Glucose tolerance in obese men is associated with dysregulation of some angiogenesis-related gene expressions in subcutaneous adipose tissue

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Obesity and its metabolic complications are one of the most profound public health problems and result from interactions between genes and environmental. The development of obesity is tightly connected with dysregulation of intrinsic gene expression mechanisms controlling majority of metabolic processes, which are essential for regulation many physiological functions, including insulin sensitivity, cellular proliferation and angiogenesis. Our objective was to evaluate if expression of angiogenesis related genes VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2, HIF1A, and EPAS1 at mRNA level in adipose tissue could participate in the development of obesity and metabolic complications. We have shown that expression level of VEGF-A, PDGFC, FGF2, and FGFRL1 genes is decreased in adipose tissue of obese men with normal glucose tolerance (NGT) versus a group of control subjects. At the same time, in this group of obese individuals a significant up-regulation of CYR61, FGF1, FGFR2, E2F8, BAI2, and HIF1A gene expressions was observed. Impaired glucose tolerance (IGT) in obese patients associates with down-regulation of CYR61 and FGFR2 mRNA and up-regulations of E2F8, FGF1, FGF2, VEGF-A and its splice variant 189 mRNA expressions in adipose tissue versus obese (NGT) individuals. Thus, our data demonstrate that the expression of almost all studied genes is affected in subcutaneous adipose tissue of obese individuals with NGT and that glucose intolerance is associated with gene-specific changes in the expression of E2F8, FGF1, FGF2, VEGF-A, CYR61 and FGFR2 mRNAs. The data presented here provides evidence that VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2, and HIF1A genes are possibly involved in the development of obesity and its complications.
Key words: mRNA expression, VEGF-A, CYR61, FGF1, FGF2, FGFR2, E2F8, HIF1A, adipose tissue, obesity, men

INTRODUCTION

Accumulating evidence raises the hypothesis that development of obesity and its metabolic complications is tightly associated with dysregulation of mechanisms controlling majority of metabolic processes, especially in adipose tissue, which is at the center of metabolic syndrome in obese individuals [1-3]. Obesity and its metabolic complications are one of the most profound public health problems and result from interactions between genes and environmental. There is data that development of obesity is connected with dysregulation of numerous gene expressions mainly through biological clock because biological rhythms are an integral component of essentially all aspects of life and regulate many physiological functions, including insulin sensitivity and cellular proliferation [4-7]. Molecular and cellular studies have demonstrated relationships between the dysfunction of circadian clocks and the development of obesity and metabolic abnormalities, including type 2 diabetes [8]. At the same time, metabolic processes feed-back into the circadian clock, affecting clock gene expression and timing of behavior [9].

The growing adipose tissue is associated with cell proliferation and angiogenesis like

other growing tissues including tumors [10-12]. It is well known that angiogenesis is mediated by numerous factors, but substantial evidence accumulating over the last decade has established the fundamental role of vascular endothelial growth factor (VEGF) as a key regulator of normal and abnormal angiogenesis evidence especially in tumors [13-15]. Importantly, VEGF is released by tumor cells and induces neovascularization in the majority of human tumors. Furthermore, VEGF is implicated in intraocular neovascularization associated with diabetic retinopathy [16]. In addition, there are factors, such as connective tissue growth factor (CTGF), which can bind VEGF and inhibits VEGF-induced angiogenesis [17]. Moreover, downregulation of CTGF by the miR-17-92 microRNA enhances neovascularization [17].

An important role in the control of angiogenesis also play cysteine-rich angiogenic inducer 61 (CYR61), brain-specific angiogenesis inhibitor 2 (BAI2), platelet derived growth factor C (PDGFC), fibroblast growth factors and their receptors, and some transcription factors like hypoxia inducible factor 1α (HIF1A), endothelial PAS domain protein 1 (EPAS1/HIF2A), and E2F8 (transcription factor of E2F family) [18-25]. Thus, CYR61 and PDGFC play an important role in proliferation and angiogenesis [18, 20]. Fibroblast growth factor proteins (FGF1 and FGF2), which also known as acidic and basic fibroblast growth hormones, are mitogenic signaling molecules that have roles in angiogenesis and function as a modifier of endothelial cell migration and proliferation, but FGF2 mainly induces lymphangiogenesis [21-23]. Moreover, FGF1 is required for adaptive adipose remodeling and metabolic homeostasis and FGF2 prevents cancer cells from endoplasmic reticulum stress-mediated apoptosis [21, 23]. Biological effect of the fibroblast growth factors is realized through the fibroblast growth factor receptors, some of which are related to VEGF signaling pathway [24, 25]. Thus, FGFR2 (fibroblast growth factor receptor 2) interacts predominantly with FGF1 and FGF2,

but FGFRL1 (fibroblast growth factor receptor-like 1) protein expression was lower in bladder tumors compared to normal tissue. Furthermore, a marked difference between FGFRL1 and the other family members is its lack of a cytoplasmic tyrosine kinase domain and potentially inhibit signaling.

There is also data that transcription factor E2F8 creates complexes with other members of E2F family of transcription factors and coordinates various cellular functions through the regulation of the expression of target genes, thereby regulating cell cycle, apoptosis, and angiogenesis, including transcriptional activation of VEGFA in cooperation with HIF1 [26-28]. There is data [29] that metabolic reprogramming of cancer cells without tumor suppressor LKB1 is realized through HIF1 [29]. Furthermore, an important role in metabolic integration plays the unfolded protein response/endoplasmic reticulum stress, which activates a complex set of signaling pathways and links obesity with insulin action and contributes to the expression profile of many regulatory genes resulting in peripheral insulin resistance and diabetic complications, acting by inhibiting insulin receptor signaling [30–33].

However, detailed molecular mechanisms of the involvement of angiogenesis-related genes in the development of obesity and its complications are not clear yet and remain to be determined. The aim of this study was to investigate the expression of genes encoded key angiogenesis-related factors in subcutaneous adipose tissue of obese men with normal and impairment glucose tolerance for evaluation of possible significance these genes in the development of obesity and its complications, particularly glucose intolerance.

METHODS

The 18 adult males participated in the study. They were divided into three equal groups: 6 lean healthy controls, 6 obese with normal glucose tolerance (NGT), and 6 obese with impaired glucose tolerance (IGT). Clinical cha-

racteristics of the study participants are shown in Table 1. All participants gave written informed consent and the studies were approved by the local research ethics committees of Institute of Experimental Endocrinology Slovak Academy of Sciences.

RNA was extracted from subcutaneous adipose tissue samples using RNasy Lipid Tissue Mini Kit (QIAGEN, Germany) according to manufacturer's protocol. The RNA pellets were washed with 75 % ethanol and dissolved in nuclease-free water. For additional purification RNA samples were re-precipitated with 95 % ethanol and re-dissolved again in nuclease-free water. QuaniTect Reverse Transcription Kit (QIAGEN, Germany) was used for cDNA synthesis according to manufacturer protocol.

The expression levels of *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2, HIF1A,* and *EPAS1* genes were measured in subcutaneous adipose tissue by quantitative PCR (qPCR). The 7900 HT Fast Real-Time PCR System (Applied Biosystems), Absolute QPCR SYBRGreen Mix (Thermo Scientific, UK) and pair of specific primers (Sigma-Aldrich, USA) were used. For amplification all splice variants of VEGF-A (vascular endothelial growth factor A) cDNA we used next forward and reverse primers: 5'– CGAAACCATGAACTTTCTGC –3' and 5'– CCTCAGTGGGCACACACTCC –3', correspondingly. The nucleotide sequences of these primers correspond to sequences 1032 – 1051 and 1333-1314 of human VEGF-A cDNA (GenBank accession number NM_003376). The size of amplified fragment is 302 bp. The amplification of VEGF-A splice variant 189 cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– CCCACTGAGGAGTCCAACAT –3' and reverse – 5'– TTTCTTGCGCTTTCGTTTTT –3'. The nucleotide sequences of these primers correspond to sequences 1317 – 1336 and 1502 – 1483 of human VEGF-A cDNA (GenBank accession number NM_003376). The size of amplified fragment is 186 bp. For amplification of E2F8 (E2F transcription factor 8) cDNA

we used next forward and reverse primers: 5'– CCACCACAGCAAATATCGTF –3' and 5'– CTTTGGCCTCAGGTAATCCA –3', correspondingly. The nucleotide sequences of these primers correspond to sequences 596 – 615 and 805 – 786 of human E2F8 cDNA (GenBank accession number NM_024680). The size of amplified fragment is 210 bp. The amplification of EPAS1 (endothelial PAS domain protein 1), also known as hypoxia-inducible factor 2alpha (HIF-2α), cDNA for real time RCR analysis was performed using two oligonucleotides primers: forward – 5'– AAGCCTTGGAGGGTTTCATT –3' and reverse – 5'– TCATGAAGAAGTCCC-GCTCT –3'. The nucleotide sequences of these primers correspond to sequences 788 – 807 and 1021 – 1002 of human EPAS1 cDNA (GenBank accession number NM_001430). The size of amplified fragment is 234 bp. For amplification of FGF1 (fibroblast growth factor 1) cDNA we used forward (5'– CTGCAGTAGCCTGGAGGTTC –3' and reverse (5'– GGCTGTGAAGGTGGT-GATTT –3') primers. The nucleotide sequences of these primers correspond to sequences 3 – 22 and 199 – 180 of human FGF1 cDNA (GenBank accession number NM_000800). The size of amplified fragment is 198 bp. For amplification of FGF2 (fibroblast growth factor 2) cDNA we used forward (5'– AGAGCGACCCTCACATCAAG –3' and reverse (5'– ACTGCCCAGTTC-GTTTCAGT –3') primers. The nucleotide sequences of these primers correspond to sequences 571 – 590 and 804 – 785 of human FGF2 cDNA (GenBank accession number NM_002006). The size of amplified fragment is 234 bp. For amplification of FGFR2 (fibroblast growth factor receptor 2) cDNA we used forward (5'– GTGCTTGGCGGGTAATTCTA –3' and reverse (5'– TACGTTTGGTCAGCTTGTGC –3') primers. The nucleotide sequences of these primers correspond to sequences 1325 – 1344 and 1566 – 1547 of human FGFR2 cDNA (GenBank accession number NM_001144918). The size of amplified fragment is 242 bp. For amplification of FGFRL1 (fibroblast growth factor receptor-like 1) cDNA we used forward

(5'– CAGCCTGAGCGTCAACTACA –3' and reverse (5'– CTCATCTTGGAGGGCTGTGT –3') primers. The nucleotide sequences of these primers correspond to sequences 340 – 359 and 489 – 470 of human FGFRL1 cDNA (GenBank accession number NM_021923). The size of amplified fragment is 150 bp. For amplification of PDGFC (platelet derived growth factor C), also known as VEGFE, cDNA we used forward (5'– CTCCTGGTTAAACGCTGTGG –3' and reverse (5'– TATCCTCCTGTGCTCCCTCT –3') primers. The nucleotide sequences of these primers correspond to sequences 1314 – 1333 and 1528 – 1509 of human PDGFC cDNA (GenBank accession number NM_016205). The size of amplified fragment is 215 bp. For amplification of BAI2 (brain-specific angiogenesis inhibitor 2) cDNA we used forward (5'– CATTGTCCTGGTGAACATGC –3' and reverse (5'– TGCACAGCAGTGATGACAAA –3') primers. The nucleotide sequences of these primers correspond to sequences 3612 – 3631 and 3959 – 3940 of human BAI2 cDNA (GenBank accession number NM_001703). The size of amplified fragment is 348 bp. For amplification of CYR61 (cysteine-rich angiogenic inducer 61), also known as insulin-like growth factor binding protein 10, cDNA we used forward (5'– CTCCCTGTTTTTGGAATGGA –3' and reverse (5'– TGGTCTTGCTGCATTTCTTG –3') primers. The nucleotide sequences of these primers correspond to sequences 852 – 871 and 1092 – 1073 of human CYR61 cDNA (GenBank accession number NM_001554). The size of amplified fragment is 241 bp. For amplification of HIF1A (fibroblast growth factor receptor like 1) cDNA we used forward (5'– GAAAGCGCAAGTCCTCAAAG –3' and reverse (5'– TGGGTAGGAGATGGAGATGC –3') primers. The nucleotide sequences of these primers correspond to sequences 2175 – 2194 and 2341 – 2322 of human HIF1A cDNA (GenBank accession number NM_001530). The size of amplified fragment is 167 bp. The amplification of beta-actin (ACTB) cDNA was performed using forward - 5'– GGACTTCGAGCAAGAGATGG –3' and reverse - 5'– AGCACTGTFTTGGCGTACAG –3' primers. These primer nucleotide sequences correspond to 747 – 766 and 980 – 961 of human ACTB cDNA (GenBank accession number NM_001101). The size of amplified fragment is 234 bp. The expression of beta-actin mRNA was used as control of analyzed RNA quantity.

An analysis of qPCR was performed using special computer program "Differential expression calculator". The values of all studied gene expressions were normalized to ACTB expression and represent as percent of control (100 %). Statistical analysis was performed as described previously [34]. All values are the means ± SEM for six different samples. P < 0.05 was considered significant in all cases.

RESULTS

As shown in Table 1, the lean healthy control participants were individuals with mean age 44 ± 3.4 years and mean body mass index (BMI) 23 ± 0.6 kg/m^2. Two obese groups with normal and impaired glucose tolerance had similar age (45 ± 3.0 and 44 ± 3.2 years), but significantly higher BMI (32 ± 0.6 and 34 ± 0.6 kg/m^2, correspondingly; P < 0.05 in both cases) versus control men. Waist circumstance was also increased in both obese groups versus control individuals (+31 and +32 %, correspondingly; P < 0.05 in both cases). In the group of obese participants with impaired glucose tolerance the levels of 2h blood glucose, insulin, and triglycerides were increased versus obese patients with normal glucose tolerance: +47%, +62%, and +60%, correspondingly; P < 0.05 in all cases (Table 1). Correspondingly, insulin sensitivity index T was decreased in obese patients with glucose intolerance versus obese patients with NGT (close to 2 fold; P < 0.05).

To test how obesity affects the expression levels of VEGF-A gene, strongly related to positive control of angiogenesis, we used two sets of primers: one for all alternative splice variants of this mRNA and other specific only for VEGF-A-189. Figure 1 demonstrates

Table 1. Characteristics of the study participants.

Variable	Lean, NGT	Obese, NGT	Obese, IGT
Age at visit (years)	44 ± 3.4	45 ± 3.0	44 ± 3.2
(*n*)	(6)	(6)	(6)
Body mass index (BMI) (kg/m^2)	23 ± 0.6	32 ± 0.6 *	34 ± 0.6 *
(*n*)	(6)	(6)	(6)
Fasting glucose (mmol/l)	4.5 ± 0.09	5.0 ± 0.22	5.5 ± 0.26
(*n*)	(6)	(6)	(6)
2h oral glucose tolerance test (OGTT) glucose (mmol/l)	5.08 ± 0.64	5.31 ± 0.88	7.83 ± 0.36 *^
(*n*)	(5)	(6)	(6)
Insulin sensitivity index (T; mg/kg/min)	7.9 ± 0.58	5.1 ± 0.67 *	2.7 ± 0.19 *^
(*n*)	(6)	(6)	(5)
Fasting triglycerides (mmol/l)	1.0 ± 0.19	1.36 ± 0.20	2.17 ± 0.44*^
(*n*)	(6)	(6)	(6)
Fasting insulin (μIU/ml)	8.0 ± 1.62	9.37 ± 0.92	15.2± 1.15 *^
(*n*)	(3)	(3)	(4)
Waist circumstance (cm)	86 ± 4.3	113 ± 3.3 *	114 ± 3.4 *
(n)	(5)	(6)	(6)

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

that expression level of VEGF-A mRNA with primers, which recognize all splice variants, is decreased (-39 %) in subcutaneous adipose tissue of obese men as compared to control individuals. The expression level of VEGF-A-189 mRNA is also down-regulated (-49 %) in this group of obese patients. At the same time, the expression level of both VEGF-A and
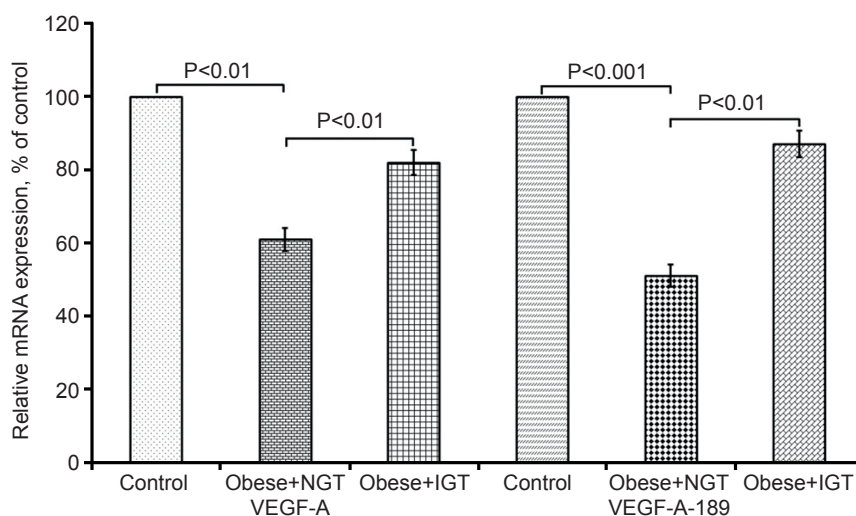

Figure 1. Expression levels of VEGF-A and its alternative splice variant VEGF-A-189 mRNA in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of VEGF-A and VEGF-A-189 mRNA were normalized to the expression of beta-actin mRNA and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6

VEGF-A-189 mRNA is increased in obese men with impaired glucose tolerance as compared with group of obese individuals, which have normal glucose tolerance: +34 % and +71 %, correspondingly (Figure 1).

We next studied the expression level of PDGFC and CYR61 mRNA in subcutaneous adipose tissue of obese men with normal and impaired glucose tolerance. As shown in Figure 2, obesity affects both of these gene expressions in opposite ways: down-regulates PDGFC mRNA level (-17 %) and strongly up-regulated the expression of *CYR61* gene (+86 %).

Figure 3 demonstrate that obesity affects the expression of two different fibroblast growth factor genes in subcutaneous adipose tissue in opposite ways: induces *FGF1* gene expression (+135 %) and down-regulates the expression level of *FGF2* gene (-36 %). At the same time, the expression level of both *FGF1* and *FGF2* genes is up-regulated in subcutaneous adipose tissue of obese patients with glucose intolerance: +27 % and +67 %, correspondingly (Figure 3). Thereafter, we tested how these changes in *FGF1* and *FGF2* gene expressions correlate with the expression of FGF receptors FGFR2 and FGFRL1. As shown in Figure 4, the expression

of *FGFR2* gene is up-regulated and *FGFRL1* gene is down-regulated in obese men with normal glucose tolerance: +61 % and -50 %, correspondingly. Moreover, glucose intolerance decreases the expression level of FGFR2 mRNA (-20 %) and does not affect FGFRL1 mRNA expression (Figure 4).

We also analyzed the expression level of brain-specific angiogenesis inhibitor 2 gene in subcutaneous adipose tissue of obese men with and without glucose intolerance. As shown in Figure 5, *BAI2* gene is expressed in adipose tissue both control and obese individuals and its expression is up-regulated in obesity (+23 %). However, no significant changes were observed in the expression of this gene in subcutaneous adipose tissue of obese men with glucose intolerance as compared to group of obese men with normal glucose tolerance. At the same time, the expression of *E2F8* gene, which encoded a key transcription factor related to the control of cell proliferation and angiogenesis, is strongly increased in adipose tissue of obese men with normal glucose tolerance (+146 %) as compared to control group (Figure 5). Furthermore, development of glucose intolerance in obesity leads to additional induction of this gene
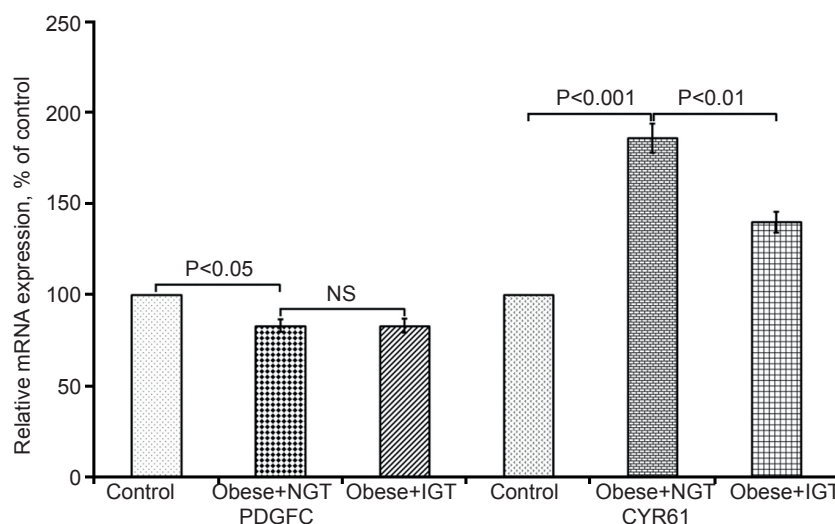

Figure 2. Expression levels of *PDGFC* and *CYR61* genes in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of these genes were normalized to the expression of beta-actin mRNA and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6
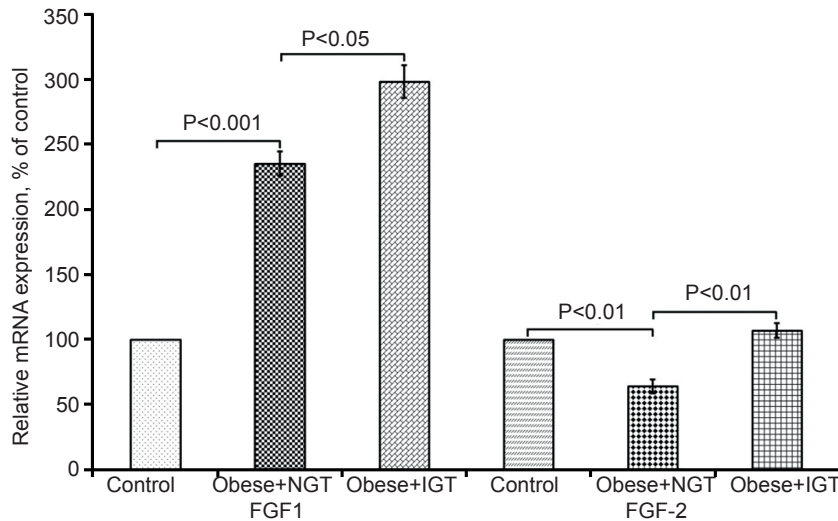
Figure 3. Expression levels of *FGF1* and *FGF2* genes in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of these *FGF* genes were normalized to the expression of beta-actin mRNA and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6

expression (+86 %) as compared to obese men with normal glucose intolerance.

Additionally, we studied how expression of transcription factors HIF1A and EPAS1/HIF2A, which mediate numerous hypoxia-induced processes including proliferation in cell-specific manner, is affected by obesity and

its complications, because there is data [24, 25] that transcription factor E2F8 can create complexes with other members of E2F family of transcription factors and in cooperation with HIF1 coordinates the regulation of the expression of target genes, thereby regulating cell cycle and angiogenesis, including transcriptional
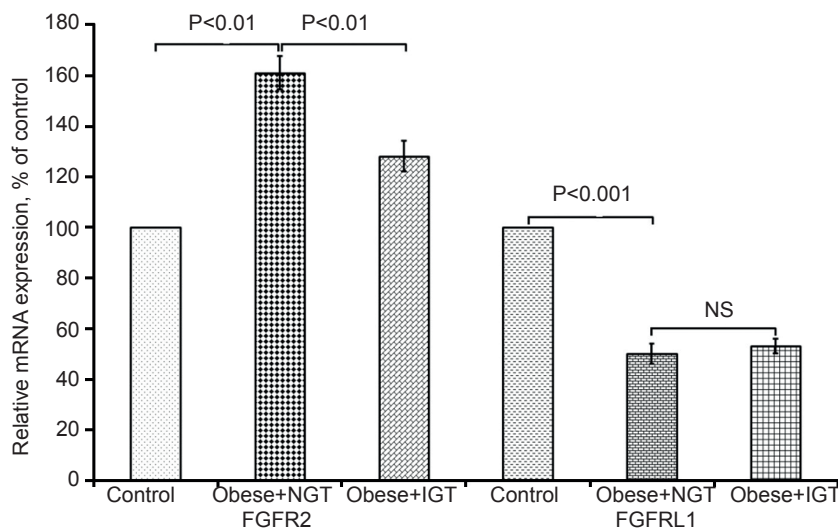

Figure 4. Expression levels of *FGFR2* and *FGFRL1* genes in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of these *FGFR* genes were normalized to the expression of beta-actin gene and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6.
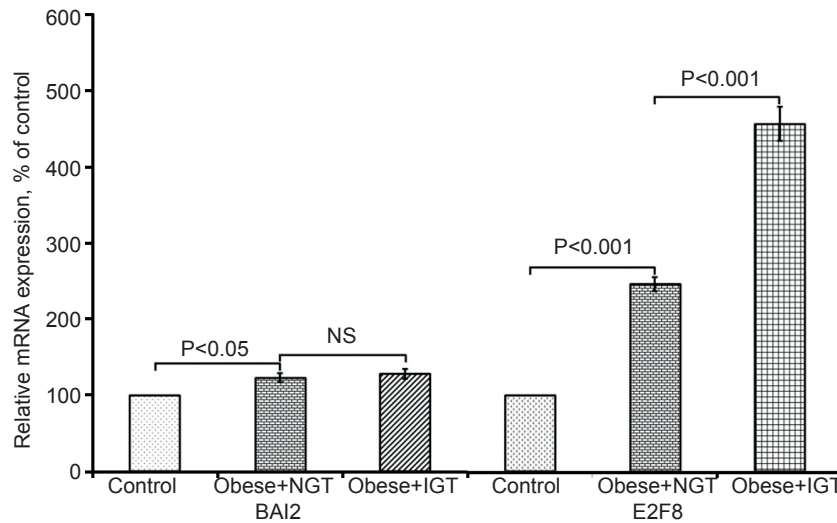
Figure 5. Expression levels of *BAI2* and *E2F8* genes in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of *BAI2* and *E2F8* genes were normalized to the expression of beta-actin mRNA and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6

activation of VEGFA. As shown in Figure 6, *HIF1A* gene expression is up-regulated in subcutaneous adipose tissue of obese individuals with NGT (+25 %) as compared to control individuals; however, glucose intolerance does not change significantly the expression of this gene as compared to obese men with NGT. Moreover, no significant changes were found in the expression level of other HIF-alpha protein – EPAS1/HIF2A in adipose tissue of both groups of obese patients (Figure 6).

Thus, the expression almost all studied genes encoded angiogenesis-related growth factors, with the exception of EPAS1, are affected by obesity in gene-specific manner, but with development of glucose intolerance is
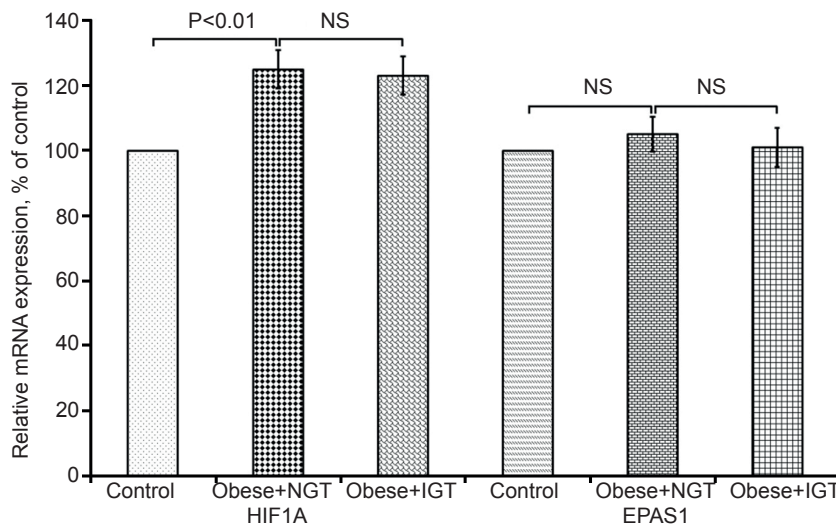

Figure 6. Expression levels of transcription factor *HIF1A* and *EPAS1* genes in subcutaneous adipose tissue of control and obese individuals with normal glucose tolerance (Obese + NGT) as well as in obese patients with impaired glucose tolerance (Obese + IGT). The values of the expression of these transcription factor genes were normalized to the expression of beta-actin gene and are represented as a percent of control (100 %). Data is expressed as mean ± SEM of values from each group; *n* = 6

associated the expression of *VEGF-A, FGF1, FGF2, FGFR2, E2F8*, and *CYR61* genes.

DISCUSSION

Results of this study clearly demonstrated that the expression level of almost all tested genes (*VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2,* and *HIF1A*) encoding key angiogenesis-related transcription factors, is affected in subcutaneous adipose tissue of obese men and possibly participate in the development of obesity and its complications, particularly glucose intolerance. Thus, angiogenesis should be also altered in both obesity and obesity-associated glucose intolerance, because growing adipose tissue needs neo-angiogenesis for cell proliferation like tumors [11, 13]. We have shown that the expression of pro-angiogenic genes *VEGF-A* and *PDGFC* in adipose tissue of obese men is down-regulated, but other pro-angiogenic genes such as *CYR61, FGFR2, FGF1, E2F8*, and *HIF1A* is up-regulated. Moreover, anti-angiogenic gene *BAI2* is also up-regulated in subcutaneous adipose tissue of obese men. It is important to note that angiogenesis is a complex network and is regulated by hundreds of pro-angiogenic and anti-angiogenic factors possibly through different mechanisms in tissue-specific manner and varies in a range of pathological conditions. Furthermore, Hose et al. [35] do not show a significantly higher median number of expressed pro-angiogenic (45) or anti-angiogenic (31) genes in CD138-purified myeloma cells from 300 untreated patients, but almost all of these myeloma cells samples aberrantly express at least one of the angiogenic factors. Thus, our results concerning deregulation of the expression of different pro-angiogenic and anti-angiogenic genes in adipose tissue of obese individuals possibly reflect specificity of angiogenesis in subcutaneous adipose tissue upon obesity and argue to data Hose et al. [35]. It is also possible that in our cohort of obese men there is stabilized or slightly suppressed angiogenesis because these individuals have increased but constant weight prolonged time and this observation correlate with decreased expression of VEGF-A as well as with increased expression of BAI2.

At the same time, development of insulin resistance and glucose intolerance in obesity significantly reprogrammed the expression of most studied angiogenesis-related genes in adipose tissue and possibly contributed to aberrant angiogenesis, which is associated with obesity complication, including type 2 diabetes and its complications. Increased expression of VEGF-A as well as FGF1 and transcription factor E2F8, which we observed in adipose tissue of obese individuals with IGT as comparted to obese men with NGT, can contribute to enhancement of angiogenesis, but development of insulin resistance is also associated with vascular endothelial growth factor resistance despite the presence of functionally active VEGF receptor 1 [36]. It is important to note that the expression of FGFR2, which predominantly interacts with both FGF1 and FGF2 and responsible for enhanced angiogenesis, is up-regulated in adipose tissue of obese individuals with NGT, but slightly down-regulated in obese patients with IGT and this down-regulation is associated with additional increase of *FGF1* gene expression. At the same time, the expression of FGFRL1, another member of FGFR family, is decreased in obese tissue upon obesity and these results correlate with its biological function as potentially inhibitor of FGF signaling, because FGFRL1 does not have a cytoplasmic tyrosine kinase domain [25]. We also analyzed the expression level of two different HIF-alpha subunits (HIF1A and EPAS1) and shown that only HIF1A is up-regulated in adipose tissue upon obesity and that development of insulin resistance/glucose intolerance does not affect it. These results agree with data Weijts et al. [28] that HIF1 in cooperation with another transcription factors (E2F8 and E2F7) promote angiogenesis through transcriptional activation of VEGFA.

However, detailed molecular mechanisms of the involvement of angiogenesis-related genes in the development of obesity and its complications are not clear yet and remain to be determined.

CONCLUSIONS

Results of this study demonstrated that the expression of almost all studied genes encoded key angiogenesis-related factors is affected in subcutaneous adipose tissue of obese individuals with NGT in gene-specific manner. Thus, the level of *VEGF-A, PDGFC, FGF2,* and *FGFRL1* gene expressions is decreased in adipose tissue of obese men with normal glucose tolerance (NGT), but is increased of *CYR61, FGF1, FGFR2, E2F8, BAI2,* and *HIF1A* gene expressions. Impaired glucose tolerance (IGT) in obese patients associates with down-regulation of CYR61 and FGFR2 mRNA and up-regulations of E2F8, FGF1, FGF2, and VEGF-A mRNA expressions in adipose tissue versus obese (NGT) individuals. Thus, our data provides evidence that *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2,* and *HIF1A* genes are possibly involved in the development of obesity and its complications; however, molecular mechanisms of the regulation of proliferation-related transcription factor genes warrant further investigation for clarification the role of these genes in obesity and its complications.

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ЕКСПРЕСІЯ ГЕНІВ, ЩО МАЮТЬ ВІДНОШЕННЯ ДО АНГІОГЕНЕЗУ, У ПІДШКІРНІЙ ЖИРОВІЙ ТКАНИНІ ЧОЛОВІКІВ З ОЖИРІННЯМ ТА НОРМАЛЬНОЮ ЧИ ПОРУШЕНОЮ ТОЛЕРАНТНІСТЮ ДО ГЛЮКОЗИ

Ожиріння та його метаболічні ускладнення є однією з найважливіших проблем охорони здоров'я. Розвиток ожиріння зумовлений взаємодією генів із факторами довкілля і тісно пов'язаний із дисрегуляцією тонких механізмів регуляції експресії генів, що контролюють більшість метаболічних процесів і є важливими для регуляції багатьох фізіологічних функцій, включаючи чутливість до інсуліну, проліферацію клітин та ангіогенез. Метою цього дослідження було оцінити роль експресії мРНК генів *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2, HIF1A* та *EPAS1,* що мають відношення до регуляції ангіогенезу, у жировій тканині в плані їх можливої участі у розвитку ожиріння та його метаболічних ускладнень. Показано, що рівень експресії генів *VEGF-A, PDGFC, FGF2* та *FGFRL1* знижується у жировій тканині чоловіків з ожирінням і нормальною толерантністю до глюкози (NGT) у порівнянні з групою контрольних суб'єктів. У той же час у цій групі чоловіків з ожирінням виявлено значне підвищення експресії генів *CYR61, FGF1, FGFR2, E2F8, BAI2* та *HIF1A.* Порушення толерантності до глюкози (IGT) у пацієнтів з ожирінням асоціюється зі зниженням експресії мРНК CYR61 і FGFR2 та збільшенням мРНК E2F8, FGF1, FGF2, VEGF-A і його сплайс-варіанту 189 у жировій тканині у порівнянні з групою чоловіків з ожирінням і NGT. Таким чином, отримані нами результати продемонстрували, що експресія більшості досліджених генів порушується у підшкірній жировій тканині чоловіків з ожирінням і NGT і що порушення толерантності до глюкози асоціюється із гено-специфічними змінами експресії мРНК E2F8, FGFR2, FGF1, FGF2, VEGF-A, CYR61 і FGFR2. Представлені тут результати свідчать про те, що гени *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2* та *HIF1A* можливо задіяні у розвитку ожиріння та його ускладнень.

Ключові слова: експресія мРНК, VEGF-A, CYR61, FGF1, FGF2, FGFR2, E2F8, HIF1A, жирова тканина, ожиріння, чоловіки.

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ЭКСПРЕССИЯ ГЕНОВ, ЧТО ИМЕЮТ ОТНОШЕНИЕ К АНГИОГЕНЕЗУ, В ПОДКОЖНОЙ ЖИРОВОЙ ТКАНИ МУЖЧИН С ОЖИРЕНИЕМ И НОРМАЛЬНОЙ ИЛИ НАРУШЕННОЙ ТОЛЕРАНТНОСТЬЮ К ГЛЮКОЗЕ

Ожирение и его метаболические усложнения являются одной из наиболее важных проблем здравоохранения. Развитие ожирения обусловлено взаимодействием генов с факторами окружающей среды и тесно повязано с дисрегуляцией тонких механизмов регуляции экспрессии генов, что контролируют большинство метаболических процессов и являются важными для регуляции многих физиологических функций, включая чувствительность к инсулину, пролиферацию клеток и ангиогенез. Целью этого исследования было оценить роль экспрессии мРНК генов *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2, HIF1A* и *EPAS1,* что имеют отношение к регуляции ангиогенеза, в жировой ткани в плане их возможной участи в развитии ожирения и его метаболических усложнений. Показано, что уровень экспрессии генов *VEGF-A, PDGFC, FGF2* и *FGFRL1* снижается в жировой

ткани мужчин с ожирением и нормальной толерантностью к глюкозе (NGT) в сравнении с группой контрольных субъектов. В то же время в этой группе мужчин с ожирением выявлено значительное повышение экспрессии генов *CYR61, FGF1, FGFR2, E2F8, BAI2* и *HIF1A*. Нарушение толерантности к глюкозе (IGT) у пациентов с ожирением ассоциируется со снижением экспрессии мРНК CYR61 и FGFR2 и увеличением мРНК E2F8, FGF1, FGF2, VEGF-A и его сплайс-варианта 189 в жировой ткани по сравнению с группой мужчин с ожирением и NGT. Таким образом, полученные нами результаты продемонстрировали, что экспрессия большинства исследованных генов нарушается в подкожной жировой ткани мужчин с ожирением и NGT и что нарушение толерантности к глюкозе ассоциируется из гено-специфиченскими изменениями экспрессии мРНК E2F8, FGF1, FGF2, VEGF-A, CYR61 и FGFR2. Представленные здесь результаты свидетельствуют о том, что гены *VEGF-A, CYR61, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, BAI2* и *HIF1A* возможно задействованы в развитии ожирения и его усложнений.

Ключевые слова: экспрессия мРНК; VEGF-A; CYR61; FGF1; FGF2; FGFR2; E2F8; HIF1A; жировая ткань; ожирение; мужчины.

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