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ORIGINAL ARTICLE

The impact of protein quantity during energy restriction on genome-wide gene expression in adipose tissue of obese humans

IPG Van Bussel [1], EMP Backx[1,2], CPGM De Groot[1,2], M Tieland[1,2], M Müller[1,3] and LA Afman[1]

BACKGROUND: Overweight and obesity is a growing health problem worldwide. The most effective strategy to reduce weight is energy restriction (ER). ER has been shown to be beneficial in disease prevention and it reduces chronic inflammation. Recent studies suggest that reducing the protein quantity of a diet contributes to the beneficial effects by ER. The organ most extensively affected during ER is white adipose tissue (WAT).
OBJECTIVE: The first objective was to assess changes in gene expression between a high-protein diet and a normal protein diet during ER. Second, the total effect of ER on changes in gene expression in WAT was assessed.
METHODS: In a parallel double-blinded controlled study, overweight older participants adhered to a 25% ER diet, either combined with high-protein intake (HP-ER, 1.7 g kg^{-1} per day), or with normal protein intake (NP-ER, 0.9 g kg^{-1} per day) for 12 weeks. From 10 HP-ER participants and 12 NP-ER participants subcutaneous WAT biopsies were collected before and after the diet intervention. Adipose tissue was used to isolate total RNA and to evaluate whole-genome gene expression changes upon a HP-ER and NP-ER diet.
RESULTS: A different gene expression response between HP-ER and NP-ER was observed for 530 genes. After NP-ER, a downregulation in expression of genes linked to immune cell infiltration, adaptive immune response and inflammasome was found, whereas no such effect was found after HP-ER. HP-ER resulted in upregulation in expression of genes linked to cell cycle, GPCR signalling, olfactory signalling and nitrogen metabolism. Upon 25% ER, gene sets related to energy metabolism and immune response were decreased.
CONCLUSIONS: Based on gene expression changes, we concluded that consumption of normal protein quantity compared with high-protein quantity during ER has a more beneficial effect on inflammation-related gene expression in WAT.

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INTRODUCTION

Overweight and obesity is a growing health problem worldwide.[1–3] One of the most effective strategies to lose weight is energy restriction (ER): restriction of food intake without malnutrition.[4] ER is also an effective strategy to diminish age-related diseases in rodents[5] and non-human primates.[6] Recent studies in rodents suggest that not the reduction of calories itself, but the reduction of dietary protein quantity contributes to the health benefits of ER.[7] In mice for example, an *ad libitum* low-protein diet seemed to be equally beneficial for health as an energy-restricted diet. Low-protein, high-carbohydrate, fed mice showed improved insulin, triglyceride and high-density lipoprotein cholesterol levels and improved Homeostasis Model Assessment, similar to ER fed mice, whereas *ad libitum* fed mice did not show this improvement.[8] Contrary to animal studies, human studies showed less-consistent findings. A meta-analysis on protein diets of periods longer than 12 weeks on health outcomes such as blood pressure, LDL, HDL and total cholesterol, triglycerides, and fasting blood glucose showed inconsistent results.[9] Other shorter, but also newer long-term intervention studies mainly focussed on insulin sensitivity and observed increased insulin sensitivity upon high-protein ER diets.[10–12] Based on these studies and this meta-analysis, no definitive conclusion can be drawn on the effect of protein versus other macronutrient ratios in an ER diet on markers of metabolic health. Markers of metabolic health are systemic markers, reflecting the total response in the body. One of the organs largely affected by ER is the white adipose tissue (WAT). Despite the important role of visceral WAT in the pathology of obesity, the role of subcutaneous WAT is becoming more clear, especially owing to the use of omics tools such as transcriptomics. It has for example been shown that subcutaneous WAT of obese individuals is characterised by hyperplasia and hypertrophy and that expression of genes involved in fat uptake and cellular differentiation are decreased in obese individuals.[13] This likely limited ability of subcutaneous WAT to store excess energy[14] leads to the compensatory ectopically storage in organs such as the liver. Nutrition may have a role in the preservation and improvement of the adequate functioning of subcutaneous WAT. Therefore, subcutaneous WAT is an interesting target to study. Transcriptomics has also been used to identify differences and overlap between the different fat depots. It has been shown that deep and superficial subcutaneous WAT depots have overlapping but also site-specific gene expression profiles.[15] Also, between epigastric and subcutaneous

[1]Division of Human Nutrition, Wageningen University & Research Centre, Wageningen, The Netherlands and [2]Top Institute Food and Nutrition, Wageningen, The Netherlands. Correspondence: Dr LA Afman, Division of Human Nutrition, Wageningen University & Research centre, PO BOX 17, NL-6700 AA, Wageningen, The Netherlands.
E-mail: lydia.afman@wur.nl
[3]Current: Norwich Medical School, University of East Anglia, NR4 7TJ, Norwich, UK.
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WAT and VAT overlap is found in expression of genes involved in inflammation, cell cycle and growth, cancer and development.[16] In addition, comparison between subcutaneous WAT and VAT gene expression revealed that macrophage-specific markers were visible in both.[17] During ER, not only the size of the adipocytes is reduced but also expression of genes involved in inflammation is decreased,[18–20] which likely also affects other organs and the whole body metabolic health status.

In this manuscript we aimed to elucidate the effects of an exchange of carbohydrates for protein during an ER diet on pathways and signalling routes in human adipose tissue by examining changes in whole-genome gene expression on subcutaneous WAT. Participants of this study were older overweight healthy men and women, following either a 12-week completely controlled normal protein ER diet (NP-ER), or a high-protein ER diet (HP-ER), in which carbohydrates of the NP-ER diet were partly replaced by protein. The diets were similar in ER, which allowed us to study potential additional effects of protein quantity over ER on gene expression changes in subcutaneous WAT.

MATERIALS AND METHODS

Study design

The current study was part of a previously published double-blind randomized study.[21] Power calculation has been described in the original study[21] and was based on the primary outcome lean body mass. Subjects were excluded if they suffered from renal insufficiency (modification of diet in renal disease estimated glomerular filtration rate >60 ml min^{-1} per 1.73 m^2), type 1 or type 2 diabetes (fasting glucose levels $\geqslant 7$ mmol l^{-1}), cancer, chronic obstructive pulmonary disease, allergy to milk products or underwent a gastric bypass. Subjects were also excluded if they had severe loss of appetite, participated in a weight loss or heavy resistance-type exercise program 3 months before the intervention or if they used supplements or drugs known to interfere with energy balance. Women could only participate if they were postmenopausal (last period 1 year previous to study start). Randomization was carried out with permuted blocks, stratified by gender and body mass index (BMI). This intervention study was highly controlled, as 90% of the daily energy intake was provided by the University. Sixty-one overweight and obese healthy women ($n=25$) and men ($n=36$), aged 55–70 years, were randomly assigned to either a high-protein diet (HP-ER; 1.7 g protein per kg per day) or normal protein diet (NP-ER; 0.9 g protein per kg per day), during a 12-week 25% energy intake restriction. A subcutaneous adipose tissue biopsy was taken before and after the intervention from 22 participants. The study protocol was approved by the Medical Ethical Committee of Wageningen University and written informed consent was obtained before study participation. The study was registered at clinicaltrials.gov as NCT01915030.

Adipose tissue biopsy

Abdominal subcutaneous WAT biopsies (~1 g) were collected by needle biopsy, 6–8 cm lateral from the umbilicus under local anaesthesia (2% lidocaine) in 22 fasted participants. For each person, the second biopsy upon the intervention was taken on the contralateral side opposite to the first biopsy taken before the intervention. After immediate washing with PBS, the tissue was snap-frozen in liquid nitrogen and stored at − 80 °C until analysis.

RNA isolation and microarray processing

Total RNA was extracted from frozen adipose tissue specimens using TRIzol reagent (Invitrogen, Breda, The Netherlands) and purified on columns using the Qiagen RNeasy Micro Kit (Qiagen, Venlo, The Netherlands). RNA integrity was checked with Agilent 2100 bioanalyzer (Agilent Technologies, Amstelveen, The Netherlands). Total RNA (500 ng per sample) was labelled using a one-cycle cDNA labelling kit (MessageAmpTM II-Biotin Enhanced Kit; Ambion Inc, Nieuwekerk aan de IJssel, The Netherlands). Sample labelling, hybridization to chips, and image scanning were performed according to the manufacturer's instructions. Total RNA (100 ng per sample) was labelled by Whole-Transcript Sense Target Assay and hybridized to human whole-genome Affymetrix Human Gene 1.1 ST arrays targeting 19715 unique genes (Affymetrix, Santa Clara, CA, USA).

Microarray data analysis

Microarray quality control and normalization were performed using Bioconductor software packages integrated in an on-line pipeline called MADMAX.[22] Microarray signals were normalized using robust multichip average (RMA).[23] Genes with normalized signals >20 on at least six arrays were defined as expressed and selected for further analysis. Significant different expression of individual genes were tested using the LIMMA R library.[24] Changes were considered significant when *P*-value was <0.05 in a paired *t*-test with Bayesian correction. Data were further analysed with gene set enrichment analysis using pre-ranked lists based on the *t*-statistic.[25] Gene sets with a false discovery rate (FDR *q*-value) <0.25 were defined as significantly regulated. A transcription factor analysis was performed on the differentially expressed genes (*P*-value <0.05) with Ingenuity Pathway Analysis (June 2012, Ingenuity Systems, Redwood City, CA, USA). Array data have been submitted to the Gene Expression Omnibus under accession number GSE84046.

Statistical analysis of clinical measurements

Data are presented as mean ± s.d. To check if there were baseline differences between the groups, an independent sample *t*-test was used. A paired *t*-test was used to check if parameters changed within groups over time. An unpaired *t*-test was used to check if changes in parameters were significant different between groups. Data were analyzed using SPSS version 22 (Released 2013, IBM SPSS Statistics for Windows, IBM Corp, Armonk, NY, USA). Results were considered statistically significant below the 0.05 level.

RESULTS

Baseline characteristics of the 22 participants volunteering a WAT biopsy are summarised in Table 1. None of the participant characteristics differed between the two intervention groups ($P>0.05$). The effect of 12 weeks of 25% ER is seen in the decrease of 9.4 kg (±3.2) body weight on average in all participants. HP-ER and NP-ER both resulted in a decrease in body weight and BMI in both groups (Supplementary Table 1). Protein quantity of the diets had no effect on weight ($P=0.67$) or BMI change ($P=0.62$).

Effect of ER on gene expression: up- and downregulation

To identify the effect of 25% ER on whole-genome gene expression in adipose tissue, we first analysed the two ER intervention groups as one group. A total of 1858 genes showed a significant change in expression upon 12 weeks 25% ER (Supplementary Figure 1). To identify potential pathways and signalling routes, Gene Set Enrichment Analysis was performed. A total of 353 gene sets was enriched upon ER (Supplementary Table 2) of which 72 up- and 281 downregulated. To merge overlapping and similar pathways, gene sets were clustered using Cytoscape. Clusters of gene sets are summarized in Table 2. Clusters of gene sets involved in energy metabolism, such as lipid

Table 1. Baseline characteristics of participants included in the microarray analysis of adipose tissue biopsies

	HP-ER n = *12*	*NP-ER* n = *10*
Age (year)	62.3 (56, 69)	61.6 (57, 68)
Gender	8♂/4♀	7♂/3♀
Height (m)	1.72 (0.11)	1.74 (0.068)
Weight (kg)	93.2 (10.2)	91.9 (6.1)
Body mass index (kg m^{-2})	31 (3)	30 (2)
Glucose (mmol l^{-1})	6.0 (0.59)	5.9 (0.54)
Waist circumference (cm)	110 (9.30)	110 (6.41)

Abbreviations: HP-ER,: high-protein energy restriction; NP-ER, normal protein energy restriction; ♂: men; ♀: women. Data represent mean and (s.d.), or median and (range).

Table 2. Summary of changes in main clusters of pathways in white adipose tissue of the total study population upon 12 weeks of 25% energy restriction (Supplementary Table 2)

Pathway cluster[a]	*25% ER (n = 22)*
Lipid metabolism and PPARα targets	↓
NRF2 targets	↓
Glucose metabolism	↓
TCA cycle	↓
Oxidative phosphorylation	↓
Adaptive immune response	↓
Immune cell infiltration	↓
Cell cycle	↓
RNA translation and processing	↑

Significantly changed pathways are determined with GSEA and clusters are based upon Cytoscape ↑: gene sets in this pathway cluster were upregulated; ↓: gene sets in this pathway cluster were downregulated. [a]Selection of these clusters based on: gene sets with a significantly different response between HP-ER and NP-ER, and significantly changed upon HP-ER (left) or NP-ER (right).

metabolism and PPARα targets, NRF2 targets, glucose metabolism and TCA cycle, as well as gene sets in oxidative phosphorylation, adaptive immune response, immune cell infiltration and cell cycle were decreased. RNA translation and processing-related gene sets were increased.

As energy metabolism-related pathways turned out to be quite prominently regulated, we visualized the robustness of the ER-induced individual changes in expression of genes related to energy metabolism by creating a heatmap, showing gene expression changes per gene per individual (Figure 1). Three out of 22 participants had a different pattern in their gene expression profiles. To evaluate whether these differences in response were owing to weight loss differences, BMI/weight loss change was also plotted, below this heatmap. Weight loss or BMI change did no show consistent change with responders or non-responder profile changes. To further analyse whether correlations were present between the gene expression changes and between weight and BMI change, we created a correlation heatmap (Supplementary Figure 2). Correlations were observed between most genes related to energy metabolism, with the strongest correlation between expression changes of genes involved in lipid metabolism. Not many correlations were observed between gene expression changes and weight or BMI change. To visualize the genes involved in energy metabolism of which the expression was changed upon ER a schematic adipocyte was created (Figure 2).

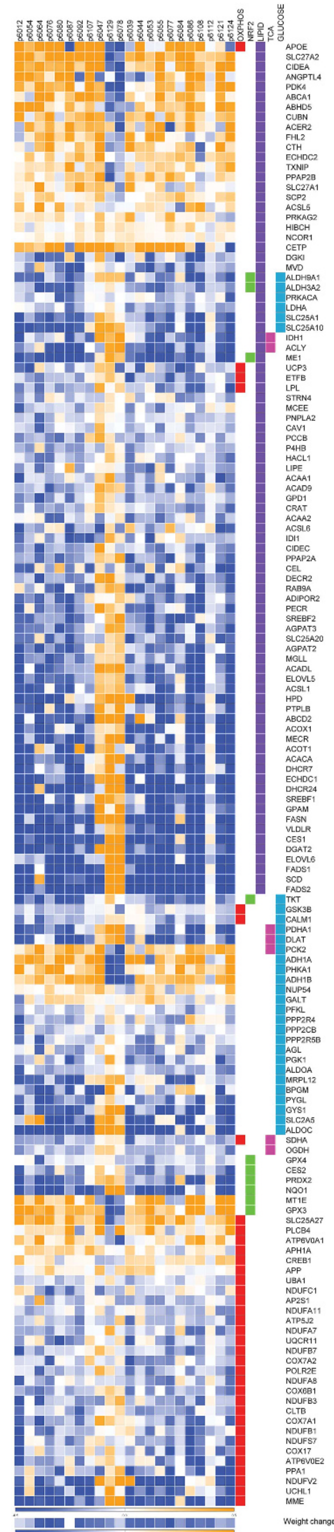

Figure 1. Heatmap of individual gene expression changes upon 25% ER of genes involved in energy metabolism. Each column represents the signal log ratio of one person; each row represents one gene. Legend: Colours in heatmap: Blue = downregulated, Orange = upregulated; Red: genes in oxidative phosphorylation; Green: genes in NRF2 targets; Purple: genes in Lipid metabolism; Pink: genes in TCA cycle; Light blue: genes in glucose metabolism.

Effect of ER on gene expression: IPA upstream regulator analysis

To identify potential upstream transcriptional regulators of genes of which expression changed upon 25% ER, IPA Upstream Regulator Analysis was used (Supplementary Table 3ab). Many upstream regulators known to control lipid metabolism were significantly predicted to be inhibited and included peroxisome proliferator-activated receptor gamma (PPARG: $z = -3.491$, $P = 8.14E-12$) and sterol regulatory element-binding proteins 1 and 2 (SREBF1: $z = -3.683$, $P = 3.33E-06$; SREBF2: $z = -3.478$, $P = 7.41E-07$). These findings fit with the strong correlation between changes in expression of their lipid-related target genes upon 25% ER (Supplementary Figure 2). Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), known to regulate oxidative phosphorylation, was predicted to be inhibited as well.

Protein quantity

Effect of protein quantity on gene expression during ER. To identify the effect of a carbohydrate-for-protein exchange in addition to ER on molecular level, we compared gene expression changes

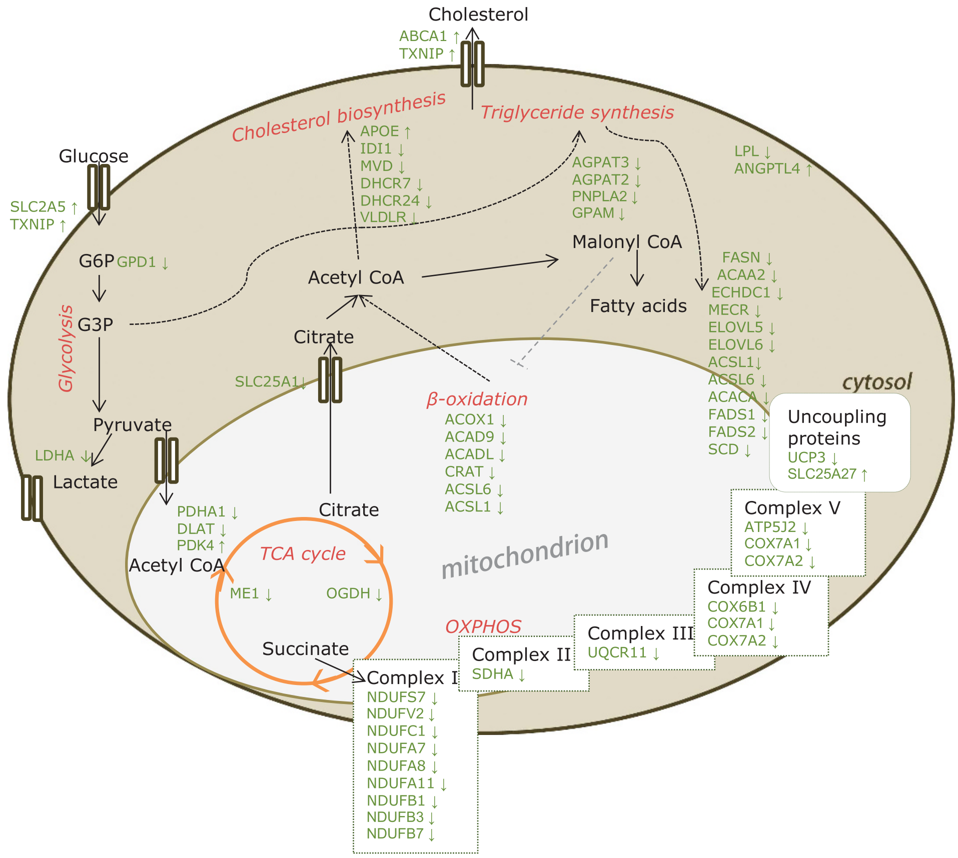

Figure 2. Schematic visualisation of adipocyte with up- or downregulated expression of genes involved in energy metabolism that changed upon 25% ER.

upon HP-ER with gene expression changes upon NP-ER. Flowchart of selection of genes is shown in Figure 3. HP-ER resulted in a significant different expression of 1869 genes and NP-ER resulted in a significant different expression of 1690 genes. A number 530 genes showed a significantly different expression change between the HP-ER and NP-ER and 500 genes showed an overlap between both diets.

Effect of ER and protein quantity on gene expression: pathway analysis

To identify in what pathways and signalling routes these genes were involved, gene set enrichment analysis was performed. A total of 371 gene sets were enriched in the NP-ER group, of which 46 up- and 325 downregulated. A total of 241 gene sets were enriched in the HP-ER group, of which 69 up- and 172 downregulated (Supplementary Table 4a–d). Comparing the response of the two diets, a number of 123 gene sets showed a significant difference between the two groups (Supplementary Table 5). Gene sets were clustered using Cytoscape as described above. A summary of the identified clusters with a differential change between the diets and a significant change upon at least one of the diets, is provided in Table 3. NP-ER diet showed a downregulation of pathways involved in inflammasome, adaptive immune response, immune cell infiltration and cell cycle, whereas HP-ER diet did not result in a downregulation of inflammatory pathways and resulted in an upregulation of cell cycle and GPCR signalling, olfactory and nitrogen metabolism-related pathways.

To visualize the individual changes in expression of genes that belonged to the downregulated clusters of pathways, we selected the genes from these clusters: immune cell infiltration, inflammasome, adaptive immune response and cell cycle. Genes with a

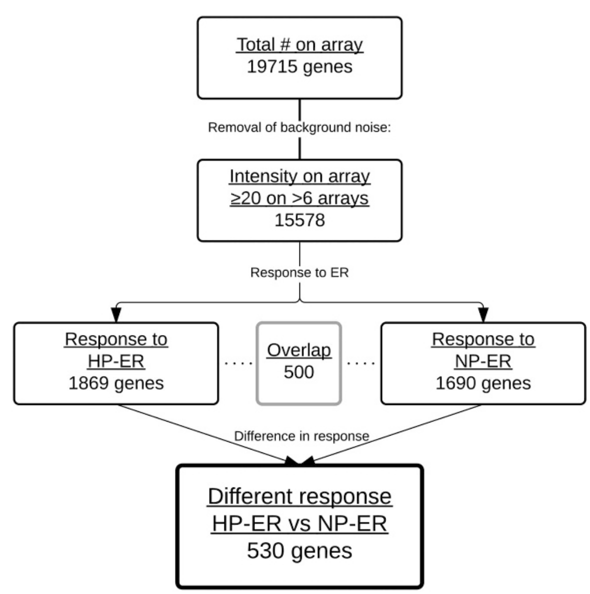

Figure 3. Stepwise selection of genes for microarray analysis: first, genes were selected for their signal intensity (≥20 in >6 arrays), and second, for a change in expression upon either normal protein energy restriction (NP-ER) diet, or upon high-protein energy restriction (HP-ER) diet ($P < 0.05$). The last block shows the number of genes that have a significantly different change in expression between HP-ER and NP-ER.

Table 3. Summary of changes in main clusters of pathways in white adipose tissue upon 12 weeks of 25% energy restriction with either high-protein (HP-ER) or normal protein (NP-ER)

Pathway cluster[a]	*HP-ER* (n = *12*)	*NP-ER* (n = *10*)
Immune cell infiltration	-	↓
Inflammasome	-	↓
Adaptive immune response	-	↓
Cell cycle	-/↑	↓
GPCR Signalling	-/↑	↓
Including olfactory signalling	↑	-
Nitrogen metabolism	↑	-

Significantly changed pathways are determined with GSEA and clusters are based upon Cytoscape ↑: gene sets in this pathway cluster were upregulated; ↓: gene sets in this pathway cluster were downregulated;— pathway cluster was not changed. [a]Selection of these clusters based on: gene sets with a significantly different response between HP-ER and NP-ER, and significantly changed upon HP-ER (left) or NP-ER (right).

significant different expression (P-value < 0.05) between the HP-ER and NP-ER diet and a significant change in expression in either the HP-ER or the NP-ER diet group, were incorporated in a heatmap (Supplementary Figure 3a). To identify potential correlations between those genes, a correlation heatmap was made (Supplementary Figure 3b). Positive correlations were observed for genes involved in immune cell infiltration and cell cycle, as is seen by the pink triangles.

Effect of protein quantity on gene expression: IPA upstream regulator analysis

IPA upstream regulator analysis was used to identify potential upstream transcriptional regulators of genes significantly different expressed between HP-ER and NP-ER diet. Only 26S proteasome was predicted to be upregulated comparing the two groups, but was not predicted to be significantly upregulated or inhibited within the HP-ER group or in the NP-ER group.

DISCUSSION

Within this study we aimed to investigate the effect of a change in protein quantity in an ER diet on the regulation of pathways and signalling routes in human WAT. Although parameters such as weight loss, glucose and waist circumference did not change owing to altered protein quantity in the 25% ER diet, whole-genome adipose tissue gene expression did change owing to the difference in protein quantity. Only the normal protein ER diet (NP-ER), and not the high-protein ER diet (HP-ER), resulted in a downregulation of expression of genes involved in inflammasome, immune cell infiltration, adaptive immune response and cell cycle-related pathways in human adipose tissue. To the best of our knowledge, no studies are known that explored the effect of an exchange of protein for carbohydrates in ER diets on whole-genome gene expression in human adipose tissue. Only one study could be identified that compared the effect of a high-protein, low glycaemic index and soluble fibre ER diet with a standard ER diet on WAT whole-genome gene expression, but this study showed no significant differences between the two diets. Changes in gene expression were only observed if both ER groups were combined.[26] This is partly in line with findings in our study, in which more changes in gene expression owing to ER than owing to protein quantity were observed. However, in contrast to the above-mentioned study, we could define a clear nutrient-specific set of genes that were either more affected upon HP-ER or more affected in the NP-ER. This deviation in outcomes can be due to differences in study design. In the before mentioned study 13 persons were included, whereas we had a larger number of 22 participants. In our study, diets were followed for a period of 12 weeks, in contrast to only 4 weeks, which could account for a more persisting effect of the diets on gene expression. Furthermore, the cross-over design had a washout period of 8 weeks between both ER diets. This period might have been too short for adipocytes to recover from ER and may have caused a carryover-effect on gene expression.

The observed reduction in expression of genes involved in immune response pathways and inflammasome-related pathways upon a normal protein diet, which was not observed on a high-protein diet, is interesting with respect to their role in inflammation in adipose tissue. Adipose tissue in obese individuals is characterized by increased expression of genes involved in the inflammasome and immune response.[27,28] Several studies have observed that caloric restriction or exercise-mediated weight loss resulted in a reduced expression of these genes.[29] Interestingly, we only observed those effects for the normal protein diet. When the protein quantity of the ER diet increased, the beneficial effects on inflammation-related gene expression were not observed. This observation points toward the potential importance of dietary macronutrient composition during ER on adipose tissue health. Despite the findings of macronutrient-specific effects, the impact of ER on gene expression was much greater. Moreover, strong correlations between changes in gene expression were observed, suggesting a potential upstream regulator responsible for this accurate regulation in expression. Especially a strong correlation between changes in expression of lipid-related genes upon ER was found. In line with this, upstream regulators PPARG and SREBPs, strong regulators of lipid metabolism, were predicted to be inhibited. Furthermore, oxidative phosphorylation related pathways were downregulated upon ER, which is in line with the finding that PGC1α was predicted to be inhibited. In addition to the pathway analysis, which identified only downregulated pathways related to energy metabolism upon ER, expression of several genes were found to be upregulated upon CR that were related to energy metabolism pathways as well. For example, PCK2 (phosphoenolpyruvate carboxykinase 2) and PDK4 (pyruvate dehydrogenase kinase 4) were upregulated. PCK2 catalyses the rate-limiting step in gluconeogenesis, when glucose is formed from lactate and other precursors derived from the TCA cycle. Upregulation of PCK2 could be due to shortage of glucose intake and the subsequent increased need of glucose formation from different precursors. PDK4 is a key inhibitor in glucose metabolism[30] and its upregulation may be explained by a decreased need for glucose oxidation. PDK4 gene expression is also known to be upregulated in human PBMCs upon fasting.[31] Genes involved in lipid metabolism were also upregulated. For example, ANGPTL4 (angiopoietin-like 4), an inhibitor of lipoprotein lipase, was upregulated and in line with this lipoprotein lipase was downregulated. This inhibition of lipoprotein lipase during ER can be explained by the assumption that fat storage is not of primary importance during ER. Also CIDEA (cell death activator), important for lipolysis, was upregulated upon ER, which can be explained by a higher demand for stored lipids as energy source. PCK2, PDK4, ANGPTL4 and CIDEA are all well-known PPAR targets pointing to two kind of effects on PPAR-target-genes, either up- or down-regulation, depending on their functional role in adipose tissue during ER. One problem in changing one macronutrient in a diet is the consequential change of another macronutrient. In our study, protein was exchanged for carbohydrates. The effects could therefore also be due to the higher amount of carbohydrates in the NP-ER diet, or due to the lower amount of carbohydrates in the HP-ER diet. One clear finding that has also been found in other transcriptome studies is the variation in response. In three out of 22 participants we observed a different gene expression response upon ER. We could not explain this variation by the amount of weight loss or weight gain. Genetic background could be important in explaining some of the variation in gene expression, as was shown before,[32] but sample sizes within this study were too small to adequately measure such effects.

A strength of our study is the highly controlled food intake. Participants were provided with all meals and always consumed their hot meal at the University, leading to a high compliance. Furthermore, effects of participants' habitual diets were largely ruled out before the start of the intervention owing to 1 week of standardised meals for the participants. However, the number of adipose biopsies was small: 10 and 12 participants per intervention arm. The aim of this study was explorative and therefore an FDR q-value of <0.25 was selected. However, examining the data with an FDR q-value of <0.1 resulted in the same clusters of gene sets and conclusion. Although findings are quite robust and provide some interesting leads, care should be taken in interpretation and translation of the findings, as results have not yet been replicated independently. Protein quantity has been studied to investigate its effect on health parameters related to muscle mass but findings show discrepancies.[21] As our findings are based on adipose tissue gene expression, caution should be taken when translating this to health advice. Further studies are needed that explore our findings in a larger population.

In conclusion, 25% ER induces a decrease in lipid and energy metabolism-related pathways, likely partly regulated via PPARG and PGC1α, in WAT in humans. The consumption of normal protein quantity compared with a high-protein quantity during ER has a more beneficial effect on inflammation-related gene expression in adipose tissue, as reflected by a decrease in inflammasome and adaptive immunity response-related pathways.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: EB, MT, CG. Performed the experiments: IB. Analyzed the data: IB, LA. Wrote the paper: IB. Critically revised the manuscript for important intellectual content: EB, MT, CG, MM, LA.

REFERENCES

1 Ogden CL, Carroll MD, Flegal KM. Prevalence of obesity in the United States. *JAMA* 2014; **312**: 189–190.

2 Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA* 2014; **311**: 806–814.

3 von Ruesten A, Steffen A, Floegel A, van der AD, Masala G, Tjonneland A *et al.* Trend in obesity prevalence in European adult cohort populations during follow-up since 1996 and their predictions to 2015. *PloS One* 2011; **6**: e27455.

4 Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. *Am J Clin Nutr* 2006; **83**: 260–274.

5 Weindruch R, Kayo T, Lee CK, Prolla TA. Microarray profiling of gene expression in aging and its alteration by caloric restriction in mice. *J Nutr* 2001; **131**: 918S–923S.

6 Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL *et al.* Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* 2012; **489**: 318–321.

7 Pamplona R, Barja G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. *Biochim Biophys Acta* 2006; **1757**: 496–508.

8 Solon-Biet SM, Mitchell SJ, Coogan SC, Cogger VC, Gokarn R, McMahon AC *et al.* Dietary protein to carbohydrate ratio and caloric restriction: comparing metabolic outcomes in mice. *Cell Rep* 2015; **11**: 1529–1534.

9 Naude CE, Schoonees A, Senekal M, Young T, Garner P, Volmink J. Low carbohydrate versus isoenergetic balanced diets for reducing weight and cardiovascular risk: a systematic review and meta-analysis. *PloS One* 2014; **9**: e100652.

10 Piatti PM, Monti F, Fermo I, Baruffaldi L, Nasser R, Santambrogio G *et al.* Hypocaloric high-protein diet improves glucose oxidation and spares lean body mass: comparison to hypocaloric high-carbohydrate diet. *Metabolism* 1994; **43**: 1481–1487.

11 Mehrabani HH, Salehpour S, Amiri Z, Farahani SJ, Meyer BJ, Tahbaz F. Beneficial effects of a high-protein, low-glycemic-load hypocaloric diet in overweight and obese women with polycystic ovary syndrome: a randomized controlled intervention study. *J AM Coll Nutr* 2012; **31**: 117–125.

12 de Luis DA, Izaola O, Aller R, de la Fuente B, Bachiller R, Romero E. Effects of a high-protein/low carbohydrate versus a standard hypocaloric diet on adipocytokine levels and insulin resistance in obese patients along 9 months. *J Diabetes Complications* 2015; **29**: 950–954.

13 Rodriguez-Acebes S, Palacios N, Botella-Carretero JI, Olea N, Crespo L, Peromingo R *et al.* Gene expression profiling of subcutaneous adipose tissue in morbid obesity using a focused microarray: Distinct expression of cell-cycle- and differentiation-related genes. *Bmc Med Genomics* 2010; **3**: 61.

14 Danforth E. Failure of adipocyte differentiation causes type II diabetes mellitus? *Nat Genet* 2000; **26**: 13–13.

15 Cancello R, Zulian A, Gentilini D, Maestrini S, Della Barba A, Invitti C *et al.* Molecular and morphologic characterization of superficial- and deep-subcutaneous adipose tissue subdivisions in human obesity. *Obesity* 2013; **21**: 2562–2570.

16 Gerhard GS, Styer AM, Strodel WE, Roesch SL, Yavorek A, Carey DJ *et al.* Gene expression profiling in subcutaneous, visceral and epigastric adipose tissues of patients with extreme obesity. *Int J Obesity* 2014; **38**: 371–378.

17 Klimcakova E, Roussel B, Kovacova Z, Kovacikova M, Siklova-Vitkova M, Combes M *et al.* Macrophage gene expression is related to obesity and the metabolic syndrome in human subcutaneous fat as well as in visceral fat. *Diabetologia* 2011; **54**: 876–887.

18 Ye J, Keller JN. Regulation of energy metabolism by inflammation: a feedback response in obesity and calorie restriction. *Aging* 2010; **2**: 361–368.

19 Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA *et al.* Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *Faseb J* 2004; **18**: 1657–1669.

20 Johansson LE, Danielsson AP, Parikh H, Klintenberg M, Norstrom F, Groop L *et al.* Differential gene expression in adipose tissue from obese human subjects during weight loss and weight maintenance. *Am J Clin Nutr* 2012; **96**: 196–207.

21 Backx EM, Tieland M, Borgonjen-van den Berg KJ, Claessen PR, van Loon LJ, de Groot LC. Protein intake and lean body mass preservation during energy intake restriction in overweight older adults. *Int J Obes* 2015; **40**: 299–304.

22 Lin K, Kools H, de Groot PJ, Gavai AK, Basnet RK, Cheng F *et al.* MADMAX - Management and analysis database for multiple ~omics experiments. *J Integr Bioinform* 2011; **8**: 160.

23 Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. *Nucleic Acids Res* 2003; **31**: e15.

24 Smyth GK. Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. Springer, pp 397–4202005.

25 Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA* 2005; **102**: 15545–15550.

26 Rizkalla SW, Prifti E, Cotillard A, Pelloux V, Rouault C, Allouche R *et al.* Differential effects of macronutrient content in 2 energy-restricted diets on cardiovascular risk factors and adipose tissue cell size in moderately obese individuals: a randomized controlled trial. *Am J Clin Nutr* 2012; **95**: 49–63.

27 Stienstra R, van Diepen JA, Tack CJ, Zaki MH, van de Veerdonk FL, Perera D *et al.* Inflammasome is a central player in the induction of obesity and insulin resistance. *Proc Natl Acad Sci USA* 2011; **108**: 15324–15329.
28 Rainone V, Schneider L, Saulle I, Ricci C, Biasin M, Al-Daghri NM *et al.* Upregulation of inflammasome activity and increased gut permeability are associated with obesity in children and adolescents. *Inte J Obes* 2016; **40**: 1026–1033.
29 Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL *et al.* The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 2011; **17**: 179–188.
30 Palomer X, Alvarez-Guardia D, Rodriguez-Calvo R, Coll T, Laguna JC, Davidson MM *et al.* TNF-alpha reduces PGC-1alpha expression through NF-kappaB and p38 MAPK leading to increased glucose oxidation in a human cardiac cell model. *Cardiovasc Res* 2009; **81**: 703–712.
31 Bouwens M, Afman LA, Muller M. Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells. *Am J Clin Nutr* 2007; **86**: 1515–1523.
32 Matualatupauw JC, Radonjic M, van de Rest O, de Groot LC, Geleijnse JM, Muller M *et al.* Apolipoprotein E genotype status affects habitual human blood mononuclear cell gene expression and its response to fish oil intervention. *Mol Nutr Food Res* 2016; **60**: 1649–1660.

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