INSULIN resistance affects the expression of the subset of proliferation-related genes in blood cells of obese boys

Dmytro O.Minchenko1,2,*, Vadym V.Davydov3, Olena A.Budreiko3, Oksana S. Moliavko1, Oleksandra V.Tiazhka2, Oleksandr H.Minchenko1
1Department of Molecular Biology, Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Kyiv, (UKRAINE)
2Department of Pediatrics, Bohomolets National Medical University, Kyiv 01601, (UKRAINE)
3SI “Institute of children and adolescent health care National Academy of Medical Science of Ukraine”, Kharkiv 61153, (UKRAINE)
E-mail: ominchenko@mail.ru

ABSTRACT

The development of obesity and its metabolic complications is associated with dysregulation of numerous intrinsic mechanisms, which control most basic metabolic processes, including insulin sensitivity and cellular proliferation. We studied the expression of genes, which responsible for control of cell proliferation, in bloodcells of obese boys with normal and impaired insulin sensitivity as well as in lean (control) individuals. It was shown that the expression level of ING1, DKK, andSH2B3genes is increased, butEXO1 and CIDEAgens is decreased in bloodcells of obese boys with normal insulin sensitivity as compared to control group. Insulin resistance in obese boys leads to up-regulation ofEXO1 andPER1gene expressions as well as to down-regulation ofCIDEA,ING1, DKK, PER2, andSH2B3genesas compared to obese patients with normal insulin sensitivity. Results of this study provide evidence that obesity affects the expression of the subset of proliferation-related genes in bloodcells and that impaired insulin sensitivity in obesity is associated with changes in the expression level ofEXO1,PER1, CIDEA, ING1, DKK, PER2, andSH2B3genes, which possibly contribute to the development of obesity, insulin resistance, and glucose intolerance and may reflect, at least in part, some changes in fat tissue.

INTRODUCTION

Biological rhythms are an integral component of essentially all aspects of life, including insulin sensitivity and cellular proliferation and the altered sleep/wake patterns are associated with development of obesity and its metabolic complications, which are the most profound public health problems. The obesity is associated with dysregulation of numerous intrinsic mechanisms,
which control the most key metabolic processes, including cellular growth, apoptosis, and insulin sensitivity as well as glucose metabolism\[^{4, 5}\]. Moreover, obesity and metabolic syndrome result from interactions between genes and environmental factors and are associated with changes in gene expressions of regulatory network in adipose tissue as well as in various organs and tissues, including blood cells\[^{6-9}\]. Adipose tissue growth is in a center of obesity and tightly associated with apoptosis and cell proliferation processes and controlled by different interconnected regulatory factors and enzymes\[^{1, 4, 8}\]. Special interest deserves the key regulatory factors and enzymes, which control cell growth and survival, especially PER1, PER2, CLOCK, CIDEA, ING1, EXO1, DKK, and SH2B3\[^{10-17}\].

PER1 is a component of the circadian clock mechanism which is essential for generating circadian rhythms by interacting with other circadian regulatory proteins and transporting them to the nucleus\[^{11, 12}\]. Rhythmic expression of genes encodes period factors PER1 and PER2 defines a critical nodal point for negative feedback within the circadian clock mechanism, including CLOCK-BMAL1 transcriptional regulation\[^{10, 12}\]. Moreover, there is data that the expression of PER1 and PER2 genes in glioma cells was much lower than in the surrounding non-glioma cells and those disturbances in PER1 and PER2 gene expressions may result in the disruption of the control of normal circadian rhythm, thus benefiting the survival of cancer cells\[^{18, 19}\]. It is interesting to note that the clock-controlled gene \(\text{PER1}\) regulates the circadian rhythm of glucose absorption in the intestine, which is mediated entirely by rhythmicity in the transcription, translation, and function of the sodium glucose co-transporter SGLT1\[^{20}\]. Circadian regulator CLOCK is a histone acetyltransferase interact with HIF-1-alpha/ARNT and activate VEGF to stimulate tumor angiogenesis and metastasis\[^{21}\]. Thus, deregulated expression of the circadian genes leads to cancer growth as well as obesity and insulin resistance\[^{3, 6, 13, 18}\].

The inhibitor of growth family, member 1 (ING1) is involved in the regulation of apoptosis, suppresses tumor growth and metastasis as well as stabilizes p53 by inhibiting polyubiquitination\[^{15, 17, 22}\]. There is data that the expression of DKK1 (dickkopf WNT signaling pathway inhibitor 1) gene is involved in development through its inhibition of the WNT signaling pathway and is associated with cancer and metastasis because it is partly involved in survival of cancer cells\[^{23, 24}\]. The exonuclease 1 (EXO1) play distinct roles in essential biological processes, are required for G1 checkpoint activation\[^{25}\]. It is interesting to note that cell death-inducing DFFA-like effect or a(CIDEA) controls apoptosis and lipolysis and has relation to development of obesity and diabetes as well as cancer\[^{26, 27}\].

The endoplasmic reticulum stress is also recognized as an important determinant of obesity, insulin resistance, and impaired glucose tolerance and contributes to the expression profile of many regulatory genes resulting in peripheral insulin resistance and other obesity complications\[^{9, 28-30}\], although detailed molecular mechanisms cannot be ruled out.

It is possible that identification of real mechanisms of metabolic abnormalities in obesity as well as its complications at molecular and cellular levels helps to better understanding why obesity develops and why only a part of the obese individuals develops secondary metabolic disorders. The main goal of this study was to clarify the role of the subset of gene expressions, encoding for important cell growth factors and enzymes, which play important role in the control of cellular growth and apoptosis, in blood cells of obese boys with and without insulin resistance for evaluation of its possible significance in development of obesity and its metabolic complications.

**MATERIAL AND METHODS**

The 15 boys participate in this study. They were divided into three equal groups (5 subjects in each group): normal individuals as control and obese patients with or without insulin resistance. All participants gave written informed consent and the studies were approved by the local research ethics committees of Institute of Children and Adolescent Health Care of the National Academy of Medical Science of Ukraine.
Clinical characteristics of the study participants are shown in TABLE 1. The normal (control) participants were individuals with mean age 14 ± 0.7 years and mean body mass index (BMI) 18.7 ± 0.12 kg/m². The obese participants with normal insulin sensitivity as well as the patients with insulin resistance were individuals with mean age (14 ± 0.6 and 14 ± 0.4 years, correspondingly) and mean BMI (31.0 ± 0.40 and 34.2 ± 2.39 kg/m², correspondingly). Thus, BMI, which is a main criteria of obesity, in these last two groups of patients was significantly higher (+66 and +83 %, correspondingly; P<0.05 in both cases) as compared to control individuals TABLE 1. Moreover, no significant changes were found in insulin resistance index in obese individuals as compared to control group, but in obese patients with insulin resistance, versus control boys as well as obese subjects with normal insulin sensitivity, the insulin resistance index is significantly increased (3.7 and 3.2 fold, correspondingly; P<0.05 in both cases). Similar results were observed in the fasting insulin levels: no significant changes in obese individuals and strong increase in obese children with insulin resistance (3.3 fold; P<0.05) as compared to control group TABLE 1.

RNA isolation. Trisol reagent (Invitrogen, USA) was used for RNA extraction from blood cells of lean (control) and obese individuals with or without insulin resistance.

Reverse transcription and quantitative real-time polymerase chain reaction analysis. The expression levels of genes related to regulation of an angiogenesis (EXO1, PER1, CIDEA, ING1, DKK, CLOCK, PER2, and SH2B3) were measured in blood cells by real-time quantitative polymerase chain reaction of complementary DNA (cDNA). QuantiTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis. The 7900 HT Fast Real-Time PCR System (Applied Biosystems), Absolute QPCR SYBRGreen Mix (Thermo Scientific, UK) and pair of primers specific for each studied gene (Sigma-Aldrich, USA; TABLE 2) were used for quantitative polymerase chain reaction.

The expression of beta-actin mRNA was used as control of analyzed RNA quantity. The amplified DNA fragments were analyzed on a 2 % agarose gel and that visualized by 5x Sight DNA Stain (EUROMEDEA). An analysis of quantitative PCR was performed using special computer program “Differential expression calculator”.

Statistical analyses were performed as described previously[31]. All values are expressed as mean ± SEM from four independent experiments; P < 0.05 was considered as significant difference.

### RESULTS AND DISCUSSION

We studied the expression of the subset of genes (EXO1, PER1, CIDEA, ING1, DKK, CLOCK, PER2, and SH2B3), which control cell proliferation and apoptosis, in blood cells of three groups: lean (control) individuals, obese boys with normal insulin sensitivity and obese patients with insulin resistance for evaluation of its possible significance to development of obesity and glucose intolerance. As shown in Figure 1, the expression levels of Exo1 and CIDEA genes are decreased in blood cells of obese boys with normal glucose tolerance as compared to the group of control children, being more significant for Exo1: 7.7 fold (P < 0.001) for Exo1 and -32 % (P < 0.01) for CIDEA.

The development of insulin resistance in obese
TABLE 2: Characteristics of the primers used for quantitative real-time polymerase chain reaction

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Primer’s sequence</th>
<th>Nucleotide numbers in sequence</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIDEA</td>
<td>cell death-inducing DNA fragmentation factor alpha-like effector A</td>
<td>F: 5’- agaccttgggagacaacag R: 5’- agaaagtgcctgcaacctg</td>
<td>337–356 628–609</td>
<td>NM_001279</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 5’- gtctctatgagctgtagcg R: 5’- tgtcgctgtaatccatcc</td>
<td>753–772 1028–1009</td>
<td>NM_003686</td>
</tr>
<tr>
<td>EXO1</td>
<td>exonuclease 1; RAD2 nuclease family member</td>
<td>F: 5’- ccaagggcaagtgtcactg R: 5’- etgcactcctatgaagga</td>
<td>1601–1620</td>
<td>NM_005537</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 5’- tccgaggaagaattgaggg R: 5’- cctgaggcagtcctgaag</td>
<td>1845–1826</td>
<td>NM_012242</td>
</tr>
<tr>
<td>ING1</td>
<td>inhibitor of growth family, member 1; p33</td>
<td>F: 5’- ccaagggcaagtgtcactg</td>
<td>591–610 747–728</td>
<td>NM_005475</td>
</tr>
<tr>
<td>DKK1</td>
<td>dickkopf WNT signaling pathway inhibitor 1</td>
<td>F: 5’- ccaagggcaagtgtcactg</td>
<td>2122–2141 2330–2311</td>
<td>NM_004898</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F: 5’- gagtttgcacacacccac R: 5’- tatatggtgttgcgttt</td>
<td>3878–3897 4086–4067</td>
<td>NM_002817</td>
</tr>
<tr>
<td>PER1</td>
<td>period circadian clock 1</td>
<td>F: 5’- ccaagggcaagtgtcactg</td>
<td>6000–6019 6207–6188</td>
<td>NM_001101</td>
</tr>
<tr>
<td>PER2</td>
<td>period circadian clock 2</td>
<td>F: 5’- ccaagggcaagtgtcactg</td>
<td>747–766 980–961</td>
<td>NM_001101</td>
</tr>
</tbody>
</table>

Individuals is associated with additional down-regulation of *cidea* gene expression (-34%; P < 0.01) in blood cells as compared to the group of obese children with and normal insulin sensitivity, being more significant versus control (more than 2 fold, P < 0.001; Figure 1). At the same time, the expression level of *EXO1* gene is up-regulated (5.6 fold; P < 0.001) in blood cells of obese children with insulin resistance versus the group of obese boys with normal insulin sensitivity, but it is significantly lower relative to control group of children (-27%; P < 0.01; Figure 1).

This data has clearly demonstrated that obesity leads to significant dysregulation of *EXO1* and *CIDEA* genes in blood cells, being more robust for *EXO1* gene and that this dysregulation of these genes is possibly contributed to cell proliferation and development of obesity as well as insulin resistance. *EXO1* plays distinct roles in essential biological processes, but functional significance of changes in *EXO1* gene expression in obesity and insulin resistance remains incompletely understood and warrants further investigation. At the same time, down-regulation of *CIDEA* in blood cells of obese children with and without insulin resistance agrees with data [26, 27] that *CIDEA* controls apoptosis and lipoly-
INSULIN resistance affects the expression of the subset of proliferation

Regular Paper

Figure 1: Relative expression level of exonuclease 1 (EXO1) also known as RAD2 nuclease family member and cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA) mRNA in blood cells of lean boys (control) as well as obese individuals with normal insulin sensitivity (Obesity - IR) and obese children with insulin resistance (Obesity + IR). The values of EXO1 and CIDEA mRNA expressions were normalized to the beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (100%); n = 5.

Figure 2: Relative expression level of inhibitor of growth family, member 1 (ING1) and dickkopf 1 homolog (Xenopus laevis) (DKK1) mRNA in blood cells of lean boys (control) and obese individuals with normal insulin sensitivity (Obesity - IR) and obese children with insulin resistance (Obesity + IR). The values of ING1 and DKK1 mRNA expressions were normalized to the beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (100%); n = 5.

Thus, it has relation to development of obesity and metabolic complications.

Next we studied the expression of ING1 and DKK1 genes in blood cells of obese boys with and without insulin resistance Figure 2.

The expression levels of ING1 and DKK1 genes is significantly increased in obese children with normal insulin sensitivity versus control: +32% (P < 0.01) and almost two fold (P < 0.001), correspondingly Figure 2. However, the development of insulin resistance induces down-regulation both of these gene expressions: -27% (P < 0.01) and -39% (P < 0.01), correspondingly. Thus, the expression of both genes is increased in obesity with normal insulin sensitivity, being more robust for DKK1 gene, but reversible changes were observed in obese children.
with insulin resistance up to control level (ING1) or close to control level (DKK1). These results agree with pro-proliferative properties of DKK1, which is involved in development through the WNT signaling pathway and is associated with cancer\textsuperscript{[23, 24]}. However, the up-regulation in obesity of ING1, which can induce cell growth arrest and apoptosis\textsuperscript{[15,16]}, has not been fully understood. It is possible that \textit{ING1} and \textit{DKK1} gene expressions are insulin-dependent and development of insulin resistance are responsible for down-regulation of these genes.

As shown in Figure 3, the expression level of \textit{SH2B3/LNK} gene is increased (+18\%; \(P < 0.05\)) in blood cells of obese boys with normal insulin sensitivity as compared to control children, but the level of \textit{CLOCK} gene expression does not change significantly in this group of obese patients.

Moreover, development of insulin resistance in obese individuals is associated with down-regulation of \textit{SH2B3} gene expression (more than 2fold; \(P < 0.001\)) in blood cells as compared to the group of
children with obesity and normal insulin sensitivity up to the level much lower than in control boys (P < 0.001; Figure 3). However, the level of \textit{CLOCK} gene expression does not change significantly in blood cells of obese participants with insulin resistance as compared to the group of obese children with normal insulin sensitivity, but this level was significantly lower than in control group (-16 \%; P < 0.05). Down-regulation of \textit{SH2B3} gene expression agrees with data Perez-Garcia et al.\cite{32} that \textit{SH2B3} as signal transduction and adapter protein plays a tumor suppressor role in the pathogenesis of acute lymphoblastic leukemia. It is possible that the level of \textit{CLOCK} gene expression in peripheral blood cells incompletely reflects the changes in fat tissue but down-regulation of this gene in obese children with insulin resistance agrees with data\cite{14,33} that \textit{CLOCK} involved in glucose homeostasis and obesity.

We next tested whether obesity as well as insulin resistance also affects the expression of circadian genes \textit{PER1} and \textit{PER2} in blood cells of obese children with normal and impaired insulin sensitivity Figure 4. The expression level of these genes does not change significantly in obesity, but development of insulin resistance leads to up-regulation of and down-regulation of gene expressions: +34\% (P < 0.01) and -22 \% (P < 0.01) versus obese children without insulin resistance.

Thus, it is possible that obesity with normal insulin sensitivity does not affect significantly the circadian clock \textit{PER1} and \textit{PER2} gene expressions in peripheral blood cells, like \textit{CLOCK} gene; however, development of insulin resistance in obese children leads to significant up-regulation of \textit{PER1} and down-regulation of \textit{PER2} gene expressions. These results agree with data\cite{1,6,12} that \textit{PER1} and \textit{PER2} genes involved in glucose homeostasis in obesity and are dependent from insulin resistance and/or participate in its development.

Results of this study provide evidence that obesity affects in blood cells the expression of the subset of genes related to cellular growth and apoptosis and that impaired glucose tolerance in obese children is associated with changes in the expression level of \textit{EXO1}, \textit{CIDEA}, \textit{ING1}, \textit{DKK1}, \textit{SH2B3}, \textit{PER1}, and \textit{PER2} genes, which possibly contribute to the development of obesity and insulin resistance as well as reflect some changes in other tissues, including fat tissue.

\section*{CONCLUSIONS}

We have shown that obesity without insulin resistance affects the expression of the subset of genes in peripheral blood cells, which can contribute in metabolic abnormalities. Most of these genes are related to control of glucose metabolism and cell proliferation and its deregulation in obese children with insulin resistance possibly responsible for development of metabolic complications. However, the molecular mechanisms explaining the role of \textit{EXO1}, \textit{CIDEA}, \textit{ING1}, \textit{DKK1}, \textit{SH2B3}, \textit{PER1}, and \textit{PER2} genes in obesity and insulin resistance are not well understood and warrant further investigations.

\section*{REFERENCES}

\begin{enumerate}
\item M. S. Bray, M. E. Young; J. Biol. Chem., \textbf{286}, 11883 (2011).
\item M. S. Bray, M. E. Young; Obes. Rev., \textbf{10}(2), 6 (2009).
\item J. Kovac, J. Hutse, H. Oster; Mol. Cells, \textbf{282}, 75 (2009).
\end{enumerate}


