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Methionine restriction improves renal insulin signalling in aged kidneys

Louise Grant[a], Emma K. Lees[a,d], Laura A. Forney[b], Nimesh Mody[a], Thomas Gettys[b], Paul A.J. Brown[c], Heather M. Wilson[a,*], Mirela Delibegovic[a,*]

[a] Institute of Medical Sciences, University of Aberdeen, College of Life Sciences and Medicine, Foresterhill Health Campus, Aberdeen, UK
[b] Nutrient Sensing and Adipocyte Signaling Department, Pennington Biomedical Research Center, Baton Rouge, LA 70808, USA
[c] Pathology Department, Aberdeen Royal Infirmary, Aberdeen, UK
[d] Liverpool Hope University, Liverpool, UK

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ABSTRACT

Dietary methionine restriction (MR) leads to loss of adiposity, improved insulin sensitivity and lifespan extension. The possibility that dietary MR can protect the kidney from age-associated deterioration has not been addressed. Aged (10-month old) male and female mice were placed on a MR (0.172% methionine) or control diet (0.86% methionine) for 8-weeks and blood glucose, renal insulin signalling, and gene expression were assessed. Methionine restriction lead to decreased blood glucose levels compared to control-fed mice, and enhanced insulin-stimulated phosphorylation of PKB/Akt and S6 in kidneys, indicative of improved glucose homeostasis. Increased expression of lipogenic genes and downregulation of *PEPCK* were observed, suggesting that kidneys from MR-fed animals are more insulin sensitive. Interestingly, renal gene expression of the mitochondrial uncoupling protein *UCP1* was upregulated in MR-fed animals, as were the anti-ageing and renoprotective genes *Sirt1*, *FGF21*, *klotho*, and *β-klotho*. This was associated with alterations in renal histology trending towards reduced frequency of proximal tubule intersections containing vacuoles in mice that had been on dietary MR for 190 days compared to control-fed mice, which exhibited a pre-diabetic status. Our results indicate that dietary MR may offer therapeutic potential in ameliorating the renal functional decline related to ageing and other disorders associated with metabolic dysfunction by enhancing renal insulin sensitivity and renoprotective gene expression.

1. Introduction

The accumulation of visceral fat is associated with ageing and is strongly linked to the development of metabolic disorders including insulin resistance (Huffman and Barzilai, 2009; Selman and Withers, 2011). Surgical removal of visceral fat or reduction in adiposity by caloric restriction (CR), are effective strategies in rodents for increasing lifespan and enhancing insulin sensitivity (Selman and Withers, 2011; Muzumdar et al., 2008; Barzilai et al., 1999). Reducing dietary levels of the essential amino acid methionine also extends lifespan (Orentreich et al., 1993; Richie et al., 1994). The advantage of dietary MR is that its benefits do not require restriction of food intake.

Our group has recently demonstrated that a MR diet can reverse age-induced metabolic dysfunction in adult mice by lipid homeostasis remodelling in the liver and white adipose tissue (WAT), and enhance insulin sensitivity in peripheral tissues (Lees et al., 2014). The occurrence of age-related glucose intolerance due to defective insulin secretion, clearance and peripheral tissue responses to insulin, is also well documented (Zhou et al., 2008). Kidney function is also known to significantly decline with age with an estimated average loss of glomerular filtration rate (GFR) of approximately 1 millilitre per year and an increased predisposition to glomerulosclerosis and interstitial fibrosis (Bitzer and Wiggins, 2016; Weinstein and Anderson, 2010).

To our knowledge, no studies have investigated the effects of MR on kidney insulin sensitivity and we hypothesised that MR dietary intervention may be a viable measure for protecting against renal dysfunction as a consequence of age-related glucose intolerance by improving renal insulin signalling. To provide proof-of-principle

* Corresponding authors.
E-mail addresses: h.m.wilson@abdn.ac.uk (H.M. Wilson), m.delibegovic@abdn.ac.uk (M. Delibegovic).

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data for further human intervention studies, we used aged mice to test this hypothesis.

2. Materials and methods

2.1. Animal studies

All animal procedures were approved by the University of Aberdeen Ethics Review Board and performed under UK Home Office project license PPL60/3951 and according to the ARRIVE guidelines. Ten-month old male and female C57BL/6J wild-type mice (Charles River, Edinburgh, UK) were singly housed and maintained at 22–24 °C on 12-h light/dark cycle with free access to food/water. Mice were maintained on a control diet (0.86% methionine) (Dyets, Bethlehem, PA, USA) for two weeks. They were then randomised into two groups based on body weight with one group given control diet and the other given MR diet containing 0.172% methionine (Dyets, Bethlehem, PA, USA), as described previously (Lees et al., 2014). The mice were maintained on each diet for 8 weeks and terminal tissues harvested after 5 h fasting. For insulin signalling experiments, male mice only were investigated. Intraperitoneal *(i.p.)* injection with saline (154 mM NaCl) or insulin (10 mU/g body weight) was administered after 5 h fast, and mice sacrificed by cervical dislocation after 10 min. Kidneys were harvested and frozen immediately in liquid nitrogen. For histological analysis, 8-week old male C57BL/6J wild-type mice were randomised to MR or control dietbased on body weight, for 190 days before kidneys were harvested and fixed in ethanol and stored at 4 °C before wax embedding and sectioning. Body composition for long-term experiment was measured by NMR using a Bruker Minispec (Billerica, MA).

2.2. Blood metabolites and whole body measurements

Tail vein blood samples were obtained after a 5 h fast and blood glucose measurements taken using glucometers (AlphaTRAK, Berkshire, UK), 6 weeks after the initiation of the diets. For the long-term MR diet study, tail vein blood samples were obtained after a 5 h fast and serum insulin levels were measured by ELISA as per the manufacturer's instructions (EMD Millipore,Temecula, CA).

2.3. Immunoblotting

Frozen kidney lysates were prepared in radioimmunoprecipitation-assay (RIPA) buffer containing fresh sodium-orthovanadate and protease-inhibitors (Agouni et al., 2010). Proteins were separated by 4–12% SDS-PAGE and transferred to nitrocellulose membranes. Immunoblots were performed using antibodies from Cell Signaling (NEB, Hitchin, UK) (unless stated otherwise) against phospho-Akt/PKB (s473), total Akt/PKB A2210 (Santa Cruz, Dallas, TX, USA), phospho-S6 ribosomal protein (s235/236), total S6 2217S, phospho-IR (tyr1162/1163) 44804G (Invitrogen, Paisley, UK), phospho-IR (tyr1158), IR-βsc-711 (Santa-Cruz, Dallas, TX, USA), β-actin (Thermo Scientific, Waltham, USA), cytochrome C, mitochondrial transcription factor (TFAM), PGC1α (Abcam, Cambridge, UK), UCP1 (Abcam, Cambridge, UK). Immunoblots were visualized using enhanced-chemiluminescence, and quantified by densitometry scanning using Bio1D-software (PeqLab, Fareham, UK).

2.4. Gene-expression analysis

Frozen kidney sections were homogenized in TriFast reagent (Peqlab, UK) (Martin and Sheaff, 2007). cDNA synthesis was carried out from 1 μg of RNA using Tetro cDNA-synthesis kit (Bioline, London, UK). Target genes were amplified by quantitative real-time PCR using gene-specific primers (Sigma, UK) and GoTaq master mix (Promega, Southampton, UK) using the Light-Cycler 480 (Roche). Relative mRNA levels were calculated using the Pfaffl method (Pfaffl, 2001) and normalised to the most stable reference gene (YWhaz, NoNo, GAPDH, HPRT or β-actin) which was identified using a web-based reference gene assessment tool (http://www.leonxie.com/referencegene.php?type=reference). Primer sequences are provided in Supplementary material.

2.5. Histological analysis

Kidney tissue was fixed in formaldehyde, embedded in paraffin, sectioned at 5 μm, and stained with haematoxylin and eosin. Slides were scored using a graticule at ×20 magnification by Dr Paul AJ Brown, consultant pathologist, Aberdeen Royal Infirmary, who was blinded to the study. 100 random intersections were examined for each kidney and a score of 0 or 1 was given for each tubular profile involving an intersection: 0 = normal histology; 1 = vacuole(s) involving that tubular profile. The total score for each kidney was calculated by addition of all 100 scores with a maximum score of 100.

2.6. Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were performed using one-way ANOVA with Tukey's multiple comparison post-tests, two-way ANOVA with Bonferroni-multiple comparisons post-tests, and two-tailed Student's *t*-tests, as appropriate, using GraphPad Prism 5 statistical-software (GraphPad Sortware Inc., San Diego, CA, USA). *P*-values <0.05 were considered significant.

3. Results

3.1. MR diet decreases fasting glucose levels, improves glucose tolerance and enhances renal insulin signalling

Eight weeks of dietary MR produced significant decreases in fasting blood glucose levels in both male and female mice when compared to control littermates (Fig. 1A). Glucose tolerance testing (GTT) revealed an improvement in glucose tolerance in MR-diet fed male mice compared to controls (Fig. 1B), in both males and females, as represented by Area Under the Curve (AUC). To test the impact of dietary MR on insulin signalling in the kidney, renal tissue (medulla and cortex) was harvested from male mice after a 5 h fast and acute injection with insulin (10 mU/g body weight) (Fig. 1C). Following insulin binding, a number of tyrosine residues including tyrosines 1158, 1162, and 1163 on the insulin receptor (IR) become autophosphorylated, leading to the phosphorylation of target substrates including IRS proteins (Saltiel and Kahn, 2001). The phosphorylation of PKB/Akt also activates mTORC1 leading to increased protein synthesis, via activation of S6K1 and its target S6 (von Manteuffel et al., 1997) Diet-induced increases in insulin-dependent PKB/Akt and S6 phosphorylation were observed with no effect on insulin activation of its receptor, suggesting that MR amplifies insulin signal downstream of IR, at the level of PKB/Akt (Fig. 1C and D). The enhancement of insulin-induced phosphorylation of PKB/Akt and S6 by dietary MR, in conjunction with diet-induced reductions in fasting glucose, are consistent with both global and tissue-specific effects of dietary MR on insulin sensitivity.

3.2. MR diet increases lipogenic gene expression in the kidney

In view of the improved glucose homeostasis and enhanced diet-dependent insulin signalling observed in the MR-diet mice,