarimobo Otobo

Useful Information

The Physiological Society team can always be found at the registration desk in the Reception Area of The Royal Society. We will be happy to help with any queries you may have but you might find an answer to your question on this page. There are also some volunteers on hand who will be happy to assist.

If you need to see us, come to the registration desk which will be open on:

Tuesday 11 December	11:00 - 17:30
Wednesday 12 December	08:00 - 17:30
Thursday 13 December	08:00 - 11:30

The Meeting Rooms

We are using four rooms for the Meeting at The Royal Society.

Wellcome Trust Lecture Hall - all oral presentations and communications.
City of London Rooms 1, 2 & 3 – poster presentations, exhibition, refreshments, lunch

Poster Sessions

Presenting authors will be accompanying their posters at the following times:
Odd numbers (e.g. PC01): Tuesday 11 December from 17.15
Even numbers (e.g. PC02): Wednesday 12 December from 15.30

Luggage Storage / Cloakroom

There is a cloakroom available, please ask at The Physiological Society registration desk for details. Please note that all belongings are left at your own risk.

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There is free Wi-Fi available at The Royal Society,
Network: rsnetwork
Password: Newton+apple

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Notices

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Mobile phones

Don't forget to turn off your mobile during all sessions at the Meeting.

Video or audio recording of presentations

Participants are reminded that the video and audio recording of any session or presentation (symposium, Oral and Poster Communications) using mobile devices or any such recording equipment is strictly prohibited.

Arriving late to sessions

We know it is not always possible to get to sessions on time (especially the morning after the Society Dinner), but we do ask that if you are running late, to take your seat quietly.

Smoking
You can't smoke inside in the UK. If you wish to smoke then you need to go outside.

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Lunch and refreshments are included in the registration package and will be available in the City of London Rooms 1 & 2.

Certificate of attendance
If you requested a certificate of attendance when you registered online, this will be available at the registration desk.

If you didn't request a certificate of attendance when you registered and now need one, please ask one of The Physiological Society team at the registration desk who will print one out for you to collect at the end of the meeting.

Changes to abstracts
We cannot make changes to abstracts.

Twitter
The official Twitter hashtag is #me2012 Make sure you are following @ThePhySoc to keep up to date on what The Society is up to.

Feedback
We want you to enjoy this meeting but are always happy to hear how we can improve. You can speak to a member of the team, or fill in your comments anonymously in a feedback questionnaire that will be emailed to you after the meeting.

Social Programme
Young Physiologists' Social

Tuesday 11 December
19.00

An area has been arranged at a local bar for any young physiologists' who would like to meet like-minded delegates on the first evening of the meeting. Ask at The Physiological Society registration desk for details. Free to attend, drinks not included.

Society Dinner
The Churchill War Rooms
Wednesday 12 December
19.00

Join us at The Churchill War Rooms, where you will receive a drink on arrival in the Harmsworth Room, which incorporates a 1940's generator on the wall with switches and dials. You'll also have the chance to explore the secret wartime headquarters which have been frozen in time and the interactive museum, followed by a three course dinner and coffee served in the HCA Auditorium surrounded by posters from the 1940's. Tickets are £60 and include exclusive use of the venue, food and drinks.

There will be a handful of tickets available for sale at the meeting, please ask at The Physiological Society registration desk.

The Churchill War Rooms
Clive Steps
King Charles St
Westminster
City of London
SW1A 2AQ

The Physiological Society Oral and Poster Competitions

The Physiological Society Oral Communication and Poster Competitions will be in operation at this Meeting.

These competitions are designed to award the best oral communication and poster presentations by an Affiliate Member of The Society.

Both will be judged on:

- the calibre and clarity of scientific data;
- the communication of the content;
- the ability of the presenter to answer questions concerning the research and findings. Invited speakers assess the competition orals and posters and discuss the science with the eligible presenter. Speakers vote on the best orals and posters, and the presenting author of the winning entrants will receive a framed certificate and an Amazon voucher after the Meeting.

Benevolent Fund

You may have noticed when registering for this Meeting, you had the option to make a donation to The Society's Benevolent Fund.

The Benevolent Fund was established in 1976 to aid Members of The Society and staff in physiology departments in times of hardship. Grants are given to assist with funeral arrangements, medical costs and childcare. Since administration costs are absorbed by The Society, every penny donated goes to people in need. For more information please visit our website: www.physoc.org/benfund.asp.

If you were able to donate, we thank you on behalf of The Physiological Society for your contribution.

If you were not able to donate, but now wish to do so, one-off donations can be submitted to the registration desk. If you are interested in donating on a more regular basis in the form of a standing order, please see a member of staff who can give you the correct form.

About The Society

The Physiological Society was founded in 1876 and is a learned society with over 2900 Members (including 21 Nobel Laureates) and Affiliates (early career scientists) drawn from over 50 countries. The majority of Members are engaged in research, in universities or industry.
The Society's charitable objectives are to promote, for the benefit of the public, the advancement of physiology, and facilitate the intercourse of physiologists both at home and abroad, and thereby contribute to the progress and understanding of biomedical and related sciences and the detection, prevention and treatment of disease, disability and malfunction of physical processes in all forms of life.

To achieve these objectives, The Society supports up to five scientific Meetings annually, organises international workshops, publishes two journals and awards grants to allow Members to travel to scientific Meetings and to carry out research collaborations. Interaction with outside bodies is encouraged through representation on various councils and committees, and active membership of the Society of Biology and the Federation of European Physiological Societies (FEPS).

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The Physiological Society

C01 and PC01

Cholesteryl Ester Transfer Protein (CETP) enhances beta-3 adrenergic receptor and UCP1 expression, inducing energy expenditure and reducing adiposity in transgenic mice

H.F. Raposo, A.A. Paiva, J.C. Christovam and H.C. F Oliveira

Department of Structural and Functional Biology, Institute of Biology, Campinas, SP, Brazil

Cholesteryl Ester Transfer Protein (CETP) is a plasma protein that mediates the exchange of triglycerides for esterified cholesterol from HDL to the apoB-lipoproteins. In this way, CETP promotes reduction of plasma HDL-cholesterol and, thus, increases the risk of atherosclerosis. Recently, we found that CETP expressing mice (CETP-Tg) present lower adipose tissue mass than non-expressing controls. In this work we investigated possible mechanisms to explain these findings. The animal protocol was approved by the State University of Campinas Committee for Ethics in Animal Research under the protocol # 1607-1. CETP-Tg and NTg control mice (C57/BL6 background) were fed with chow diet from weaning. Samples were obtained post euthanasia, when mice were 5 month old. CETP-Tg mice had reduced perigonadal (-29%) and subcutaneous (-27%) fat depots that could not be explained by differences in fat intake and excretion. Adipose tissue (perigonadal and subcutaneous) and liver lipogenesis rates (estimated using 3H2O and 14C-acetate) were similar in both CETP-Tg and control mice. Lipid retention and glucose uptake by liver, muscle, white and brown adipose tissues (WAT, BAT), estimated after an oral dose of 3H-triolein and 3H-deoxiglucose, respectively, showed no significant differences between groups. In the fed state, CETP group showed higher (~50%) basal lipolysis in vivo and isoproterenol stimulated lipolysis by isolated adipocytes. In accordance, visceral adipose HSL and ATGL mRNA expression were elevated. Beta 3 adrenergic receptor protein levels were upregulated in visceral and brown adipose tissues. Regarding gene expression: UCP1, ATGL, FATP1 were increased in BAT, whilst PPARa, PPARy, PGC1a, HSL, Perilipin, AMPK, CD36, CPT1, RIP140 were not changed. In addition, whole body energy expenditure (measured by respirometry) was found to be elevated in CETP-Tg mice (10%). After fasting, no significant differences were detected in lipolysis and in energy expenditure between groups. These data suggest that CETP could decrease adipose triglycerides availability and change lipolysis and thermogenic related gene expression. The increased whole body energy expenditure results in the reduction of body fat content. These findings disclose a novel anti-adipogenic role for CETP.
Key-words: CETP, UCP1, B3AR, lipolysis, adiposity

Financial support: Fapesp, CNPq and Capes (Brazilian agencies).

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C02 and PC02

Expression of Zinc finger of the cerebellum 1 suggests a possible role in development and/or function of brown adipose tissue

J. de Jong[1], T.B. Waldén[2], J.A. Timmons[3], N. Petrovic[1], I.R. Hansen[1], B. Cannon[1] and J. Nedergaard[1]

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Introduction - Since the discovery of brown adipose tissue in adult humans, the interest in brown adipocyte development and function has gained great interest. Whereas white adipocytes have the capacity to store energy in the form of triglycerides, brown adipocytes are able to use fatty acids as fuel to produce heat. It is of great interest to study which factors contribute to these different functions.
Candidates for such genes could be found among those that are differentially expressed between brown and white adipocytes. One such a gene is Zic1 (Zinc finger of the cerebellum 1), which is found selectively in brown adipose tissue.
Methods - Male NMRI mice were kept at either 30°C, room temperature or 4°C for 3 weeks starting at 6 weeks of age. Mice were euthanized and adipose tissues were analyzed for gene expression. For primary cultures, male NMRI mice (3-4 weeks old, kept at room temperature) were euthanized and adipose depots were collected and prepared for culture.
Results - In different adipose depots analyzed (brown, white and brite,) Zic1 expression is clearly restricted to brown adipose depots. Its expression was not detectable in white and brite adipose depots (Fig. 1a).
This restriction to brown adipocytes is maintained in primary adipose cultures. Both in undifferentiated brown preadipocytes and differentiated brown adipocytes Zic1 expression can be detected, whereas it is absent in cultured epididymal (pre)adipocytes (Fig. 1b).
Results obtained in brown primary cultures also indicate that Zic1 expression is (negatively) regulated by treatment with the PPARγ-agonist rosiglitazone (Fig .1c).
Zic1 expression analysis in interscapular brown adipose tissue does not seem to be affected by diet or temperature (Fig. 2a and 2b).
In Zic1 knockdown experiments in primary brown adipose cultures, Ucp1 expression induced by acute norepinephrine treatment is blunted when Zic1 is knocked down (Fig. 2c).
Conclusions - Zic1 clearly shows selective brown adipose tissue expression compared to other adipose depots suggesting a possible role in the development or function of brown adipose tissue. The blunted Ucp1 expression upon knockdown of Zic1 in brown adipose culture suggests a functional role for Zic1 in regulation of Ucp1 expression downstream of norepinephrine signaling. Together, these findings ask for follow-up experimental investigation of Zic1 as a functional player in the development and or function of brown adipose tissue.

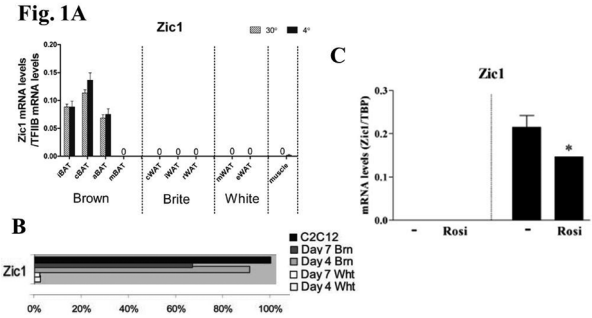

Figure 1. Zic1 is a brown-specific adipose marker

(A) Zic1 shows BAT-specific expression when compared to white and brite adipose depots (Waldén et al., 2012)

(B) Zic1 is expressed in C2C12 muscle cell line and brown (Brn) preadipocytes, but not in white (Wht) preadipocytes (Timmons et al., 2007).

(C) Zic1 expression is decreased in primary cultures of brown adipose upon 5 days of treatment with the PPARγ-agonist Rosiglitazone. Again, Zic1 is not detected in white primary cultures (Petrovic et al, 2010). The significance of rosiglitazone treatment was determined with a paired t test performed on the logarithmized expression levels of control and rosiglitazone-stimulated white and brown adipocyte cultures. *, p <0,05

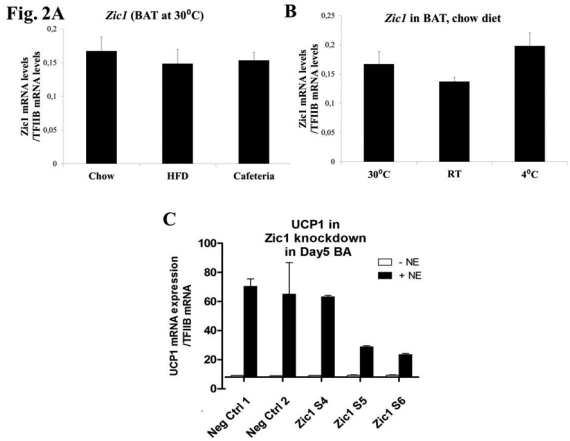

Figure 2. Zic1 expression is not influenced by diet or temperature, but may still be involved in regulation of Ucp1 expression

(A) Zic1 expression in iBAT is not influenced by. Mice were fed different diets at 30°C for 3 weeks. n=6

(B) Temperature does not affect Zic1 expression levels. Mice were kept at indicated temperatures for 3 weeks on chow diet. n=6

(C) Ucp1 expression upon 2h 1μM NE treatment in primary brown adipocytes on day 5 of culture. Three different siRNAs were used, two of which blunt Ucp1 expression upon NE treatment (S5 and S6). n=2

Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 285: 7153–7164, 2010.

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Walden TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am J Physiol Endocrinol Metab 302: E19–E31, 2012.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C03 and PC03

Imaging brown adipose tissue at work

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Objective: Brown adipose tissue is a powerful organ metabolizing vast amounts of circulating lipoproteins and glucose. Here we visualise and analyse the delivery of lipophilic nutrients to brown and brown-in-white ("brite") adipocytes in vivo. We use state-of-the-art multi-modal imaging technologies based on lipoprotein Trojan horses engineered with superparamagnetic or fluorescent nanocrystals.
Methods:All experiments with mice as well as their sacrifice afterwards were conducted under anaesthesia (intraperitoneal injection of 90mg/kg xylazine and 1.28 mg/kg ketamine). Magnetic resonance imaging (MRI) was performed using a clinical 7T scanner with FVB wild-type mice. Real-time intravital imaging was performed using a confocal fluorescence microscope equipped with a resonant scanner with mice expressing green fluorescent protein under control of the Uncoupling protein-1 (Ucp1) promoter.
Results: Using MRI we could detect lipoprotein catabolism non-invasively in brown adipose tissue as well as in brite adipocytes in inguinal white adipose tissue for the

first time, indicated by a strong increase in T2* contrast. Using intravital imaging we could visualize lipoprotein and lipid uptake into brown adipocytes in real-time. This process comprised a two-step mechanism: initial binding followed by endocytosis. Interestingly, in electron microscopy studies we detected nanocrystals labelling the lipoproteins in endocytic vesicles of endothelial cells. When analysing brite adipocytes in inguinal white adipose tissue, only brite but not their neighbouring white adipocytes display endocytosis. This finding was supported by intravital imaging where only brite but not white adipocytes are characterized by lipoprotein accumulation at their access site to the capillary.
Conclusion: In this project we were able to non-invasively image and quantify the activity of brown but also of brite adipocytes for the first time using MRI. Our mechanistic studies using intravital imaging and electron microscopy suggest that brown as well as brite adipocytes are able to manipulate their endothelial microenvironment, resulting in increased local influx of lipoproteins and nutrients for combustion or storage.

We acknowledge expert technical assistance provided by Hendrik Herrmann. O. T. Bruns is supported by a European Molecular Biology Organization Long-Term Fellowship, Alexander Bartelt was supported by a Post-doctoral Fellowship Award from the European Atherosclerosis Society.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C04 and PC04

Quantification of de novo lipogenesis by room calorimetry

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Thermoneutral environments are defined as those in a temperature zone where a determined warm-blooded animal does not have to expend extra energy on heating. This same zone is known for facilitating accumulation of adipose tissue and decreasing brown adipose tissue activity to its minimal thermogenic level. Although high-fat diets have clearly a higher impact on body composition at this temperature zone, particularly interesting is the situation where a low-fat diet causes significant increase in the whole body lipid content.
Assuming that de novo lipogenesis is exacerbated at the thermoneutral zone, our study is focused on the creation a model to indirectly predict an organism's lipid weight gain by de novo lipogenesis over several weeks, based on a calorimetry measurement for 24 hours and the knowledge of food composition.
For this study, male C57Bl6 mice living at thermoneutrality were fed a low or high-fat diet during 12 weeks at thermoneutrality (30 °C). Total lipid weight of mice was quantified by magnetic resonance imaging (EchoMRI) at each second week of experiment. Mice are gently restrained and remain conscious during the meas-

urement, which takes around 45 seconds. At week 12, indirect calorimetry was performed during 24 h (mice remained in their home cages), when respiratory gases and exchange rate were measured. An equation was developed based on the respiratory quotient (RQ) expected for the food in comparison with the measured RQ, where the shifts towards higher values were considered proportional changes in whole-body metabolism towards de novo lipogenesis. The percentage of RQ shift was assumed to be the same percentage of O2 used in the reaction, and the conversion rate of glucose to lipids could be determined based on classic stoichiometric values. MRI analysis demonstrated that fat weight gain occurred in both food groups, being higher in the high-fat group. During room calorimetry measurement, the RQ shift towards de novo lipogenesis was only seen in low-fat-fed mice, demonstrating our basic assumptions for the calculations were correct. The equation accurately indicated the lipid weight gain by de novo lipogenesis during the first 6 weeks of experiment, but failed to predict the lack of linearity from week 6 onward. The fat weight accumulated under low-fat diet seems to originate from carbohydrates and de novo lipogenesis is absent in mice fed with a high-fat diet. Our results indicate that de novo lipogenesis probably can be quantified by the combination of room calorimetry and food composition data. Deeper understanding of each part of process is necessary for a more accurate prediction to be made and an equation to be fully accepted.

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C05 and PC05

Central prolactin-releasing peptide is required for cold- and leptin-induced thermogenesis in mice

S.M. Luckman

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The RFamides are so called because they all have common C-terminal arginine and phenylalanine residues. Through evolution, they have had a role in feeding behaviour and, in rodents, brainstem neurones containing one RFamide, prolactin-releasing peptide (PrRP), mediate the effects of the gut-brain satiety signalling. Thus, PrRP can mimic satiation and reduction in gastric emptying, whilst the actions of cholecystokinin are absent in mice lacking either PrRP or its receptor (GPR10). Both of these transgenic models display late-onset obesity. However, GPR10 receptor knock-out mice are obese primarily because they display lower than normal energy expenditure. In this study, we use mice fitted with intraperitoneal radiotelemetric devices and with indwelling lateral ventricular cannulae. Surgery is carried out under general inhalation anaesthesia (2% isoflurane in 1L/min oxygen). Animals are given an intraperitoneal (i.p.) injection of 0.01mg kg-1 buprenorphine before recovery and maintained at 37oC for four hours post surgery. Animals remained singly housed post surgery, and were monitored daily for 7 days before further procedures were

carried out. Intracerebroventricular injection of PrRP (4 nmol) increases both energy expenditure and core-body-temperature in wild-type, but not in GPR10 knock-out mice (analysis of variance with Bonferroni post hoc tests used in all analyses). When later kept at a lower ambient temperature (15oC for five days), the mice maintain their core-body temperature by shivering, as they are unable to activate brown adipose tissue (BAT), even though their BAT has thermogenic capacity. Similarly, the mice cannot initiate non-shivering thermogenesis in response to leptin (5 mg/kg, i.p.). We have demonstrated that a population of PrRP neurones in the dorsomedial nucleus of the hypothalamus, are an integral part of the central circuitry involved in sympathetic activation of brown adipose tissue.

The author would like to acknowledge the funding of the BBSRC and support from Eli Lilly.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C06 and PC06

LR11 negatively regulates thermogenesis

A. Whittle[1], V. Peirce[1], M. Jiang[2], H. Bujo[2] and A. Vidal-Puig[1]

[1]Institute of Metabolic Science, University of Cambridge, Cambridge, UK and [2]Department of Genome Research, Chiba University, Chiba, Japan

LR11 is a neuronal apolipoprotein E (APOE) receptor implicated in the pathogenesis of Alzheimer's disease, potentially through its dual function in vesicular trafficking [1]. There is also evidence that a portion of the LR11 peptide is proteolytically cleaved and this enters the nucleus to regulate transcription [2]. We observed that mice lacking LR11 were less susceptible to diet induced obesity and that LR11 expression was reduced in response to high fat diet feeding, suggesting a potential role for LR11 in energy balance.

Methods: Wild type and LR11-/- C57Bl6/J mice were housed at either 21°C or thermoneutrality (30°C) and fed high fat diet (HFD) from 8 weeks old. Adipose tissues were collected at sacrifice. Indirect calorimetry (MetaTrace – Creative Scientific UK) was performed after 8 weeks of diet administration and adipose tissues were explanted at sacrifice for gene expression analysis (TaqMan – ABI). In a parallel study primary preadipocytes were extracted from wild type and LR11-/- mice aged 8 weeks old for differentiation in vivo and characterisation of thermogenic gene profiles. Similarly, immortalised brown adipocytes from a C57Bl6/J murine cell line were transfected with control and LR11 over-expression vectors (pCDNA – Promega) and differentiated for 8 days in vitro before gene expression profiling.

Results: LR11-/- mice showed reduced weight gain compared to wild type mice when housed at thermoneutrality (28 g ± 2.2 g vs 44g ± 2.5g, $p < 0.05$) and were found to be hypermetabolic (30 j/min ± 0.8 vs 27.5 j/min ± 0.75, $p < 0.05$), a phenotype which was not observed at lower environmental temperatures. Expression

of thermogenic genes was significantly increased in brown adipose tissue (BAT) and subcutaneous white adipose tissue (WAT) depots. Conversely, LR11 overexpression in a BAT cell line repressed expression of thermogenic genes and transcription factors. LR11 expression was also found to be negatively regulated in the hypothalamus in response to high fat diet feeding and cold exposure in wild type animals.
Conclusions: LR11 acts as a negative regulator of thermogenesis in brown and subcutaneous adipose tissues. Downregulation of LR11 allows increased thermogenic gene expression and increased energy expenditure, conferring protection from diet-induced obesity. In vivo, this effect is independent of adrenergic stimulation as it penetrates the phenotype only at thermoneutrality, where sympathetic stimulation of BAT is absent. These results highlight LR11 as a new player in the regulation of thermogenic capacity in conditions more translatable to the human living environment (thermoneutrality) and suggest an additional mechanism of brown fat regulation.

Rogaeva, E., et al. (2007)

Böhm, C., et al. (2005)

The immortalised brown adipocyte cell line was a gift from Prof. Johannes Klein

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C07 and PC07

Effect of novel thiazolidinadiones on expression of UCP1 and mitochondrial respiratory chain proteins in brown and white adipose tissues

A. Kalinovich, G. Abreu-Vieira, I.G. Shabalina, N. Petrovic, B. Cannon and J. Nedergaard

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Rosiglitazone is a clinically approved anti-diabetic thiazolidinadione which now is not used anymore due to negative side effects. It acts as proliferator-activated receptor γ (PPARγ) agonist, which is associated with its stimulating effect on brown adipose tissue (BAT) recruitment. The latter effect could be interesting due to the anti-obesity role of BAT. However, the same ability of rosiglitazone to activate PPARγ leads to weight gain. Two novel thiazolidinadiones, MSDC-0602 and MSDC-0160, have been suggested as potent anti-diabetic drugs acting by a mechanism independent of PPARs and potentially lacking the negative side effect on weight gain (1).
In this work we studied in vivo effects in mice of these two novel agents in comparison to the effect of rosiglitazone. Mice were kept in thermoneutrality and on high fat diet to stimulate obesity. Drugs were administered with food (rodent diet with 45 kcal% of fat and 300 ppm of Rosiglitazone, MSDC-0160 or MSDC-0602, ad libitum). We confirmed that rosiglitazone results in slight body weight gain in com-

parison to control mice. The same effect was shown for MSDC-0160. However, another drug, MSDC-0602, actually decreased body weight and was thus considered as the most promising.
Weight of inguinal white adipose tissue (iWAT) was consistent with body weight: mice treated with rosiglitazone or MSDC-0160 had larger iWAT depot in comparison to control mice, whereas MSDC-0602 decreased the weight of the tissue.
We also measured effects of the drugs on expression of UCP1 and mitochondrial respiratory chain proteins in interscapular brown (iBAT) and iWAT. Examination of iBAT showed that none of the drugs increased the amount of UCP1 per mg of tissue protein. However due to the fact that rosiglitazone and MSDC-0160 resulted in brown adipose tissue recruitment, total UCP1 amount in the tissue was doubled. However, neither rosiglitazone nor MSDC-0160 resulted in significant increase of total amount of respiratory chain proteins in tissue. No effect of the most promising drug MSDC-0602 on proteins levels in iBAT was observed.
All drugs had prominent effects on iWAT. Levels of UCP1 and respiratory chain proteins were significantly increased with all drugs, although rosiglitazone had more profound effect. However, the browning of iWAT in treated mice did not increase UCP1 amount to more than 5 % of the untreated level of iBAT.
Thus, considering the absence of effect on body weight gain and significant increase of brite adipose tissue in MSDC-0602 treated mice, the novel tiazolidinedione, MSDC-0602 could be suggested as a potential anti-diabetic and anti-obesity drug.
Chen Z et al. (2012). JBC 287(28), 23537-48.

We would like to thank Rolf F. Kletzien from Metabolic Solutions Development Company (USA) for useful advices and discussions.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C08 and PC08

Pyruvate kinase M2 deficiency promotes a brown fat-like program in white adipocytes

A. Bettaieb[1], J. Bakke[1], N. Nagata[1], A. Tomilov[1], C. Lyssiotis[2], J. Asara[2], G. Cortopassi[1], L. Cantley[2] and F.G. Haj[1]

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Brown adipocytes are specialized to dissipate chemical energy as heat and are associated with improved metabolic phenotypes. Herein we demonstrate that pyruvate kinase M2 (PKM2) is expressed in brown and white adipose depots. shRNA-mediated depletion of PKM2 in white preadipocytes promotes the development of a brown fat-like thermogenic program. Reconstitution of knockdown cells with PKM2 abrogates thermogenic gene induction that is caused by PKM2 deficiency. In addition, metabolomic profiling of PKM2 deficiency revealed notable metabolic

similarities with bona fide brown adipocytes. Importantly, PKM2 deficiency in white adipocytes increases ATP turnover, and enhances basal and maximal mitochondrial respiration. Notably, subcutaneous injection of PKM2-deficient preadipocytes into mice (following established protocols) gives rise to ectopic fat pads with morphological and biochemical characteristics of brown adipocytes with enhanced glucose uptake in vivo. Collectively, these findings identify PKM2 as a novel component of the molecular circuit that contributes to adipocyte plasticity and adaptive thermogenesis, which may have potential therapeutic implications.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C09 and PC09

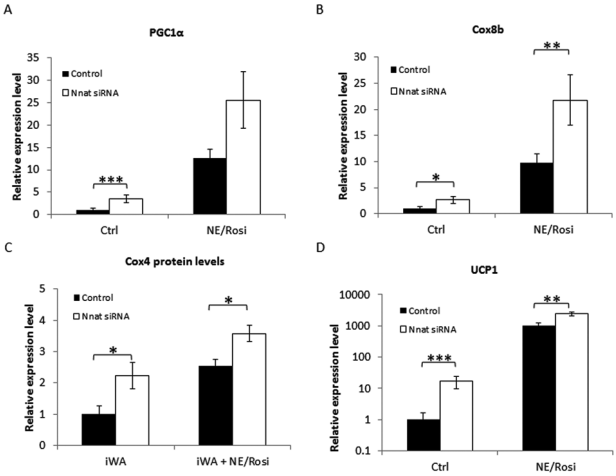
Loss of neuronatin promotes 'browning' of primary mouse adipocytes

V. Gburcik[1], B. Cannon[3,1], M.E. Cleasby[1] and J.A. Timmons[2,1]

[1]Comparative Biomedical Sciences, Royal Veterinary College, London, UK, [2]Sports, Exercise and Health Sciences, Loughborough University, Loughborough, UK and [3]The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

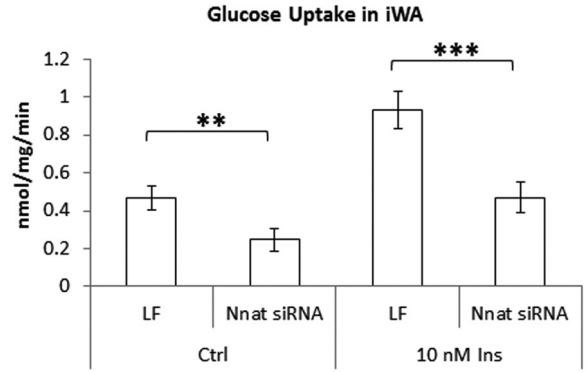
Failure of white adipose tissue to appropriately store excess metabolic substrate seems to underpin obesity-associated type 2 diabetes (1,2). Encouraging 'browning' of white adipose has been suggested as a therapeutic strategy to dispose of excess stored lipid and ameliorate the resulting insulin resistance (3). Genetic variation at the DNA locus encoding the novel proteolipid neuronatin has been associated with obesity, and we recently observed that neuronatin expression is reduced in subcutaneous adipose tissue from obese humans (these data can be found, along with the transcript profiles, at NCBI (GSE27951)). Thus, to explore the function of neuronatin further, we used RNAi to silence expression (>90% of protein) in murine primary adipocyte cultures and examined the effects on adipocyte phenotype and glucose disposal. Adipocyte progenitors were isolated from the 129/Sv strain of mice (Harlan, UK). We found that primary adipocytes only express the longer, α-isoform, of neuronatin. Loss of neuronatin led to 10 times increased expression of uncoupling protein-1 mRNA, 2-3 fold increased expression of the mitochondrial genes peroxisome proliferator-activated receptor gamma coactivator 1α and cytochrome c oxidase (Cox) 8b mRNA, and a two-fold induction of Cox4 protein expression in primary subcutaneous white adipocytes, indicative of a 'browning' effect. This was accompanied by increased phosphorylation of AMP-activated protein kinase and acetyl coA carboxylase, suggestive of increased fatty acid utilisation. Similar, but less pronounced, effects of neuronatin silencing were also noted in primary brown adipocytes. In contrast, loss of neuronatin caused a two-fold reduction in both glucose uptake and glycogen synthesis without altering insulin signalling (n=4). All statistical analysis was performed using two-way ANOVA RM followed by Newman-Keuls multiple comparison tests. Thus, neuronatin appears

to be a novel regulator of 'browning' and metabolic substrate utilisation in white adipocytes.



Neuronatin silencing potentiates mitochondrial biogenesis and a BAT-like phenotype in brown and inguinal white adipocytes. Expression levels of thermogenic marker genes in control and NE/Rosi-stimulated cultures of brown and inguinal white adipocytes were measured with and without Neuronatin silencing. PGC-1α (A), Cox8b (B) and UCP1 (note logarithmic y-axes) (D) mRNA levels are shown in control and NE/Rosi-treated brown adipocytes and inguinal white adipocytes ± neuronatin siRNA pool. Expression levels in the brown adipocyte cultures were set in each experiment to 1.0 and the levels in the other cultures were expressed relative to this value in each experiment. The values represent means ± S.E. of 5 independent experiments. (C) Protein levels of Cox4 in control and NE/Rosi-treated brown adipocytes and inguinal white adipocytes ± neuronatin siRNA were analysed by Western blotting and the graph represents the means ± S.E. of 4 independent experiments.

A



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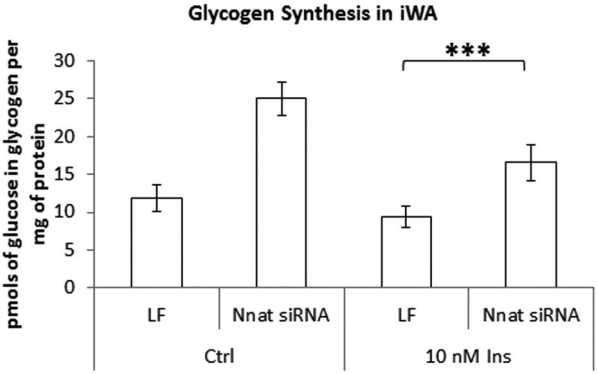

Effect of Neuronatin silencing on glucose uptake and glycogen synthesis in primary adipocytes. (A) Glucose uptake in inguinal white primary adipocytes ± insulin stimulation and ± neuronatin siRNA. The values represent means ± S.E. of 5 independent experiments, each conducted in triplicate. (B) The effect of neuronatin silencing on glycogen synthesis in control and insulin-stimulated inguinal white adipocyte cultures. The values represent means ± S.E of 4 independent experiments, each conducted in triplicate (pmoles of glucose incorporated in glycogen per mg of protein).

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This study was supported by a grant from the BBSRC, UK. MEC is supported by a Wellcome Trust University Award.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C10 and PC10

Brown adipose tissue in epicardial adipose tissue of adult humans

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Adipose tissue present between the heart and visceral layer of the pericardium, i.e. in direct contact with the heart, is known as epicardial adipose tissue (EAT) and is located primarily near the coronary vessels. It has been proposed that it has a role in lipid storage as well as limiting the impact of torsion forces through the cardiac cycle (Keegan et al 2004). The brown adipose tissue (BAT) specific gene uncoupling protein (UCP)1, and other BAT related genes, are all highly expressed in EAT (Sacks et al. 2009). The aim of the present study was to quantify the amount of UCP1 within EAT and to compare this with paracardial and subcutaneous adipose tissue (SAT) depots from the same individuals.
Method: Adipose tissue samples were obtained from 6 females and 2 males aged between 58-74 years and with a body mass index between 26.6-38.3 kg/m2. Mitochondria were prepared from ~200mg of adipose tissue (Symonds et al. 1992) and abundance of UCP1 was determined by immunoblotting using a rabbit polyclonal antibody to human UCP1 (Abcam, Cambridge, U.K.). Specificity of the detection was confirmed using non-immune rabbit serum and a range of molecular-weight markers was included on all gels. All gels were run in duplicate and reference samples (i.e. from either BAT or the liver (negative control) of a 6 hour old sheep) were included on each gel to allow comparison between each group.
Results: UCP1 was highly abundant in all EAT samples but was absent from paracardial fat and was only found in the sternal depot of subcutaneous fat. The mean ± SEM optical density of from all EAT samples was 0.77 ± 0.0.28 compared with 0.23 ± 0.11 arbitrary units for sternal SAT ($p<0.05$). UCP1 protein was undetectable in paracardial fat as well as upper abdominal and lower extremity SAT.
Discussion: UCP1 is present in significant amounts within human EAT, thereby confirming it is a BAT depot in adult humans. This raises the possibility that EAT can have a thermoregulatory function in the heart, possibly protecting against hypothermia. BAT also influences vascular lipoprotein homeostasis by inducing triglyceride rich lipoprotein turnover and channelling lipids (Bartlet el al. 2011) and could protect the heart against toxic levels of fatty acids in the coronary circulation.

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The authors would like to acknowledge Mrs. Vicky Wilson for technichal assistance.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C11 and PC11

Unequivocal identification of brown adipose tissue in an adult human using Magnetic Resonance Imaging

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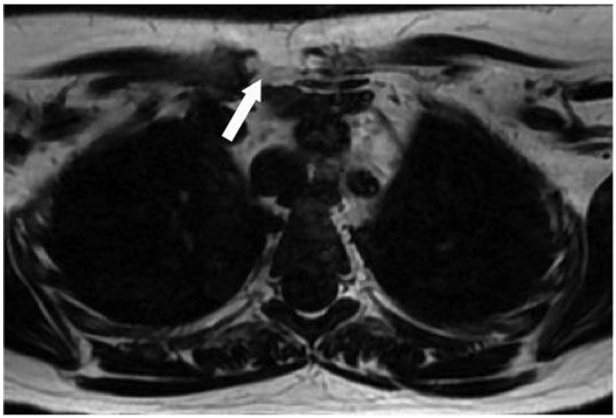
Aim: Manipulation of human brown adipose tissue (BAT) represents a novel therapeutic option for type 2 diabetes mellitus and obesity. The aim of our study was to develop and test a novel Magnetic Resonance Imaging (MR) based method to identify human BAT and delineate it from white adipose tissue (WAT), and validate it by providing histological and immunohistochemical confirmation of BAT.
Methods: Initial scanning with 18F-FDG PET-CT radiotracer uptake on a 25-year old Caucasian female with primary hyperparathyroidism, showed avid uptake within the mediastinum, neck, supraclavicular fossae and axillae, consistent with BAT. Subsequently, serial MR scans were performed using 3-echo IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation) sequence. Retrospectively, regions of interest (ROIs) were identified on MR corresponding to PET-CT images. Prospectively, ROIs were identified on MR images based on signal intensity and appearance, and compared with PET-CT. Immunohistochemical staining using uncoupling protein-1 antibody was performed on fat samples corresponding to low MR-signal, obtained during parathyroidectomy.
Results: Of the 111 retrospectively identified ROIs from PET-CT scans, 88 (79%) showed corresponding low signal on the MR images: 100% in mediastinum, 29/31 (93.5%) in neck, 31/41 (75.6%) supraclavicular, and 8/14 (57%) in axillae. Prospectively, 87% of ROIs identified on MR scans corresponded to increased areas of uptake on PET-CT scans. Histology and immunohistochemistry confirmed BAT.
Conclusion: We provide the first ever report that MR can be used reliably to identify BAT in a human adult, with histological and immunohistochemical confirmation. Our data demonstrate proof of concept to support the development of MR, a safe and reproducible imaging modality, as a biomarker for human BAT.

Anatomical region	Number of BATretro ROIs with lower signal with lower MR signal then adjacent fat	Number of BATretro ROIs with similar MR signal to adjacent fat	Total number of ROIs	Total area of ROIs (mm2)
All regions	93	18	111	9,030
Mediastinum	25	0	25	2.348
Supraclavicular	31	10	41	3,313
Neck	29	2	31	1,451
Axillae	8	6	14	1,918

For each anatomical region the number of BATretro ROIs(regions of interest) with low and normal signal intensity on MR are tabulated. The ROIs were retrospectively drawn on MR from areas of corresponding high 18F-FDG uptake on the PET-CT images



fat:IDEAL MR image demonstrating low signal area within the mediastinal fat, between the left common carotid and subclavian arteries (arrowhead) and inferior to the right subclavian artery (white arrow) corresponding to BAT on PET-CT

We acknowledge Sean James, department of Histopathology and the Radiographers, University Hospital of Coventry and Warwickshire, for their assistance in the study.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

C12 and PC12

Brown adipose tissue function and premature ageing: Impaired thermogenesis in mice expressing defective mitochondrial DNA polymerase

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Impaired thermogenesis is one of the features of normal ageing. MtDNA mutator mice expressing defective mtDNA polymerase exhibit several features of premature aging and reduced lifespan [1]. The observed phenotype in mtDNA mutator mice is a direct consequence of the accumulation of mtDNA point mutations, leading to a decreased assembly of the respiratory chain complexes and thus to mitochondrial dysfunction [2]. The aim of the present study was to explore the brown adipose tissue (BAT) function and related thermogenesis in this mouse model of premature ageing.

MtDNA mutator mice had low body temperature measured rectally with a Microprobe Thermometer (as in [3]). Low body temperatures were evident at both room environment and thermoneutrality suggesting an anapyrexia (regulated decrease in defended body temperature). The hypothermia (inability to compensate for heat loss) was also identified as the reason for lowered body temperature of these mice. During gradually decreasing the environmental temperature in the metabolic chamber, wild type (WT) mice responded by adequate increase in thermogenesis, whereas mtDNA mutator mice were unable to further increase their metabolism at environmental temperatures below 24 °C and went into torpor. We also examined potential recruitment of BAT by measuring oxygen consumption in mice in response to sympathetic stimulus, norepinephrine (NE), subcutaneously injected in dose 1 mg per kg body weight as it was described in [3]). MtDNA mutator mice at 30 °C responded to NE with a lower level as compared with the level of NE response at 22 °C, indicating preserved central regulation of recruitment in these mice. However, magnitudes of NE-response in mtDNA mutator mice were much lower as compared with response in WT mice. Normal sympathetic signalling was confirmed by normal changes in morphology of BAT at 30 °C (few large unilocular lipid droplets in cytoplasm of brown adipocytes) as compared with 22 °C (numerous small multilocular lipid droplets in cytoplasm). However, the thermogenic capacity of BAT was significantly reduced in mtDNA mutator mice: the total protein content in BAT was 41% lower in mtDNA mutator mice than in WT mice. As compared with wild-type mitochondria, on all 3 substrates investigated (pyruvate, palmitoyl-l-carnitine and glycerol-3-phosphate), UCP1-dependent oxygen consumption was significantly reduced in isolated mtDNA mutant mitochondria as was maximal oxidative capacity (FCCP-response) indicating impaired thermogenesis on the level of

brown-fat mitochondria in mtDNA mutator mice, although UCP1 content was preserved.

Thus, mtDNA mutation led to lower activity of brown adipose tissue and impaired thermogenesis; i.e. also in this respect, mtDNA mutator mice mimicked normal ageing.

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This study was supported by the Swedish Research Council, the Knut and Alice Wallenberg foundation and the European Project DIABAT.

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PC13

Actions of L-citrulline on gastric mucus secretion and gastric mucous cell counts in albino rats

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Oral L-citrulline is efficiently converted to L-arginine, the precursor for endothelial nitric oxide (NO) synthesis.Nitric oxide NO enhances gastric mucus production. This study investigates the role of nitric oxide as up regulator of mucus secretion and mucous cell count in the stomach. Sixty Adult male albino rats, weighing between 180-210 g were used for the experiment. They were divided into two study groups, (gastric mucus secretion study group, gastric mucus cell count study group), of thirty rats per group and each group was further subdivided into five groups with six (n=6) rats in each group. Group (I) served as control. Group (II) was treated with L-Citrulline (600mg/kg) for five days. Group (III) received L-Citrulline (900mg/kg) for five days. Group (IV) received L-Arginine (200mg/kg) and group (V) was treated with misoprostol (100μg/kg). At the end of treatments, the animals were fasted for 24 hours but allowed free access to water until an hour before they were sacrificed by cervical dislocation. Gastric mucus wall content was determined by Alcian blue method and the rate of secretion was determined by the quantity of Alcian blue extract per gram wet stomach(mg/g). The tissues were fixed in 10% formalin and embedded in Paraffin wax, then sectioned with microtome at 4microns. The mucus cell count was counted using calibrated microscope in five randomly selected areas of the gastric mucosal tissue on each slide. The results show that in the first study group, L-citrulline increases the rate of gastric mucus secretion from 7.4±1.58mg/g tissue in the control to12.7±5.05mg/g tissue in the 600mg/kg treated in group 2,while group 4 treated with L-arginine the secretion is about 11.30±2.64mg/g per tissue while in the second study group, the mucus cell count in the control is 14.50±2.56 cells/mm2 and it decreases to 10.75±1.80 and 14.20±3.80 cells/mm2 in 600mg/kg and 900mg/kg l-citrulline treated group while it increases to 15.60±2.27 cells/mm2 and 17.96±2.73cells/mm2in the L-arginine and misoprotol treated group. It is therefore concluded that L- citrulline increases gastric mucus secretion which could be as a result of up-regulation of endothelial Nitric oxide via the citrulline-NO cycle.

Analysis of actions of L-citrulline on gastric mucous secretion and mucous cell count of the stomach of albino rats

grouping	no of animals	treatment	gastric mucus secretion g/tissue	Gastric mucus counts(cells/mm2
I	5	control	7.4±1.58*	14.50±2.56***
II	5	600mg/kg of L-citrullline	12.7±5.05**	10.75±1.80
III	5	900mg/kg of L-citrulline	10.11±1.83**	14.20±3.80**
IV	5	L-arginine 200mg/kg	11.30±2.64**	15.60±2.27***
V	5	Misoprostol 100ug/kg	8.71±2.0*	17.96±2.73***

All values are expressed as mean± SEM, ***P<0.05,*p>0.05**=highly significant compared with the control,

*= level of significance is not very high compared with control

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC14

Application of high content image analysis to identify factors that encourage the brown-like phenotype in human adipose-derived stem cells

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There are at least two distinct types of adipose cells and tissue in mammals, white and brown. White adipocytes store fat as triacylglycerol during caloric excess and release free fatty acid during fasting. By contrast, brown adipocytes are highly adapted to expend energy as heat via the action of Uncoupling protein-1 (UCP1) in the mitochondria. Brown adipocytes burn substantial amounts of glucose and lipid and can thus counteract obesity, diabetes and dyslipidemia. Significant amounts of brown adipose tissue have been recently identified in adult humans raising the possibility that variations in brown fat function may profoundly influence body weight and systemic metabolism. In addition, a subpopulation of progenitor cells is present within white adipose tissue that is capable of differentiating into brown like adipocytes or “beige/brite” adipocytes. We aim to establish a set of phenotypic analysis for identifying and developing small or large molecules

that enhance differentiation of human adipose stem cells (hASCs) into "beige/brite" cells. Molecule(s) with such properties could potentially be beneficial for body weight as well as glycemic control for diabetic patients.
A large scale isolation procedure has been established to obtain and bank hASCs from human adipose tissue biopsies in order to provide sufficient number of cells for screening. We focused on optimization of image-based medium thought-put assays using hASCs and UCP-1/neutral lipid as readout to screen and characterize new chemical entities (NCE's) that encourage the "beige/brite" phenotype.These cells could be differentiated in 96-384 well plates into adipocytes with different UCP-1 content, which is detected by immunohistochemsitry using UCP-1-specific antibodies. This allows us to utilize high content imaging technology to quantitatively measure UCP-1 content and other morphological features. We will present results of using this technology to assess a set of reference
compounds that potentially regulate the "browning" of adipose-derived stem cells. Positive hits from such screen will then been further characterized using Seahorse Extracellular Flux (XF) Analyzer in order to understand the effects of compound(s) on the bioenergetics.
Patient consent:
Samples of adipose tissue is collected from patients undergoing elective surgery at Sahlgrenska University Hospital in Gothenburg, Sweden.
All study subjects received written and oral information before giving written informed consent for the use of the tissue. The studies were approved by The Regional Ethical Review Board in Gothenburg, Sweden

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PC15

Association of circulating peroxiredoxins levels with indicators of glycemic control in patients with type II diabetes mellitus

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Oxidative stress is an important pathophysiological mechanism in the development and complications of type II diabetes mellitus (1). To protect against oxidative stress

the body cells developed an active enzyme system; the peroxiredoxins (Prdxs). To date six isoforms have been identified (2). Although recent reports demonstrated an increase in red blood cell concentration of Prdx 1 and 2 (3) it is unrecognized whether diabetic state and/or glycemic control affects circulating plasma levels of Peroxiredoxins. Fifty three type II diabetic patients and 25 healthy controls were recruited for this study. Fasting blood samples were collected, blood pressure measurement taken, and body mass index calculated.Plasma levels of Prdx1,2,4 and 6 as well as insulin were measured by ELISA kits . Glycosylated hemoglobin (HbA1c), Fasting sugar and lipids were measured. Insulin resistance index (HOMA-IR) was calculated (4). Data were analyzed using SPSS 18.0 program. Results are expressed as mean ± SD.Results: Clinical and laboratory data are shown in table 1. Higher plasma levels of Prdx1,2,4 and 6 were reported in diabetic subjects. A negative associations of plasma Prdx2 and Prdx6 with fasting blood sugar (Fig-1 a&b) and HbA1c (Fig-2 a&b) were detected in diabetic subjects. Conclusions: Although diabetics demonstrated higher levels of the Peroxiredoxins as a sort of adaptations to increased oxidative stress that supervenes diabetic status, failure of this kind of adaptations occurs with poorer glycemic control.
All subjects recruited in the study signed a consent after approval of the Institutional Review Board at King Khalid University Hospital.

Table 1: Demographic and clinical data of control subjects and all diabetic patients:

	Control (non-diabetic) n=25	Diabetic n= 53	P-value
Age (years)	49.5±5.1	61.33± 11.14	
Gender (F/M)	8/17	28/25	
BMI	25.26± 4.06	26.79± 4.32	0.12
SBP (mmHg)	117.56± 3.9	135.59± 21.95	0.001
DBP (mmHg)	75.89± 6.277	75.85± 9.06	0.986
Serum Insulin (IU/L)	13.17± 5.86	17.75± 14.94	0.206
FBS (mmol/L)	4.71± 0.544	9.67± 4.09	0.001
HOMA-IR	2.82± 1.437	7.14± 6.2	0.005
Total Cholesterol (mmol/L)	4.49± 0.617	4.76± 1.38	0.362
Serum Triglyceride (mmol/L)	0.92± 0.39	1.86± 1.07	0.001
HDL (mmol/L)	0.99± 0.314	1.17± 1.16	0.46
LDL (mmol/L)	2.7± 0.657	3.64± 1.45	0.009
Plasma Prdx1 (pg/ml)	16.77± 3.899	21.92± 5.77	0.001
Plasma Prdx2 (pg/ml)	20.37± 8.61	36.61± 14.966	0.001
Plasma Prdx4 (ng/ml)	2696± 1972	3835± 1454	0.005
Plasma Prdx6 (ng/ml)	238± 111	311± 111	0.008

Variables are mean± SD, $P<0.05$ is significant. BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBS: fasting blood sugar, LDL: low density lipoproteins, HDL: high density lipoproteins.

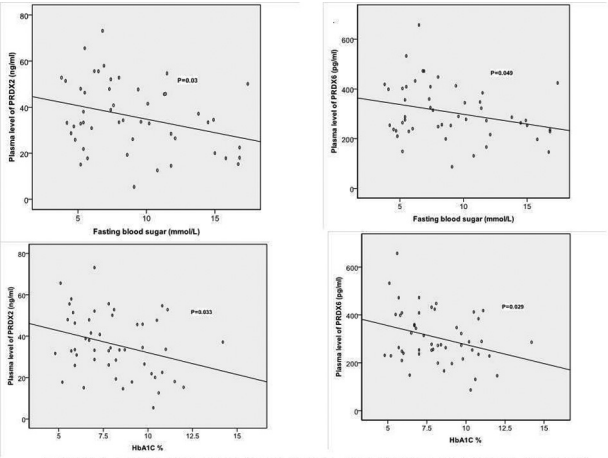

Figure-1. Effect of fasting blood sugar and HbA1C on plasma levels of PRX2 and PRDX6.

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The authors are thankful to Deanship of Scientific Research at King Saud University.

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PC16

Candidate Genes involved in cAMP-stimulated cell proliferation of brown preadipocytes

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Brown preadipocyte proliferation is one of the crucial events during brown adipose tissue recruitment in response to cold exposure and relevant physiological challenges [1]. β1-adrenoceptor signalling through cAMP has been revealed as the hypertrophic effector in norepinephrine-stimulated cell proliferation of primary cultured brown preadipocytes [2]. Proto-oncogene regulation is implicated in this recruitment process as seen with the discovery of mitogenic proteins regulation in a β-adrenoceptor dependent manner, including the angiogenic protein VEGF [3] and the proto-oncogene c-fos [4]. To explore functional signalling proteins involved in this process, a microarray study was carried out to identify candidate genes possibly involved in cAMP induced cell proliferation. We have further exam-

ined these candidate gene expression with reverse transcriptase quantitative PCR (RT-qPCR).

NMRI mice weighing around 13-15 g were sacrificed with CO2 euthanasia followed by cervical dislocation, as approved by the Northern Stockholm Animal Ethics Committee. Brown adipocyte precursors are separated from the interscapular, cervical and axillary brown adipose tissue depots, as described by Néchad et al.[5]. To validate NE regulation of gene expression, RNA was isolated from newly cultured brown preadipocytes, which were stimulated with norepinephrine at a series of time points: 1 h, 2 h, 4 h and 8 h. RT-qPCR was used for gene expression analysis, and 8 of the 19 genes are validated as upregulated with the stimulation of 1 μM of norepinephrine, with the most prominent effect at 2 h or 4 h for most genes. To check the effect of cAMP on the expression of these genes, RNA isolated from samples with 1 μM of forskolin stimulation for 4 h was used for RT-qPCR analysis. 18S was used as endogenous control for gene expression analysis and the expression level of all other genes were expressed as the percentage of 18S. Data shown in table 1 are mean ± SEM, compared with Student t-test. * $p<0.05$; ** $p< 0.01$.

Here we report that 8 out of 19 genes derived from microarray study has been confirmed with RT-qPCR to be upregulated in brown preadipocytes at mRNA levels with both NE and FSK stimulation: Adora 2B, ARHGAP8, Fgf18, Gpr133, Prr5, Tmem37, Twist 2 and Ube2S. We propose that these protein-encoding genes are possibly functionally involved in cAMP-promoted cell proliferation.

cAMP regulation of gene expression in brown preadipocytes

Gene name	Adora 2b	ARHGAP8	Fgf18	Gpr133	Prr5	Tmem 37	Twist 2	Ube 2S
DMSO	0.28 ± 0.01	0.029 ± 0.002	0.23 ± 0.04	0.33 ± 0.05	0.23 ± 0.05	0.48 ± 0.02	0.95 ± 0.04	15 ± 2
FSK	0.80 * ± 0.07	0.11 * ± 0.01	0.57 * ± 0.03	1.18 ** ± 0.09	0.40** ± 0.06	1.02 ** ± 0.03	2.18 * ± 0.24	22 * ± 3

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We thank Dr James A Timmons for assitance in data analysis on microarray study.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC17

Coffee attenuates induction of insulin resistance by high sucrose-diet in rats

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Numerous epidemiology reports suggested that consumption of coffee is associated with lower risk of diabetes mellitus (Natella & Scaccini, 2012). These epidemiological observations have not been evaluated experimentally. The present study therefore employs an animal model to examine the relationship between intake of coffee and risk of type 2 diabetes mellitus (in this case, insulin resistance). In the study, high-sucrose feeding (30% w/v) was used to induce insulin resistance (Riberio et al, 2005). Sprague-Dawley rats (male, 120-150g) were maintained for 12 weeks on a normal diet (ND, n=6), normal diet supplemented with coffee (ND+COF, 300 mg/kg BW, n=6), high sucrose-diet (HSD, n=6) and high sucrose-diet supplemented with coffee (HSD+COF, n=6). Glucose tolerance and insulin resistance were measured by performing the oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) respectively; and for both tests, the area under curve (AUC) was calculated. Blood sampling was carried out by tail tipping for rats loosely restrained in hand towel for the determination of blood glucose level at intervals of 0, 30, 60, 90, 120 and 180 min. The serum insulin was estimated using ELISA while an automatic blood chemical analyzer was employed for the measurement of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). Malondialdehyde (MDA), glutathione reduced (GSH) and superoxide dismutase (SOD) were measured using previously described standard methods. For these measurements, blood sample was obtained at once by retro-orbital bleeding under anaesthesia with pentobarbital (50 mg/kg; I.P.). Data were expressed as mean±SEM, compared by ANOVA. HSD rats showed reduced glucose tolerance (AUC: 2016.8±119.2 vs. 1017.5±59.9, $p<0.05$) and insulin sensitivity (AUC: 535.88±36.41 vs. 414.5±29.08, $p<0.05$) when compared with ND rats but intragastric administration of coffee prevented these metabolic abnormalities in HSD+COF (AUCOGTT: 1132.3±64.5; AUCITT: 388.75±26.27). In addition, insulin concentration was markedly increased in HSD rats compared with ND rats (12.25±0.51 vs. 7.69±0.22, $p<0.05$), but was suppressed in HSD+COF rat treated with coffee (9.21±0.66, $p<0.05$). Whereas compared with the HSD rats, the levels of TG and LDL (0.44±0.06 vs. 1.43±0.32; 0.75±0.03 vs. 0.91±0.06 respectively, $p<0.05$) decreased; HDL (1.10±0.06 vs. 0.69±0.02, $p<0.05$) increased significantly in HSD+COF rats. Additionally, MDA level was reduced (21.4±2.36 vs. 37.4±3.59, $p<0.05$), GSH (0.53±0.03 vs. 0.24±0.01, $p<0.05$) and SOD (4.38±0.47 vs. 1.63±0.01, $p<0.05$) activities were increased in HSD+COF when compared with HSD rats. Collectively, our data indicate that coffee consumption confers protection against the risk of contracting non-insulin dependent diabetes in rats and lends support to the idea that coffee consumption is associated with a lower risk of type 2 dia-

betes. Understanding some aspect of the relationship between coffee and diabetes is a desirable step in the context of healthy eating habits and disease control.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

PC18

Decreased fatty acid uptake and metabolism during acute and chronic cardiac ischemia are mediated by different mechanisms

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The hearts obtains 70% of its energy for contraction from the oxidation of fatty acids, derived from adipose, hepatic and dietary sources. Myocardial ischemia is accompanied by increased adipose lipolysis and elevated fatty acid supply to the heart. We investigated the effects of acute and chronic ischemia on cardiac fatty acid uptake and metabolism.

Wistar rat hearts were perfused in Langendorff contracting mode during pre-ischemia, low-flow ischemia and reperfusion, using 3H-substrates for measurement of substrate metabolism. During 30 mins of ischemia, there was a 32% decrease in sarcolemmal location of the predominant fatty acid transporters, FAT/CD36, as it translocated into intracellular endosomes to limit cardiac fatty acid uptake. This was accompanied by a 95% decrease in fatty acid oxidation rates, with no change in intramyocardial lipids. Following 30 mins of reperfusion, decreased sarcolemmal FAT/CD36 persisted, but fatty acid oxidation rates returned to pre-ischemic levels in line with contractile function, but resulted in a 35% decrease in myocardial triglyceride content. To investigate the longer term metabolic effects of ischemia, changes in cardiac fatty acid metabolism were investigated in the chronically ischemic rat heart, 6 months after in vivo myocardial infarction. Fatty acid oxidation and myocardial lipid incorporation were decreased by 30% and 25%, respectively, correlating positively with the in vivo cardiac ejection fraction. In contrast to acute ischemia, chronic ischemia decreased total protein levels of FAT/CD36, accompanied by decreased levels of other fatty acid transporters and fatty acid oxidation proteins.

In conclusion, during acute ischemia, FAT/CD36 moved away from the sarcolemma, associated with a shift from fatty acid oxidation to glycolysis, whilst intramyocardial lipid accumulation was prevented. In contrast, following chronic ischemia, fatty acid utilisation is downregulated, achieved by downregulating multiple proteins involved in fatty acid metabolism. In light of the increased adipose lipolysis during

acute and chronic ischemia, the cardiac metabolic changes are likely a protective response to prevent fatty acid uptake under conditions of limited oxidation.

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PC19

Defective regulation of energy expenditure in IL10 knockout mice

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Obesity results from an imbalance between caloric intake and energy expenditure. Non-shivering thermogenesis in brown adipose tissue (BAT) is primarily linked to maintenance of body temperature, however the observation that BAT is stimulated by consumption of a high fat diet (HFD) in rodent models, a phenomenon referred to as diet-induced thermogenesis, suggests that BAT activation can be a mechanism of protection against obesity development. Since BAT thermogenesis may be important to whole body energy balance, disruption on diet-induced thermogenesis may lead to obesity. Sub-clinical inflammation is a hallmark of human and experimental obesity. However, no previous study has addressed the connection between BAT thermogenesis and inflammatory pathways. In the present study, we used IL10-/- mice exposed to a HFD in order to investigate the role of this important anti-inflammatory cytokine on diet-induced thermogenesis and its consequences on the development of obesity. Eight week old male IL10-/- and C57BL/6 mice were fed on a HFD (IL10H and CH groups, respectively) or chow (IL10C and CC groups, respectively). All experiments were conducted in accordance with the principles and procedures described by "Guide for the Care and Use of Laboratory Animals", approved by the University of Campinas Ethical Committee. The results described were confirmed in, at least, two independent experiments. Data was analyzed by ANOVA and Students t Test, as appropriate.

After 8 weeks, IL10-/- and C57BL/6 mice fed on chow had similar body mass gain, and when exposed to a HFD both showed higher weight gain than those on chow diet ($p<0,05$) . However, IL10H mice had a significantly higher weight gain than CH mice ($p<0,05$). Since no difference on caloric ingestion was detected between IL10-/- and C57BL/6 mice, we assessed O2 consumption/CO2 production by indirect calorimetry. IL10C consumed less oxygen than CC mice ($p<0,05$) and the HFD ingestion resulted in increased oxygen consumption (VO2) for both animal models ($p<0,05$) but there was no difference between IL10H and CH. Although statistically the VO2 is not different between the IL10H and CH groups, IL10H mice consumed slightly less oxygen. The impact of these phenomena may be cumulative through the 8 weeks and contribute to differences in body weight. In addition, the expression of UCP1 in BAT ($n=6$) was significantly lower in IL10-/- mice as compared to C57BL/6 mice on the chow diet ($p<0,01$). When exposed to the HFD, both ani-

mals increased UCP1 levels (p<0,01), but IL10-/- mice had a lower increase than C57BL/6 (p<0,05). Thus, in the absence of IL10 there is an impairment of diet-induced thermogenesis leading to increased body mass gain, highlighting the key role of inflammation on energy balance disruption that results in obesity.

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PC20

Effect of (+)-snic acid as a fat burner on the rat hepatocyte; correlated histological and biochemical *in vivo* study

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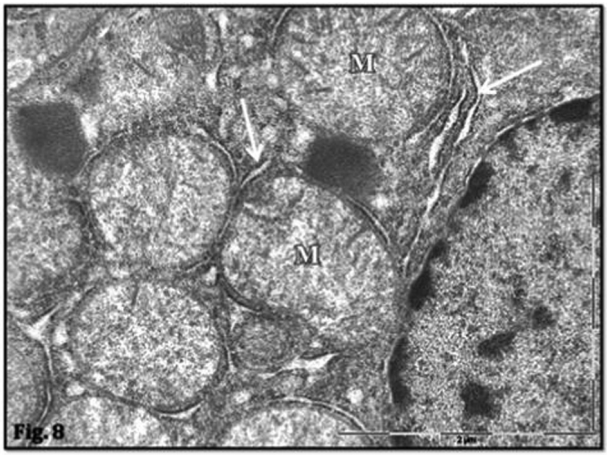
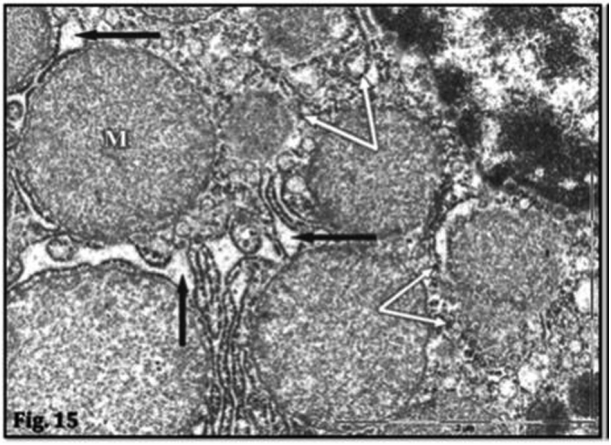
Appetite and metabolic control are the main factors controlling body weight gain and could be the keys for controlling the problem of obesity. Usnic acid (UA), an uncoupler of oxidative phosphorylation, was known for its use in many products to control body weight. It was reported to be hepatotoxic. Hence the aim of the present study was to test its safety dose using experimental rats. Forty adult male lean rats (200-250 g) were divided into four groups ten animals each (n=10); control received standard diet, group1 received 1% carboxymethyl cellulose water solution, group 2 received 100 mg usnic acid/kg and group 3 received 300 mg usnic acid/kg, 5 days for 7 weeks using gastric gavages. Serum glucose, liver functions, lipid profile, lipase, leptin and Insulin were estimated. Liver was processed for electron microscope studies and results were analyzed using SPSS. The liver index was increased significantly (P≤0.05) in high-dose usnic acid compared to the control. Hepatocytes showed an increase in lipid droplets, swollen mitochondria, fragmented rough endoplasmic reticulum cisterns, abundant smooth endoplasmic reticulum and focal damage of hepatocyte membranes near bile canaliculi, all these changes were dose dependent. There was significant increase in total protein, albumin and total bilirubin in group received low-dose of Usnic acid (P≤0.05). Glucose, magnesium, total protein, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and total bilirubin were significantly increased in group received Usnic acid at high-dose (P≤0.05). Serum cholesterol and high density lipoprotein were significantly increased in all treated groups (P≤0.05) while triglycerides were slightly increased. In conclusion, usnic acid in controlled low dose is potentially non hepatotoxic in lean rats and could be used safely in future weight loss products especially if formulated as adipocytes targeted nanoparticles.



Carboxymethyl cellulose 1% (CMC) control mitochondria with preserved cristae



High dose of usnic acid: Showing swollen mitochondria and ill defined cristae

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The authors are grateful to King Abdulaziz City for Science and Technology for funding this project (No.13-98), King Fahad medical research center (KFMRC) at King Abdulaziz University, where most of this work carried out.

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PC21

Fetal macrosomia of diabetic rats: histological and histochemical studies of skin and brown fat

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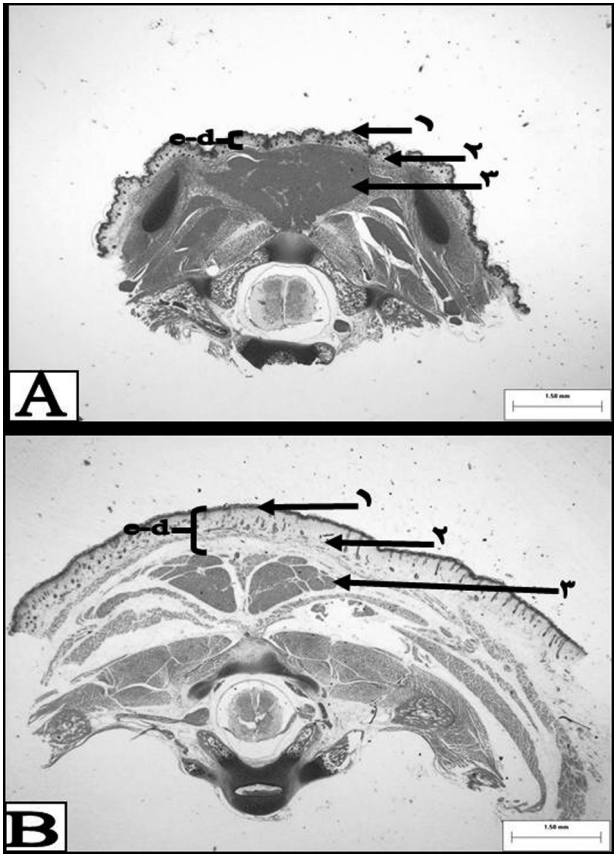
Macrosomia or increased fetal size than normal is one of the major clinical problems that carry health hazards for both mother and fetus .Macrosomia has different causes, however mostly associated with mild hyperglycemia or gestational diabetes .In the present study the histological and histochemical structure of back skin with the underlying brown fat was studied in macroscomic fetuses of mildly diabetic rats and compared to control. The main objectives were to correlate structural changes in such organs with the increase in fetal body weight above average normal. Animal care and experimental protocols and procedures were approved by the Institutional Animal Care King Fahed medical research center Jeddah Saudi Arabia.

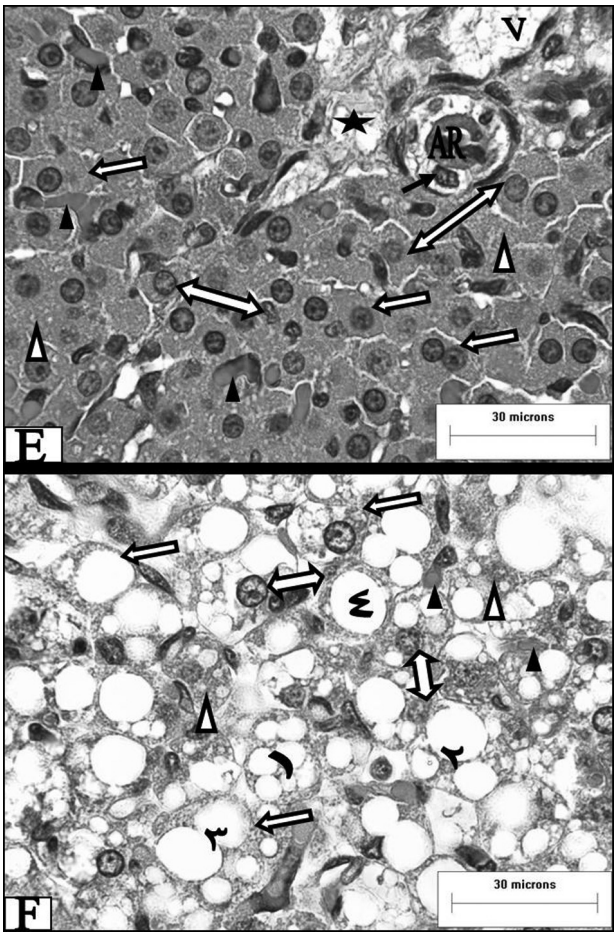
Mild diabetes was induced in female rat prior to pregnancy via intra peritoneal injection of Alloxan in a dose equal to 100 mg/kg body weight) dissolved in citrate buffer .Three days latter blood sugar was checked and animals with blood glucose ranging from 130-250 mg/ ml (mild diabetes) were divided into subgroups (4 animals per cage) at day 20 gestation, animals were anaesthetized with ether, abdomen was opened and uterine horn were cut longitudinally. Fetuses with a weight that ranged between (6.5 -6.7 g) were considered macrosomic compared to normal weight fetuses(3.9 -5.8 gm.). The results showed insignificant increase in the number and thickness of epidermal cell layers, significant increase in dermal thickness mostly due to edema, and increase in surface area occupied by brown fat lobules, Most cells of the latter tissue showed transformation to white type adiopocytes. Histochemistry proved increased lipid accumulation within dermal fibroblasts, scanty accumulation of lipid droplets in epidermal cell layers and significant lipid accumulation in the brown fat adipocytes. Insignificant increase in polysaccharides content was observed in examined tissues.

CONCLUSIONS: The present results could be preliminary to demonstrate histological and histochemical changes in cutaneous and adipose brown tissue that may explain the increasing of body weight and size in macrocosmic fetuses of mildly diabetic animals. The results were discussed in view of available literature.

Key words : macrosomia – diabetes- histology and histochemistry -inter scapular skin- brown fat body.



B-increase lobules area of brown fat body in macrosomia compared to normal weight fetus (A).tif



B-Transformation of brown adipocytes to white type in macrosomia.tif

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thanks to staff members for their technical supporting for preparing histological slides

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PC22

Hypothalamic BMP8B induces thermogenesis in BAT in a manner dependent of AMPK activation

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The key factor for development of obesity is a positive energy balance, in which excess energy is stored in white adipose tissue. Accumulated lipids are burned by brown adipose tissue (BAT) in the process of thermogenesis. Bone morphogenetic proteins (BMP's) are known to have an important role in the development of adipose tissue, where BMP's 2 and 4 contribute to white adipogenesis (1) and BMP7 induces commitment of preadipocytes into a brown adipocyte lineage (2). The ventromedial hypothalamic nucleus (VMH) is involved in the control of energy homeostasis, where it has been shown that activation of AMP-activated protein kinase (AMPK) and fatty acid metabolism promotes energy conservation and control of BAT activation (3). The aim of our study was to investigate the role of hypothalamic Bmp8b on energy balance and thermogenesis in BAT.
To perform the experiments female C57Bl6/J mice and Sprague-Dawley rats were used. Surgical procedures were performed with mice and rats under tribromoethanol (480 mg/kg) and ketamine (100mg/kg)/xylazine (15mg/kg) anaesthesia (IP), respectively. Animals were implanted in the lateral ventricle with intracerebroventricular (ICV) cannulae as previously described (3, 4), and received an ICV injection of Bmp8b or vehicle. Before Bmp8b treatment rats were treated with AMPKα dominant-negative (DN) or constitutively active (CA) expressing isoforms, or GFP as a control, targeted to the VMH using stereotaxic adenoviral delivery (3, 4). After Bmp8b treatment body temperature was recorded with a rectal probe and BAT skin temperature was recorded with an infrared thermal camera. Hypothalamic protein levels were analyzed by western blot using specific antibodies (3). Neuronal activation in specific nuclei was measured by immunohistochemistry (3).

Compared to vehicle, mice treated with Bmp8b showed an increase in core body temperature and increased phosphorylation of hypothalamic AMPKα and ACCα. Also, these animals displayed augmented neuronal activation in VMH and lateral hypothalamic area and in raphe pallidus and inferior olive nuclei, which coordinate sympathetic tone to BAT. Bmp8b's effect on core temperature was increased in presence of AMPKα-DN and was completely inhibited by expression of AMPKα-CA in comparison to vehicle treated animals. BAT's relative activation after Bmp8b ICV treatment, measured by thermal imaging, correlated extremely well with changes in body temperature in a given animal.
Our data show that central Bmp8b induces thermogenesis in BAT through neuronal activation in hypothalamic regulatory nuclei and medulla oblongata. Also, we suggest that AMPK in the hypothalamus acts in opposition to Bmp8b creating a conterregulatory mechanism that modulates thermogenesis in BAT to control energy balance (5). Thus, modulation of central Bmp8b action could offer new strategies to counter obesity.

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PC23

Identification of a clavicular brown adipose tissue depot in the sheep

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Multiple evidence from human studies indicates that the primary sites for brown adipose tissue in humans are the bilateral clavicular depots [1]. In this study we aimed to identify an analogous region in the young sheep in order to profile changes in gene expression of critical brown (e.g. uncoupling protein (UCP)1) and white (e.g. RIP 140) adipose tissue markers during early life[2]. These were compared with perirenal-abdominal adipose tissue which represents the major fat depot in young sheep.
Methods: Four triplet-bearing mothers were entered into the study and a randomly selected triplet euthanased with an intra-venous injection of barbiturate at 1, 7 or 28 days of age for adipose tissue sampling. All procedures were conducted with Home Office Approval under UK legislation. Gene expression for target genes was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-

PCR). Their relative expression was calculated using the ΔΔCt method, relative to the geometric mean of 2 reference genes (18S and RPL19) and expressed in arbitrary units. Data are presented as mean ± SE and the effect of age assessed by ANOVA.
Results: As expected gene expression of UCP1 in perirenal adipose tissue significantly decreased with age (1d: 0.744±0.133: 28d: 0.004±0.001 ($p<0.001$)) and this was accompanied by comparable trends in DIO2 and PDK4 within the clavicular depot. In addition, there was an increase in gene expression of RIP140 with age in both the perirenal and clavicular depots (1d: 0.22±0.03: 28d: 0.76±0.11 ($p<0.01$)).
Conclusion: Our study shows that in the sheep during early life the changes in gene expression with the clavicular adipose tissue expression are very similar to those seen within the perirenal depot of the same animals. Taken together these are indicative of a brown depot transforming to become white or possibly a beige depot, in accord with recent suggestions in adult humans [3]. The young sheep may, therefore, provide a novel model for examining the developmental process of adipose transformation in early life.

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PC24

Inhibitory effect of visfatin and leptin on human and rat myometrial contractility

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Adipokines, active substances secreted from adipocytes influence metabolic functions. Their levels have been shown to alter in pregnancy and it has been suggested that their dysregulation could be associated with complications of pregnancy, including gestational diabetes. Visfatin, is a novel adipokine (originally known as pre-B cell colony-enhancing factor) with multiple functions including insulin mimetic effects, and it has been found to have altered maternal plasma levels in obese women and during gestation. Leptin, one of the best characterised adipokines, has been reported to decrease contractility of the myometrium. We have therefore investigated the effect of visfatin on myometrial contractility and compared them to leptin.

Myometrial strips from either term pregnant women having a caesarean section (with informed consent and ethical approval) or humanely killed rats were dissected, superfused with Krebs and the effects of visfatin or leptin at 1-100 nM studied. After establishment of regular contractions the adipokines were added for 20 minutes. In pregnant human myometrium, visfatin at 1nM had little effect on contractility but 10 nM produced a significant (paired T Test) decrease in the amplitude and integral of spontaneous contractions (47±18 % and 50±19 % respectively, mean + sem, control 100%; n= 4). Leptin at this concentration (10 nM) had only a small inhibitory effect (~ 5 – 10%) on contractions of rat or human myometrium. These data are the first to show that visfatin can inhibit myometrial contractility and that it may do so more potently than leptin. Increased output of adipokines associated with maternal adiposity, along with cholesterol, which we have previously demonstrated to inhibit contractility, may impair force production in uterine myocyte and contribute to uterine dystocia and the need for unplanned caesarean delivery in obese mothers.

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PC25

Late gestation maternal nutrient restriction reprogrammes the transcriptional control of uncoupling protein 1 in neonatal brown adipose tissue

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Non-shivering thermogenesis in brown adipose tissue (BAT) of precocial newborns, such as sheep, is essential for effective adaptation to cold exposure of the extrauterine environment. A suboptimal nutritional environment in utero can reprogramme adipose tissue development and function[1] and, is associated with excess adiposity in later life although the precise mechanism(s) by which this occurs are not fully established. Maternal nutrient restriction in late gestation, coincident with appearance of large amounts of the BAT specific uncoupling protein (UCP)1, restricts its synthesis [2]. The aim of the present study was, therefore, to examine the extent to which factors associated with enhancing, or suppressing, UCP1 gene expression maybe reset in newborn sheep born to nutrient restricted (NR) mothers.
Methods: Twin bearing Border Leicester cross Swaledale sheep of similar age and body fat distribution were individually housed at 110 days of gestation and randomly assigned to receive either 100% of metaboliseable energy (ME) requirements for body weight and stage of gestation (i.e. controls; n=8) or 60% of this amount (i.e. NR; n=9). Then, within 12 hours of birth, one twin from each mother was randomly selected and humanely euthanased with an intra-venous injection of barbiturate for tissue sampling. All procedures were conducted with Home Office Approval under UK legislation. Gene expression for regulators of UCP1 synthesis

was determined using qPCR. Their expression was calculated using the ΔΔCt method and expressed relative to the geometric mean of 3 reference genes (IPO8, RSP2 and YWAZ), normalised to the control group and expressed in arbitrary units. Data are presented as mean ± SE and were compared between groups using a Mann-Whitney test.
Results: In accord with previous findings[2], there was a trend towards a reduction in UCP1 gene expression in offspring born to NR mothers (C: 1 ± 0.19; NR: 0.6 ± 0.12 (p=0.07)) this was accompanied by reduced mRNA abundance for type II iodothyronine deiodinase (DIO2) (C: 1 ± 0.23; NR: 0.44 ± 0.10 (p <0.05)). In contrast, the expression of RIP140 was increased (C: 1 ± 0.14; NR: 1.77± 0.20 (p<0.05)) as was that of PPARY (C: 1± 0.10; NR: 1.60 ± 0.17). The expression of other genes associated with adipogenesis such as Cidea and TWIST I was unchanged.
Conclusion: Reduced UCP1 abundance following maternal nutrient restriction coincident with the time of its rapid appearance results in a complex set of adaptations linked to multiple pathways controlling UCP1 transcription. As UCP1 is lost through postnatal life, these would be predicted to subsequently promote white adipose tissue deposition and thus may contribute to excess adiposity in later life.

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PC26

Mechanisms of carbamoylcholine action on respiration and oxidative phosphorylation of isolated pancreatic acini

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The relationship between cellular metabolism and signalling is an important problem in physiology. Acetylcholine as one of the main secretagogues modulates mitochondrial functions in acinar pancreacytes (Voronina et al., 2002; 2004; 2010), presumably due to increase of ATP hydrolysis or Ca^{2+}-transport into mitochondria. The aim of this work was to investigate mechanisms of acetylcholine analogue, carbamoylcholine (CCh), action on respiration and oxidative phosphorylation of isolated pancreatic acini. Male rats (200–300 g) were anaesthetised with chloroform, decapitated and following the removal of the pancreas suspensions of isolated acini (cell viability ≥ 95%) were obtained (Williams et al., 1978). Oxygen consumption was measured using a Clark electrode at 37°C in a standard extracellular solution (140.0 mM NaCl, 4.7 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 10.0 mM HEPES, 10.0

mM glucose, 2.5 mg ml^{-1} BSA and 0.1 mg ml^{-1} soybean trypsin inhibitor at pH 7.4). Respiration of digitonin-permeabilized cells was measured in sucrose-based solution (Manko et al., 2012). Statistical significance was determined by paired t-test. Values are given as mean±S.E.M. Resting respiration rate of isolated acini was 0.26±0.02 nmol O_2 min^{-1} 10^{-6} cells. CCh (10 μM), added into respiration chamber (rapid but transient effect), intensified respiration by 23.4 % for 33±3 s (P≤0.001, n=15). This effect was completely prevented by preceding application of 10 μM 2-aminoethoxydiphenyl borate (2-APB), 6 μM oligomycin or 0.5 μM FCCP (n=3-4). Ruthenium red (10 μM) attenuated rapid CCh effect (~5%, P≤0.01, n=3), however it was prolonged to 74±10 s. Incubation (5 min) with 1 or 10 μM CCh (slow long-term effect) elevated respiration rate by 11.4 or 12.4%, respectively (n=3). 0.1 μM CCh did not influence respiration rate. Preincubation (10 min) with 2-APB or ruthenium red did not affect resting respiration rate and prevented slow CCh effects (n=3–4). Oligomycin inhibited respiration rate by 62.7% (P≤0.001, n=4), and abolished slow effects of CCh. FCCP increased respiration rate by 48.12% in control (P≤0.001, n=4). After preincubation with 1 μM CCh, FCCP-uncoupled respiration rate increased by 8.75%. Also, preincubation with CCh prior to cell permeabilization increased respiration rate at pyruvate+malate oxidation by 10.5% (P≤0.05, n=5), but not at succinate oxidation. In contrast, preincubation with 10 μM CCh did not influence FCCP-uncoupled respiration and decreased respiration at pyruvate+malate oxidation by 12% (P≤0.05, n=5). Thus, mediocre CCh dose (1 μM) intensifies respiration and oxidative phosphorylation of acinar pancreacytes by feed-forward mechanism via Ca^{2+}-transport into the mitochondria and activation of Ca^{2+}-sensitive mitochondrial dehydrogenases. Prolonged action of high CCh dose (10 μM) seems to impair mitochondrial functions.

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PC27

Mercury chloride-induced glucose intolerance in rats: role of oxidative stress

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Although mercury is recognized as a hazardous chemical, its commercial importance and applications has led to an increase in occupational and environmental exposures in many parts of the world. This study evaluated the impact of HgCl2

on glucose homeostasis and the possible involvement of oxidative stress. In addition, the potential protective effects of the antioxidant Alpha Lipoic Acid (ALA) were investigated. Thirty Sprague Dawley rats were divided into five equal groups of six animals: Group 1 (control) received 0.5 ml distilled water; Group 2 (ALA) received 100 μg/kg of ALA; Group 3 (LDM) and Group 4 (HDM) received 250 and 500 μg/kg body weight of HgCl2, respectively; and Group 5 (HDM-ALA) received 500 μg/kg of HgCl2 and 100 μg/kg of ALA. Both HgCl2 and ALA were administered orally for 14 days. 1-2 drops of blood were collected six times with a minimum of 30 mins interval on glucose strips from the rat tail vein to determine fasting blood glucose and oral glucose tolerance test (OGTT). 3hrs post OGTT; the rats' were anaesthetized with ketamine (90mg/kg b.w) and Xylazine (10mg/kg b.w) as a single intraperitoneal injection and the rats' livers were removed for determination of the activities of reduced glutathione GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation, indexed as Malondialdehyde (MDA) levels. Values are means ± SEM, compared by one way ANOVA. The fasting blood glucose (mmol/L) for Control, ALA, LDM, HDM and HDM-ALA were as follows; 4.02 ± 0.35, 4.6 ± 0.26, 7.23 ± 0.48, 8.03 ± 0.64 and 4.45 ± 0.41 respectively. After thirty minutes of glucose load (2ug/kg body weight) blood glucose levels were 6.62 ± 0.85, 7.22 ± 0.64, 9.43 ± 1.07, 9.43 ± 1.05 and 7.03 ± 0.52 in the Control, ALA, LDM, HDM and HDM-ALA respectively. Subsequent values were obtained every 30 minutes and after 3 hours, the blood glucose values were 2.32 ± 0.43, 4.26 ± 0.23, 7.29 ± 0.29, 8.28 ± 0.33 and 3.97 ± 0.21 in the Control, ALA, LDM, HDM and HDM-ALA respectively. The results on MDA in the Control, ALA, LDM, HDM and HDM-ALA groups are; 0.0318 ± 0.005, 0.0242 ± 0.004, 0.069 ± 0.004, 0.104 ± 0.011 and 0.045 ± 0.006 respectively. Superoxide dismutase activity was; 46.74 ± 4.85, 43.16 ± 3.68, 24.42 ± 3.17, 19.62 ± 2.51, 35.32 ± 3.38. Reduced glutathione values were; 0.31 ± 0.04, 0.32 ± 0.04, 0.15 ± 0.02, 0.13 ± 0.03, 0.21 ± 0.02 and catalase activity were; 224.60 ± 15.03, 230.98 ± 14.98, 155.11 ± 17.63, 139.38 ± 16.44, 162.94 ± 15.62 in the Control, ALA, LDM, HDM and HDM-ALA respectively (values are in units/mg protein). The results showed changes in glucose tolerance and oxidative indices in rats exposed to HgCl2. However, treatment with ALA attenuated all HgCl2 triggered changes. This study shows the induction of glucose intolerance by HgCl2 and suggests the involvement of oxidative stress as an important regulator of glucose homeostasis during HgCl2 exposure.

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PC28

Modification of PPARα activity is detrimental to cardiac function during chronic hypoxia – critical role of PPARα on cardiac substrate metabolism

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PPARα plays a central role in the regulation of cellular lipid metabolism in tissues with high capacity for fatty acid oxidation, such as heart, brown adipose tissue, slow-twitch skeletal muscle, and liver. We postulated that PPARα plays is pivotal to cardiac metabolic response to chronic hypoxia and used high fat (HF) feeding and gene deletion to alter PPARα expression. Control mice (129EvSv) and PPARα-/- mice were exposed to 3 weeks of normobaric hypoxia at 11% oxygen (n=70), or to normoxia (n=84). To stimulate the PPARα pathway, half the mice were fed a HF diet (55% fat), and controls were chow-fed (7.5% fat). Mice were anesthetised with 1.5% isoflurane in O2 and in vivo cardiac function was measured using cine MRI. Hearts were isolated and perfused to measure palmitate oxidation and glycolytic flux using 3H labelling. Cardiac function in control mice was unaffected by chronic hypoxia, but palmitate oxidation was reduced by 23% ($p<0.05$) and glycolytic flux increased 2-fold ($p<0.01$). PPARα mRNA expression was decreased by 31% ($p<0.05$) in hypoxic chow fed mice, which reduced protein levels of UCP3 by 37% ($p<0.05$) and MTE-1 by 39% ($p<0.01$). HF feeding during hypoxia decreased cardiac output by 24% ($p<0.01$), decreased ejection fraction by 9% ($p<0.05$), and increased palmitate oxidation by 30% ($p<0.01$) compared with normoxic controls. HF feeding increased PPARα mRNA expression 4-fold ($p<0.05$) and doubled UCP3 and MTE-1 ($p<0.05$) levels, and were unchanged by hypoxia. PPARγ and PPARβ/δ protein levels were unaffected by hypoxia or HF feeding. Hypoxic PPARα-/- hearts had 19% ($p<0.01$) lower cardiac output than normoxic controls, with cardiac metabolism and UCP3 level unaltered by hypoxia and/or HF feeding. In conclusion, feeding a high fat diet under hypoxic conditions prevented metabolic adaptation to hypoxia, with cardiac substrate metabolism similar to that of normoxic high fat fed mice. With scarce oxygen supply, the increase in UCP3 may have decreased the efficiency of mitochondrial ATP synthesis and impaired cardiac function following chronic hypoxia. Deletion of PPARα also impaired cardiac function as metabolic adaptation was prevented. Therefore, cardiac metabolic adaptation via PPARα is essential to maintain cardiac function following chronic hypoxia.

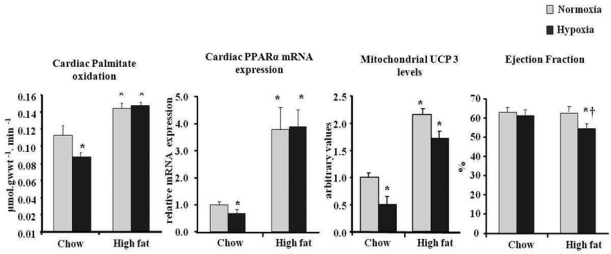

Figure 1. Effects of hypoxia and high fat feeding in mouse hearts

* p < 0.05 relative to normoxic control on chow
† p < 0.05 relative to normoxic control on high-fat

British Heart Foundation

Malaysian Ministry of Higher Education

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PC29

Role of retinoic acid, rosiglitazone and forskolin in the expression of human and mouse UCP1 reporter constructs in differentiated and undifferentiated HIB1B brown adipocytes

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Introduction: Expression of the brown adipocyte specific gene, uncoupling protein 1 (UCP1), is regulated by a distal enhancer interacting with the proximal promoter. Expression from the human promoter is stimulated by isoproterenol and thiazolidinediones but only in the present of retinoic acid whereas expression of the mouse UCP 1 promoter can be stimulated without retinoic acid.
To establish the molecular mechanism for this species difference we have stably transduced mouse UCP1 promoter reporter constructs and examined their activity in the differentiated mouse cell line.
Methods: Mouse and human enhancer-proximal promoter luciferase pLenti reporter constructs were transiently transfected into undifferentiated HIB-1B cells, or used to produce lentiviral particles which were used to produce stably transduced cells that were differentiated for 7 days using a standard protocol of IBMX, dexamethasone, triiodthyronine and insulin. HIB1B cells were then cultured in the presence and absence of retinoic acid, forskolin (to stimulate cAMP) and the thiazolidinedione, rosiglitazone. Luciferase assays were performed up to 48h later.
Results: Transcription from both mouse and human UCP1 promoter luciferase reporter constructs transiently transfected into undifferentiated HIB1B cells was strongly upregulated by the separate addition of forskolin, retinoic acid or rosigli-

tazone to the media, although combined addition of these drugs enhanced luciferase activity. In differentiated HIB1B cells stably transduced with the mouse construct, addition of forskolin only weakly stimulated transcription but strongly upregulated luciferase activity, when combined with retinoic acid or rosiglitazone,
Conclusions: Transcription of a UCP1 enhancer-promoter reporter construct stably transduced into differentiated HIB1B cells is only fully responsive to cAMP simulation in the presence of retinoic acid or rosiglitazone and this may have importance in the strategy to treat human obesity by increasing the activity of UCP1 in adipose tissue.
Conflict of Interest: None
Funding: Saudi Arabian government and Nottingham University

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PC30

Severe respiratory chain defects cause increased recruitment of brown adipose tissue

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Increased recruitment of brown adipose tissue has been reported to occur during hyperphagia and chronic cold exposure in animal models. However, few studies have focused on the investigation of this phenomenon in humans. We studied patients with a mitochondrial encephalopathy, Myoclonic Epilepsy with Ragged Red Fibres (MERRF) who develop large masses of adipose tissue known as lipomas specifically in the interscapular, subscapular and cervical regions. These patients harbour a single nucleotide substitution (m.8344A>G) within the mitochondrial genome and develop neurological features such as dementia, ataxia and myoclonic jerks. We have established that lipomas consist of brown adipose tissue which contains high levels of mutant mtDNA and are significantly deficient in key mitochondrial respiratory chain subunits. Proliferating adipocytes were found in lipomas based on Ki67 protein expression and increased macrophage infiltration was detected by CD68 immunohistochemistry suggesting that cellular hypertrophy and hyperplasia are involved in tissue expansion. We propose that the lack of functional respiratory chain subunits hinders uncoupling by UCP1 leading to the generation of a negative feedback loop to increase recruitment of brown adipose tissue. Increased recruitment of brown adipose tissue was investigated by studying the cAMP-PKA signalling pathway in lipoma tissues. An upregulation of β3 adrenergic receptors, along with the detection of P-CREB exclusively in patient tissues and preferential expression of PGC1α was indicative of increased cAMP-PKA signalling involved in lipoma formation. The main aim of this work is to develop treatment strategies to prevent the formation of these disfiguring and uncomfortable lipomas in patients harbouring the m.8344A>G mutation.

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PC31

Short-chain 3-L-hydroxyacyl-CoA Dehydrogenase (SCHAD) and its role in the regulation of thermogenesis

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Dysregulation of fatty acid oxidation plays a pivotal role in the pathophysiology of obesity, insulin resistance, and in impaired thermoregulation. We generated a gene-trap knockout mouse (*Hadh*$^{-/-}$ mouse; gene name: *Hadh*, protein name: SCHAD) to disrupt one of the last steps in mitochondrial beta-oxidation. Deletion of *Hadh* results in a reduced body weight due to a loss of acylcarnitines via the urine and a compensatory elevated beta-oxidation (Schulz et al, Endrocrinology 2011). To study the link of beta-oxidation and thermogenesis *in vivo* we carried out cold exposure analysis with and without exogenous energy supply. To study cold tolerance of *Hadh*$^{+/+}$- and *Hadh*$^{-/-}$ mice, animals were kept single housed in a climate cabinet for 3 hours at 4°C to measure their rectal temperature every 30 minutes. Moreover, we isolated brown adipocytes of *Hadh*$^{+/+}$- and *Hadh*$^{-/-}$ mice, immortalized the adipocytes with a SV40 virus and performed *in vitro* studies with differentiated adipocytes. *Hadh*$^{-/-}$ mice exhibited significantly higher body temperature than their wild-type littermates under control conditions. In contrast, acute cold exposure led to a significant reduction of body temperature of *Hadh*$^{-/-}$ mice due to their inability to use fat as an energy source under these conditions. After cold exposure levels of phosphorylated hormone-senstive lipase (pHSL) were also lower in brown adipose tissue (BAT) of *Hadh*$^{-/-}$ mice than in BAT of *Hadh*$^{+/+}$ mice. Moreover, preliminary data of immortalised brown adipocytes of *Hadh*$^{-/-}$ mice displayed reduced isoproterenol-induced lipolysis and exhibited lower amounts of pHSL. Hadh is required for maintaining thermogenesis by influencing lipolysis in brown adipocytes.

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PC32

Transcriptomic profiles in epididymal, inguinal, and brown adipose depots

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[Introduction]: Fat tissues have been classified as either white adipose tissue (WAT) or brown adipose tissue (BAT). WAT accumulates excess energy in form of triglyceride. Conversely, BAT dissipates excess energy through adaptive thermogenesis by brown adipocyte-specific uncoupling protein (UCP) 1. Recently, a third type of adipocyte, name brite has been identified in WAT. These brite cells express many of genes specifically expressed in brown adipocytes, including Ucp1. Some WAT depots are more prone to induction of brite adipocytes. This study aimed to identify genes whose might be a key factor related to the difference in brite inducing ability of WAT depots.
[Methods]: Epididymal WAT (EWAT), inguinal WAT (IWAT), and interscapular BAT were obtained from 6-wk-old C57BL/6 mice that were kept at 4°C or 26°C for 16 days. . All mice were anesthetized with seroflurane and then killed by cervical dislocation before harvesting adipose depots. The difference in brite inductive activity of each depot was determined by western blotting of UCP1. Gene expression was measured using Agilent SurePrint G3 Mouse microarray. Validity the cDNA microarray was determined by qRT-PCR. Microarray data was analyzed by Rank products method (n=3).
[Results]: Cold accumulation did not significantly affect body weight of mice. However, elevating in BAT weight and decreasing in IWAT and EWAT weights were observed ($p<0.05$). Significant induction of UCP1 protein expression was found in BAT (1.8 times) and IWAT (25 times) ($p<0.05$). No UCP1 protein expression in EWAT was observed. Gene expressing profiles of IWAT and EWAT were similar to each other and much different from that of BAT, even when these tissues were taken from cold exposed mice. EWAT was found to exhibit 100 and 3000 times lower Ucp1 than that in IWAT and BAT. Up-regulation of Ucp1 in IWAT and BAT responding to cold exposure was detected, while the level of Ucp1 expression in EWAT was not altered. The expressions of brown fat-specific genes were highest in BAT and their expression level elevated after cold exposure in all tissues, except Pgc1α. Elevation of adipogenic gens aP2 and Pparg was detected in all tissues of cold exposed mice. Number of genes that their expression levels were significantly up-regulated (> 2 fold, false discovery rate < 20%) by cold-exposure is 1474 genes. Within this group of genes, 12 genes are receptor genes. By comparing between tissues, there were 22 receptor genes that are higher express in IWAT than EWAT. After gene expression validity by PCR, eight genes were selected for study their function related to Ucp1 expression. The highly expressing and significantly inducible by cold exposure genes (especially receptor gene), in IWAT and BAT, but not in EWAT, identi-

fied in this study might be used as a target in a strategy for increase energy expenditure that may also provide opportunities for the development of new therapeutics for obesity.

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PC33

Dead in bed syndrome: a misnomer?

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Introduction: 'Dead-in-bed' syndrome was identified as a cause of death in young male diabetic patients in 1991. The aetiology of the syndrome however, has been elusive since then. Although a possible mechanism of Prolongation of the QT interval during hypoglycaemic events in sleep has arisen.
Aims: Here the current theories of the so called 'dead-in-bed' syndrome are reviewed and compared with other sudden death syndromes to gain insight into the aetiology.
Methods: A literature review of 'dead-in-bed' articles as well as articles on other sudden death syndromes.
Results: DBS occurs at a rate 46 times higher than the general population (p=0.0205). When one includes all sudden unexplained deaths in diabetics this rises to a standard mortality ratio of 103 (p=0.0138). Around 75% of these deaths are in young men and hypoglycaemia during one of these deaths has been confirmed. However there is a possibility of genetic predisposition to QT interval prolongation and candidate genes include SCN5A and others associated with long QT and Brugada syndrome.
Conclusion: Dead-in-bed syndrome has similarities to other sudden death syndromes and more research into the genetic mechanisms of it are needed in which SCN5A is a candidate due to its mutations often causing death during sleep. Due to its similarities with other sudden death syndromes; it is concluded that 'dead-in-bed' syndrome should be renamed sudden unexplained death in diabetes (SUDD); so that any mortalities due to this syndrome are not unnecessarily excluded.

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Professor Colin Dayan, Cardiff University

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PC35

Sweating and thirst perception in premenopausal, perimenopausal and postmenopausal women during moderate exercise

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Sweating and increased thirst are common symptoms of menopause (Minkin et. al., 1997; Cadena, 2010) and also important factors during exercise (Francesconi, 1988), although thirst sensation decreases with ageing (Ferry, 2006).
In this study, we examined the sweat rate (SR), sweat volume (SV), sweat sodium concentration (S[Na+]) and changes in thirst perception (TP), in premenopausal (preM), perimenopausal (periM) and postmenopausal (postM) women after a moderate exercise.
Thirty (30) healthy women comprising of preM (aged 22.5±0.8yrs, n=10), periM (aged 46.5±1.1yrs, n=10) and postM (aged 52.2±0.9yrs, n=10) participated in the study. Participants were adequately informed of the experimental procedures and they all consented to it. TP was rated using the Visual Analogue Scale, VAS. Sweat was obtained with the sweat suction apparatus from a 120cm2 circular area marked on the skin of the face and neck (Ugwu and Oyebola, 1996), after a moderate exercise (a 15min walk on the treadmill) at a speed of 4.2 km/h at 27 0C, with the start time noted and SV and [Na+] measured. SR was determined using the formula: (volume of sweat collected)/(exercise time) .
Exercise induced a significant change in TP in all groups- preM, periM and postM (2.1±0.5 cm vs 2.2±0.2 cm vs 0.99±0.2 cm), with the postM women exhibiting the least thirst ratings ($p<0.05$). Although there was no significant difference in SV (1.3±0.4 ml vs 1.7±0.2 ml vs 0.9±0.1 ml) and S[Na+] (125.4±18.7 mmol/l vs 77.2±8.8 mmol/l vs 101.3±10.6 mmol/l) between the groups, the periM women showed a significantly higher SR (0.07±0.02 ml/min vs 0.12±0.01 ml/min vs 0.06±0.01ml/min) ($p<0.05$). A significant ($p<0.0001$) and positive correlation existed between TP, SR, SV and S[Na+] .
In conclusion, these data are consistent with the earlier reports that older adult women exhibit diminished thirst sensation and further indicate that increase in sweat rate and volume produce a concomitant increase in thirst perception in women during moderate exercise.

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We appreciate the support of Prof Ugwu AC. and Prof Obika LFO. as well as the Department of Chemical Pathology, University of Benin Teaching Hospital, Benin City,Nigeria.

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PC36

The effect of dietary fat and glycaemic index on metabolic profiles of human urine

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Modification of dietary fat (1) and carbohydrate (2) have been associated with lower metabolic syndrome risks. This study aimed to examine the changes in metabolic profile of people with higher risk of metabolic syndrome after a dietary intervention.

Only samples collected at Imperial College London, one site of the multi-centred RISCK trial (3) were analysed. Subjects (n=70) were initially fed a high saturated fat diet (HS) for four weeks, before randomly assigned to one of the experimental diets for 24 weeks: high monounsaturated fat (MUFA)/high glycemic index (GI) (HM/HGI); high MUFA/low GI (HM/LGI) and low fat/high GI (LF/HGI). The urinary metabolome was measured using proton NMR spectroscopy and analysed with multilevel Partial Least Squares Discriminant Analysis (4). The prediction accuracy of the models was assessed by the difference in correct classification rate between the model and a null model (permutation) using an in-house developed MATLAB script.

Figure 1 represents one of the specra obtained, i.e. the HM/HGI group. Overall, the results showed that proline betaine (from citrus), tartaric (from grapes) and glycine (protein sources) increased with the LGI treatment while cis-aconitate increased with the HGI treatment. Additionally, urinary ethanol, metabolites indicative of protein consumption (creatine, carnosine, trimethylamine) and proline betaine could be linked to the LF diet while cis-aconitate and acetaminophen glucuronides could be linked to the HM diet. Application of a data treatment methodology based on a paired data structure has improved the data interpretability and enables the investigation of the effect of dietary modification on human metabolism despite the large between subject variations.

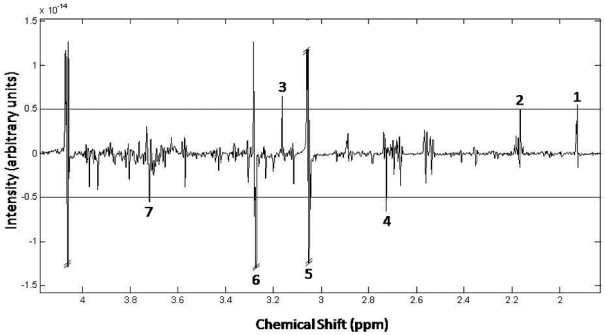

Figure 1. Proton NMR spectra of the HM/HGI group. Metabolites above the top red line (at the intensity of $0.5*10^{-14}$) are significantly increased with the diet, while the metabolites below the bottom red line (at the intensity of $-0.5*10^{-14}$) are significantly decreased with the diet. 1: acetate; 2: acetaminophen glucuronide; 3: cis-aconitate; 4: dimethylamine; 5: creatine; 6: Trimethylamine N-oxide; 7: anserine; ppm: parts per million.

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The RISCK study was funded by the Food Standards Agency, UK (NO2031). NAI is awarded a PhD studentship from the Malaysian Ministry of Rural and Regional Development. The Section of Investigative Medicine is funded by an Integrative Mammalian Biology (IMB) Capacity Building Award, an FP7-HEALTH-2009-241592 EurOCHIP grant and funding from the NIHR Biomedical Research Centre Funding Scheme.

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PC37

The effects of constituents of garlic on uterine contractility

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Garlic (Allium sativum) is rich in organosulphur compounds, which are considered responsible for most of its beneficial physiological effects that reduce the risk of cardiovascular disease. Allicin (diallyl thiosulfinate), the main organosulfur compound, is produced from the amino acid alliin by alliinase when garlic is crushed. Allicin rapidly decomposes mainly to diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and ajoene. After consumption, neither allicin nor its metabo-

lites have been found in blood or urine, indicating that these compounds are rapidly metabolized. We have recently shown that H2S reduces uterine contractility. We were therefore interested to investigate if garlic and its constituents could reduce contractility, with a long term goal of preventing preterm labour.
Myometrial strips from term pregnant humanely killed rats were dissected, superfused with Krebs and the effects of adding for 15-30 mins, garlic powder,(Rajah brand) garlic oil (Sigma, which contains 30-50 wt % DAS,
10-13 wt % DATS) and DATS (Santa Cruz) were studied.
The garlic powder studied from 0.01 to 1 mg/ml had little effect on contractions, although a significant decrease in amplitude was found at 0.7 mg/ml (73±6% versus 100% control, mean +sem, n=3). Garlic oil, at 1:1000, greatly reduced or abolished contractions. DATS dose dependently reduced uterine contractions, particularly their amplitude. Thus at 750 μM amplitude was significantly reduced to 77± 7%, n = 6, paired t-test.
Our data suggest that the organosulphur compounds in garlic can inhibit uterine contractions. Given our earlier findings that H2S releasing compounds significantly reduce uterine contractions and Ca transients, we suggest that these effects of garlic organosulphur compounds will be via H2S production. These preliminary data suggest that garlic may be a useful supplement to help prevent preterm delivery.

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SA01

Diet-induced thermogenesis and brown adipose tissue

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That brown adipose tissue is activated when mammals need to defend their body temperature is well established; also that prolonged exposure to cold leads to a recruitment of the tissue (i.e. increased amounts of the uncoupling protein UCP1). It is also generally accepted that it is this process that constitutes nonshivering thermogenesis, and that no other organ, protein or process is involved in nonshivering thermogenesis.

Concerning the response of mammals to overnutrition, the situation is unsettled. The discussion centers on the phenomenon of "diet-induced thermogenesis"; it is, however, not established what this thermogenesis experimentally entails. In all animals, eating leads to a metabolic increase due to the cost of the digestive process as such ("obligatory diet-induced thermogenesis"). However, an additional component, occurring as a direct or indirect effect of overnutrition, is what is referred to as diet-induced thermogenesis (or better: "facultative diet-induced thermogenesis"). Although discussed for more than 30 years, the existence of this thermogenesis has repeatedly been questioned.

The methodological shift from rats to mice, caused by the development of transgenic techniques, has had the generally unrealized effect that the metabolism of the experimental animal (i.e. of mice) is mainly governed by the need for the animal to defend its body temperature: mice under normal animal house conditions are in reality cold-exposed. Therefore, only in mice at thermoneutrality can the issue of the existence of metaboloregulatory thermogenesis be settled. We find that under such conditions, feeding mice an obesogenic diet (i.e. a high-fat diet or a cafeteria diet) does induce a recruitment of brown adipose tissue, accompanied by an increase in the metabolic response to norepinephrine. This response is fully dependent on UCP1. Moreover, mice that lack UCP1 become obese on a normal diet – but even more obese on a high-fat diet. Thus, it would seem that diet-induced thermogenesis does exist, and that in its absence, obesity easily develops. This must also mean that there must be signalling processes that, based either on food amount or quality - or on the effect of overfeeding, i.e. on obesity - govern the recruitment and activity of brown adipose tissue. Probably the mediatory signal is leptin. The blood levels of leptin increase not only with increasing lipid stores in the body but also as an acute response to feeding, and all leptin-induced thermogenesis is derived from brown adipose tissue. Mice without leptin are unable to develop diet-induced thermogenesis - whereas exposure of such mice to cold still leads to (some) classical nonshivering thermogenesis.

Why this energy-wasting response to overnutrition has developed is still unknown. The idea that it may allow animals to "extract" e.g. protein from a poor

quality diet without making them suffer from problems of overweight is appealing – but experimental evidence for this is still scarce.

The present insight that also adult humans possess brown adipose tissue expands the interest in a metabolic defence against overnutrition from being an animal adaptation to being a possible factor in human health.

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SA02

Brown adipose tissue (BAT) development and new insights into its role in energy balance in early life

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In precocial newborns such as sheep and humans, the onset of non-shivering thermogenesis through activation of the BAT specific uncoupling protein (UCP)1 is essential for effective adaptation to cold exposure of the extra-uterine environment (Symonds, et al. 2012c). For both species, significant depots of BAT are present both around central organs, such as the kidney and heart, but also around the neck or supraclavicular region. The extent to which these BAT depots are replaced by white adipose tissue, or are transformed to a mix of beige and white adipocytes, remains to be fully established. Over the first month of life in the sheep, the majority of the BAT depots laid down in the fetus undergo rapid depot-specific transition adopting white or beige characteristics. This process can be manipulated by environmental challenges to the fetus and/or neonate which, therefore, offer the potential to promote BAT function (Symonds, et al. 2010).

We have developed the technique of thermal imaging in order to quantify potential changes in BAT activity through the life cycle in free-living subjects (Symonds, et al. 2012a). This has shown that BAT activity can be much greater in children than adults and is negatively correlated to body mass index. We are now beginning to further explore the impact of other modulators of BAT function including diet and genetic factors (Symonds, et al. 2012b). There is now a new opportunity to quantify and manipulate BAT development in early life in order to both promote survival of the newborn and also to prevent excess adiposity in later life (Symonds and Budge 2012).

Symonds ME & Budge H 2012 How promising is thermal imaging in the quest to combat obesity? Imaging in Medicine.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA03

Transcriptional control of brown adipocyte development

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The differentiation of both brown (heat-producing) and white (energy storing) adipocytes is regulated by the master transcription factor Pparγ. While Pparγ can explain the characteristics that are common to the two types of fat cells, it remains unclear whether Pparγ also controls lineage-specific gene programs. Here, we show that Early B-Cell Factor-2 (Ebf2) determines brown versus white adipocyte identity. A binding motif for Ebf was highly enriched within brown adipose-specific Pparγ binding sites that we identified by genome-wide ChIP-Seq. Of the Ebf isoforms, Ebf2 was selectively expressed in brown relative to white adipocytes and was bound at brown fat-specific Pparγ target genes. Strikingly, Ebf2 expression in myoblasts or white pre-adipose cells recruited Pparγ to brown-selective binding sites and reprogrammed cells to a brown fat fate. Notably, Ebf2 and Pparγ cooperated to directly and powerfully activate transcription of Prdm16, a key regulator of thermogenic genes. In brown fat cells, Ebf2 expression was essential for establishing and maintaining a brown fat-specific gene program. Finally, the presumptive brown adipose tissue in Ebf2-deficient mice was completely devoid of brown character and had a molecular profile resembling white fat. Taken together, these results show that Ebf2 determines brown adipocyte identity.

This work was supported by the NIH Director's New Innovator Award DP2 OD007288 (P.S.) and NIDDK/NIH award R00 DK081605 (P.S.)

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SA04

A role for brown fat in energy balance?

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The predominant model for a role of brown fat (BAT) is based upon the notion that an animal with a higher level of UCP1-BAT will burn off more calories than an animal with lower levels of BAT. Thus, we have the conclusions in the literature that elevated UCP/BAT levels reduce obesity based upon experiments in animals and in humans in which a negative correlation is observed between PET/BAT and obesity. The underlying assumption is that humans living at thermoneutrality in the absence of BAT have increased obesity. While there is some evidence for experiments in mice that such a behavior occurs, other evidence indicates that it is not always so, that is, Ucp1 KO mice at thermoneutrality are not always more susceptible to diet-induced obesity than wild-type mice. Accordingly, if you have the fatalistic view that humans are and will predominantly live at thermoneutrality, it is reasonable to pursue an anti-obesity strategy based upon increasing UCP1 levels. If you believe that a pharmaceutical approach will be essential to reduce obesity, than it is also reasonable to target drugs to cellular and molecular mechanisms that induce BAT levels and UCP1 expression. However, given that the molecular mechanisms for inducing Ucp1 and BAT are not specific, but participate broadly in the control of embryonic differentiation (and in cancer), then it behooves us to develop alternatives to taking drugs until such time as safe targets are developed.
Much published evidence from studies of mice with reduced thermogenic capacity from inactivation of Ucp1 and other components of non-shivering thermogenesis shows the huge benefits from reducing the ambient temperature, even a few degrees below thermoneutrality. While inactivation of Ucp1 was unexpectedly and paradoxically found to augment the combustion of calories at reduced ambient temperature, the benefits for wild type mice are such that reduction of ambient temperature from 28 to 20°C reduced metabolic efficiency by half in mice fed a high fat diet. At lower temperatures the effects of the obesogenic environment are totally neutralized and the adiposity index of mice fed a standard low fat (11Kcal %) remains at a normal healthy level. An interesting aspect of the effects of the cool environment on body weight regulation is that they may be independent of UCP1/BAT, rather they are the response of systemic energy balance, which would include UCP1/BAT, but not be dependent on high levels of BAT. Thus, humans, with or without BAT, can benefit from the “cool” environment.

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SA05

Insights into brown adipose tissue function in cold

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In mammals living within the thermoneutral zone basal heat production is sufficient to compensate for heat loss and defend a drop in body temperature. Moving to ambient temperatures below this zone the thermal conductance is decreased to a minimum and thermoregulatory heat production is activated to compensate for increased heat loss. In small mammals two mechanisms, nonshivering thermogenesis in brown adipose tissue and shivering in skeletal muscle, contribute to thermoregulatory heat production. Nonshivering thermogenesis in brown adipose tissue is based on the unique capacity of brown adipocyte mitochondria to dissipate proton motive force as heat without ATP synthesis. This process is catalyzed by the mitochondrial carrier Uncoupling Protein 1 (UCP1) (for review see (1)). Non-UCP1-mediated mechanisms for nonshivering thermogenesis in tissues other than brown adipose tissue have been suggested, but their physiological relevance and biochemical mechanisms are not clear.

The power for nonshivering thermogenesis in brown adipose tissue is around 160 mW/g at thermoneutrality and attains up to 330 – 480 mW/g in the cold (reviewed in (2)). This recruitment of heating power in the cold, also termed adaptive thermogenesis, is mainly accomplished by hyperplastic growth of brown adipose tissue, acceleration of mitochondrial biogenesis in brown adipocytes, and increased UCP1 synthesis. Moreover, angiogenesis improves blood supply to fuel high metabolic rate and to distribute the heat in the body. This dynamic remodelling is controlled by the sympathetic innervation of brown adipose tissue. In the control of adaptive thermogenesis in brown adipocytes adrenergic signaling pathways target complex transcriptional machineries composed of nuclear hormone receptors, cAMP response element binding proteins and coactivators. However, the cell-type specificity of these transcriptional responses are far from being understood. A brown adipocyte specific enhancer has been well characterised in the Ucp1 gene (1), and most recently our laboratory has identified another enhancer in the Ucp3 gene conveying brown adipocyte specific gene transcription (unpublished data). Further analysis of the transcription factor modules and epigenetic modifications at such cis regulatory genomic enhancer regions will help to understand the molecular basis of adaptive thermogenesis. Endocrine or paracrine mediators such as prostaglandins, bile acids, natriuretic peptides and fibroblast growth factors may also contribute to recruitment, either downstream or parallel to neuronal adrenergic signaling. The physiological relevance of these factors in the context of cold acclimation is not clear.

In mice acclimated to thermoneutrality, acute cold exposure elicits strong shivering as the capacity for nonshivering thermogenesis is low and not sufficient to defend normothermia. During prolonged exposure for several days shivering is sub-

sequently replaced by nonshivering thermogenesis. In fact cold acclimated rodents refrain from shivering unless their maximal power for nonshivering thermogenesis approaches exhaustion. A major question is whether this recruitment of thermogenic heating power can be completely ascribed to the recruitment of thermogenic capacity in brown adipose tissue.
To address this question we compared the maximal cold induced heating power (HPmax) of wildtype and UCP1 ablated C57BL/6J mice, including basal metabolic rate, shivering and nonshivering thermogenesis (3). All mice were preacclimated to moderate cold at 18°C before acclimation to 5°C. In wildtype mice acclimated close to thermoneutrality (27°C) HPmax was 757 mW, but increased to ~1230 mW (+473 mW) after cold acclimation whereas in UCP1-KO mice HPmax had increased from 714 mW to 941 mW (+227 mW). This reduction of adaptive thermogenesis by 52 % in UCP1-KO mice underlines the important role of brown adipose tissue for improved cold resistance. However, this observation also demonstrates that significant recruitment of additional heating power can occur in the absence of functional brown adipose tissue. In white adipose tissues of cold acclimated UCP1-KO mice the mitochondrial density, the activity of cytochrome c oxidase and the expression of the brown adipocyte marker CIDEA are increased as compared to wildtype (3). The trigger and the utility of this remodeling is unclear but the extra metabolic power in white adipose tissue may contribute to adaptive thermogenesis in UCP1-KO mice.

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SA06

Brown adipose tissue as an endocrine organ

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In the last decades, the recognition of an endocrine role for white adipose tissue constituted a breakthrough in the understanding of the cross-talk among tissues for regulation of metabolism and for many other processes, from inflammation to the control of feeding behaviour. In contrast to white adipose tissue, the capacity

of brown adipose tissue (BAT) to synthesize and release most of the currently recognized adipokines, is very low. In fact, the capacity to produce adipokines such as adiponectin or even leptin is considered a marker sign of white-versus-brown identity in adipose cells (1). Retinol binding protein-4, a protein with adipokine properties, is released both by white and brown adipose tissue, but there is a specific regulation in brown adipocyte in response to noradrenergic and PPARalpha-mediated signals (2). An exception to the traditional concept of a poor secretory capacity of brown adipocytes is triiodothyronine (T3). Differential expression of 5'-deiodinase in brown but not in white adipocytes makes brown adipose tissue an active site of T3 synthesis by deiodination of T4. In the late 80' it was shown that locally generated T3 is not only involved in intracellular regulation of brown adipocyte thermogenesis but also makes brown adipose tissue a quantitatively relevant site of production of systemic T3 in conditions of highly recruited brown adipose tissue thermogenesis (3). Other signalling molecules, such as several interleukins and IGF-1, have been claimed to be synthesized and released to circulation by brown adipose tissue (1). We have found that brown adipose tissue is an active site of synthesis and release of the hormonal factor fibroblast growth factor-21 (FGF21). Noradrenergic-mediated thermogenic activation of brown fat induces a cascade of intracellular signalling involving activation of p38-MAP kinase that ultimately results in activation of FGF21 gene transcription through phosphorylation of ATF2 and binding to the FGF21 gene promoter. This results in the induction of FGF21 synthesis and FGF21 release by brown adipocytes. Direct assessment of FGF21 release "in vivo" by determination of arterio-venous differences in FGF21 concentration across interscapular BAT evidenced a significant FGF21 output in conditions of thermogenic activation of BAT (4). Calculations based on the FGF21 turn-over rate and FGF21 total output release by interscapularar BAT, indicate that BAT is a substantial source of systemic FGF21 in conditions of enhanced thermogenesis. Recognition of an endocrine role of BAT for the FGF21 system suggest that some of the beneficial actions of BAT activation on systemic metabolism may be mediated by an active release of FGF21 and/or other yet unidentified hormonal factors that favour glucose homeostasis and a healthy metabolic profile. Moreover, an active endocrine role of BAT may explain the association between BAT activity and systemic metabolism even in conditions in which BAT amounts are scarce (e.g. adult humans) and exclusive attribution of consequences of BAT activity to overall changes in energy balance are unlikely. The combination of multiple experimental approaches, from candidate-based experimentation to high throughput screening, is expected to lead to the identification of the brown adipocyte secretome and its biological significance.

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SA07

BAT sensitisers: Role of BMP8b

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Thermogenesis in brown adipose tissue (BAT) is fundamental to energy balance and seems to be relevant for humans. Bone morphogenetic proteins (BMPs) regulate adipogenesis, and we will discuss a role of BMP8B in the direct regulation of thermogenesis. Specifically we will examine the regulation of BMP8B by nutritional and thermogenic factors in mature BAT, its role as adrenergic sensitizer and its global effect controlling peripheral and central mechanisms of energy dissipation. Finally we will address the advantages posed by therapeutic strategies focused on modulation of sensitivity to sympathetic tone over other strategies direct to increase brown fat mass.

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SA08

RIP140 and the relationship between brown and white adipocytes

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The primary role for RIP140 in fat is to repress brown fat genes in white fat depots. Thus, RIP140 blocks the expression of the BRITE (Brown-in-White) program in white adipose tissues to maintain the integrity of this energy storage depot.
Adipose tissues constitute a remarkably dynamic organ that responds to stimuli ranging from nutrients and hormones to external temperature. Brown and white adipose tissues (BAT and WAT) differ in their appearance and function due to distinct gene expression profiles. The transcriptional coregulator RIP140, encoded by the NRIP1 gene, is present in adipose tissues and enables white and brown adipocytes to express unique sets of genes that determine their very different functions. Genetically modified mice that lack the NRIP1 gene are lean and resistant to diet-induced obesity. Although RIP140 is detected in both brown and white adipose depots it has different roles in each tissue. RIP140-null white adipocytes show

profound alterations in the transcriptome in contrast to brown adipocytes that display few changes in gene expression. The function of RIP140 as a transcriptional corepressor correlates with the elevated expression of many genes when ablated in white adipocytes. In WAT, it represses genes normally expressed at high levels in BAT, including UCP1, CIDEA, FABP3, PPARα, L-PGDS and Gyk. In wild type mice, under conditions of cold exposure, these genes are switched on in BRITE adipocytes within WAT depots. Interestingly, discrete WAT depots show differential responses to cold with subcutaneous being most responsive and visceral mesenteric being largely unresponsive. RIP140 also represses genes associated with early events in brown and BRITE adipocyte differentiation including PRDM16, Tbx1, CD137 and Tmem26.

Whereas WAT store lipids that are released into the circulation when required, BAT uses its stored fat to generate heat by oxidation of fatty acids to maintain body temperature. These functions are reflected in tissue morphology, as white adipocytes usually contain a single giant lipid droplet (LD) occupying most of the cytoplasm (unilocular), while brown adipocytes are filled with many smaller LDs (multilocular). Smaller LDs, with higher surface/volume ratio, facilitate the release of stored lipids given the extensive surface accessible to lipases. LD-associated proteins regulate the storage and release of triacylglycerol from LDs and are generally more highly expressed in BAT compared to WAT. The RIP140-regulated gene CIDEA is an important regulator of LD dynamics that is cold-induced in WAT depots. The CIDEA protein coats the surface of the droplets and facilitates their enlargement by lipid transference between adjacent LDs. With the use of specific deletions and point mutations, we have refined its mechanism of action and determined the discrete protein regions involved in each step: LD targeting, dimerization, LD clustering and lipid transference.

Thus, RIP140, by repressing genes required for BAT differentiation and function, is important for preventing the inappropriate expression of the BRITE phenotype in WAT depots and represents a valuable target for the activation brown adipocytes in white fat.

RIP140-targeted repression of gene expression in adipocytes.

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SA09

Lessons from PET-CT imaging of brown adipose tissue

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Until several years ago there was a widely held belief that there is no functional BAT in adult humans. However, in 2009 several studies using the modern scanning technique of positron emission tomography (PET) did show active cold induced brown adipose tissue in young adult humans.
PET scanning can assess the metabolic activity of brown adipose tissue in humans indirectly. This scan is in most studies combined with computed tomography (CT) scan for anatomical details. The most widely used PET tracer for this purpose is 18F-fluorodeoxyglucose (18F-FDG) for the measurement of glucose uptake. 18F-FDG scanning can be static or dynamic. Static scans provide information about the (relative) presence and activity of BAT. Dynamic scans provide actual glucose uptake rate. Glucose uptake rate however, does not provide information about the total oxidative metabolism. In fact the most important fuel of BAT are fatty acids. Therefore, the contribution of fatty acids must be estimated.
Alternatively, several recent studies combined FDG-PET/CT measurements with the use of other tracers and dynamic scanning techniques: 15O-H2O for blood perfusion, 18F-THA for nonesterified fatty acid uptake, 11C-acetate for oxidative metabolism, and 15O2 for oxygen extraction.
For this symposium several of the recent PET studies will be presented. First, intervention studies using static and dynamic FDG-PET/CT scans will be shown. Next, results from studies using alternative tracers will be discussed with respect to fuel oxidation, oxidative metabolism and perfusion of BAT. An estimate will be made on the actual contribution of BAT to whole body non-shivering thermogenesis. And finally, expectations on near future scanning possibilities, such as MRI, will be presented.

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SA10

Brown adipose tissue and longevity

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Different theoretical considerations suggest that brown adipose tissue (BAT) and uncoupling proteins (UCPs) could affect aging and longevity. BAT activation leads to an increased energy expenditure and substrate oxidation which could prevent the development of obesity and related life shortening health disorders such as the metabolic syndrome. The hallmark of brown adipose tissue is the presence of UCP1 which uncouples mitochondrial respiration from ATP synthesis, thus generating heat. According to the "uncoupling-to-survive" hypothesis, an increased mitochondrial proton conductance could lead to decreased production of reactive oxygen species (ROS) which in turn could minimize oxidative damage to DNA and thus slow ageing. This hypothesis is supported by recent reports that genetic variations in human UCP genes are associated with longevity and also more directly by animal studies. We have shown that transgenic mice with ectopic expression of UCP1 in skeletal muscle mitochondria (HSA-UCP1 mice) displayed a markedly increased longevity under high fat diet feeding which was linked to a delayed obesity development and improved glucose homeostasis. We further observed a positive correlation of total energy expenditure with lifespan. Although isolated muscle mitochondria from HSA-UCP1 mice showed a decreased ROS production in vitro, we did not find evidence for decreased oxidative stress in vivo. On the contrary, we observed an increased fat metabolism in skeletal muscle of HSA-UCP1 mice which was linked to an increase in lipid peroxidation products, indicating an increased oxidative stress. This was paralleled by an induction of endogenous antioxidant defense systems and increased redox signaling. This argues for the mitochondrial hormesis hypothesis suggesting that ROS are not always detrimental to health but essential signaling molecules for health and longevity.

The research leading to these results has received funding from the European Union's Seventh Framework Program FP7 2007-2013 under grant agreement # 244995 (BIOCLAIMS Project) and from the German Research Foundation (DFG: KL613/14-2).

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SA11

UCP1-independent unmasking of mitochondrial oxidative capacity in adipocytes: Amelioration of obesity

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We are facing a revival of the strategy to counteract obesity and associated metabolic disorders by inducing thermogenesis mediated by mitochondrial uncoupling protein-1 (UCP1). Thus, the main focus is on the adaptive non-shivering thermogenesis occurring both in adipocytes contained in the typical depots of brown adipose tissue (BAT) and in UCP1-containing cells that could be induced in white adipose tissue (WAT) and could represent a separate cell lineage. A possibility to reduce adiposity based on the induction of energy expenditure in classical white adipocytes is largely neglected. Nevertheless, the contribution of WAT to resting metabolic rate in lean human subjects is close to 5% and it doubles in obesity (1), while in adult mice reared at 20oC, the total oxidative capacity of WAT represents ~30-50% of BAT oxidative capacity (2). Data will be presented, which support a notion that induction of energy expenditure in WAT may influence total energy balance and reduce obesity: (i) studies in both humans and rodents document negative associations between oxidative capacity of mitochondria in WAT and obesity; (ii) pharmacological activation of AMPK in rats (3) as well as cold-acclimation of the UCP1-ablated mice (4) confers obesity resistance, which is associated with the increased oxidative capacity in WAT; and (iii) combined intervention using long-chain n-3 polyunsaturated FA (omega 3) and mild calorie restriction exerted synergism in the prevention of obesity in mice fed HF diet (5), which was associated with a strong prevention of low-grade obesity-associated inflammation of WAT, hypolipidemic and insulin-sensitizing effects, and synergistic induction of mitochondrial oxidative phosphorylation (OXPHOS) in epididymal WAT, an effect that could not be detected either in other fat depots including interscapular BAT or in non-adipose tissues. These changes in WAT metabolism occurred in the absence of UCP1 induction and resulted in a significant stimulation of palmitate oxidation measured ex vivo in both tissue fragments and adipocytes liberated from epididymal WAT of mice subjected to the combined intervention. Whole-body effects could not be explained by changes either in food intake or physical activity (5). Results document the involvement of a futile substrate cycle (6) in white adipocytes, which is based on lipolysis and re-esterification of free fatty acids and it is associated with the induction of mitochondrial OXPHOS capacity, β-oxidation, and energy expenditure in WAT. Quantitatively, the degree of induction of lipid catabolism in WAT in response to the combined intervention is similar to that observed in the transgenic mice rendered resistant to obesity by ectopic expression of UCP1 in WAT (7). Thus, the induction of UCP1-independent energy expenditure in WAT in response to a combination of the two physiological stimuli could be involved in the

induction of obesity resistant phenotype. New combination treatments for obesity may be designed using naturally occurring micronutrients like omega 3 or plant polyphenols, reduced calorie intake, and pharmacological compounds, which are based on the induction of UCP1-independent energy expenditure in adipocytes.

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SA12

Systemic metabolic effects of brown adipose tissue

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Brown adipose tissue (BAT) has recently been rediscovered in humans and burns fatty acids for the production of heat in order to defend the body against cold. We revealed an exciting new aspect of brown adipose tissue biology, deeply impacting the general concepts of lipid and lipoprotein metabolism. Using state-of-the-art nanotechnology we decipher the molecular mechanism how nutrients are delivered to BAT and how activation of BAT can be used to correct elevated blood lipids and how to combat obesity. Recently, by comparing gene expression signatures from human biopsies it was demonstrated that UCP1-positive “BAT” cells resembled those of murine “brite” adipocytes more closely than classical brown adipocytes. However, it remains unclear whether so called “brite” adipocytes arising also in white adipose tissue (WAT) of mice are as powerful as their brown adipocyte relatives. We were able to show that the conversion of white to brite adipocytes creates a cell type metabolically as powerful as classical brown adipocytes. This process is associated with a shift in the lipidomic landscape from dietary to endogenously produced fatty acids in WAT and also in plasma. Thus, the formation and activation of brite adipocytes in WAT initiates a metabolic reprogramming of glucose and lipid metabolism in WAT within days, ameliorating whole body metabolic health.

*All experiments with mice as well as their sacrifice afterwards were conducted under anaesthesia (intraperitoneal injection of 90mg/kg xylazine and 1.28 mg/kg ketamine). All experiments All experiments were performed with approval from

Animal Welfare Officers of the University Medical Center Hamburg-Eppendorf and Behörde für Soziales, Familie, Gesundheit und Verbraucherschutz Hamburg, Germany.

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