

The Expression of *TIMP1*, *TIMP2*, *VCAN*, *SPARC*, *CLEC3B* and *E2F1* in Subcutaneous Adipose Tissue of Obese Males and Glucose Intolerance

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Received December 1, 2012; revised January 2, 2013; accepted January 17, 2013

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ABSTRACT

We investigated the expression of *TIMP1*, *TIMP2*, *SPARC*, *VCAN*, and *CLEC3B* genes, encoded matricellular proteins with pleiotropic functions, and glucose intolerance in obese male subjects with normal and impaired glucose tolerance. The purpose of this study was to examine the association between the gene expressions and glucose intolerance in obesity. The results indicate that obesity leads to significant increase of *TIMP1*, *TIMP2*, *E2F1* and *CLEC3B* gene expressions in subcutaneous adipose tissue, especially *TIMP2* gene. However, more significant increase of the expression of *TIMP1* and *TIMP2* was found in adipose tissue of obese patients with glucose intolerance. No significant changes were found in the expression of *VCAN* and *SPARC* genes in adipose tissue of obese subjects with normal glucose tolerance but increased in the group of obese subjects with glucose intolerance. At the same time, the *E2F1* and *CLEC3B* gene expressions were decreased in adipose tissue of obese patients with glucose intolerance. Results of this study provide evidence that changes in the expression of genes encoded *TIMP1*, *TIMP2*, *VCAN*, *SPARC*, *E2F1* and *CLEC3B* in subcutaneous adipose tissue of obese individuals associate with glucose intolerance.

Keywords: Obesity; Glucose Intolerance; Men; Gene Expression; *TIMP1*; *TIMP2*; *VCAN*; *SPARC*; *CLEC3B*; *E2F1*

1. Introduction

Obesity is one of the most profound public health problems. Much has been learned regarding the regulation of body weight and adiposity [1,2]. Like many other medical conditions, obesity, metabolic syndrome and type 2 diabetes are the results of interaction between environmental and genetic factors, including biological rhythms which control metabolism and are intrinsically and interdependently linked [3,4].

Circadian rhythms govern or contribute to regulation of a large array of metabolic and physiological functions, including insulin sensitivity, energy homeostasis, satiety signaling, cellular proliferation, and cardiovascular function [5-7]. Recent studies have demonstrated relationships between the dysfunction of circadian clock system and the development of metabolic abnormalities, including adiposity and excessive levels of circulating lipids [8-11].

Adipose tissue growth is tightly associated with cell proliferation processes and angiogenesis which is an important component of different proliferative processes. In particular, fat tissue growth is regulated by different tightly interconnected factors [12,13]. Special interest deserve the matrix proteins with pleiotropic roles, such as the tissue inhibitor of matrix metalloproteinase (*TIMP1* and *TIMP2*) [14,15], versican (*VCAN*) [16] and *SPARC* (secreted protein acidic and rich in cysteine, also known as osteonectin) [17]. These proteins are linked to human obesity and diabetes complications as they to reveal anti-angiogenic properties. Moreover, they seem to regulate cell growth and apoptosis, particularly through affecting the vascular endothelial growth factor (*VEGF*), at resulting in *VEGF* resistance in diabetes mellitus despite the presence of the functionally active VEGF receptor 1 [18, 19].

TIMPs are multifunctional and can act either directly through cell surface receptors or indirectly through modulation of protease activity to direct cell fate [20]. Thus,

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TIMP1 is able to promote cell proliferation in a wide range of cell types, and may also have an anti-apoptotic function [14]. However, at the same time, *TIMP1* is a potent inhibitor of tumor growth and angiogenesis and is involved in cell adhesion and migration [14]. *TIMP1* is a negative regulator of adipogenesis both in mice and in humans [15]. *TIMP2* thought to be a metastasis suppressor and has a unique role among *TIMP* family members in its ability to directly suppress the proliferation of endothelial cells [14]. Nevertheless, *TIMP-2* has been shown to induce gene expression, to promote G(1) cell cycle arrest, and to inhibit cell migration [14]. It was shown that *TIMP-2* involved in binding to the receptor integrin $\alpha3\beta1$, and mediates angio-inhibitory and tumor suppressor activity [20].

It was also observed that enhanced neovascularization correlated with down regulation of anti-angiogenic connective tissue growth factor (CTGF). The latter binds VEGFA and its angiogenic activity are inhibited in the VEGFA-CTGF complex form [12]. But, stability of this complex as well as the angiogenic activity of VEGF depends from the matrix metalloproteinases and its inhibitors [12,21].

SPARC (secreted protein acidic and rich in cysteine), also known as osteonectin or BM-40, is a widely expressed profibrotic protein with pleiotropic roles, is linked to human obesity, insulin resistance, diabetes mellitus and diabetes complications such as diabetic retinopathy and nephropathy because it has anti-angiogenic properties and appears to regulate cell growth [17]. Elevated plasma levels of this protein were observed in patients with newly diagnosed type 2 diabetes mellitus [22]. Furthermore, evidence suggests that adipose tissue becomes increasingly fibrotic in obesity and SPARC as a regulator of the extracellular matrix also contributes to this adipose-tissue fibrosis [17,23]. Fibrosis of subcutaneous adipose tissue may restrict accumulation of triglycerides in this type of tissue. These are, therefore, deposited as ectopic lipids in other non-adipose tissues such as the liver or as intramyocellular lipids in skeletal muscle and predisposes to insulin resistance [17].

Versican (*VCAN*), also known as chondroitin sulfate proteoglycan 2 (*CSPG2*), is one of the main components of the extracellular matrix and is considered to be crucial to several key cellular processes involved in development and disease; *i.e.* cellular adhesion, proliferation, differentiation, migration, and angiogenesis, as well as, in intercellular signaling and connecting cells with the extracellular matrix [16,24,25]. Recently, the Wnt/ β -catenin/TCF response elements in the human versican promoter, which is essential for activation of versican transcription, was identified [25]. Moreover, the versican was identified among six proteins at the center of the angiogenesis-associated network [26]. Hence, the angiogenesis

phenomena are governed by complex signaling networks.

The transcription factor *E2F1* regulates the expression of genes involved in a wide range of cellular processes; it is needed for regulation of nuclear receptor networks by retinoblastoma and via the CDK4-pRB-E2F1 regulatory pathway is involved in glucose homeostasis, defining a new link between cell proliferation and metabolism [27].

The endoplasmic reticulum stress is recognized as an important determinant of type 2 diabetes and contributes to the expression profile of many regulatory genes resulting in peripheral insulin resistance and diabetic complications, acting by inhibiting insulin receptor signaling [28,29]. However, detailed molecular mechanisms of the involvement of the endoplasmic reticulum stress in the development of obesity and its complications with different degree of dysglycemia are not yet clear.

Because the function of some matricellular proteins with pleiotropic roles is closely linked to metabolic homeostasis and these proteins can modulate the intracellular signaling network and lead to development of obesity and its complications (insulin resistance and glucose intolerance), the main goal of this work was to study the role of gene expressions encoding for the matricellular proteins with pleiotropic roles, in subcutaneous adipose tissue of obese individuals for evaluation of its possible significance to development of human obesity and glucose intolerance.

2. Materials and Methods

2.1. Patients

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. Patients clinical characteristics are shown in **Table 1**. The 18 male subjects participate in the study. They were divided into three groups (6 men in each group): 1) clinically healthy lean individuals, 2) patients with obesity and normal glucose tolerance (NGT) and 3) obesity and impaired glucose tolerance (IGT).

The lean (control) participant groups were individuals with mean age 44 ± 3 years and mean body mass index (BMI) 23 ± 0.6 kg/m². The obese participant groups were individuals with mean age (45 ± 3 and 44 ± 3 years, respectively) and mean BMI (32 ± 0.6 and 34 ± 0.6 kg/m², respectively). Thus, BMI in these groups of patients was significantly higher as compared to lean individuals (**Table 1**).

In the group of obese patients with impaired glucose tolerance 2 h glucose level in the blood was 7.8 mmol/l during an OGTT as compared with 5.3 mmol/l in obese participants (NGT) (**Table 1**). Moreover, in this group of obese patients with glucose intolerance is decreased in-

sulin sensitivity index T (2.70 ± 0.19 vs 5.09 ± 0.67 mg/kg/min in an obese patients) and increased the level of fasting triglycerides (2.17 ± 0.44 vs 1.36 ± 0.2 mmol/l in an obese patients) as well as fasting insulin (15.2 ± 2.3 vs 9.37 ± 1.6 μ IU/ml in an obese patients (NGT), as shown in **Table 1**.

2.2. RNA Isolation

RNasy Lipid Tissue Mini Kit (QIAGEN, Germany) was used for RNA extraction from subcutaneous adipose tissue.

2.3. Reverse Transcription and Quantitative Real-Time PCR Analysis

The expression levels of genes encoded *TIMP* metalloproteinase inhibitor 1 (*TIMP1*), *TIMP2*, *SPARC* (secreted protein, acidic, cysteine-rich) and versican (*VCAN*) 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 SYBR Green Mix (Thermo Scientific, UK) and pair of primers specific for each studied gene (Sigma, USA) were used for quantitative real-time polymerase chain reaction (qPCR). The primers sequences are shown in **Table 2**. The expression of beta-actin mRNA was used as control of analyzed RNA quantity.

An analysis of quantitative real-time polymerase chain reaction was performed using special computer program "Differential expression calculator" and statistical analysis—in Excel program. The values of different gene expressions were normalized to the expression of beta-actin mRNA and represent as percent of control (lean individuals, 100%) and are the means \pm SEM for up to six different samples.

3. Results

In this study, we analyzed the expression levels of genes

Table 1. Characteristics of the study participants.

Variable	Lean, NGT	Obese, NGT	Obese, IGT
Age at visit (years) (<i>n</i>)	44 \pm 3.4 (6)	45 \pm 3 (6)	44 \pm 3.2 (6)
Body mass index (BMI) (kg/m ²) (<i>n</i>)	23 \pm 0.6 (6)	32 \pm 0.6* (6)	34 \pm 0.6* (6)
Fasting glucose (mmol/l) (<i>n</i>)	4.5 \pm 0.09 (6)	5.0 \pm 0.22* (6)	5.5 \pm 0.26 (6)
2 h oral glucose tolerance test (OGTT) glucose (mmol/l) (<i>n</i>)	5.08 \pm 0.64 (5)	5.31 \pm 0.88 (6)	7.83 \pm 0.36** (6)
Insulin sensitivity index (T; mg/kg/min) (<i>n</i>)	7.9 \pm 0.58 (6)	5.1 \pm 0.67* (6)	2.7 \pm 0.19** (5)
Fasting triglycerides (mmol/l) (<i>n</i>)	1.0 \pm 0.19 (6)	1.36 \pm 0.2 (6)	2.17 \pm 0.44** (6)
Fasting insulin (μ IU/ml) (<i>n</i>)	8.0 \pm 2.8 (3)	9.37 \pm 1.6 (3)	15.2 \pm 2.3* (4)

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

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	Gen Bank accession number
<i>TIMP1</i>	tissue inhibitor of matrix metalloproteinase 1	F: 5'-ATTCGACCTCGTCATCAGR: 3'-GCAGTTTTCCAGCAATGAG	301 - 320 530 - 511	NM_003254
<i>TIMP2</i>	tissue inhibitor of matrix metalloproteinase 1	F: 5'-GATGCACATCACCTCTGTGR: 3'-GTCGAGAAACTCCTGCTTGG	665 - 684 950 - 931	NM_003255
<i>SPARC</i>	Secreted protein acidic and rich in cysteine; osteonectin	F: 5'-TGCCTGATGAGACAGAGGTG R: 3'-AAGTGGCAGGAAGAGTCGAA	176 - 195 479 - 460	NM_003118
<i>VCAN (CSPG2)</i>	versican;chondroitin sulfate proteoglycan 2	F: 5'- CCTTCTGGGGGAAGAACTCC R: 3'-GGTCACATAGGAAGCGTGGT	102 - 121 272 - 253	NM_004385
<i>CLEC3B</i>	C-type lectin domain family 3, member B, tetranectin	F: 5'-ACCCAGAAGCCCAAGAAGATR: 3'-GAAGGTCTTCGTCTGGGTGA	169 - 188 369 - 350	NM_003278
<i>E2F1</i>	E2F transcription factor 1	F: 5'-GGGCTCTAACTGCACTTTCGR: 3'-AGGGAGTTGGGTATCAACC	1830 - 1849 2096 - 2077	NM_005225
<i>ACTB</i>	beta-actin	F: 5'-GGACTTCGAGCAAGAGATGG R: 3'-AGCACTGTGTTGGCGTACAG	747 - 766 980 - 961	NM_001101

encoding tissue inhibitor of matrix metalloproteinase 1 and 2, *SPARC*, versican, *CLEC3B* and *E2F1* in subcutaneous adipose tissue from three groups of participants: lean (control), obese with normal glucose tolerance and obese with impaired glucose tolerance. As shown in **Figure 1**, the expression level of *TIMP1* and *TIMP2* mRNA in subcutaneous adipose tissue of obese individuals with normal glucose tolerance increases as compared to the control (lean) subjects: +56% and +183%, correspondingly. Moreover, in obese patients with impaired glucose tolerance (IGT) the expression of these genes was significantly higher as compared both to the lean subjects and the obese individuals with normal glucose tolerance.

As shown in **Figure 2**, the expression level of *SPARC* and *VCAN* mRNA did not change significantly in subcutaneous adipose tissue of the obese individuals, compared with lean participants. At the same time, the expression level of *SPARC* and *VCAN* mRNA is increased in subcutaneous adipose tissue of the obese individuals with impaired glucose tolerance, when compared to the groups of obese subjects with normal glucose tolerance or lean participants, being much more intense for the *SPARC* gene (**Figure 2**).

As shown in **Figure 3**, the expression level of *CLEC3B* and *E2F1* mRNA significantly increased in subcutaneous adipose tissue of the obese individuals with NGT, compared with lean participants, being much more intense for the *CLEC3B* gene. At the same time, the expression level both of these genes in adipose tissue of the obese individuals with impaired glucose tolerance was

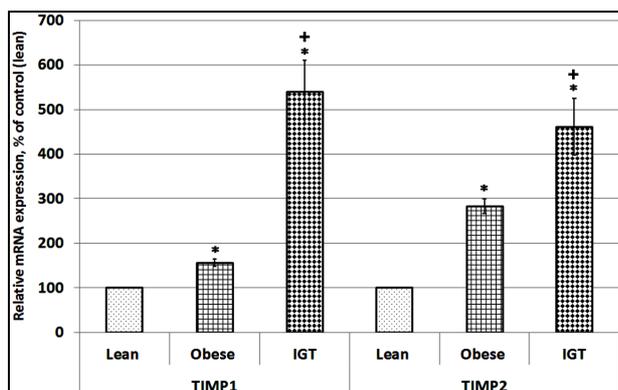


Figure 1. The expression level of TIMP metalloproteinase inhibitor 1 and 2 (*TIMP1* and *TIMP2*) mRNA in subcutaneous adipose tissue of lean (Lean) and obese individuals with normal glucose tolerance (Obese) as well as in obese patients with glucose intolerance (IGT). The values of *TIMP1* and *TIMP2* mRNA expressions were normalized to the expression of beta-actin mRNA and are represented as a percent of control (Lean, 100%). Data is expressed as mean \pm SEM of values from each group; * $P < 0.05$ vs group of lean individuals; $^+P < 0.05$ vs group with obesity and normal glucose tolerance test (NGT).

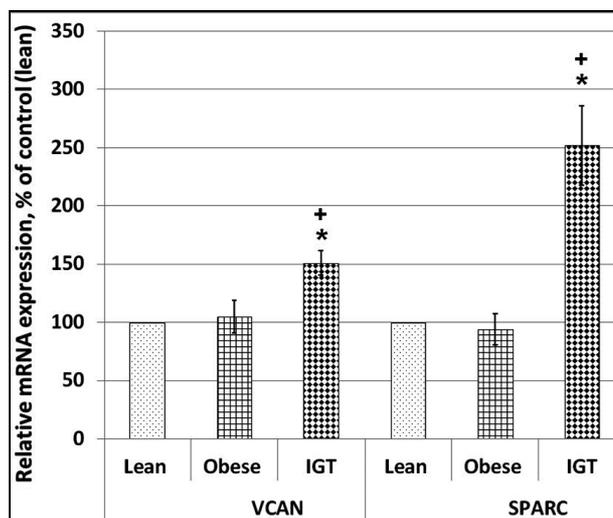


Figure 2. Versican (*VCAN*) and secreted protein acidic and rich in cysteine (*SPARC*) mRNA expression level in subcutaneous adipose tissue of lean and obese individuals with normal glucose tolerance (Obese) as well as in obese patients with impaired glucose tolerance (IGT). The values of *VCAN* and *SPARC* mRNA expressions were normalized to the expression of beta-actin mRNA and are represented as a percent of control (Lean, 100%). Data is expressed as mean \pm SEM of values from each group; * $P < 0.05$ vs lean group; $^+P < 0.05$ vs obese group.

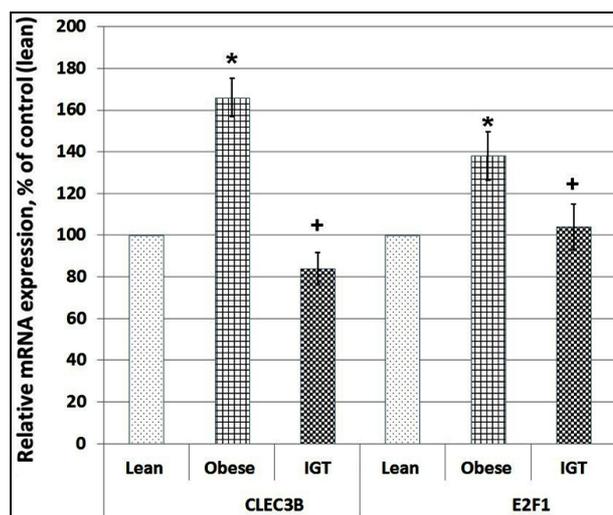


Figure 3. C-type lectin domain family 3, member B (*CLEC3B*) and transcription factor *E2F1* mRNA expression level in subcutaneous adipose tissue of lean and obese individuals with normal glucose tolerance (Obese) as well as in obese patients with impaired glucose tolerance (IGT). The values of *VCAN* and *SPARC* mRNA expressions were normalized to the expression of beta-actin mRNA and are represented as a percent of control (Lean, 100%). Data is expressed as mean \pm SEM of values from each group; * $P < 0.05$ vs lean group; $^+P < 0.05$ vs obese group.

decreased when compared to the groups of obese subjects with normal glucose tolerance (**Figure 3**).

We also analyzed the correlation between different clinical characteristics and gene expressions. There is positive correlation between increased BMI and *TIMP1* ($r = 0.93$), *TIMP2* ($r = 0.95$), *E2F1* ($r = 0.89$) and *CLEC3B* ($r = 0.92$) gene expressions in the obese men with normal glucose tolerance versus lean subjects (**Figure 4**). Moreover, as shown in **Table 1**, obese individuals with glucose intolerance versus obese subjects with normal glucose tolerance have significantly higher the 2 h oral glucose tolerance test (OGTT), fasting triglycerides and fasting insulin levels, but decreased insulin sensitivity index. We observed negative correlation between this insulin sensitivity index and *TIMP1* ($r = -0.70$), *TIMP2* ($r = -0.64$), *SPARC* ($r = -0.64$), *VCAN* ($r = -0.66$), and *CLEC3B* ($r = 0.75$) gene expressions in the obese individuals with glucose intolerance versus obese men with normal glucose tolerance (**Figure 5**).

4. Discussion

In this study, we have demonstrated that obesity is asso-

ciated with dysregulation of the *TIMP1*, *TIMP2*, *VCAN*, *CLEC3B*, *E2F1* and *SPARC* genes, which encode the important regulatory factors with pleiotropic functions, in particular, control angiogenesis and growth processes [14,15,17,25]. Moreover, these factors are involved in the development of the obesity, glucose tolerance and type 2 diabetes, the most profound public health problems [15,17,27]. Angiogenesis is an important component of different proliferative processes, in particular, fat tissue growth; however, angiogenesis can also contribute to the development of complications associated with obesity, insulin resistance and glucose intolerance. In this study we have shown that obesity leads to a significant increase of *TIMP1* and *TIMP2* gene expressions in men subcutaneous adipose tissue, besides that, the most significant increase was shown for *TIMP2* gene. It is possible that proteins encoded by these genes are involved the development of obesity, because both the *TIMP1* and *TIMP2* genes are implicated in direct regulation of cell growth, apoptosis and angiogenesis [14].

However, the tissue inhibitors of metalloproteinases

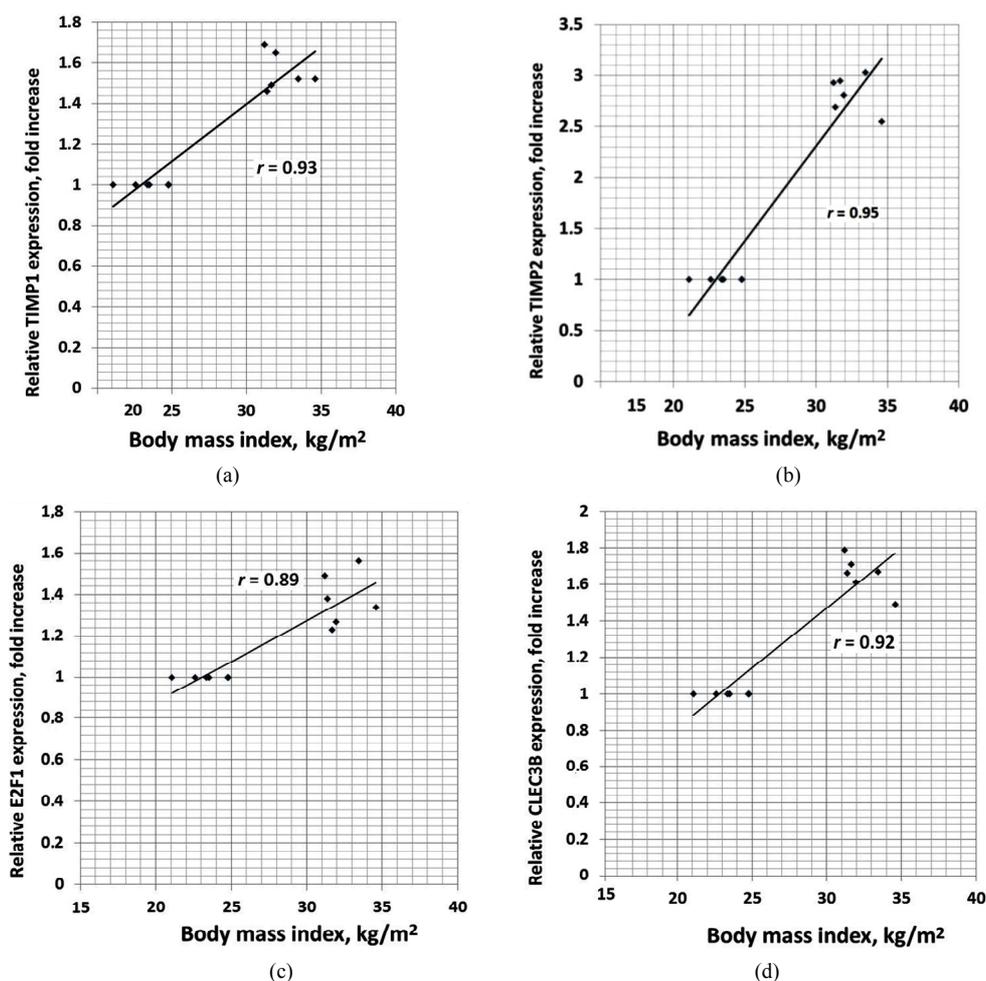


Figure 4. The correlation between body mass index (BMI) and *TIMP1* (a); *TIMP2* (b); *E2F1* (c) and *CLEC3B* (d) gene expressions in the obese men with normal glucose tolerance versus lean subjects.

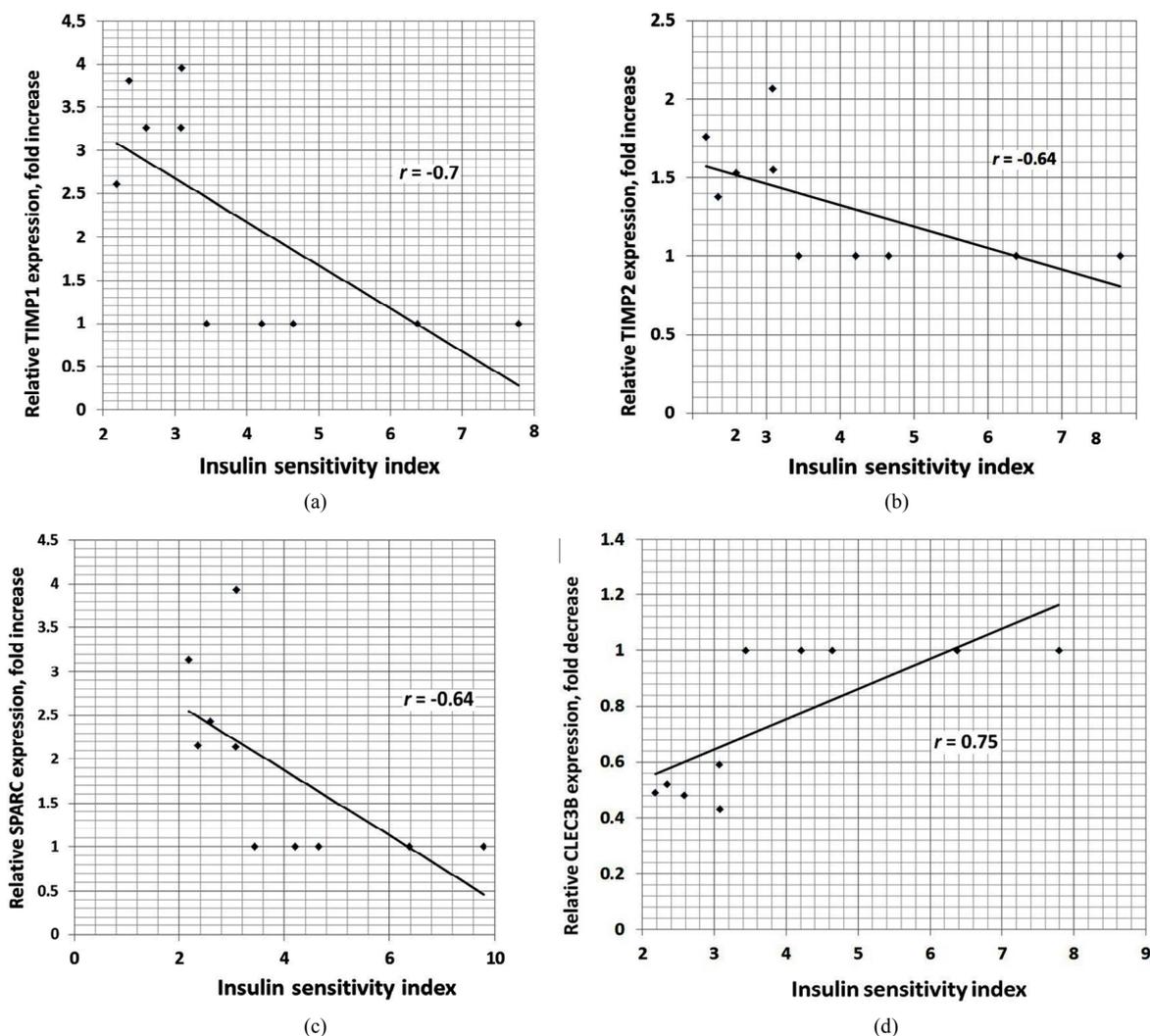


Figure 5. The correlation between insulin sensitivity index and *TIMP1* (a); *TIMP2* (b); *SPARC* (c) and *CLEC3B* (d) gene expressions in the obese individuals with glucose intolerance versus obese men with normal glucose tolerance.

(*TIMPs*) are multifunctional and can both promote and inhibit of cells growth in cell specific manner via different signaling pathways [14]. *TIMPs* can act either directly through cell surface receptors or indirectly through modulation of protease activity to direct cell fate. Thus, Meissburger *et al.* [15] have shown that tissue inhibitor of matrix metalloproteinase 1 controls adipogenesis in obesity in humans. At the same time, *TIMP1* can inhibit cell growth and apoptosis via binding to CD63 [14]. Tissue inhibitors of metalloproteinases suppress matrix metalloproteinase activity critical for extracellular matrix turnover associated with both physiologic and pathologic tissue remodeling; however, anti-proliferative as well as anti-angiogenic effects of *TIMP2* are independent of metalloproteinase inhibition [20]. It was also shown that these effects of *TIMP2* require alpha 3 beta 1 integrin-mediated binding of this tissue inhibitor of metalloproteinases to endothelial cells. Further, *TIMP2* induces a

decrease in total protein tyrosine phosphatase activity associated with beta1 integrin subunits as well as dissociation of the phosphatase SHP-1 from beta 1; however, *TIMP2* also results in a concomitant increase in protein tyrosine phosphatase activity associated with tyrosine kinase receptors FGFR-1 and KDR [20]. Furthermore, *TIMP2* has been shown to induce gene expression, to promote cell cycle arrest and to inhibit cell migration [14]. Thus, these findings establish an unexpected, metalloproteinase-independent mechanism for *TIMP2* inhibition of endothelial cell proliferation and reveal an important component of the anti-angiogenic effect of this metalloproteinase inhibitor [20].

Moreover, it is possible that these genes are also involved the development of obesity associated complications, like glucose intolerance, because we have shown significant up-regulation of both *TIMP1* and *TIMP2* as well as *SPARC* and *VCAN* gene expressions in adipose

tissue of subjects with impaired glucose intolerance versus the obese individuals with normal glucose tolerance. This observation agrees with data which recently has been shown that transcription factor retinoid-related orphan receptor gamma as a negative regulator of adipocyte differentiation modulates insulin sensitivity in obesity through expression of metalloproteinase-3 and its inhibitors [30]. It is also known that *SPARC* expression is predominant in subcutaneous adipose tissue, its expression contributing to metabolic dysregulation in obesity and is associated with insulin resistance and glucose intolerance [17,22]. The augmented expression of *SPARC* gene in the obese participants with glucose intolerance is possibly associated with hyperinsulinemia, because insulin as well as leptin can induce its expression [23].

It is possible that changes in *TIMP1*, *TIMP2*, *SPARC*, *CLEC3B*, *E2F1*, and *VCAN* gene expressions in obese individuals with impaired glucose tolerance are probably a result, at least partly, of endoplasmic reticulum stress mediated by the insulin resistance and elevated level of glucose. Recently, the endoplasmic reticulum stress is recognized as an important determinant of obesity and type 2 diabetes as well as a central feature of peripheral insulin resistance and glucose homeostasis [28,31,32].

Furthermore, *SPARC* as a regulator of the extra cellular matrix may contribute to metabolic dysregulation in obesity, insulin resistance and diabetes complications such as diabetic retinopathy and nephropathy, conditions that are ameliorated in the *SPARC*-knockout mouse model, via adipose-tissue fibrosis [17]. Hence, our results agree with this data that the *SPARC* protein is associated with diabetes complications, because *SPARC* protein plays an important role in angiogenesis [33]. At the same time, the primary function of *SPARC* in angiogenesis remains unclear, because *SPARC* activity in some circumstances promotes angiogenesis, while in others is more consistent with an anti-angiogenic activity [34]. Undoubtedly, the mercurial nature of *SPARC* belies a redundancy of functional proteins in angiogenesis. Moreover, fibrosis of subcutaneous adipose tissue may restrict accumulation of triglycerides in this type of tissue which are, therefore, diverted and deposited as ectopic lipids in other tissues such as the liver or as intramyocellular lipids in skeletal muscle, which predisposes to insulin resistance [17].

Results of this study have also shown enhanced level of *VCAN* expression in subcutaneous adipose tissue of obese individuals with glucose intolerance, which possibly contributes to proliferative processes, angiogenesis, metabolic dysregulation, and insulin resistance, because this chondroitin sulfate proteoglycan as an one of the main components of the extracellular matrix participates in cell adhesion, proliferation, migration, apoptosis and angiogenesis [16,25]. Most of these effects of versican

are possibly mediated through the Wnt/ β -catenin/TCF response elements in the *VCAN* promoter [35].

We also observed significant reduction of *E2F1* gene expression in adipose tissue of obese patients with glucose intolerance. This data is consistent with results of Blanchet *et al.* [36] concerning functional activity of transcription factor E2F1 which regulate both proliferative and metabolic genes and coordinates cellular responses by acting as a regulatory switch between cell proliferation and metabolism. This reduction of the expression of *E2F1* gene is possibly associated with activation of pro-proliferative and suppression of anti-proliferative gene expressions. Thus, tumor suppressor kinase LKB 1 is turning out to be a key regulator of the body's metabolic activities, including its handling of glucose body's level via activation of AMPK [37]. Moreover, knockout of AMPK related gene *NUAK2* leads to developing of obesity, metabolic syndrome as well as cancer [38].

It is interesting to note that angiogenesis like many other biological processes is regulated by complex network of different factors which are tightly interconnected [12,19,21,39]. Thus, thrombospondin-1, a matrix-bound adhesive glycoprotein, has been shown to modulate tumor progression and up-regulates tissue inhibitor of metalloproteinase-1 production in human tumor cells and also up-regulates matrix metalloproteinases MMP-2 and MMP-9 [39]. This data suggested that the balance between matrix metalloproteinases and tissue inhibitors of metalloproteinases is a key determinant in different biological effects of THBS1, including tumor cell invasion, and may provide an explanation for the divergent activities reported for thrombospondin-1 in tumor progression. Thus, the THBS1 is involved in influencing the critical balance between MMPs and their inhibitors, maintaining the controlled degradation of the extracellular matrix needed to support metastasis and possibly in obesity as well as in obesity with different degree of dysglycemia. Moreover, different cells express a lot of pro-angiogenic and anti-angiogenic genes (e.g., *TIMP1*, *TIMP2*); however, detailed analysis of melanoma cells from different patients do not show a significantly higher median number of expressed pro-angiogenic or anti-angiogenic genes, but 97% of these cell samples aberrantly express at least one of the angiogenic factors [40]. Thus, the angiogenesis is regulated by complex network of pro-angiogenic and anti-angiogenic factors and aberrant expression at least one of these factors can modify an angiogenesis.

It is possible that *TIMP1*, *TIMP2*, *VCAN*, *CLEC3B*, *E2F1* and *SPARC* are also included in this network and the balance between different regulatory factors which participate in the control of angiogenesis, including vascular endothelial growth factor, a key pro-angiogenic factor, insulin, insulin-like growth factors, leptin and

others, really determinate an angiogenesis both in obesity and in obesity with glucose intolerance.

Results of this study also provide strong evidence that expression of *TIMP1*, *TIMP2*, *VCAN* and *SPARC* genes encoded the key regulatory factors with pleiotropic functions in subcutaneous adipose tissue of the obese individuals with glucose intolerance is deregulated and that these changes can be determined both by insulin resistance and endoplasmic reticulum stress. Collectively, the results of this study underscore the crucial role of *TIMP1*, *TIMP2*, *SPARC* and *VCAM* regulatory factors with pleiotropic function in the developing the obesity and its complications, especially glucose intolerance via participation in the intracellular signaling network, responsible for regulation of angiogenesis and glucose metabolism.

5. Acknowledgements

We thank Prof. Iwar Klimes (Institute of Experimental Endocrinology, Slovakia) for support and interest in this work as well as the gift of RNA from men's adipose tissue and clinical characteristics of patients.

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