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Expression of genes, which participate in the control cell proliferation, in subcutaneous adiposet issue of the obese men with glucose intolerance

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ABSTRACT

The development of obesityand its metabolic complications, the most profound public health problems, is associated with dysregulation of different intrinsic mechanisms which control most basic metabolic processes. We have studied the expression levels of mRNA of CTGF, MYLK, MEST, PLAU, PLAT, PLAUR, SERPINE1, TPD52, ITGA3, ITGB1, ITGAM, and ITGAVfactorsrelated to the control of proliferation processes in subcutaneous adipose tissueof obese menwith and withoutimpaired glucose tolerance. It was shown that the expression levelall of these genes significantly increase in subcutaneous adipose tissue in obese menwithout impaired glucose tolerance as compared tocontrol group of lean subjects. Magnitude of obesity-induced changes in expression levels of different factorswas gene specific and more robust in the case of *CTGF* and*SERPINE1* genes. Further increase of the expression levels of PLAU, PLAUR, SERPINE1, ITGA3, andITGAV mRNAsin subcutaneous adipose tissue was found in obese patients with impaired glucose tolerance. At the same time, development of glucose intolerance in obese individuals leads to down-regulation of the expression of genes which encode forCTGF, MYLK, PLAT, and TPD52. Results of this study provide strong evidence that expression of genes mostly related to the regulation of proliferationisup-regulated in adipose tissue of obese individuals and possiblycontribute to fat tissue storage. Glucose intolerance-associated changes in expression levels of mRNA of studied genesin obese patients were more prominent in the case of CTGF, PLAUR, and SERPINE1 and possibly contribute to the development of obesity complications. ! 2014 Trade Science Inc. - INDIA

KEYWORDS

mRNA expression;
CTGF;
MYLK;
MEST;
PLAU;
PLAT;
PLAUR;
SERPINE1;
TPD52;
Integrin;
Human adipose tissue;
Obesity;
Glucose intolerance.

INTRODUCTION

Obesity is one of the most profound public health problems. Accumulating evidence raises the hypothesis that dysregulation of intrinsic clock mechanisms controlling majority of metabolic processes are involved in

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the development of obesity, metabolic syndrome and type 2 diabetes[1–4]. In obese individuals adipose tissue is at the center of metabolic complications, includingdecreased insulin sensitivity[5,6]. Obesity as well as its metabolic complicationsresult from interactions between genes and environmental. It is possible that dysregulation of different intrinsic mechanisms which control most metabolic processes are involved in the development of obesity and its metabolic complications[4,5]. Moreover, molecular and cellular studies have demonstrated that obesity is associated with enhanced cellular proliferation and is a major risk factor for cancer[7]. Obesity is inducedendoplasmic reticulum stress signaling, which contributes to the expression profile of many regulatory genes resulting in peripheral insulin resistance and obesity complications, acting by inhibiting insulin receptor signalingresponsible for augmentation of cell proliferation[8–11]. Moreover, the endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes[5,8].

Adipose tissue growth is tightly associated with increased expression and activity ofmultiple growth factors. Special interest represents growth factors and its receptors as well as matrix proteins,which are linked to human obesity and metabolic complications because to regulate cell growth[12-20]. Thus, it was shown that tumor growth and neovascularization correlated with connective tissue growth factor (CTGF)and thrombospondin-1 (THBS1), besides that both CTGF and THBS1 are predicted targets for repression by the miR-17-92 microRNA[14]. At the same time, connective tissue growth factor can binds vascular endothelial growth factor-A (VEGFA) and angiogenic activity of this VEGF is inhibited in the VEGFA-CTGF complex form; however, stability of this complex as well as the angiogenic activity of VEGF depends from matrix metalloproteinases and its inhibitors[15].

Moreover, mesoderm specific transcript (MEST) may play a role in development and the loss of imprinting of this gene has been linked to certain types of cancer[18]. The expression levels of miR-335 significantly correlated with those of MEST, supporting the notion that the intronic miR-335 is co-expressed with its host gene and may be due to promoter switching. Multifunctional serine/threonine protein kinase MYLK (myosin light chain kinase, MLCK) has been shown to control the growth initiation and promotes cell migration. Myosin light chain kinase functions downstream of Ras/ERK to promote migration of urokinase-type plasminogen activator-stimulated cells in an integrin-selective manner[19]. This protein kinase is responsible for high proliferative ability of breast cancer cells and plays a role in the regulation of cell survival; however, no data concerning fat tissue.

Recently was shown that the expression of urokinase-type plasminogen activator (PLAU) and plasminogen activator inhibitor-1 (SERPINE1 or PAI-1) is up-regulated in co-cultures with squamous cell carcinoma cells but not with normal oral keratinocytes[16]. These results demonstrate that head and neck squamous cell carcinoma cells regulate the expression of PLAU and SERPINE1. Moreover, domain 2 of PLAUR regulates single-chain urokinase-mediated angiogenesis through beta1-integrin and VEGF receptor-2 (VEGFR2)[17].

Integrins are heterodimeric integral membrane proteins composed of an alpha chain and a beta chain, and function as cell surface adhesion molecules. Integrin alpha3 subunit joins with beta 1 subunit to form an integrin that interacts with many extracellular-matrix proteins. Integrin alpha-3/beta-1 is a receptor for fibronectin, laminin, collagen, epiligrin, and thrombospondin andcontributes to the invasive nature of glioma stem-like cell via ERK1/2[20]. Integrin alpha5/beta1 overexpression is associated with lymph node metastasis and vascular invasion in cervical cancer[21].

Tumor protein D52 (TPD52), which also known asprostate leucine zipper (PrLZ),is expressed in multiple cancers and elevates the phosphorylation of AKT and STAT3 as well asupregulates BCL2 expression[22].

However, a detailed molecular mechanism of the development of obesity and its complications in some representatives of obese individuals is not yet clear and remains to be determined.

The main goal of this work was to study the role of the expression of genes related to the regulation of proliferation (*CTGF, MYLK, MEST, PLAU, PLAT, PLAUR, SERPINE1, TPD52, ITGA3, ITGB1, ITGAM*, and*ITGAV*)in subcutaneous adipose tissue of obese individuals as well as obese patients with impaired glucose tolerance for evaluation of its significance to the development of human obesity and its metabolic

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complications.

MATERIALS AND METHODS

Patients' characteristics

The 18 male subjectsparticipate in this study. They were divided into three equal groups (6 men in each group): lean individuals as control and patients with obesity and with or withoutglucose intolerance.

Subjects studied were recruited from the patients' cohort within the LipidomicNet Project at Institute of Experimental Endocrinology Slovak Academy of Sciences. All participants gave written informed consent and the studies were approved by the local research ethics committees of Institute of Experimental Endocrinology.

Clinical characteristics of the study participants are shown in TABLE 1. The lean (control) participants were individuals with mean age 45 ± 8 years and mean body mass index (BMI) 23 ± 1.4 kg/m^2. The obese participants with normal glucose tolerance as well as the patients with glucose intolerancewere individuals with mean age (45 ± 8 and 44 ± 7 years, correspondingly) and mean BMI (32 ± 1.4 and 34 ± 1.4 kg/m^2, correspondingly). Thus, BMI, which is a main criteria of obesity, in these last two groups of patients was significantly higher (+39 and +48 %, correspondingly; P<0.05) as compared to lean individuals (TABLE 1).

Moreover, obese individuals have significantly lower insulin sensitivity index (-35 %; P<0.05). In obese patients with impaired glucose tolerance, versus obese subjects with normal glucose tolerance, the 2h oral glucose tolerance test (OGTT) andfasting insulin levels significantly increased (+47 and +62 %, correspondingly; P<0.05), but decreased insulin sensitivity index (almost two fold; P<0.05) (TABLE 1). This data clearly demonstrated that a group of obese patients with impaired glucose tolerancehas insulin resistance.

RNA isolation

RNasy Lipid Tissue Mini Kit (QIAGEN, Germany) was used for RNA extraction from subcutaneous adipose tissue of lean and obese individuals with normal or impaired glucose tolerance.

Reverse transcription and quantitative real-time polymerase chain reaction analysis

The expression levels of genes related to regulation of an angiogenesis (*CTGF, MYLK, MEST, PLAU, PLAT, PLAUR, SERPINE1, TPD52, ITGA3, ITGB1, ITGAM*, and*ITGAV*)were measured in subcutaneous fat tissue 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, USA) were used for quantitative polymerase chain reaction. Sequences of these primers are shown in TABLE 2.

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".

TABLE 1 : Characteristics of the study participants.

Variable	Lean, NGT	Obese, NGT	Obese, IGT
Age at visit (years)(*n*)	45 ± 8.3 (6)	45 ± 7.4 (6)	44 ± 7.8 (6)
Body mass index (BMI) (kg/m^2); (*n*)	23 ± 1.4 (6)	32 ± 1.4 * (6)	34 ± 1.4 * (6)
Fasting glucose (nmol/l) (*n*)	4.5 ± 0.22 (6)	5.0 ± 0.55 (6)	5.5 ± 0.65 (6)
2h oral glucose tolerance test (OGTT) glucose (nmol/l) (*n*)	5.08 ± 1.40 (5)	5.31 ± 1.26* (5)	7.83 ± 0.9*^ (6)
Insulin sensitivity index (T; mg/kg/min) (*n*)	7.9 ± 1.41 (6)	5.1 ± 1.65 * (6)	2.7 ± 0.42 *^ (5)
Fasting triglycerides (nmol/l) (*n*)	1.0 ± 0.47 (6)	1.36 ± 0.49 (6)	2.2 ± 1.07 (6)
Fasting insulin (IU/ml) (*n*)	8.0 ± 2.8 (3)	9.37 ± 1.6 (3)	15.2± 2.3 *^ (4)

Data are means ± SEM; NGT – normal glucose tolerance; IGT –impaired glucose tolerance; * - P < 0.05 vs control (lean group); ^ - P < 0.05 vs obese (NGT) group

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

Gene symbol	Primer's sequence	Nucleotide numbers in sequence	GenBank accession number
CTGF	F: 5'- ACTGTCCCGGAGACAATGAC R: 5'- TGCTCCTAAAGCCACACCTT	1189 – 1208 1527 – 1508	NM_001901
MYLK	F: 5'- CCCGTGCTAGGAACTGAGAG R: 5'- TTCTCGCTGTTCTCCACCTT	1249 – 1268 1487 – 1468	NM_005965
MEST	F: 5'- TTGGCTTCAGTGACAAACCG R: 5'-TGACAGCACACCTCCATCTT	576 – 595 859 – 840	NM_002402
TPD52	F: 5'- TGTTGGCTCAGTCATCACCA R: 5'-TTTTCTGGAAGAGGCTCCGT	506 – 515 694 – 675	NM_005079
PLAU	F: 5'- TCACCACCAAAATGCTGTGT R: 5'- AGGCCATTCTCTTCCTTGGT	1210 – 1229 1432 – 1413	NM_002658
PLAUR	F: 5'- GCCTTACCGAGGTTGTGTGT R: 5'- TGTTGCAGCATTTCAGGAAG	486 – 505 809 – 790	NM_002659
PLAT	F: 5'- CAGCAGGCCCTGTACTTCTC R: 5'- GGCTTTGAGTCTCGATCTGG	501 –520 772 – 753	NM_000930
SERPINE1	F: 5'-CTCTCTCTGCCCTCACCAAC R: 5'- GTGGAGAGGCTCTTGGTCTG	954 – 973 1165 – 1146	NM_000602
ITGA3	F: 5'-GCCTGCCAAGCTAATGAGAC R: 5'-AGAAGCTTTGTAGCCGGTGA	2547 – 2566 854 – 835	NM_005501
ITGB1	F: 5'- CGAGGTCATGGTTCATGTTG R: 5'- CAGTGTTGTGGGATTTGCAC	2354 – 2373 2793 – 2774	NM_002211
ITGAM	F: 5'- ATCTCAACTTCACGGCCTCA R: 5'-ACGGGATGTCACACTGGATT	2911 – 2930 3204 – 3185	NM_000632
ITGAV	F; 5'- GTGGTGCTGTCTACCTCTGT R:5'-TCAGTGGCTCCTTCTCTGTG	346 – 365 576 – 557	NM_002205
ACTB	F; 5'- GGACTTCGAGCAAGAGATGG R: 5'-AGCACTGTGTTGGCGTACAG	747 – 766 980 – 961	NM_001101

Statistical analysis

Statistical analyses were performed according to Student's *t*-test using OriginPro 7.5 software. All values are expressed as mean ± SEM from six independent experiments;$p < 0.05$ was considered as significantdifference.

RESULTS

In this study we have analyzed the expression levels of genes encoded different factors, which participate in the regulation of cell proliferation:CTGF (connective tissue growth factoralso known as insulin-like growth factor-binding protein 8, IGFBP8) andMYLK (myosin light polypeptide kinase), TPD52 (tumor protein D52also known as prostate leucine zipper, PrLZ)and MEST (mesoderm specific transcript), PLAU (plasminogen activator, urokinase)and PLAUR (plasminogen activator, urokinase receptor), PLAT (tissue plasminogen activator)and SERPINE1 (serpin peptidase inhibitor, clade E also known as nexin and plasminogen activator inhibitor type 1) as well as several subunit of integrin: ITGA3 (integrin, alpha 3 or antigen CD49C)and ITGB1 (integrin, beta 1 or antigen CD29),ITGAM (integrin, alpha M)and ITGAV (integrin, alpha 5)in adipose tissue from three groups of participants: lean (control), obese with normal glucose tolerance test (NGT) and obese with impaired glucose tolerance (IGT).

Expression of *CTGF, MYLK, PLAU,* and *PLAUR* genes in subcutaneous adipose tissue of obese individuals with normal and impaired glucose tolerance

As shown in Figure 1, the expression level of CTGF and MYLKmRNA in subcutaneous adipose tissue of obese individuals with normal glucose tolerance significantly increases as compared to the control (lean) subjects: +445 % and +58 %, correspondingly ($P < 0.05$). At the same time, in obese patients with impaired glucose tolerance (IGT) the expressionof CTGF and MYLK mRNA was also higher ascompared to the lean subjects (+445 % and +58 %, correspondingly; $P < 0.05$), but loweras compared to the obese individuals

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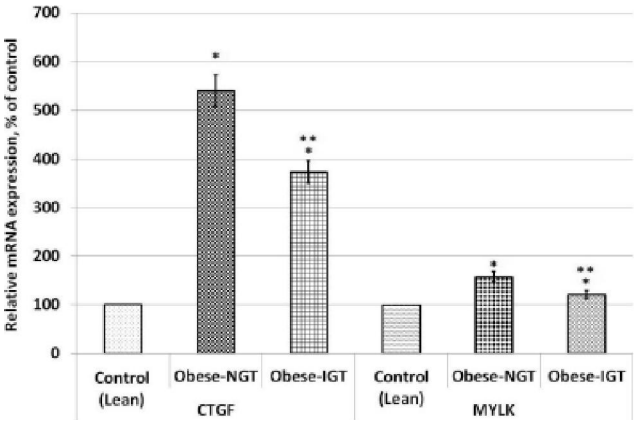

Figure 1 : The expression level of CTGF (connective tissue growth factor also known as insulin-like growth factor-binding protein 8, IGFBP8) and MYLK (myosin light polypeptide kinase) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of CTGF and MYLK mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of control individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

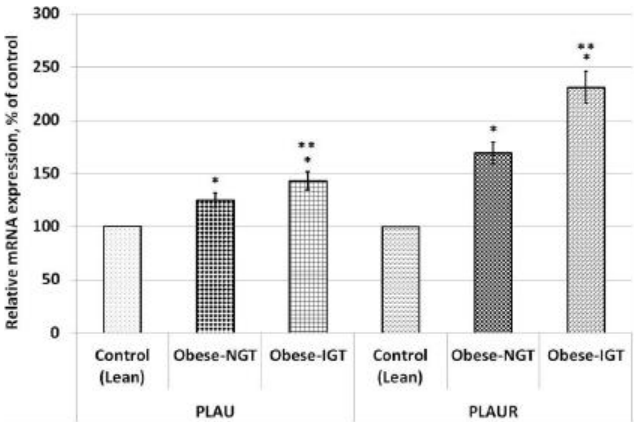

Figure 2 : The expression level of PLAT (tissue plasminogen activator) and MEST (mesoderm specific transcript) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of PLAT and MEST mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of control individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

with normal glucose tolerance(-31 % and -23 %, correspondingly; P < 0.05).

Significantly less changes were observed in the expression level of PLAU mRNA in subcutaneous adipose tissue of obese patients with normal glucose tolerance (+25 %; P < 0.05) as compared to control group; however, in obese individuals with impaired glucose tolerance the increase of this gene expression was more pronounced (+43 %; P < 0.05)as compared to lean patients and +24 % (P < 0.05) as compared to obese individuals with NGT (Figure 2). At the same time, more significant changes were found inPLAURmRNA expression in adipose tissue of obese individuals with normal glucose tolerance(+69 %; P < 0.05) as compared to the control subjects (Figure 2). Moreover,impaired glucose tolerance in obese men is associated with more pronounced (+131 %; P < 0.05) induction of PLAUR mRNA expression in adipose tissue as compared to control men and (+37 %; P < 0.05) versus obese individuals with NGT.

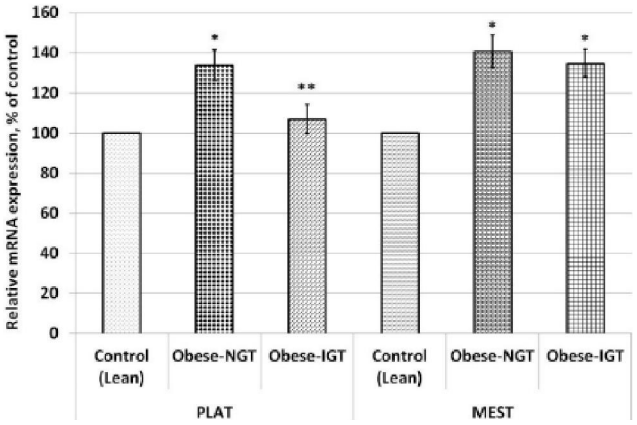

Figure 3 : The expression level of PLAU (plasminogen activator, urokinase)and PLAUR (plasminogen activator, urokinase receptor) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of PLAU and PLAUR mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of control individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

Expression of *PLAT, MEST, TPD52*, and *SERPINE1* genes insubcutaneous adipose tissue of obese individuals with normal and impaired glucose tolerance

As shown in Figure 3, in subcutaneous adipose tissue of obese men with normal glucose tolerancethe expression level of *PLAT* and *MEST*genes is also increases (+34 % and +41 %, correspondingly; P < 0.05) as compared to lean individuals; however, in obese pa-

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tients with impaired glucose tolerance the expression level of *PLAT* gene decreases (-20 %; P < 0.05) in comparisonto the group of obese subjects with normal glucose tolerance. At the same time, no significant changes were found in *MEST*gene expression in subcutaneous adipose tissue of obese individuals with glucose intoleranceversus obese men with normal glucose tolerance (Figure 3).

We have also studied the expression level of a gene, which encodesfor SERPINE1,and have shown that its expression strongly increases in subcutaneous adipose tissue both in the group of obese men with normal glucose tolerance (+440 %, P < 0.05) and glucose intolerance: (+621 %, P < 0.05, as compared to the group of lean individuals (Figure 4).

Thus, development of glucose intolerance in obese individuals is associated with additional increase of SERPINE1 mRNA expression (+33 %, P < 0.05). In Figure 4 is also shown that in subcutaneous adipose tissue of obese men with normal glucose tolerance the expression level of TPD52 mRNA is significantly increased(+70 %, P < 0.05). At the same time, in obese individuals with impaired glucose tolerance we have found that TPD52 mRNA expression level is significantly less as compared to obese men with normal glucose tolerance (-12 %, P < 0.05).

Expression of integrin genes in subcutaneous adipose tissue of obese individuals with normal and impaired glucose tolerance

We have also shown that obesity affects the expression of different integrin genesin subcutaneous adipose tissue (Figure 5 and 6). Thus, the expression level of *ITGA3*and *ITGB1* genesis significantly increased in obese patients with normal glucose tolerance versus lean individuals(+63 % and +48 %, correspondingly, in both cases P < 0.05). Development of glucose intolerance in obese individuals is associated with additional increase of ITGA3 mRNA expression (+27 %, P < 0.05) in subcutaneous adipose tissue without significant changes in *ITGB1* gene expression (Figure 5).

It was also found that changes in*ITGAV* geneexpression are similar to that for*ITGA3* gene (Figure 5 and 6). Thus, the expression level of *ITGAV* gene is significantly increased in obese patients with normal glucose tolerance (+68 %, P < 0.05) versus lean individuals. Moreover, development of glucose intolerance in obese patients leads to additional increase in the expression level of ITGAV mRNA in adipose tissue as

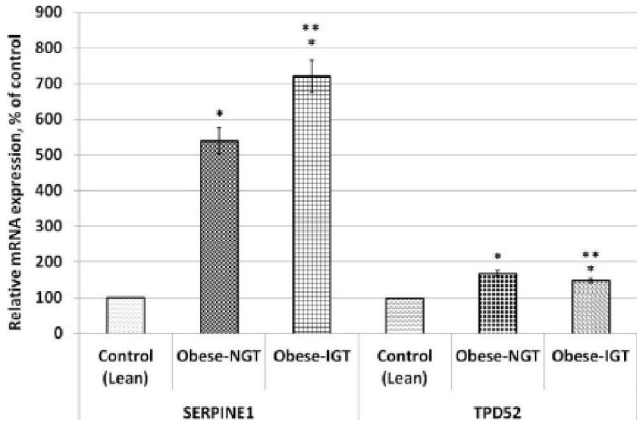

Figure 4 : The expression level of TPD52 (tumor protein D52 also known as prostate leucine zipper, PrLZ) and SERPINE1 (serpin peptidase inhibitor, clade E also known as nexin and plasminogen activator inhibitor type 1) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of TPD52 and SERPINE1 mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of l control individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

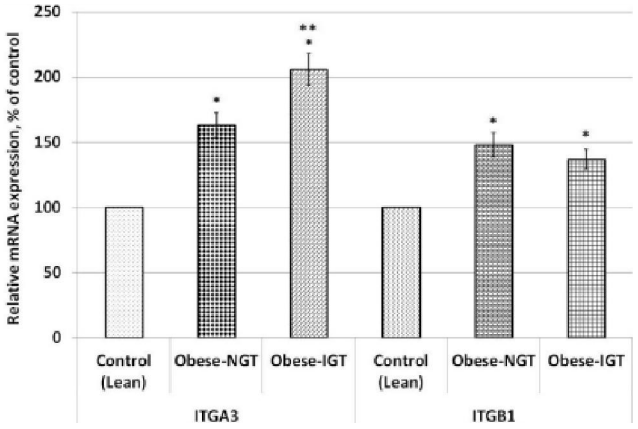

Figure 5 : The expression level of fibronectin receptor subunit ITGA3 (integrin, alpha 3 or antigen CD49C) and ITGB1 (integrin, beta 1 or antigen CD29) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of ITGA3 and ITGB1 mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of lean individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

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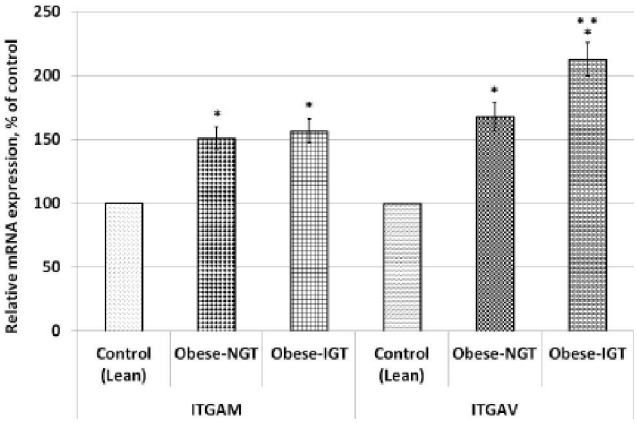

Figure 6 : The expression level of fibronectin receptor subunit ITGAM (integrin, alpha M) and ITGAV (integrin, alpha 5) mRNA in subcutaneous adipose tissue of lean men (control) and obese individuals with and without impaired glucose tolerance. The values of ITGAM and ITGAV mRNA expressions were normalized to the expression of beta-actin mRNA and are expressed as mean ± SEM and represented as a percent of control (Lean, 100 %); *n* = 6; * - P < 0.05 vs group of control individuals; ** - P < 0.05 vs group with obesity and normal glucose tolerance test (NGT).

compared to group of obese individuals with normal glucose tolerance (+25 %, P < 0.05). At the same time,the changes in *ITGAM* gene expression, other member of integrin gene family, is similar to that for *ITGB1* gene (Figure 5 and 6). Thus, the expression level of *ITGAM* gene is increased in subcutaneous adipose tissue of obese patients with normal glucose tolerance (+51 %, P < 0.05) as compared to control individuals; however, no significant changes were found in this gene expression in obese individuals with glucose intoleranceversus obese men with normal glucose tolerance (Figure 6).

DISCUSSION

In this study, we have demonstrated that obesity is associated with up-regulation of genes, which encodeimportant regulatory factors involved in the development of the obesity and its complications, the most profound public health problems. We have studied the expression of genes mostly related to up-regulation of proliferative processes in subcutaneous adipose tissue of obese individuals with normal glucose tolerance as well as obese patients with glucose intolerance. The results of our investigationclearly demonstrated that obesity leads to a significant increase of the expression of *CTGF, MYLK, MEST, PLAU, PLAT, PLAUR, SERPINE1, TPD52, ITGA3, ITGB1, ITGAM,* and *ITGAV*genes in subcutaneous adipose tissue, besides that, the most significant increase was shown for *CTGF*and*SERPINE1*genes. These findings are largely consistent with data from other studies about the involvement of these genes in the regulation of different proliferative processes and angiogenesis[7,15,16,18,19,21]. Hence, it is possible that these genes are also involved the development of obesity, metabolic complications as well as in the development of insulin and VEGF resistance[8,13,14,17,20,23]. Moreover, the endoplasmic reticulum stress also plays an important role in the regulation of different metabolic and physiological processes including proliferation as well asthe development of obesity, insulin resistance and glucose intolerance[5,7,9,11,24,25].

It is interesting to note that proliferation, like many other biological processes, is regulated by complex network of different factors which are tightly interconnected. Thus, MYLK, a multifunctional serine/threonine protein kinase, has been shown to control the growth initiationand functions downstream of RAS/ERK as well as promotes cell migration[19]. We have shown that MYLK mRNA expression is increased in subcutaneous fat tissue of obese men and this data correlates with fat tissue growth and increasing of BMI. Moreover, development of glucose intolerance in obese individuals is not accompanying by an additional increasing of BMI as well as additional increasing of MYLK mRNA expression. Thus, the MYLK is possibly involved in obesity but not inthe development of impaired glucose tolerance.

It is possible thatCTGF, MESTand TPD52are also included in this network and the balance between different regulatory factors which participate in the control of proliferationin obesity, becausethese factors regulate cell growth and the expression of its mRNA is also increased in adipose tissue as well ascorrelate with fat tissue growth and increasing of BMI. Moreover, in obese individuals with glucose intolerance no significant changes in MEST mRNA expression as well as in BMI were found. At the same time, the expression level of CTGFand TPD52 mRNA is decreased in obesity with glucose intolerance and associated withinsulin resistance. It is possible that the expression of these genes as well as *MYLK* gene is regulated by insulin and development

of insulin resistance leads to suppression of these gene expressions.

We have also shown that the expression of PLAU, PLAUR, SERPINE1 and integrins, which are associated with enhanced proliferation[17,20,21], is increased in adipose tissue of obese men and possibly contributes to fat tissue growth upon obesity; however, this regulatory mechanisms remains not clear yet and warrants further investigation. Moreover,an additional increase of *PLAU, PLAUR, SERPINE1* as well as *ITGA3* and *ITGAV* gene expressions was shown for subcutaneous adipose tissue of obese individuals with impaired glucose tolerance. This dataclearly demonstrates that the expression of these genes is associated with glucose intoleranceas well asinsulin resistance and possibly contributes to the development of obesity complications. Collectively, these results demonstrate the important role of CTGF,SERPINE1 andother regulatory growth factors with pleiotropic function in developing of obesity and its complications, especially glucose intolerance and insulin resistance.

CONCLUSIONS

Results of this study provide strong evidence that expression of genes encodedthe important regulatory factors, which related to the control of proliferation, is up-regulated in subcutaneous adipose tissue of the obese individuals with normal glucose tolerance and possibly contributes to fat tissue storage. At the same time, in obese patients with insulin resistance and glucose intolerance the changes in the expression of studied genes are different. In obese individuals with impaired glucose tolerance was found an additional increase insubcutaneous adipose tissuethe expression of*PLAU, PLAUR, SERPINE1*, *ITGA3*, and *ITGAV* genes only. Collectively, results of this study underscore the crucial role of someregulatory growth factors in developing of obesity and its complication: insulin resistance and glucose intolerance.

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