Short-term high-fat meal intake alters the expression of circadian clock-, inflammation-, and oxidative stress-related genes in human skeletal muscle

Zsófia Budai, László Balogh and Zsolt Sarang

Department of Biochemistry and Molecular Biology Faculty of Medicine, University of Debrecen, Debrecen, Hungary; Institute of Sport Sciences University of Debrecen, Debrecen, Hungary

ABSTRACT

Dietary food, depending on timing, amount and composition can influence gene expression in various tissues. Here, we investigated the effect of high-fat meal diets of different compositions on the gene expression pattern of human skeletal muscle. Gene expression data of skeletal muscle samples from human volunteers prior and 4 h after the consumption of high lipid-containing meal consisting of either saturated-, monounsaturated- or polyunsaturated fatty acids were downloaded from the public repository. List of 843 differently expressed genes (DEGs) was generated. Functional analysis revealed that circadian rhythm-, inflammation- and oxidative stress-related genes are highly overrepresented among the DEGs. The magnitude of gene expression changes significantly increases with the saturation level of the dietary fatty acids and the majority of the DEGs are upregulated. We propose that, by altering circadian clock gene expression and inducing inflammation and oxidative stress, high lipid intake can contribute to muscle function decay in the long run.

ARTICLE HISTORY

Received 18 October 2018
Revised 30 November 2018
Accepted 6 December 2018

KEYWORDS

Circadian rhythm; high-fat diet; inflammation; oxidative stress; skeletal muscle

Introduction

Several clinical studies investigated the effect of dietary saturated-, monounsaturated- or polyunsaturated fatty acid (SFA, MUFA and PUFA) intake on the human physiology with special focus on prevention of type 2 diabetes, obesity, cardiovascular diseases and inflammation. High amount of SFA intake was related to postprandial upregulation of genes associated with pro-inflammatory pathways in peripheral blood mononuclear cells in comparison with MUFA or PUFA intake (Calder et al. 2011). It also increases the risk of cardiovascular diseases and decreases glucose tolerance and insulin sensitivity (Hammad et al. 2016; Imamura et al. 2016). In addition to these effects, dietary fatty acids showed influence on the circadian rhythm in animals (Kohsaka et al. 2007; Liu et al. 2017).

Simple circadian clock, composed of two genes, evolved initially in unicellular algae and reached a complex system in mammals where it regulates several aspects of life including biological and behavioural processes such as sleep/wake cycle, body temperature, metabolism, temporal feeding pattern and hormone levels (Bhadra et al. 2017). The master regulator of the mammalian clock is regulated by the light cycle and is located in the hypothalamic suprachiasmatic nucleus (SCN) (Buhr and Takahashi 2013). The SCN neurons regulate further hypothalamic nuclei and the pineal gland to adjust body temperature and production of hormones such as cortisol and melatonin (Ebadi and Govitrapong 1986; Sage et al. 2004). In SCN neurons the molecular mechanism of circadian clock regulation is carried out by several core clock genes such as the transcription factor brain and muscle Arnt-like protein 1 (BMAL1), circadian locomotor output cycles kaput (CLOCK), Period (PER1-3) and Cryptochrome (CRY1,2) genes. The proteins encoded by these genes establish a negative feedback loop where BMAL1:CLOCK heterodimer auto-represses Bmal1 expression and induces the production of PER and CRY proteins which in turn repress the activity of BMAL1:CLOCK complex (Cho et al. 2012; Liberman et al. 2017). The expression of core clock genes is influenced by REV-ERBa/NR1D1 and BHLHe40/DEC transcription factors (Crumbley and Burris 2011). Besides transcriptional control, PER1 and PER2 proteins are also regulated by phosphorylation by casein kinase 1 epsilon (CK1ε) leading to
their increased ubiquitylation and degradation. CRY1 is phosphorylated and destabilised by AMP-activated protein kinase (Lamia et al. 2009).

The circadian gene expression is not restricted only to SCN neurons but nearly all peripheral cells express clock genes and have oscillators which are influenced by the master regulator centre in SCN but entrained primarily by food and feeding time (Schibler et al. 2003). Food restriction or feeding at the wrong time points can desynchronise peripheral oscillators but does not affect the SCN clock (Hara et al. 2001). As soon as 2 h following food intake, Per2 and Dec1 were induced in rat liver desynchronising circadian rhythm enforcing the dominant role of feeding time in peripheral oscillator entrainment (Wu et al. 2010). Not only the feeding time but also the type of diet has an impact on peripheral oscillators. In experimental animal models, long-term high-fat diet (HFD) was shown to attenuate the amplitude of core clock gene expression in liver, muscle and adipose tissues but not in SCN (Kohsaka et al. 2007; Liu et al. 2017). Perturbation of peripheral circadian rhythm manifests in serious physiological alterations in animal models. For example, CLOCK or BMAL1 deletion in pancreas results in impaired glucose tolerance and reduced insulin secretion while ablation of the same genes in skeletal muscle leads to the diminished force due to decreased mitochondrial function (Andrews et al. 2010; Marcheva et al. 2010). Human cohort studies have revealed that altered circadian rhythm due to sleep disorders, night shift work or jetlag is associated with the development of obesity and metabolic syndrome (Parsons et al. 2015).

As being the centres for fat metabolism several studies describe the effect of dietary fatty acids on the liver and adipose tissue (Do et al. 2011; Almon et al. 2012; Nishikawa et al. 2012; Voigt et al. 2013; Choi et al. 2015; Muthulakshmi et al. 2015; Li et al. 2017) but less data is available on its effect on skeletal muscle (Sparks et al. 2005; de Wilde et al. 2009, Jans et al. 2012). In the present paper, using data mining, global gene expression and gene set enrichment analysis, we aimed to characterise the gene expression changes induced by high fatty acid intake in human skeletal muscle. We have found that single short-term high fat intake alters skeletal muscle gene expression pattern and SFAs induced more prominent gene expression changes than MUFAs or PUFAs. Dietary fatty acids altered the expression of 4 core clock genes and 21 circadian rhythm regulation related genes possibly desynchronising the skeletal muscle circadian clock system. Moreover, we detected a rapid increase of inflammatory- and oxidative stress response-related gene expression following high-fat meal consumption in muscles.

Materials and methods

Data processing

Gene expression data, generated from Affymetrix Human Gene 1.0 ST Arrays, corresponding for 56 vastus lateralis muscle biopsy were retrieved from public Gene Expression Omnibus repository (GEO series GSE31901). The 56 biopsy samples were derived from a single-blind, randomised, crossover study where 10 insulin-resistant, adult, obese men consumed 3 high-fat mixed meals and samples were collected prior and 4 h after SFA, MUFA or PUFA containing meal consumption. The meal provided 2.6 MJ (equivalent to 621 kcal), consisting of 61% of energy as fat, 33% of energy as carbohydrates and 6.3% of energy as protein. The source of MUFA in the HFD meal was 40 g olive oil. The PUFA meal consisted of 20 g safflower oil and 20 g fish oil (18% EPA and 12% DHA) (Bioriginal).

For detailed description of participants and study group details, sample preparation and microarray hybridisation protocol; see original paper, Jans et al. 2012. Gene expression data from human vastus lateralis muscle samples prior and 4 h following high-fat meal intake was compared using the GEO2R platform (https://www.ncbi.nlm.nih.gov/geo/geo2r/). Equal value intensity distribution and cross-comparability of individual microarrays were confirmed using box-and-whisker plots. The significance of fold changes (FC) between control and high-fat meal samples was tested with Moderated t-test adjusted by Benjamini–Hochberg False Discovery Rate (FDR) correction for multiple testing and two-way ANOVA followed by Bonferroni post hoc test. Data were imported in Microsoft Access and list of differently expressed genes (DEGs) was generated by removing non-significantly changed transcripts based on 0.05 multiple testing adjusted p-value cut-off value and twofold FC cut-off value.

Functional profiling

In order to gain insight in the biological function of the given transcripts, we used the PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System (http://pantherdb.org/) to identify canonical pathways (PANTHER and Reactome pathways) and biological processes and functions.
Gene Ontology) with over-representation of DEGs. Duplicated transcripts were removed from the input list of DEGs and statistically significant enrichment of genes within PANTHER and Reactome pathways and GO categories was determined using Fisher’s Exact test with FDR multiple test correction (FDR < 0.05).

Clustering and visualization of data

DEGs were clustered with Cluster software (M. Eisen, 1999 http://rana.lbl.gov/EisenSoftware.htm) using a self-organising map (SOM) with 100,000 iterations and 2 nodes. The log2 transformed FC values of genes in selected PANTHER analysis terms and the result of SOM clustering were visualised using heat maps generated by TreeView software (M. Eisen, http://rana.lbl.gov/EisenSoftware.htm). Kyoto Encyclopedia of Genes and Genomes (KEGG) was used to visualise the role of DEGs related to circadian rhythm (Kanehisa Laboratories, http://www.kegg.jp/) and Search Tool for the Retrieval of Interacting Genes v10 (STRING), covering both physical interactions and functional associations between proteins, was used to display protein-protein interactions of 25 circadian rhythm genes at medium confidence range (JensenLab, https://string-db.org/) (Szklarczyk et al. 2017). The prediction methods that were activated are the following: neighbourhood, gene fusion, co-occurrence, co-expression, experiments, databases and text mining.

Results

DEGs following high-fat meal intake

Out of the 19,793 genes present on the microarray, 14,424 genes were defined as expressed in skeletal muscle samples (Jans et al. 2012). From the 6182 transcripts which showed significantly altered expression (adjusted p value < 0.05) after lipid meal at least in one of the samples we identified 843 DEGs (based on at least twofold change present in any of the samples) following high fatty acid consumption in the analysed GEO data series. One hundred and forty-three transcripts showed decreased, and 700 transcripts showed increased gene expression at 4h after lipid intake (Figure 1(A)). The top 50 downregulated and upregulated genes are listed in Supplementary Tables 1 and 2, respectively. The mean FC of decreased transcripts in the SFA group (−2.37 ± 1.21) was significantly lower than that of the PUFA (−1.93 ± 1.21) and MUFA groups (−2.09 ± 1.2) (Figure 1(B)). The median FC value of decreased transcripts was −1.84, −2.02 and −2.25 in PUFA, MUFA and SFA groups, respectively.
The mean FC of increased transcripts in the SFA group \((2.99 \pm 1.56)\) was significantly higher than that of the PUFA \((2.58 \pm 1.51)\) and MUFA groups \((2.67 \pm 1.57)\) (Figure 1(B)). The median FC value of increased transcripts was 2.31, 2.35 and 2.63 in PUFA, MUFA and SFA groups, respectively. Taken together, our results indicate that HFD increases rather than decreasing gene expression in skeletal muscle, and SFAs induce significantly higher gene expression changes than unsaturated fatty acids.

Functional profiling of differently expressed genes

To give an insight into biological processes that might be affected by lipid intake in skeletal muscle we carried out gene set enrichment analysis using the PANTHER Classification System tool. Our analysis revealed significant overrepresentation of circadian rhythm-, external stimulus-response- and nitrogen compound metabolism-related GO-Slim Biological Processes among the genes that were downregulated following high-fat meal consumption (Table 1). The same analysis revealed 10 PANTHER Pathways (Table 2), for example, “Inflammation mediated by chemokine and cytokine signaling pathway” and “Oxidative stress response”, and 31 Reactome pathways (Table 3), such as “Interleukin-6 signaling”, “Metallothioneins bind metals”, “Dissolution of Fibrin Clot” and “Circadian Clock” among the upregulated genes.

Short-term high-fat meal intake desynchronises circadian clock

By investigating the genes belonging to significantly overrepresented categories we have identified 13 genes associated with the circadian rhythm of cells. Four of them were among the downregulated genes (NR1D1, NR1D2, PER1 and PER3), and nine in the upregulated genes (ARNTL, BHLHE40, CRY1, HIF1A, NAMPT, NOCT, NRIP1, SERPINE1 and SIK1). To identify possible other candidate genes related to circadian clock system we extended the search using the 207 genes containing “Circadian rhythm related genes” category found in PathCards database (Weizmann Institute of Science). The extended search resulted in 12 more DEGs associated with the circadian clock. Heat map and protein-protein interactions map of the 25 circadian rhythm associated DEGs are shown in Figure 2(A,B), respectively. Eight out the 25 DEGs were also found in KEGG Mapper “Circadian rhythm” pathway (Figure 2(C)).

Short-term high-fat meal intake induces inflammation and oxidative stress

Functional profiling of DEGs revealed that genes related inflammation and chemotactic migration of leukocytes are overrepresented in both Reactome and Panther pathway databases. We have identified 21 DEGs in cytokine, chemokine receptor and ligand families (Figure 3(A)). The oxidative stress response metallothionein (MT) gene family was one of the highest overrepresented gene category found by our analysis. We have identified 15 DEGs, including MTs, members of dual specificity phosphatases (DUSP) and superoxide dismutase 2, which play a role in the protection of cells against oxidative stress (Figure 3(B)).

Discussion

In the present study, we describe the acute gene expression changes taking place in the skeletal muscle of obese, insulin-resistant men 4 h following intake of three types of high-fat mixed meals with different fatty acid compositions. We have found that high-fat meal consumption alters muscle gene expression having a major impact on several genes including circadian rhythm, inflammatory- and antioxidant/stress response. Several experiments have shown that diet can influence peripheral clocks but only a limited number of studies are available addressing the effect of food intake on skeletal muscle circadian clock gene expression and those are carried out in animals (Cardoso et al. 2017; Liu et al. 2017). The present study found that fat consumption, regardless of composition, damped the expression of transcriptional

| Table 1. PANTHER GO-Slim Biological Process overrepresentation test of downregulated genes following HFD. |
|---------------------------------------------------|-----------------|----------------|-----------------|-----------------|
| PANTHER GO-Slim biological process                  | REF# | F#      | Expected | Fold enrichment | FDR              |
| Circadian rhythm                                   | 14   | 4       | 0.1     | 42.6            | 1.3E-03          |
| Rhythmic process                                   | 14   | 4       | 0.1     | 42.6            | 6.5E-04          |
| Response to external stimulus                      | 351  | 10      | 2.4     | 4.3             | 1.3E-02          |
| Nitrogen compound metabolic process                | 2524 | 32      | 16.9    | 1.9             | 2.3E-02          |

REF#: number of reference genes in the given category; F#: number of genes found among the downregulated genes following HFD in muscle tissue; FDR: False Discovery Rate value calculated by Benjamini-Hochberg procedure for multiple test correction (FDR < 0.05).
Table 2. PANTHER Pathway overrepresentation test of upregulated genes following HFD.

| PANTHER pathways                                      | REF# | F# | Expected | Fold enrichment | FDR   |
|-------------------------------------------------------|------|----|----------|----------------|-------|
| Plasminogen activating cascade                        | 18   | 6  | 0.6      | 10.9           | 1.58E-03 |
| Blood coagulation                                     | 46   | 7  | 1.4      | 5.0            | 1.54E-02 |
| p53 pathway                                           | 89   | 13 | 2.7      | 4.8            | 5.75E-04 |
| Interleukin signalling pathway                        | 89   | 13 | 2.7      | 4.8            | 4.31E-04 |
| Toll receptor signalling pathway                      | 59   | 8  | 1.8      | 4.4            | 1.49E-02 |
| CCKR signalling map                                   | 174  | 23 | 5.3      | 4.3            | 2.07E-06 |
| Oxidative stress response                             | 58   | 7  | 1.8      | 3.9            | 4.65E-02 |
| Apoptosis signalling pathway                          | 120  | 13 | 3.7      | 3.5            | 3.55E-03 |
| Inflammation mediated by chemokine and cytokine signalling pathway | 255  | 22 | 7.8      | 2.8            | 1.04E-03 |
| Gonadotropin-releasing hormone receptor pathway       | 237  | 20 | 7.3      | 2.8            | 2.22E-03 |

| REF#: number of reference genes in the given category; F#: number of genes found among the upregulated genes following HFD in muscle tissue; FDR: False Discovery Rate value calculated by Benjamini-Hochberg procedure for multiple test correction (FDR < 0.05).

Table 3. Reactome pathway overrepresentation test of upregulated genes following HFD.

| Reactome pathways                                      | REF# | F# | Expected | Fold enrichment | FDR   |
|-------------------------------------------------------|------|----|----------|----------------|-------|
| Metallothioneins bind metals                           | 11   | 6  | 0.3      | 17.8           | 1.6E-03 |
| Response to metal ions                                 | 11   | 6  | 0.3      | 17.8           | 1.8E-03 |
| Metabolism                                            | 1968 | 87 | 60.4     | 1.4            | 5.0E-02 |
| Dissolution of Fibrin Clot                             | 13   | 6  | 0.4      | 15.0           | 2.4E-03 |
| Hemostasis                                             | 586  | 50 | 18.0     | 2.8            | 2.4E-07 |
| Organic cation transport                               | 9    | 4  | 0.3      | 14.5           | 3.4E-02 |
| SLC-mediated transmembrane transport                   | 264  | 20 | 8.1      | 2.5            | 3.8E-02 |
| Interleukin-6 signalling                               | 10   | 4  | 0.3      | 13.0           | 4.1E-02 |
| Interleukin-6 family signalling                        | 26   | 7  | 0.8      | 8.8            | 5.8E-03 |
| Signalling by Interleukins                             | 383  | 30 | 11.8     | 2.6            | 2.4E-03 |
| Cytokine Signaling in Immune system                    | 606  | 54 | 18.6     | 2.9            | 2.1E-08 |
| Immune System                                          | 1594 | 99 | 48.9     | 2.0            | 5.0E-08 |
| Growth hormone receptor signalling                     | 21   | 6  | 0.6      | 9.3            | 1.4E-02 |
| Regulation of TLR by endogenous ligand                | 19   | 5  | 0.6      | 8.6            | 4.2E-02 |
| Toll-Like Receptors Cascades                           | 148  | 14 | 4.5      | 3.1            | 2.9E-02 |
| Innate Immune System                                   | 784  | 43 | 24.1     | 1.8            | 3.7E-02 |
| BMAL1:CLOCK, Npas2 activates circadian gene expression | 42   | 9  | 1.3      | 7.0            | 2.9E-03 |
| Circadian Clock                                        | 62   | 9  | 1.9      | 4.7            | 2.3E-02 |
| Amino acid transport across the plasma membrane        | 30   | 6  | 0.9      | 6.5            | 4.0E-02 |
| Cell surface interactions at the vascular wall         | 101  | 18 | 3.1      | 5.8            | 6.0E-06 |
| TP53 Regulates Transcription of Cell Cycle Genes       | 48   | 8  | 1.5      | 5.4            | 2.3E-02 |
| Chemokine receptors bind chemokines                    | 57   | 9  | 1.8      | 5.1            | 1.5E-02 |
| Peptide ligand-binding receptors                       | 193  | 17 | 5.9      | 2.9            | 1.9E-02 |
| Class A/1 (Rhodopsin-like receptors)                   | 323  | 23 | 9.9      | 2.3            | 2.8E-02 |
| Interferon gamma signalling                            | 91   | 13 | 2.8      | 4.7            | 2.6E-03 |
| Interferon Signalling                                  | 192  | 19 | 5.9      | 3.2            | 3.0E-03 |
| Signalling by PTK6                                     | 64   | 9  | 2.0      | 4.6            | 2.7E-02 |
| tRNA modification in the nucleus and cytosol           | 59   | 8  | 1.8      | 4.4            | 5.0E-02 |
| Integrin cell surface interactions                     | 85   | 11 | 2.6      | 4.2            | 1.5E-02 |
| Extracellular matrix organisation                      | 292  | 24 | 9.0      | 2.7            | 4.2E-03 |
| Cellular Senescence                                    | 154  | 16 | 4.7      | 3.4            | 6.4E-03 |

| REF#: number of reference genes in the given category; F#: number of genes found among the upregulated genes following HFD in muscle tissue; FDR: False Discovery Rate value calculated by Benjamini-Hochberg procedure for multiple test correction (FDR < 0.05).

repressors PER1, PER3, NR1D1/Rev-erbα and NR1D2/Rev-erbβ, and circadian associated repressor of transcription (CIART) representing the negative regulatory arm of the circadian clock. In accordance with this, the expression of BMAL1, CRY1 and several other circadian rhythm associated genes was upregulated following a high-fat meal intake. The molecular clock in muscle cells regulates the expression of more than one thousand genes mainly involved in metabolic processes and disturbance of this clock leads to arrhythmicity, decreased muscle insulin sensitivity and premature aging characterised by myopathy, and muscle wasting, called sarcopenia (Hodge et al. 2015; Liu et al. 2016). Moreover, excess lipid intake desynchronises not only muscle but liver and adipose tissue clocks as well and leads to obesity which in turn further disrupts circadian rhythm establishing a vicious cycle in obese individuals (Kohsaka et al. 2007; Kaneko et al. 2009; Wang et al. 2015).

HFD can increase inflammatory responses in muscle tissue. As short as 3 d of HFD increased pro-inflammatory leukocyte infiltration and inflammatory marker Ly6B, tumour necrosis factor alpha (TNFα), chemokine (C-C motif) ligand (CCL) 2 and C-C chemokine receptor (CCR) 2 expression in mouse skeletal muscle (Fink et al. 2014). Nine days of HFD induced
increased inflammatory and innate immune response gene expression in human skeletal muscle (Laker et al. 2017). In various human studies, consumption of SFAs was found to be related to postprandial upregulation of genes associated with pro-inflammatory pathways in peripheral blood mononuclear cells, in comparison with MUFA or PUFA intake. Moreover, acute high-SFA meal consumption induced a postprandial pro-inflammatory gene expression in blood monocytes and subcutaneous adipose tissue (Rocha et al. 2017). In line with these, we observed postprandial upregulation of several proinflammatory cytokines, for example, interleukin (IL) 1B and IL6, and chemokine signalling genes, such as CCL2, 8, C-X-C motif chemokine ligand (CXCL) 1, CXCL2, 8 and 10, CCR1, C-X-C motif chemokine receptor (CXCR) 1

Figure 2. Expression pattern and the protein–protein interaction of circadian rhythm related genes. (A) Heat map of DEGs. Based on Weizmann Institute of Science PathCards database, 25 DEGs were identified in DEGs in skeletal muscle samples following SFA, MUFA and PUFA intake. Heat map displays the log2 transformed FC values of SFA, MUFA and PUFA samples compared to control samples. Numbers inside the heat map indicates the log2 transformed FC values of the given transcripts. FC: fold change. (B) Protein–protein interaction network of 25 differently expressed circadian rhythm-related genes. STRING tool was used to visualise protein–protein interaction network between circadian rhythm-related genes at medium confidence level. Nodes are the genes, and edges between genes indicate the confidence of interaction. Line thickness indicates the strength of data support. (C) Modified “Circadian rhythm” pathway from KEGG. The pathway displays 8 out of the total 25 DEGs in skeletal muscle samples following SFA, MUFA and PUFA intake related to circadian clock system. Protein symbols were replaced by gene symbols for the eight DEGs to reflect gene-based data. Arrows next to the genes indicate the up- or downregulation of the gene. u: ubiquitination; p: phosphorylation.
and CXCR2, and CXCR4, indicating development of inflammation in skeletal muscle tissue following only 4 h of high-fat meal consumption regardless of FA composition of the meal. Interestingly, a disturbed circadian rhythm is also implicated in muscle inflammatory processes. Overexpression of Rev-erbα or inhibition of Rev-erbβ increased IL-6 expression in vascular smooth muscle and skeletal muscle cells, respectively (Delerive et al. 2001; Migita et al. 2004). Moreover, the inflammatory cytokine TNFα was shown to upregulate BMAL1 and CRY1 expression rheumatoid synovial cells further enforcing the link between inflammation and circadian rhythm (Yoshida et al. 2013). While temporal inflammation is required for muscle stem cell activation and proliferation during regeneration, continuous inflammation depletes stem cell pool, triggers protein catabolism and impairs anabolic processes of skeletal muscle leading to sarcopenia on the long run (Costamagna et al. 2015).

Another significantly overrepresented category among the upregulated DEGs was the MT gene family with 6 members: MT1A, E, G, M, X and MT2A. MTs are members of the stress protein family and play a role in toxic heavy metal and, as antioxidants, in ROS neutralisation in cells (Manuel et al. 1992; Sato and Bremner 1993). Their expression is induced by many factors including metals, inflammatory cytokines, steroids and stress (Chiaverini and De Ley 2010; Lynes et al. 2014). In contrast, MT expression was found to be downregulated following HFD in the spleen of mice (Cui et al. 2012). MTs were also shown to have a protective effect against high-fat-diet-induced

---

**Figure 3.** Expression pattern of inflammation and oxidative stress related genes. (A) Expression pattern of 21 DEGs belonging to cytokine, chemokine receptor and ligand families. (B) Expression pattern of 15 oxidative stress related genes. Heat maps display the log2 transformed FC values of DEGs in skeletal muscle samples following SFA, MUFA and PUFA intake. Significance of FCs was tested with moderated t-test adjusted by Benjamini-Hochberg correction for multiple testing using 0.05 adjusted p value and two-fold FC cut-off values.
obesity in mice (Sato et al. 2010). In our case, the increased \textit{IL1B} and \textit{IL6} expression and excess oxidative stress caused by fatty acid overload (Muioio and Neufer 2012) can lead to the observed overexpression of MT family members in the fat-loaded muscle. The increased inflammation and oxidative stress were shown to activate the mitogen-activated protein kinase (MAPK) pathway (Pearson et al. 2001). The DUSP proteins are playing a role in the oxidative stress response of the cells by dephosphorylating and deactivating the MAPKs (Theodosiou and Ashworth 2002). We found that \textit{DUSP5}, \textit{DUSP6} and \textit{DUSP14} were also upregulated after lipid consumption. \textit{DUSP5} and \textit{DUSP14} are known to exert an anti-inflammatory effect through deactivation of MAPK pathway (Zheng et al. 2013; Seo et al. 2017). The early upregulation \textit{MTs} and DUSP family members in muscle tissue may provide immediate protection mechanism against inflammation and elevated ROS formation following high-fat meal ingestion.

**Conclusion**

Here, we describe that high-fat containing meal intake leads to profound acute gene expression changes in skeletal muscle involving disturbance of circadian clock system, and induction of inflammation-, and stress response-related genes which can result in impaired regeneration, decreased insulin sensitivity and enhanced catabolism in muscle tissue in the long run. Decreased dietary SFA, and, to a lesser extent MUFA and PUFA, intake can contribute to the prevention of harmful inflammation and can delay muscle function decay.

Regarding the limitation of the study, the microarray technology detects RNA transcripts, and has a decreased sensitivity in detecting low-abundance genes; therefore, future validation of the gene expression data with more sensitive quantitative PCR and protein detecting methods would increase the reliability of the results. In spite of these limitations, our study describes potentially important aspects of human nutrition and muscle physiology.

**Disclosure statement**

Zsolt Sarang was a recipient of Lajos Szodoray fellowship given by the University of Debrecen. The other authors report no conflict of interest.

**Funding**

This work was supported by the National Research, Development and Innovation Office [124244], European Union project titled: Institutional Developments for Intelligent Specialization programme [grant number: EFOP-3.6.1-16-2016-00022 “Debrecen Venture Catapult Program”] and by the GINOP-2.3.2-15-2016-00006 project and EFOP-3.6.3-VEKOP-16-2017-00009 (co-financed by the European Union and the European Regional Development Fund).

**ORCID**

Zsolt Sarang http://orcid.org/0000-0003-4965-266X

**Data availability**

The raw microarray data used in the article can be found deposited in the Gene Expression Omnibus repository (GSE31901). The top 50 up- and downregulated transcripts are included within the supplementary tables.

**References**

Almon RR, Dubois DC, Sukumaran S, Wang X, Xue B, Nie J, Jusko WJ. 2012. Effects of high fat feeding on liver gene expression in diabetic goto-kakizaki rats. Gene Regul Syst Bio. 6:151–168.

Andrews JL, Zhang X, McCarthy JJ, McDearmon EL, Hornberger TA, Russell B, Campbell KS, et al. 2010. CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. Proc Natl Acad Sci USA. 107:19090–19095.

Bhadra U, Thakkar N, Das P, Pal Bhadra M. 2017. Evolution of circadian rhythms: from bacteria to human. Sleep Med. 35:49–61.

Buhr ED, Takahashi JS. 2013. Molecular components of the mammalian circadian clock. Handb Exp Pharmacol. 217:3–27.

Calder PC, Ahluwalia N, Brouns F, Bueter T, Clement K, Cunningham K, Esposito K, et al. 2011. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 106:S5–S78.

Cardoso TF, Quintanilla R, Tibau J, Gil M, Mármol-Sánchez E, González-Rodríguez O, González-Prendes R, et al. 2017. Nutrient supply affects the mRNA expression profile of the porcine skeletal muscle. BMC Genomics. 18:603.

Chiarverini N, De Ley M. 2010. Protective effect of metallothionein on oxidative stress-induced DNA damage. Free Radic Res. 44:605–613.

Cho H, Zhao X, Hatori M, Yu RT, Barish GD, Lam MT, Chong LW, et al. 2012. Regulation of circadian behaviour and metabolism by REV-ERB-α and REV-ERB-β. Nature. 485:123–127.

Choi MS, Kim YJ, Kwon EY, Ryoo JY, Kim SR, Jung UJ. 2015. High-fat diet decreases energy expenditure and expression of genes controlling lipid metabolism, mitochondrial function and skeletal system development in
the adipose tissue, along with increased expression of extracellular matrix remodelling- and inflammation-related genes. Br J Nutr. 113:867–877.

Costamagna D, Costelli P, Sampaolesi M, Penna F. 2015. Role of inflammation in muscle homeostasis and myogenesis. Mediators Inflamm. 2015:805172.

Crumbley C, Burris TP. 2011. Direct regulation of CLOCK by EB. PLoS One. 6:e17290.

Cui J, Xiao Y, Shi YH, Wang B, Le GW. 2012. Lipoic acid attenuates high-fat-diet-induced oxidative stress and B-cell-related immune depression. Nutrition. 28:275–280.

de Wilde J, Smit E, Mohren R, Boekschoten MV, de Groot P, van den Berg SA, Bijland S, et al. 2009. An 8-week high-fat diet induces obesity and insulin resistance with small changes in the muscle transcriptome of C57BL/6J mice. J Nutrigenet Nutrigenomics. 2:280–291.

Delerive P, Monté D, Dubois G, Trottein F, Fruchart-Najib J, Mariani J, Fruchart JC, et al. 2001. The orphan nuclear receptor ROR alpha is a negative regulator of the inflammatory response. EMBO Rep. 2:42–48.

Do GM, Oh HY, Kwon EY, Cho YY, Shin SK, Park HJ, Jeon SM, et al. 2011. Long-term adaptation of global transcription and metabolism in the liver of high-fat diet-fed C57BL/6J mice. Mol Nutr Food Res. 55:S173–S185.

Ebadi M, Govitrapong P. 1986. Neural pathways and neurotransmitters affecting melatonin synthesis. J Neural Transm Suppl. 21:125–155.

Fink LN, Costford SR, Lee YS, Jensen TE, Bilan PJ, Oberbach A, Blüher M, et al. 2014. Pro-inflammatory macrophages increase in skeletal muscle of high fat-fed mice and correlate with metabolic risk markers in humans. Obesity (Silver Spring). 22:747–757.

Hammad S, Pu S, Jones PJ. 2016. Current evidence supporting the link between dietary fatty acids and cardiovascular disease. Lipids. 51:507–517.

Hara R, Wan K, Wakamatsu H, Aida R, Moriya T, Akiyama M, Shibata S. 2001. Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. Genes Cells. 6:269–278.

Hodge BA, Wen Y, Riley LA, Zhang X, England JH, Harfmann BD, Schroder EA, et al. 2015. The endogenous molecular clock orchestrates the temporal separation of substrate metabolism in skeletal muscle. Skelet Muscle. 5:17.

Imamura F, Michia R, Wu JH, de Oliveira Otto MC, Otite FO, Abioye AI, Mozaffarian D. 2016. Effects of saturated fat, polyunsaturated fat, monounsaturated fat, and carbohydrate on glucose-insulin homeostasis: a systematic review and meta-analysis of randomised controlled feeding trials. PLoS Med. 13:e1002087.

Jans A, Konings E, Goossens GH, Bouwman FG, Moors CC, Boekschoten MV, Afman LA, et al. 2012. PUFAs acutely affect triacylglycerol-derived skeletal muscle fatty acid uptake and increase postprandial insulin sensitivity. Am J Clin Nutr. 95:825–836.

Kaneko K, Yamada T, Tsukita S, Takahashi K, Ishigaki Y, Oka Y, Katagiri H. 2009. Obesity alters circadian expressions of molecular clock genes in the brainstem. Brain Res. 1263:58–68.

Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, et al. 2007. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. Cell Metab. 6:414–421.

Laker RC, Garde C, Camera DM, Smiles WJ, Zierath JR, Hawley JA, Barrès R. 2017. Transcriptomic and epigenetic responses to short-term nutrient-exercise stress in humans. Sci Rep. 7:15134.

Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, et al. 2009. AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. Science. 326:437–440.

Li Y, Chu Y, Yu L, Kang H, Zhou L. 2017. Transcriptomic analysis of Bama pig’s liver in various nutritional states reveals a metabolic difference of fatty acids. Food Funct. 8:3480–3490.

Liberman AR, Kwon SB, Vu HT, Filipowicz A, Ay A, Ingram KK. 2017. Circadian clock model supports molecular link between PER3 and human anxiety. Sci Rep. 7:9893.

Liu J, Zhou B, Yan M, Huang R, Wang Y, He Z, Yang Y, et al. 2016. CLOCK and BMAL1 regulate muscle insulin sensitivity via SIRT1 in male mice. Endocrinology. 157:2259–2269.

Liu L, Jiang G, Peng Z, Li Y, Li J, Zou L, He Z, et al. 2017. The effect of high fat diet on daily rhythm of the core clock genes and muscle functional genes in the skeletal muscle of Chinese soft-shelled turtle (Trionyx sinensis). Comp Biochem Physiol B Biochem Mol Biol. 213:17–27.

Lynes MA, Hidalgo J, Manso Y, Devischier L, Laukens D, Lawrence DA. 2014. Melatonin and stress combine to affect multiple organ systems. Cell Stress Chaperones. 19:605–611.

Manuel Y, Thomas Y, Pellegrini O. 1992. Melatonin and tissue damage. IARC Sci Publ. 118:231–237.

Marcheva B, Ramsey KM, Buhr ED, Kobayashi Y, Su H, Ko CH, Ivanova G, et al. 2010. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinemia and diabetes. Nature. 466:627–631.

Migita H, Morser J, Kawai K. 2004. Rev-erba upregulates NF-kappaB-responsive genes in vascular smooth muscle cells. FEBS Lett. 561:69–74.

Muolio DM, Neuffer PD. 2012. Lipid-induced mitochondrial stress and insulin action in muscle. Cell Metab. 15:595–605.

Muthulakshmi S, Chakrabarti AK, Mukherjee S. 2015. Gene expression profile of high-fat diet-fed C57BL/6J mice: in search of potential role of azelaic acid. J Physiol Biochem. 71:29–42.

Nie J, DuBois DC, Xue B, Jusko WJ, Almon RR. 2017. Effects of high-fat feeding on skeletal muscle gene expression in diabetic Goto-Kakizaki rats. Gene Regul Syst Bio. 11:177625017710009.

Nishikawa S, Sugimoto J, Okada M, Sakairi T, Takagi S. 2012. Gene expression in livers of BALB/C and C57BL/6J mice fed a high-fat diet. Toxicol Pathol. 40:71–82.

Parsons MJ, Moffitt TE, Gregory AM, Goldman-Mellor S, Nolan PM, Poulton R, Caspi A. 2015. Social jetlag, obesity and metabolic disorder: investigation in a cohort study. Int J Obes (Lond). 39:842–848.

Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K, Cobb MH. 2001. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev. 22:153–183.
Rocha DM, Bressan J, Hermesdorf HH. 2017. The role of dietary fatty acid intake in inflammatory gene expression: a critical review. Sao Paulo Med J. 135:157–168.

Sage D, Ganem J, Guillaume F, Lafarge-Anglade G, Françoise-Bellam AM, Bosler O, Becquet D. 2004. Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. J Biol Rhythms. 19:144–156.

Sato M, Bremner I. 1993. Oxygen free radicals and metallothionein. Free Radic Biol Med. 14:325–337.

Sato M, Kawakami T, Kondoh M, Takiguchi M, Kadota Y, Himeno S, Suzuki S. 2010. Development of high-fat-diet-induced obesity in female metallothionein-null mice. Faseb J. 24:2375–2384.

Schibler U, Ripperger J, Brown SA. 2003. Peripheral circadian oscillators in mammals: time and food. J Biol Rhythms. 18:250–260.

Seo H, Cho YC, Ju A, Lee S, Park BC, Park SG, Kim JH, et al. 2017. Dual-specificity phosphatase 5 acts as an anti-inflammatory regulator by inhibiting the ERK and NF-kB signaling pathways. Sci Rep. 7:17348.

Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, Smith SR. 2005. A high-fat diet coordinately down-regulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. Diabetes 54:1926–1933.

Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, Santos A, et al. 2017. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 45:D362–D368.

Theodosiou A, Ashworth A. 2002. MAP kinase phosphatases. Genome Biol. 3:REVIEWS3009.

Voigt A, Agnew K, van Schothorst EM, Keijer J, Klaus S. 2013. Short-term, high fat feeding-induced changes in white adipose tissue gene expression are highly predictive for long-term changes. Mol Nutr Food Res. 57:1423–1434.

Wang D, Chen S, Liu M, Liu C. 2015. Maternal obesity disrupts circadian rhythms of clock and metabolic genes in the offspring heart and liver. Chronobiol Int. 32:615–626.

Wu T, Ni Y, Kato H, Fu Z. 2010. Feeding-induced rapid resetting of the hepatic circadian clock is associated with acute induction of Per2 and Dec1 transcription in rats. Chronobiol Int. 27:1–18.

Yoshida K, Hashiramoto A, Okano T, Yamane T, Shibanuma N, Shiozawa S. 2013. TNF-α modulates expression of the circadian clock gene Per2 in rheumatoid synovial cells. Scand J Rheumatol. 42:276–280.

Zheng H, Li Q, Chen R, Zhang J, Ran Y, He X, Li S, et al. 2013. The dual-specificity phosphatase DUSP14 negatively regulates tumor necrosis factor-α and interleukin-1-induced nuclear factor-κB activation by dephosphorylating the protein kinase TAK1. J Biol Chem. 288:819–825.