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RESEARCH PAPER

Transcriptome analysis of peripheral blood mononuclear cells in human subjects following a 36 h fast provides evidence of effects on genes regulating inflammation, apoptosis and energy metabolism

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Abstract There is growing interest in the potential health benefits of diets that involve regular periods of fasting. While animal studies have provided compelling evidence that feeding patterns such as alternate-day fasting can increase longevity and reduce incidence of many chronic diseases, the evidence from human studies is much more limited and equivocal. Additionally, although several candidate processes have been proposed to contribute to the health benefits observed in animals, the precise molecular mechanisms responsible remain to be elucidated. The study described here examined the effects of an extended fast on gene transcript profiles in peripheral blood mononuclear cells from ten apparently healthy subjects, comparing transcript profiles after an overnight fast, sampled on four occasions at weekly intervals, with those observed on a single occasion after a further 24 h of fasting. Analysis of the overnight fasted data revealed marked inter-individual differences, some of which were associated with parameters such as gender and subject body mass. For example, a striking positive association between body mass index and the expression of genes regulated by type 1 interferon was observed. Relatively subtle changes were observed following the extended fast. Nonetheless, the pattern of changes was consistent with stimulation of fatty acid oxidation, alterations in cell cycling and apoptosis and decreased expression of key pro-inflammatory genes. Stimulation of fatty acid oxidation is an expected response, most likely in all tissues, to fasting. The other processes highlighted provide indications of potential mechanisms that could contribute to the putative beneficial effects of intermittent fasting in humans.

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Introduction

There is growing interest in the potential health benefits of diets that involve regular periods of fasting (Robertson and Mitchell 2013; Trepanowski et al. 2011). It is postulated that this pattern of eating better reflects the periods of feast and famine that the human genome has evolved to cope with (Halberg et al. 2005). Studies in animals suggest that certain patterns of feeding such as alternate-day fasting may offer similar health benefits to long-term caloric restriction, in terms of increased longevity and reduced risks of developing chronic diseases such as cardiovascular disease (CVD), dementia and certain cancers (Trepanowski et al. 2011). Additionally, intermittent fasting might be considered more readily achievable by human subjects than long-term caloric restriction, although considerable personal commitment and self-control is required to sustain this regimen voluntarily over protracted periods (Heilbronn et al. 2005b).

A range of possible mechanisms underlying the apparent benefits of intermittent fasting has been proposed. These include increased efficiency of metabolic fuel usage, decreased systemic inflammation, reduced circulating IGF and increased cell survival via modulation of apoptosis and enhanced cytoprotection (Robertson and Mitchell 2013). However, the key molecular mechanisms are currently poorly defined. Moreover, the evidence to support health benefits of intermittent fasting in humans is currently rather limited. Studies of individuals choosing to undergo periods of fasting for religious reasons have often provided conflicting findings, perhaps due to limited experimental control in these types of study, or because of inadequately long periods of fasting (Trepanowski et al. 2011). Comparatively few studies of intermittent fasting have been performed with human subjects using well defined and controlled dietary interventions and, even in these cases, the findings are not always consistent (Heilbronn et al. 2005a, b; Johnson et al. 2007; Klempel et al. 2012; Kroeger et al. 2012; Soeters et al. 2009).

The study described here examined the acute effects of a single extended fast on gene transcript profiles in peripheral blood mononuclear cells (PBMC). PBMC have been used as an accessible source of cells for a wide range of human studies. A compromise is often made in selecting these cells, as they are frequently not the primary target for clinical or nutritional interventions. Nonetheless, the comparative ease with which PBMC can be obtained from blood samples and the scope for serial sampling continue to make them a prime target for future studies and, in particular, for gene/protein expression-based biomarker development. Indeed, current evidence suggests that PBMC may prove useful in biomarker development, through the application of functional genomic techniques, for an increasingly wide range of clinical conditions (Mohr and Liew 2007). Some of these are conditions in which PBMC are directly involved in the disease process. In other cases, PBMC are not known to be directly involved in the pathology of the condition but nonetheless appear to exhibit characteristic responses as the disease develops and progresses. Such responses may reflect the role of PBMC in the body as sentinels (Mohr and Liew 2007). Alternatively, PBMC also exhibit some ectopic expression of tissue-specific genes that may follow a pattern of response indicative of that occurring in less accessible tissues (Liew et al. 2006).

With this in mind, it is important to understand the degree of inter- and intra-individual variation and to characterise both the patterns and magnitude of responses observed in PBMC compared with those in the less accessible target tissues. This information is required not only for assessing the validity of PBMC for biomarker development but also for developing designs of future studies employing functional genomic techniques to ensure appropriate statistical power. We and others have previously described variations in gene transcript profiles of PBMC from apparently healthy human subjects (Eady et al. 2005; Radich et al. 2004; Whitney et al. 2003). Such studies consistently demonstrate substantial inter-individual variation. On the other hand, there is typically much less day-to-day variation between samples from the same individual. In the study described here, the analysis has been extended to compare baseline inter- and intra-individual transcriptome variation with the scale of response to a simple nutritional challenge; in this case an extended (36 h) fast. This work was undertaken as part of the NuGO Proof of Principle Study in which metabolomic and proteomic techniques were also applied to components of blood, urine and saliva to obtain a comprehensive overview of normal phenotypic variation between and within individuals and the response to extended fasting (Baccini et al. 2008; Bouwman et al. 2011; Rubio-Aliaga et al. 2011).

Materials and methods

The design of the study has been described in detail previously (Baccini et al. 2008). The study was performed in accordance with the principle of the Declaration of Helsinki. Ethical permission for the study was obtained from the North of Scotland Research Ethics Services prior to the

start of the study, and all volunteers gave informed consent. Ten healthy volunteers (3 males and 7 females, body mass index (BMI) range 18.5–39.7 kg/m^2 and age range 25–56 years) were enrolled at the Rowett Institute of Nutrition and Health, University of Aberdeen. The volunteers were asked to come to the Human Nutrition Unit once a week on different days each week during a 4-week period after an overnight fast to provide a blood, saliva and 24 h urine samples. After the fourth sampling day, volunteers were fasted for an additional 24 h (total of 36 h) followed by a final sample collection. Blood was collected into vacutainers containing potassium EDTA anticoagulant. PBMC were isolated using the Optiprep™ method as described previously (de Roos et al. 2008). A portion of the PBMC from each sample was transferred immediately to lysis buffer (Qiagen RNEasy Midi kit) and the cells ruptured by passing the lysate through a 21G needle. The lysates were snap frozen and stored at −80 °C. RNA was prepared from the lysates using RNEasy Midi kits according the manufacturer's instructions and stored at −80 °C. RNA purity and yield were assessed using a Nanodrop 1000 spectrophotometer. Aliquots of the purified RNA samples were used to prepare biotin labelled cDNA using GeneChip Expression 3′ Amplification OneCycle Target labelling kits (Affymetrix) and hybridised to Affymetrix human GeneChip® arrays custom made for NuGO (www.nugo.org) by ServiceXS (Leiden, Netherlands) using the manufacturer's standard protocols.

Array data analysis

Quality control of the array data was performed using a dedicated software pipeline (De Groot et al. 2008). The data were normalised using the GC-RMA method with "full model" option. Only genes with a normalised signal ≥20 on at least two arrays were considered to be expressed and included in the subsequent statistical analysis. The array data and sample information have been made available in MIAME-compliant format in the ArrayExpress database (accession number E-MTAB-2922).

Data for expressed genes for all 50 samples were analysed on a log2-scale within the R software suite using a linear model including effects for subject, BMI, fasting and the interactions of fasting with gender and BMI as well as the three-way interaction of fasting, gender and BMI. The main effects of gender and BMI were analysed separately using the expressed genes dataset for the 40 overnight fasted (baseline) samples arrays only. Probability values were adjusted for multiple testing using the Benjamini Hochberg method for false discovery rate control. Apparent functional relationships between genes identified through these analyses were investigated further using DAVID Bioinformatic Resources 6.7 (da Huang et al. 2009a, b), GO-Elite (Zambon et al. 2012) to test for enrichment of pathways included in Wikipathways (www.wikipathways.org) using Cytoscape (www.cytoscape.org) for visualisation and BiblioSphere literature mining software (Genomatix Software GmbH, Munich, Germany).

Real-time RT-qPCR

The expression profiles of a number of genes, selected on the basis of array data analysis, were checked by real-time RT-qPCR. The RT-qPCR was performed using an ABI Prism 7300 (Applied Biosystems) for each of four test genes (CDKN1A, IER3, CLU and IFI44L) plus the reference gene RNA polymerase II. Expression of the test genes was determined using pre-designed TaqMan® Gene Expression Assay kits specific for each gene (Life technologies products Hs00355782_m1, Hs004187506_g1, Hs00156548_m1 and Hs00915292_m1). The probe and primers used for determination of RNA polymerase II mRNA levels were as described previously (Hurst et al. 2008). A one-step RT-PCR was employed for all genes. Real-time RT-qPCRs were carried out in a 96-well plate using TaqMan® 1-step RT-PCR Master Mix Reagent Kits (Life Technologies) in a total volume of 25 µl/well consisting of 250 nM probe, 900 nM forward and reverse primers. Test samples (30 ng of total RNA per reaction) were analysed by comparison with a standard curve, included on each plate, consisting of serial dilutions (120, 60, 30, 15 and 7.5 ng/well) of a pooled PBMC RNA reference sample (r^2 for log linear fit of standard curves was >0.99 in all cases). The real-time RT-qPCR conditions were as follows: 48 °C for 30 min, then 95 °C for 10 min, followed by 40 amplification cycles of 95 °C for 15 s and 60 °C for 1 min. Reactions were carried out in triplicate, and data were analysed by the ABI PRISM 7300 detection system software. Within plate intra-assay variation, based on the average coefficient of variations for the triplicate analyses of all test samples, ranged from 5 to 9 % for all genes analysed. The data generated for each of the test genes, using the relative standard curve method, were normalised against the mean value obtained in the same way for RNA polymerase II (Radonic et al. 2004). Pearson correlation analysis was used to compare the array and real-time RT-qPCR data for these genes and linear regression to analyse the relationship between IFI44L mRNA levels and BMI. Paired *t* tests were used to compare mRNA levels for each of the genes, based on the real-time RT-qPCR data, in overnight fasting versus extended fast samples.

Results and discussion

The quality control analysis (De Groot et al. 2008) suggested that high quality consistent data were obtained from

all arrays with one possible exception. Standard quality metrics for Affymetrix GeneChip arrays (scaling factors, ratio of signal for 3′ and 5′ GAPDH and β-actin probes, percentage of genes called "present" and background levels) were all within standard acceptable ranges, except for the one possible outlier (the array for the extended fast sample from volunteer 7) for which the ratio of signal for 3′ and 5′ GAPDH and β-actin probes were high and both the array–array intensity correlation and Ward cluster analyses performed after data normalisation highlighted this array as a marginal outlier. Based on this, subsequent statistical analyses were performed initially both with and without the data from this array. Comparison of the outputs suggested that exclusion of this array had little effect on the overall findings. Therefore, the full dataset was employed.

Of the 23,941 probesets represented on the array, 10,449 or 10,397 passed the criteria for representing expressed genes (i.e. normalised signals of ≥20 on at least two of the arrays) in the full set of 50 arrays or the subset of 40 baseline arrays, respectively.

Baseline data: association of expression profiles with gender, subject and BMI

The linear model analysis of the 40 baseline array subset incorporating data from the 10,397 probesets for expressed genes suggested significant differences (FDR < 5 %) between the genders in the expression of genes represented by 3,013 probesets (29 % of expressed genes) and between subjects in the expression of genes represented by 2,468 probesets (24 % of expressed genes) (online resource 1). As might be expected, many of the genes that exhibited the most marked differences between the genders were found to be located on the X or Y chromosomes (e.g. 16 of the 20 genes with the lowest p values) and there was notable overlap with genes identified previously as exhibiting highly gender-specific expression (e.g. X- and Y-linked ribosomal proteins S4, Jumonji, AT rich interactive domain 1D, Y-linked DEAD box polypeptide 3, chromosome Y open reading frame 15B, X-linked eukaryotic translation initiation factor 1A and X-linked zinc finger protein) (Eady et al. 2005).

The linear model analysis of the 40 baseline arrays also suggested that BMI was significantly associated with the expression of genes represented by 343 probesets (FDR < 5 %; 3 % of expressed genes) (online resource 1). This contrasts with only three genes [CD151 antigen, transforming growth factor beta-induced 68 kDa protein and interferon-induced protein 44-like (IFI44L)] for which significant associations with BMI were observed in our previous study (Eady et al. 2005). Of the three BMI-associated genes identified previously, only IFI44L was represented in the list of genes corresponding to the 343 probesets from the current study. Based on this observation, we selected IFI44L as one of the genes for further analysis by real-time RT-qPCR. The real-time RT-qPCR data obtained closely matched the array data for this gene ($r = 0.905$, $p < 0.0001$ for the Pearson correlation analysis) and reconfirmed the observation of a positive association between IFI44L mRNA levels and BMI ($r^2 = 0.405$, $p < 0.0001$) (Fig. 1).

Relatively little is known about the function of IFI44L. However, it is one of a group of seven genes that have been put forward as potential biomarkers specific for type 1 IFN bioactivity (Malhotra et al. 2011). All of these genes have IFN-stimulated response element sequences in their promoters close to the sites of initiation of transcription and their expression is increased in vivo in PBMC following INFβ treatment in multiples sclerosis patients. Transcript levels for all these genes also have been shown to exhibit dose dependent increases in primary PBMC treated in vitro with INFβ but not with INFγ (Malhotra et al. 2011). Interestingly, of these seven genes, four were present in the list of genes exhibiting significant association with BMI in the current study [IFI44L, myxovirus resistance 1 (MX1), radical S-adenosyl methionine domain-containing 2 (RSAD2) and HECT domain and RCC1-like domain-containing protein 5 (HERC5)] all exhibiting a positive association between BMI and expression levels ($p < 10^{-5}$). A fifth [interferon-induced protein with tetratricopeptide repeats 1 (IFIT1)] fell just outside the criteria set for statistical significance (adjusted p value = 0.076). The remaining 2 genes (interferon, alpha-inducible protein 27

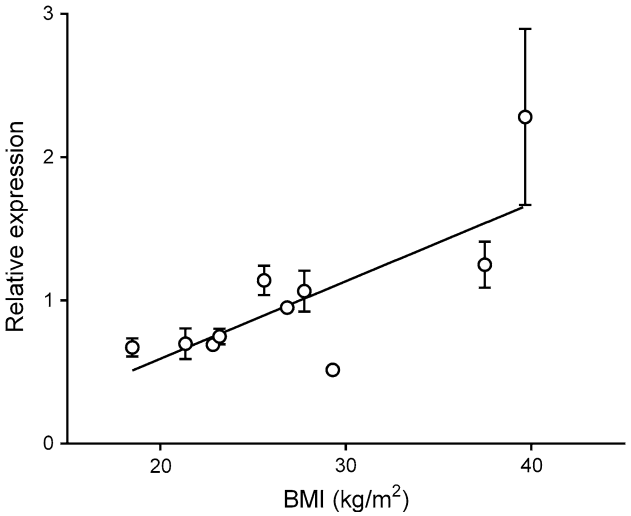

Fig. 1 Association of IFI44L mRNA levels in peripheral blood mononuclear cells with BMI of the donors. Real-time RT-qPCR analysis of IFI44L mRNA levels in PBMC samples normalised against expression of the reference gene RNA polymerase II. *Error bars* indicate mean ± SEM for overnight fasted samples ($n = 4$) from each donor. Linear regression analysis results: $r^2 = 0.405$, 95 % confidence interval for slope 0.03225–0.07605, $p < 0.0001$)

(IFI27) and ubiquitin-specific peptidase 18 (USP18)] from the set of seven had not been included in the statistical analysis as they failed to meet the criteria set for expressed genes. A number of other genes shown previously to be up-regulated by type 1 IFN in endothelial cells were also present in the list of genes significantly associated with BMI (Indraccolo et al. 2007). These were OASL, OAS1, OAS2, OAS3, RTP4/IFRG28, HERC6, ISG15, SP110 and PSMB9 and the expression of each of these also exhibited an apparent positive association with BMI.

Transcript levels of IFN-regulated genes have been highlighted as exhibiting significant inter-individual variation in PBMC in a number of studies (Eady et al. 2005; Radich et al. 2004; Whitney et al. 2003). To our knowledge, no direct association between increased type 1 IFN activity and obesity in humans has been reported to date. However, in mice, transcription of the IFNβ gene in white adipose tissue has been shown to be positively associated with body mass (Weisberg et al. 2003), and interferon regulatory factor 7, a master regulator of type 1 IFN response, is up-regulated in white adipose tissue, liver and gastrocnemius muscle in response to high fat diet-induced or genetic obesity (Wang et al. 2013b). IFNα-treated mice exhibit changes in fatty acid metabolism that appear to arise from impacts both on fatty acid synthesis in the liver and lipolysis in fat cells (Feingold et al. 1989, 1992; Margalit et al. 2006) and other members of the IFN regulatory factor family appear to regulate metabolic processes and may play functional roles in some of the pathological conditions associated with obesity (Wang et al. 2013a).

In humans, obese individuals undergoing type 1 IFN therapy for chronic viral hepatitis often exhibit reduced or no response to the treatment and this is associated with lower induction of genes such as MX1, MX2, RSAD2, USP18, IFI6, IFI27, OAS1 and OAS2 in PBMC following the therapy (Aggarwal et al. 2008). This appears to align with observations that PBMCs from individuals with elevated baseline expression of type 1 IFN-regulated genes exhibit reduced responsiveness to IFNα treatment ex vivo, as measured by induction of MX1 mRNA levels (Radich et al. 2004) and PBMC isolated from obese individuals exhibit an impaired type 1 IFN response to toll-like receptor ligands (Teran-Cabanillas et al. 2013), suggesting that obesity may lead to abnormal type 1 IFN signalling.

Effects of extended fast on PBMC transcription profiles

In contrast to the marked gender, subject and BMI-associated differences in PBMC gene transcript profiles, the effects of the extended fast (36 h), as examined using the full set of arrays, were much more difficult to detect. In fact, using the same criteria (FDR < 5 %) with a linear model also taking into account, gender, BMI and subject effects, no genes would be considered to be significantly affected by the extended fast. However, using less conservative statistical criteria (unadjusted $p < 0.05$ and >1.4-fold change in expression), as employed previously in similar human studies (Bouwens et al. 2007), genes represented by 48 probesets would be considered to be significantly affected by the extended fast (Table 1). Of these, 15 had been identified as being differentially expressed in the only previous study of gene expression in PBMCs in vivo following varying periods of fasting in human subjects (Bouwens et al. 2007). Expression of 13 of these 15 changed in the same direction as reported previously, including increases also confirmed previously by real-time RT-qPCR in certain PPARα-regulated genes such a pyruvate dehydrogenase kinase isoform 4 (PDK4), carnitine palmitoyltransferase 1 (CPT1) and carnitine/acylcarnitine translocase (SLC25A20) (Bouwens et al. 2007). Increases in PDK4 and CPT1 are also observed in response to fasting in skeletal muscle where these changes correspond to metabolic shifts to accommodate the fasting state: the increase in PDK4 inhibiting pyruvate dehydrogenase thereby acting to conserve glucose and the increase in CPT1 indicating the up-regulation of fatty acid import into mitochondria for β-oxidation (Pilegaard et al. 2003).

Entrez gene identifiers were obtained for 44 of the 48 probesets identified as apparently being regulated following the extended fast. Of these, 42 mapped to unique identifiers. Gene annotation enrichment analysis, performed with this list of 42 genes using the DAVID Bioinformatic Resources 6.7 (da Huang et al. 2009a, b), identified statistically significant enrichment for a small number of functional annotation categories most of which related to cytokine/chemokine activity (Table 2). In a similar manner, we also used the GO-Elite package to perform over-representation analysis of pathways in WikiPathways (applying the following cutoff: z-score ≥ 1.96, permuted p value ≤ 0.05 and a minimum of 3 changed genes). A network visualisation the pathways identified and the genes involved in them also emphasised the regulation of certain cytokines and inflammatory responses, or pathways with an important inflammatory component, in response to the extended fast. Additionally, this analysis highlighted over-representation of genes involved in adipogenesis and senescence and autophagy pathways (online resource 2).

Potential functional associations between these genes were investigated further using the BiblioSphere literature mining tool. Of the 42 unique Entrez genes identifiers, 38 genes passed the initial co-citation filter (i.e. 38 of the genes were co-cited in PubMed at the abstract level with at least one other gene in the list either directly or via a common transcription factor). The software was used to generate a network of the 24 genes that were directly co-

Table 1 Genes affected by the extended fast according to the criteria uncorrected p value <0.05 and magnitude of fold change >1.4 as determined using a linear model analysis also taking into account, gender, BMI and subject effects

Probeset ID	GeneName	Fold change	Adjusted p value
207113_s_at	TNF	−1.74	0.00002
202887_s_at	DDIT4	1.64	0.00002
203633_at	CPT1A	1.64	0.00003
203821_at	HBEGF	2.57	0.00005
225207_at	PDK4	2.26	0.00019
207850_at	CXCL3	−1.47	0.00025
201631_s_at	IER3	−1.95	0.00058
202284_s_at	CDKN1A	1.70	0.00060
223217_s_at	NFKBIZ	−1.57	0.00071
203658_at	SLC25A20	1.42	0.00077
207156_at	HIST1H2AG	1.49	0.00126
205239_at	AREG	2.31	0.00173
206283_s_at	TAL1	1.66	0.00198
205114_s_at	CCL3	−2.11	0.00220
224856_at	FKBP5	−1.41	0.00353
201059_at	CTTN	1.50	0.00476
39402_at	IL1B	−2.04	0.00482
206167_s_at	ARHGAP6	1.41	0.00559
202729_s_at	LTBP1	1.49	0.00655
206655_s_at	GP1BB	1.76	0.00723
209774_x_at	CXCL2	−2.06	0.00777
1555938_x_at	VIM	−1.72	0.00783
223717_s_at	ACRBP	1.45	0.01097
207815_at	PF4V1	1.56	0.01284
226188_at	HSPC159	1.55	0.01452
206493_at	ITGA2B	1.47	0.01458
206110_at	HIST1H3H	1.63	0.01473
208791_at	CLU	1.55	0.01535
235739_at	n/a	1.45	0.01675
241036_at	n/a	1.47	0.01763
229943_at	TRIM13	−1.45	0.01846
203574_at	NFIL3	1.50	0.02137
230170_at	OSM	1.55	0.02277
201058_s_at	MYL9	1.52	0.02642
NuGO_eht0298527_at	CLEC1B	1.55	0.02726
NuGO_eht0299289_at	C6orf25	1.40	0.02753
205442_at	MFAP3L	1.55	0.02858
241824_at	n/a	1.46	0.03054
227180_at	ELOVL7	1.51	0.03600
204622_x_at	NR4A2	1.78	0.03866
203936_s_at	MMP9	1.72	0.03998
207826_s_at	ID3	−1.44	0.04005
230097_at	GART	−1.43	0.04148
224823_at	MYLK	1.51	0.04278
229778_at	C12orf39	1.41	0.04288
236610_at	n/a	1.47	0.04456
220496_at	CLEC1B	1.41	0.04753
235885_at	n/a	1.49	0.04801

cited at the abstract level (Fig. 2). This highlighted two main clusters of genes centred on (a) the cyclin-dependent kinase inhibitor CDKN1A and (b) tumour necrosis factor alpha (TNF).

In line with the known function of TNF, many of the genes in the cluster around it are associated with inflammation. In fact, the simultaneous down-regulation of TNF, interleukin 1β (IL1B), the chemokine CXCL2, the immediate early genes IER3 and NFKBIZ is suggestive of a moderate anti-inflammatory response to the extended fast. Both TNF and IL1B are known to stimulate the expression of CXCL2, NFKBIZ and IER3 at the level of the gene transcripts in a variety of cells (Shen et al. 2005; Sparna et al. 2010). As reported previously, multiplex immunoassay analysis of plasma samples from the same subjects showed a small but significant reduction in circulating IL1B protein concentrations that parallels the change in PMBC IL1B mRNA levels observed here (Bouwman et al. 2011). The data from the multiplex immunoassay also identified moderate but significant reductions in certain other inflammatory cytokines and chemokines such as interleukins 8 and 16 and monocyte chemotactic protein-1 (MCP-1/CCL2) (Bouwman et al. 2011). TNF levels also appeared to decrease, but this did not achieve statistical significance. However, virtually all of the analytes detected with this multiplex platform as being significantly increased in plasma following the extended fast were pro-inflammatory molecules (e.g. EN-RAGE, MIP-1β) or well-established biomarkers of inflammation (e.g. C-reactive protein, ferritin, vascular cell adhesion molecule-1, TIMP-1 and complement 3), suggesting that the extended fast elicited a systemic pro-inflammatory response that may be quite distinct from the effects observed in the PBMC.

The second cluster of genes centred on CDKN1A. This is not one of the genes that were previously identified as being affected by fasting in PBMC from human volunteers (Bouwens et al. 2007). However, CDKN1A mRNA levels have been reported to increase in a range of tissues (muscle, liver, brain and kidney) in fasted mice (Ebert et al. 2010; Hakvoort et al. 2011; Mitchell et al. 2010; Swindell 2008; Zhang et al. 2011). The cluster of genes around CDKN1A in the network includes a number of genes that regulate cell cycling/proliferation and cellular senescence. This reflects the pivotal role CDKN1A plays in blocking cell cycling and regulating apoptosis following cellular stresses and DNA damage (Jung et al. 2010). Many of the genes in this cluster are known to regulate directly the transcription of CDKN1A, exhibit co-ordinated expression together with CDKN1A or be regulated at the level of transcription through the actions of CDKN1A. For example, ID3, for which the mRNA levels were down-regulated

Table 2 Gene annotation enrichment analysis, performed using the DAVID Bioinformatic Resources 6.7, of genes identified as being regulated by the extended fast

Category	Term	Gene count	% of list	Raw p value	Adjusted p value[a]
GOTERM_MF_FAT	Cytokine activity (GO:0005125)	7	16.7	6.96×10^{-6}	9.88×10^{-4}
SP_PIR_KEYWORDS	Cytokine	6	14.3	3.98×10^{-5}	5.87×10^{-3}
GOTERM_CC_FAT	Extracellular region part (GO:0044421)	10	23.8	4.23×10^{-4}	2.55×10^{-2}
GOTERM_CC_FAT	Extracellular space (GO:0005615)	9	21.4	2.18×10^{-4}	2.62×10^{-2}
INTERPRO	Small chemokine, C-X-C, conserved site (IPR018048)	3	7.1	7.10×10^{-4}	3.19×10^{-2}
INTERPRO	Small chemokine, C-X-C/ Interleukin 8 (IPR002473)	3	7.1	4.77×10^{-4}	3.22×10^{-2}
GOTERM_BP_FAT	Response to wounding (GO:0009611)	9	21.4	3.88×10^{-5}	3.22×10^{-2}
PIR_SUPERFAMILY	CXC chemokine (PIRSF002522)	3	7.1	1.22×10^{-3}	3.84×10^{-2}

[a] Benjamini and Hochberg adjusted p value

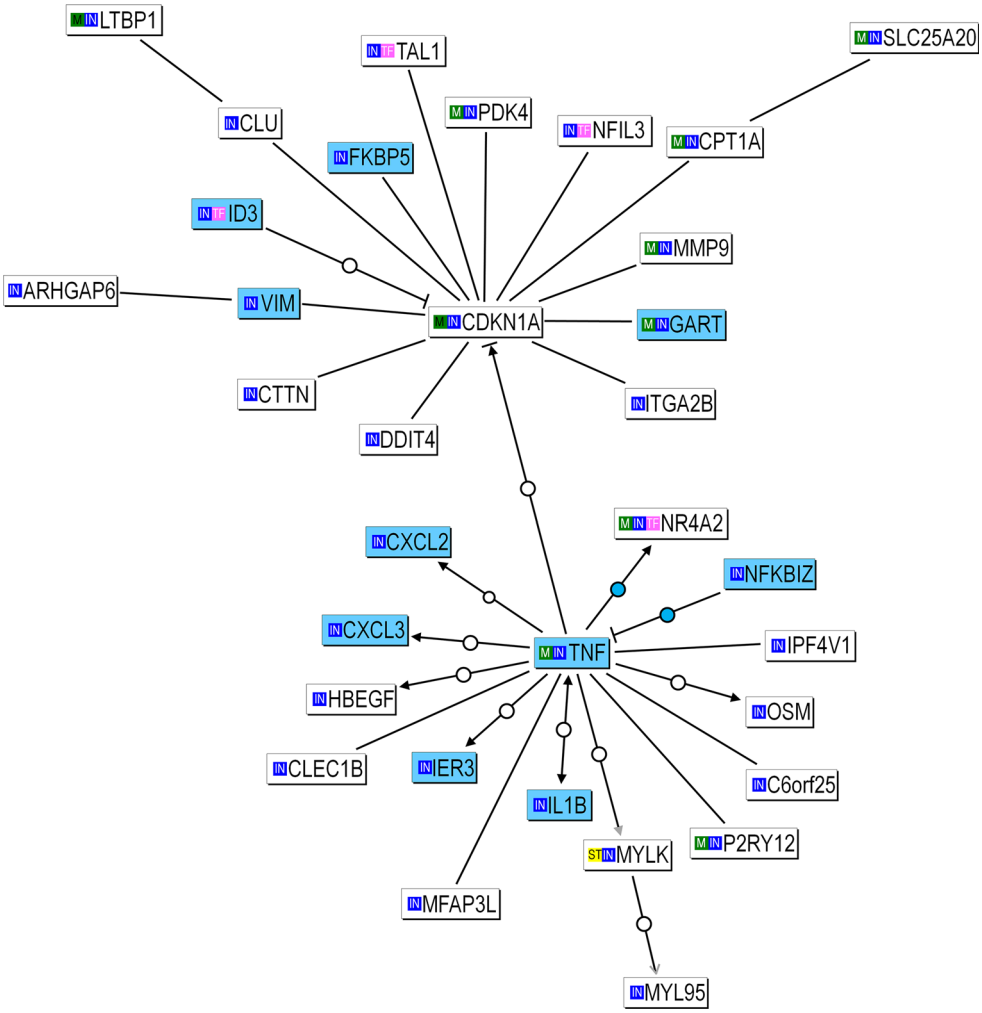

Fig. 2 Shortest path co-citation network of genes for which transcript levels were significantly altered following extend fast generated using BiblioSphere software. *Open* and *shaded boxes* indicate significant up and down-regulation of transcript levels, respectively. *Lines* indicate genes co-cited within PubMed at the abstract level. *Open arrowheads* indicate evidence of regulation. *Closed arrowheads* indicate evidence of activation. *Blocked arrowheads* indicate evidence of inhibition. *Grey arrowheads* indicate enzymatic modification. *Open circles* indicate connection annotated by Molecular Connections experts. *Shaded circles* indicate connection annotated by Genomatix experts

following the extended fast, inhibits expression of CDKN1A while the transcription factor TAL1, for which the mRNA levels where up-regulated following the extended fast, is known to bind to the CDKN1A promoter and induce its expression (Lacombe et al. 2010). Expression of DDIT4, for which mRNA levels were up-regulated

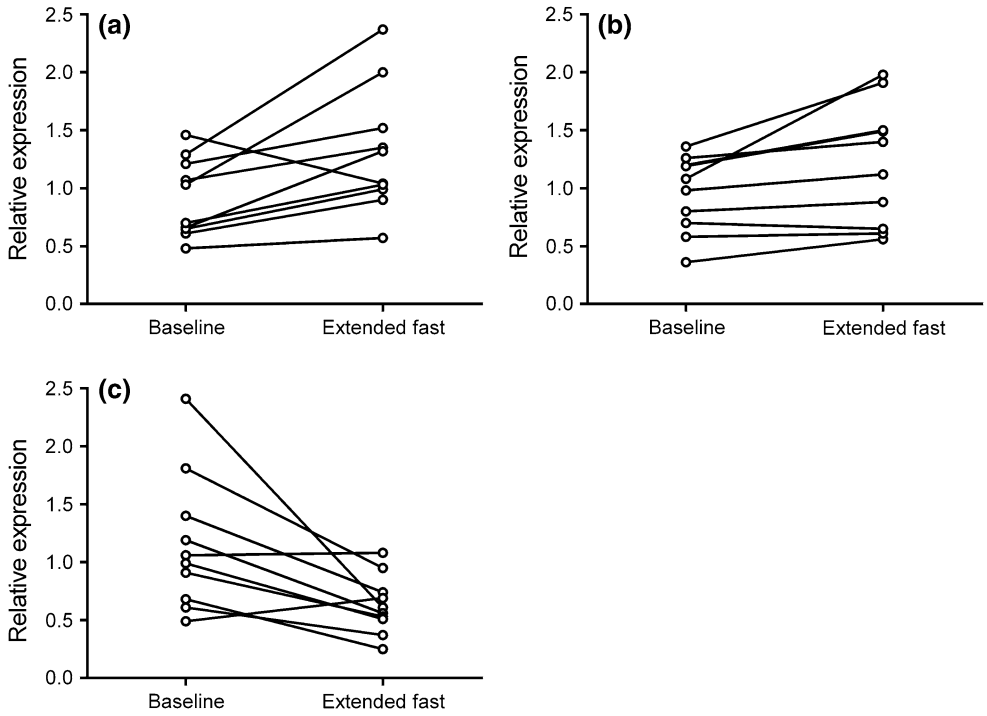

Fig. 3 Effects of an extended fast on the levels of **a** CDKN1A, **b** clusterin and **c** IER3 mRNAs. *Each line* indicates the relative mRNA levels for a different individual after overnight (baseline) and extended (36 h) fasts. Real-time RT-qPCR data for each gene were normalised first against levels of the reference gene RNA polymerase II and then against the mean normalised value for the baseline values

following the extended fast, is increased in parallel with increased CDKN1A in response to oxidative and other cellular stresses (Han et al. 2008) and in liver following treatment with glucocorticoids; hormones that help to regulate blood glucose during fasting (Wong et al. 2010).

Expression of clusterin at the level of mRNA is, at least in part, dependent on CDKN1A (Chen et al. 2004). Clusterin, for which the mRNA levels increased following the extended fast, is a regulator of apoptosis and its expression is used as a marker of cell senescence (Petropoulou et al. 2001; Trougakos et al. 2006). However, care must be taken in interpreting clusterin mRNA levels as the nuclear form of the protein is pro-apoptotic, whereas the secreted form is anti-apoptotic (Petropoulou et al. 2001; Shannan et al. 2006).

There is also complex interplay between TNF and CDKN1A in the regulation of lymphocyte proliferation, inflammation and apoptosis. Mitogen activation of human primary lymphocytes in vitro results in an increase in both TNF and CDKN1A mRNA levels, whereas treatment with the immunosuppressant cyclosporin, which inhibits T lymphocyte proliferation, reduces TNF mRNA levels but increases expression of CDKN1A both at the level of mRNA and protein (Khanna 2005). Treatment of human PBMC with TNF in vitro increases apoptosis and necrosis via a process that involves increases in levels of CDKN1A protein (Ryazantseva et al. 2010). TNF has also been shown to stimulate CDKN1A expression, both at the level of mRNA and protein in murine alveolar cells and macrophages, while CDKN1A can suppress TGF-β induced TNF expression (Yamasaki et al. 2008).

Real-time RT-qPCR analysis

To assess the validity of the findings from the array data, mRNA levels for three of the genes identified from the array analysis as being affected by the extended fast (CDKN1A, clusterin and IER3) were also analysed by real-time RT-qPCR. In all three cases, the real-time RT-qPCR data corresponded closely to the values obtained from the arrays ($r > 0.9$, $p < 0.001$ for Pearson correlation analysis of the real-time RT-qPCR and array data for each gene) and there were significant changes ($p < 0.05$) in the mRNA levels measured by real-time RT-qPCR for all three genes, that matched the changes detected with the array data (Fig. 3).

Conclusions

This study provides new insights into effects of prolonged fasting on gene transcript profiles in PBMC in vivo. In particular, in addition to confirming changes in the expression of genes involved in fatty acid oxidation and

glucose metabolism that have been described previously (Bouwens et al. 2007), we also observed changes in a number of key genes involved in inflammatory, cell cycle and apoptotic processes. These align well with some of the mechanisms proposed, but not yet conclusively demonstrated, to play a role in the increased longevity and reduced incidence of chronic disease associated with caloric restriction and intermittent fasting in animal models and with changes in biomarkers of these conditions in humans (Robertson and Mitchell 2013; Trepanowski et al. 2011). In human subjects, longer term intermittent fasting, undertaken for Ramadan or as part of a weight loss study, has been reported to attenuate circulating levels of certain pro-inflammatory cytokines such as IL1B, TNF and IL6 and inflammatory markers such as CRP (Aksungar et al. 2007; Faris et al. 2012; Kroeger et al. 2012). The transcriptomic analysis performed here, together with the targeted proteomic analysis described previously, suggest that moderate reductions in inflammatory cytokines can be detected following just one extended fast. However, plasma concentrations of certain other inflammatory markers, such as C-reactive protein, were noted to be elevated following the extended fast. This suggests a more complex mechanism than simply a broad spectrum attenuation of inflammatory signals.

The magnitudes of the changes in gene transcript levels brought about by the extended fast were generally rather small, particularly when considered in relation to the substantial inter-individual differences observed. Using relatively low stringency statistical criteria, only 48 genes were found to be significantly affected by the extended fast. This contrasts with one previous study of the effect of fasting on gene transcription profiles in PBMC from human subjects, which identified 1,200 and 1,386 genes that changed, according to the same criteria, after 24 and 48 h of fasting, respectively (Bouwens et al. 2007). This apparent discrepancy is likely to be due largely to two key differences in the study designs. Firstly, Bouwens and co-workers compared transcript profiles after 24 or 48 h of fasting with those obtained immediately after a meal (i.e. the fed state), whereas the comparison made here was between overnight fasted and 36 h fasted samples. Secondly, the group of subjects in the previous study was much more homogeneous (males subjects aged 19–22 years with BMI > 19 and <25 kg/m^2) than that in the current study (both male and female subjects aged 25–56 years with BMI ranging from 18.5 to 39.7 kg/m^2).

One of the key aims of the current study was to evaluate the scale of response to a simple nutritional intervention, detected using transcriptomic, proteomic and metabolomic methods, in relation to the normal biological background variation. With this in mind, we decided to examine what might be considered the worst case scenario, in which a minimal number of inclusion/exclusion criteria were applied, thus creating the potential for a highly heterogeneous study group. Nonetheless, one of the conclusions from this work is that all three functional genomic approaches are capable of detecting responses to a simple nutritional challenge, even on a background of substantial inter-individual variation. Moreover, the array and real-time RT-qPCR data described here highlight that, in addition to the inter-individual differences at baseline, there also appear to be significant inter-individual differences in the response to the nutritional challenge. Use of more homogeneous study populations may well help to reduce such variability and could, therefore, be considered beneficial when employing functional genomic methods for detecting the often subtle effects of nutritional interventions although such studies would need to be followed up to determine whether or not equivalent effects are observed in other groups of subjects.

The counter side to this argument is that use of heterogeneous study populations can itself provide opportunities for new findings. For example, we observed a striking positive association between subject BMI and the expression of type 1 IFN-regulated genes that we would have been unlikely to see in a group with less variation in BMI. This is the second study in which we have observed a positive association between BMI and mRNA levels for IFI44L, a gene considered as a potential marker of type 1 IFN bioactivity (Malhotra et al. 2011), in human PBMCs (Eady et al. 2005). Direct measurement of INFα activity is very difficult, which is why the transcript levels of genes regulated by IFNα are currently widely used as markers of bioactivity (Malhotra et al. 2011). The evidence obtained in this study strongly suggests a relationship between BMI and type 1IFN activity that merits further investigation. However, any future studies would need to take into account the observation that the transcript levels of many IFN-regulated genes vary not only between individuals, but also between multiple samples from the same individual. Thus, repeated sampling from subjects, as performed here, may be required to overcome problems with intra-individual variability and enable the association to be tested rigorously.

In summary, PBMC gene transcriptome analysis of the baseline samples, collected after an overnight fast on four separate occasions, again highlighted the marked degree of inter-individual variation and provided new insights into potential associations between parameters such as BMI and gene transcript profiles. In particular, a positive association between BMI and the expression of genes regulated by type 1 IFN was observed. The scale of response to the extended fast (36 h) at the level of the transcriptome within PBMC appeared to be rather subtle and difficult to detect against the background of substantial inter-individual

variation. Nonetheless, the pattern of changes described here is consistent with stimulation of fatty acid oxidation, alterations in a number of genes involved in cell cycling and apoptosis, and a decrease in the expression of a number of key pro-inflammatory genes. This last observation contrasts with evidence of a systemic pro-inflammatory response to the extended fast reported previously (Bouwman et al. 2011) and may provide the first evidence for a more fundamental anti-inflammatory response to fasting that could contribute to the putative beneficial effects of intermittent fasting in humans.

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Conflict of interest Ruan Elliott, Baukje de Roos, Susan Duthie, Freek Bouwman, Isobel Rubio-Aliaga, Katie Crosley, Claus Mayer, Abigael Polley, Carolin Heim, Susan Coort, Chris Evelo, Francis Mulholland, Hannelore Daniel, Edwin Mariman and Ian Johnson declare that they have no conflict of interest.

Ethical standard All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all volunteers included in the study.

References

Aggarwal V, Palmer C, Nguyen N, Lloyd A, Zekry A (2008) Differential expression of interferon stimulating genes in obese subjects receiving treatment for chronic hepatitis C. J Gastroen Hepatol 23:A282–A282

Aksungar FB, Topkaya AE, Akyildiz M (2007) Interleukin-6, C-reactive protein and biochemical parameters during prolonged intermittent fasting. Ann Nutr Metab 51:88–95

Baccini M et al (2008) The NuGO proof of principle study package: a collaborative research effort of the European Nutrigenomics Organisation. Genes Nutr 3:147–151

Bouwens M, Afman LA, Muller M (2007) 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 86:1515–1523

Bouwman FG et al (2011) 2D-electrophoresis and multiplex immunoassay proteomic analysis of different body fluids and cellular components reveal known and novel markers for extended fasting. BMC Med Genomics 4:24. doi:10.1186/1755-8794-4-24

Chen T, Turner J, McCarthy S, Scaltriti M, Bettuzzi S, Yeatman TJ (2004) Clusterin-mediated apoptosis is regulated by adenomatous polyposis coli and is p21 dependent but p53 independent. Cancer Res 64:7412–7419. doi:10.1158/0008-5472.CAN-04-2077

da Huang W, Sherman BT, Lempicki RA (2009a) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37:1–13. doi:10.1093/nar/gkn923

da Huang W, Sherman BT, Lempicki RA (2009b) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57. doi:10.1038/nprot.2008.211

De Groot PJ, Reiff C, Mayer C, Muller M (2008) NuGO contributions to GenePattern. Genes Nutr 3:143–146. doi:10.1007/s12263-008-0093-2

de Roos B et al (2008) Proteomic methodological recommendations for studies involving human plasma, platelets, and peripheral blood mononuclear cells. J Proteome Res 7:2280–2290

Eady JJ, Wortley GM, Wormstone YM, Hughes JC, Astley SB, Foxall RJ, Doleman JF, Elliott RM (2005) Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. Physiol Genomics 22:402–411. doi:10.1152/physiolgenomics.00080.2005

Ebert SM, Monteys AM, Fox DK, Bongers KS, Shields BE, Malmberg SE, Davidson BL, Suneja M, Adams CM (2010) The transcription factor ATF4 promotes skeletal myofiber atrophy during fasting. Mol Endocrinol 24:790–799. doi:10.1210/me.2009-0345

Faris MA, Kacimi S, Al-Kurd RA, Fararjeh MA, Bustanji YK, Mohammad MK, Salem ML (2012) Intermittent fasting during Ramadan attenuates proinflammatory cytokines and immune cells in healthy subjects. Nutr Res 32:947–955. doi:10.1016/j.nutres.2012.06.021

Feingold KR, Soued M, Serio MK, Moser AH, Dinarello CA, Grunfeld C (1989) Multiple cytokines stimulate hepatic lipid synthesis in vivo. Endocrinology 125:267–274

Feingold KR, Doerrler W, Dinarello CA, Fiers W, Grunfeld C (1992) Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis. Endocrinology 130:10–16

Hakvoort TB et al (2011) Interorgan coordination of the murine adaptive response to fasting. J Biol Chem 286:16332–16343. doi:10.1074/jbc.M110.216986

Halberg N, Henriksen M, Soderhamn N, Stallknecht B, Ploug T, Schjerling P, Dela F (2005) Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol 99:2128–2136. doi:10.1152/japplphysiol.00683.2005

Han ES et al (2008) The in vivo gene expression signature of oxidative stress. Physiol Genomics 34:112–126. doi:10.1152/physiolgenomics.00239.2007

Heilbronn LK, Civitarese AE, Bogacka I, Smith SR, Hulver M, Ravussin E (2005a) Glucose tolerance and skeletal muscle gene expression in response to alternate day fasting. Obes Res 13:574–581. doi:10.1038/oby.2005.61

Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E (2005b) Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. Am J Clin Nutr 81:69–73

Hurst R, Elliott RM, Goldson AJ, Fairweather-Tait SJ (2008) Se-methylselenocysteine alters collagen gene and protein expression in human prostate cells. Cancer Lett 269:117–126. doi:10.1016/j.canlet.2008.04.025

Indraccolo S et al (2007) Identification of genes selectively regulated by IFNs in endothelial cells. J Immunol 178:1122–1135

Johnson JB et al (2007) Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. Free

Radic Biol Med 42:665–674. doi:10.1016/j.freeradbiomed.2006.12.005

Jung YS, Qian Y, Chen X (2010) Examination of the expanding pathways for the regulation of p21 expression and activity. Cell Signal 22:1003–1012. doi:10.1016/j.cellsig.2010.01.013

Khanna AK (2005) Reciprocal role of cyclins and cyclin kinase inhibitor p21WAF1/CIP1 on lymphocyte proliferation, allo-immune activation and inflammation. BMC Immunol 6:22. doi:10.1186/1471-2172-6-22

Klempel MC, Kroeger CM, Bhutani S, Trepanowski JF, Varady KA (2012) Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. Nutr J 11:98. doi:10.1186/1475-2891-11-98

Kroeger CM, Klempel MC, Bhutani S, Trepanowski JF, Tangney CC, Varady KA (2012) Improvement in coronary heart disease risk factors during an intermittent fasting/calorie restriction regimen: relationship to adipokine modulations. Nutr Metab (Lond) 9:98. doi:10.1186/1743-7075-9-98

Lacombe J, Herblot S, Rojas-Sutterlin S, Haman A, Barakat S, Iscove NN, Sauvageau G, Hoang T (2010) Scl regulates the quiescence and the long-term competence of hematopoietic stem cells. Blood 115:792–803. doi:10.1182/blood-2009-01-201384

Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA (2006) The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. J Lab Clin Med 147:126–132. doi:10.1016/j.lab.2005.10.005

Malhotra S et al (2011) Search for specific biomarkers of IFNbeta bioactivity in patients with multiple sclerosis. PLoS ONE 6:e23634. doi:10.1371/journal.pone.0023634

Margalit M, Shalev Z, Pappo O, Sklair-Levy M, Alper R, Gomori M, Engelhardt D, Rabbani E, Ilan Y (2006) Glucocerebroside ameliorates the metabolic syndrome in OB/OB mice. J Pharmacol Exp Ther 319:105–110. doi:10.1124/jpet.106.104950

Mitchell JR et al (2010) Short-term dietary restriction and fasting precondition against ischemia reperfusion injury in mice. Aging Cell 9:40–53. doi:10.1111/j.1474-9726.2009.00532.x

Mohr S, Liew CC (2007) The peripheral-blood transcriptome: new insights into disease and risk assessment. Trends Mol Med 13:422–432. doi:10.1016/j.molmed.2007.08.003

Petropoulou C, Trougakos IP, Kolettas E, Toussaint O, Gonos ES (2001) Clusterin/apolipoprotein J is a novel biomarker of cellular senescence that does not affect the proliferative capacity of human diploid fibroblasts. FEBS Lett 509:287–297

Pilegaard H, Saltin B, Neufer PD (2003) Effect of short-term fasting and refeeding on transcriptional regulation of metabolic genes in human skeletal muscle. Diabetes 52:657–662

Radich JP et al (2004) Individual-specific variation of gene expression in peripheral blood leukocytes. Genomics 83:980–988

Radonic A, Thulke S, Mackay IM, Landt O, Siegert W, Nitsche A (2004) Guideline to reference gene selection for quantitative real-time PCR. Biochem Biophys Res Commun 313:856–862

Robertson LT, Mitchell JR (2013) Benefits of short-term dietary restriction in mammals. Exp Gerontol. doi:10.1016/j.exger.2013.01.009

Rubio-Aliaga I et al (2011) Metabolomics of prolonged fasting in humans reveals new catabolic markers. Metabolomics 7:375–387. doi:10.1007/s11306-010-0255-2

Ryazantseva NV, Novitskii VV, Zhukova OB, Biktasova AK, Chechina OE, Sazonova EV, Radzivil TT, Wice AN, Chasovskikh NY (2010) Role of NF-kB, p53, and p21 in the regulation of TNF-alpha mediated apoptosis of lymphocytes. Bull Exp Biol Med 149:50–53

Shannan B, Seifert M, Boothman DA, Tilgen W, Reichrath J (2006) Clusterin and DNA repair: a new function in cancer for a key player in apoptosis and cell cycle control. J Mol Histol 37:183–188. doi:10.1007/s10735-006-9052-7

Shen F, Ruddy MJ, Plamondon P, Gaffen SL (2005) Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17- and TNF-alpha-induced genes in bone cells. J Leukoc Biol 77:388–399. doi:10.1189/jlb.0904490

Soeters MR, Lammers NM, Dubbelhuis PF, Ackermans M, Jonkers-Schuitema CF, Fliers E, Sauerwein HP, Aerts JM, Serlie MJ (2009) Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. Am J Clin Nutr 90:1244–1251. doi:10.3945/ajcn.2008.27327

Sparna T, Retey J, Schmich K, Albrecht U, Naumann K, Gretz N, Fischer HP, Bode JG, Merfort I (2010) Genome-wide comparison between IL-17 and combined TNF-alpha/IL-17 induced genes in primary murine hepatocytes. BMC Genom 11:226. doi:10.1186/1471-2164-11-226

Swindell WR (2008) Comparative analysis of microarray data identifies common responses to caloric restriction among mouse tissues. Mech Ageing Dev 129:138–153. doi:10.1016/j.mad.2007.11.003

Teran-Cabanillas E, Montalvo-Corral M, Caire-Juvera G, Moya-Camarena SY, Hernandez J (2013) Decreased interferon-alpha and interferon-beta production in obesity and expression of suppressor of cytokine signaling. Nutrition 29:207–212. doi:10.1016/j.nut.2012.04.019

Trepanowski JF, Canale RE, Marshall KE, Kabir MM, Bloomer RJ (2011) Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. Nutr J 10:107. doi:10.1186/1475-2891-10-107

Trougakos IP, Petropoulou C, Franceschi C, Gonos ES (2006) Reduced expression levels of the senescence biomarker clusterin/apolipoprotein j in lymphocytes from healthy centenarians. Ann N Y Acad Sci 1067:294–300. doi:10.1196/annals.1354.039

Wang XA et al (2013a) Interferon regulatory factor 9 protects against hepatic insulin resistance and steatosis in male mice. Hepatology. doi:10.1002/hep.26368

Wang XA et al (2013b) Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance. Am J Physiol Endocrinol Metab. doi:10.1152/ajpendo.00505.2012

Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr (2003) Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808

Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, Brown PO (2003) Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci USA 100:1896–1901. doi:10.1073/pnas.252784499

Wong S, Tan K, Carey KT, Fukushima A, Tiganis T, Cole TJ (2010) Glucocorticoids stimulate hepatic and renal catecholamine inactivation by direct rapid induction of the dopamine sulfotransferase Sult1d1. Endocrinology 151:185–194. doi:10.1210/en.2009-0590

Yamasaki M, Kang HR, Homer RJ, Chapoval SP, Cho SJ, Lee BJ, Elias JA, Lee CG (2008) P21 regulates TGF-beta1-induced pulmonary responses via a TNF-alpha-signaling pathway. Am J Respir Cell Mol Biol 38:346–353. doi:10.1165/rcmb.2007-0276OC

Zambon AC, Gaj S, Ho I, Hanspers K, Vranizan K, Evelo CT, Conklin BR, Pico AR, Salomonis N (2012) GO-Elite: a flexible solution for pathway and ontology over-representation. Bioinformatics 28:2209–2210. doi:10.1093/bioinformatics/bts366

Zhang F, Xu X, Zhou B, He Z, Zhai Q (2011) Gene expression profile change and associated physiological and pathological effects in mouse liver induced by fasting and refeeding. PLoS ONE 6:e27553. doi:10.1371/journal.pone.0027553