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

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ABSTRACT

The development of obesity and its metabolic complicationsis 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 asin lean (control) individuals. It was shown that the expression level of *ING1, DKK,* and*SH2B3*genes is increased, but*EXO1* and *CIDEA*genes is decreased in bloodcells of obese boys with normal insulin sensitivity as compared to control group. Insulin resistance in obese boys leads toup-regulation of *EXO1* and*PER1*gene expressions as well as to down-regulation of *CIDEA, ING1, DKK, PER2,*and*SH2B3*genesas 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 of *EXO1,PER1, CIDEA, ING1, DKK, PER2,*and*SH2B3*genes, 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.

KEYWORDS

Gene expressions;
Proliferation-related genes;
Human blood cells;
Obesity;
Insulinresistance.

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[1-3]. 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 andcell proliferation processes and controlled by different interconnected regulatory factors and enzymes[1, 4, 8]. Special interest deserves the key regulatory factorsandenzymes, which control cell growth and survival, especiallyPER1, PER2, CLOCK, CIDEA, ING1, EXO1,DKK,andSH2B3[10–17].

PER1 is a component of the circadian clock mechanism which is essential for generating circadian rhythms by interacting with other circadianregulatory proteins and transporting them to the nucleus[11, 12]. Rhythmic expression of genes encodes period factors PER1 and PER2defines a critical nodal point for negative feedback within the circadian clock mechanism, includingCLOCK-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 andthose 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 *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-1alpha/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 asstabilizes p53 by inhibiting polyubiquitination[15-17,22]. There is data that the expression of*DKK1*(dickkopf WNT signaling pathway inhibitor 1)geneisinvolved in development through its inhibition of the WNT signaling pathwayand 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 lipolys is and has relation to development of obesity and diabetesas 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 15boysparticipate in this study. They were divided into three equal groups (5subjectsin each group): normal individuals as control and obesepatients 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.

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TABLE 1 : Characteristics of the study participants

Variable	Control	Obesity	Obesity + IR
Age at visit (years)	14 ± 0.73	14 ± 0.6	14 ± 0.38
Body mass index (BMI) (kg/m^2)	18.7 ± 0.12	31 ± 0.40 *	34.2 ± 2.39 *
Insulin resistance index (HOMA)	2.36 ± 0.17	2.70 ± 0.28	8.70 ± 1.41*^
Fasting insulinIU/ml)	13.0 ± 0.95	14.1 ± 1.35	43.4 ± 6.70 *^
Fasting glucose (mmol/l)	4.1 ± 0.22	4.3 ± 0.14	4.5 ± 0.08
2h oral glucose tolerance test (OGTT) glucose (mmol/l)	4.28 ± 0.08	5.08 ± 0.11	5. 36 ± 0.18 *

Data are means ± SEM; IR – insulin resistance; *n* = 5;* - P < 0.05 versus control group; ^ - P < 0.05 versus obese group.

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/m^2. 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/m^2, 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<0.05in 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 (3.7and 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). QuaniTect 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 fourindependent 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*SH2B*), 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 resistancefor evaluation of its possible significance to development of obesity and glucose intolerance. As shown in Figure 1, the expression levels of *Exo1*and *CIDEA* genesaredecreasedin blood cells of obese boys with normal glucose tolerance as compared to the group of control children, being more significant for*Exo1*:7.7fold(P < 0.001) for *Exo1*and -32 %(P < 0.01) for *CIDEA*.

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TABLE 2 : Characteristics of the primers used for quantitative real-time polymerase chain reaction

Gene symbol	Gene name	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
CIDEA	cell death-inducing DNA fragmentation factor alpha-like effector A	F: 5'-agaccttgggagacaacacg R: 5'-agaaactgtcccgtcacctg	337–356 628–609	NM_001279
EXO1	exonuclease 1; RAD2 nuclease family member	F: 5'-gctccctatgaagctgatgc R: 5'-tgcctttgctaatccaatcc	753–772 1028–1009	NM_003686
ING1	inhibitor of growth family, member 1; p33	F: 5'-ccaagggcaagtggtactgt R: 5'-ctgccatccctatgaaagga	1601–1620 1845–1826	NM_005537
DKK1	dickkopf WNT signaling pathway inhibitor 1	F: 5'-tccgaggagaaattgaggaa R: 5'-cctgaggcacagtctgatga	591–610 747–728	NM_012242
SH2B3 (LNK)	SH2B adaptor protein 3 (lymphocyte-specific adapter protein Lnk)	F: 5'-aggagcacaggcagaagtgt R: 5'-agcggcatcctctaacaaga	2122–2141 2330–2311	NM_005475
CLOCK	circadian locomotor output circuits kaput	F: 5'-aggtttgatcacagcccaac R: 5'-tatcatgcgtgtccgttgtt	1605–1624 1949–1930	NM_004898
PER1	period circadian clock 1	F: 5'-caccctgatgacccactctt R: 5'-ggtaaggctggactggatga	3878–3897 4086–4067	NM_002616
PER2	period circadian clock2	F: 5'-cgtgccaagcagttgactta R: 5'-cagcaaggctcaacaaatca	6000–6019 6207–6188	NM_022817
ACTB	beta-actin	F: 5'-ggacttcgagcaagagatgg R: 5'-agcactgtgttggcgtacag	747–766 980–961	NM_001101

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 2fold, $P < 0.001$; Figure 1). At the same time, the expression level of *EXO1*gene is up-regulated (5.6fold; $P < 0.001$) 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 contributedtocell 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-

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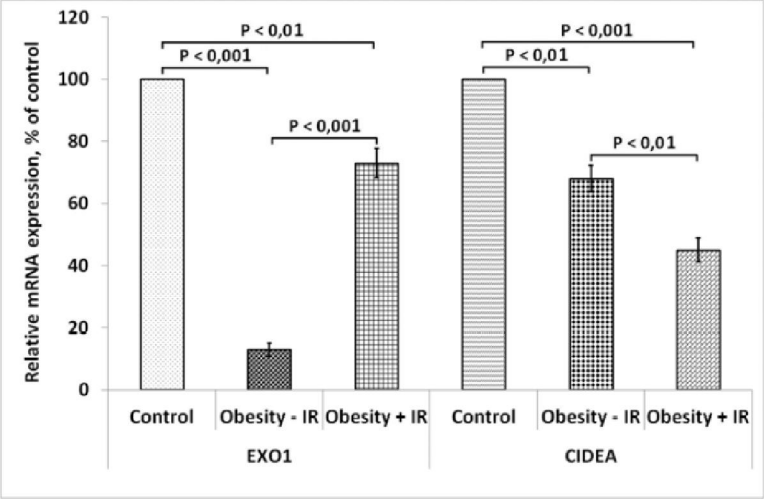

Figure 1 : Relative expression level of exonuclease 1(EXO1)also known as RAD2 nuclease family memberandcell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA) mRNA in blood cells of lean boys (control) as well as obese individuals with normal insulin sensitivity (Obesity - IR)and obese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

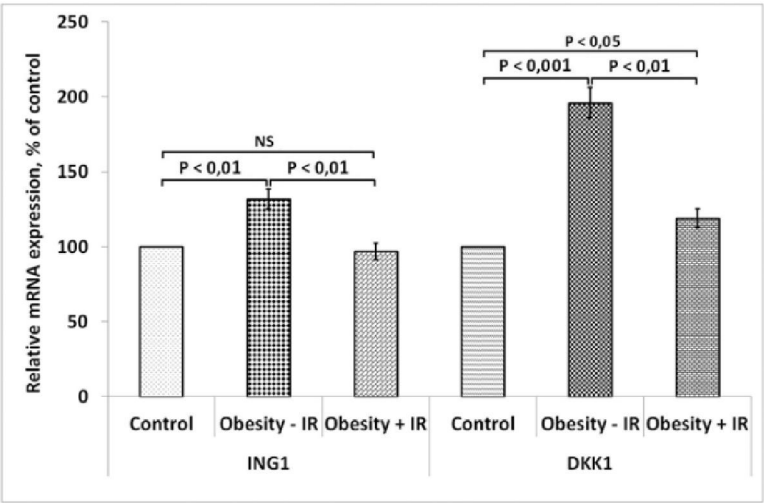

Figure 2 : Relative expression level of inhibitor of growth family, member 1(ING1)anddickkopf 1 homolog (Xenopus laevis) (DKK1) mRNA in blood cells of lean boys (control) and obese individuals with normal insulin sensitivity (Obesity - IR) and obese children with insulin resistance (Obesity + IR). The values of ING1 and DKK1mRNA expressions were normalized to the beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (100 %); *n* = 5

sis. 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 withand 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

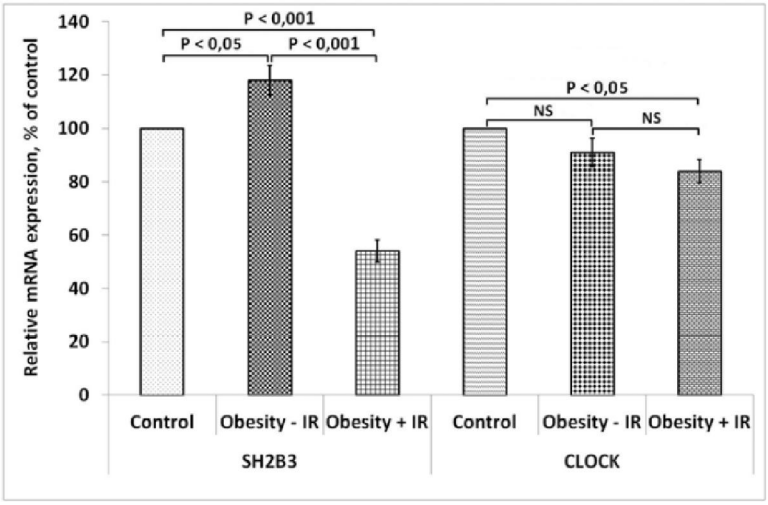

Figure 3 : Relative expression level of SH2B adaptor protein 3 (SH2B3)also known as lymphocyte-specific adapter protein LNK (LNK) andcircadian locomotor output circuits kaput(CLOCK) mRNA in blood cells of lean boys (control) and obese individuals normal insulin sensitivity (Obesity - IR) and obese children with insulin resistance (Obesity + IR). The values of SH2B3 and CLOCKmRNA expressions were normalized to the beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (100 %); *n* = 5

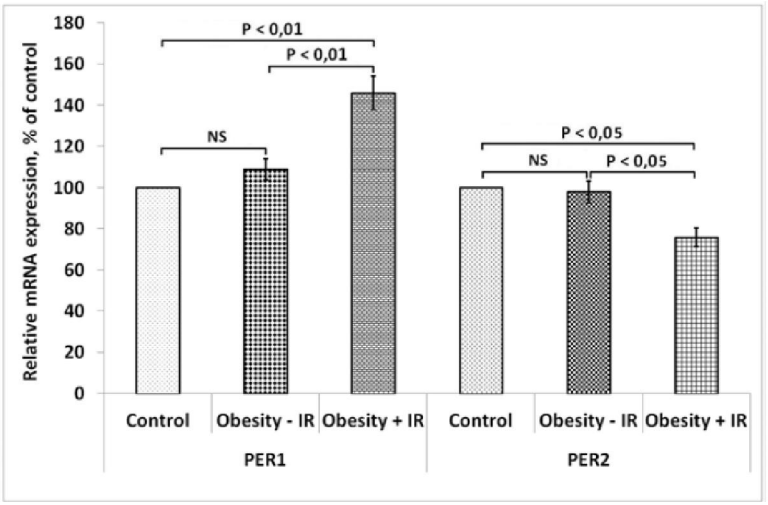

Figure 4 : Relative expression level of period 1(PER1)andperiod 2 (PER2) mRNA in blood cells of lean boys (control) and obese individuals with normal insulin sensitivity (Obesity - IR) and obese children with insulin resistance (Obesity + IR). The values of PER1 and PER2mRNA expressions were normalized to the beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (100 %); *n* = 5

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[23, 24]. However, the up-regulation in obesity of ING1, which can induce cell growth arrest and apoptosis[15,16],has not been fully understood. It is possible that *ING1* and *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 *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 *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 *SH2B3* gene expression (more than 2fold; P < 0.001) in blood cells as compared to the group of

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children with obesity and normal insulin sensitivityup to the level much lower than in control boys ($P < 0.001$; Figure 3). However, the level of *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 *SH2B3* gene expression agrees with data Perez-Garcia et al.[32] that 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 *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[14,33] that CLOCK *involved in glucose homeostasis* and obesity.

We next tested whether obesityas well as insulin resistance also affects the expression of circadian genes *PER1* and *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 *PER1* and *PER2* gene expressions in peripheral blood cells, like *CLOCK* gene; however, development of insulin resistance in obese children leads to significant up-regulation of *PER1* and down-regulation of *PER2* gene expressions. These results agree with data[1,6,12] that *PER1* and *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 *EXO1, CIDEA,ING1, DKK1, SH2B3, PER1,*and *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.

CONCLUSIONS

We have shown that obesity withoutinsulin resistance affectsthe expression of the subset of genes in peripheral bloodcells, 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 *EXO1, CIDEA,ING1, DKK1, SH2B3, PER1,*and *PER2*genes in obesity and insulin resistance are not well understood and warrant further investigations.

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