EXPRESSION MARKERS OF HUMAN SKELETAL MUSCLE ASSOCIATED WITH DISORDERS OF GLUCOSE METABOLISM IN THE BASAL AND POSTPRANDIAL STATE

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BACKGROUND. Skeletal muscles play a key role in the organism's carbohydrate metabolism. Dysregulation of insulin-dependent glucose uptake in skeletal muscle disrupts carbohydrate metabolism in the organism and can lead to the development of obesity and type 2 diabetes.
AIM. To identify expression markers (genes) of human skeletal muscle associated with disorders of glucose metabolism in the basal state and after a mixed meal normalized for body mass.
MATERIALS AND METHODS. The study involved three groups of 8 people: healthy volunteers, obese patients without and with type 2 diabetes. Venous blood samples were taken in the morning (09:00) after an overnight fast and 30 min, 60 min, 90 min, 120 min, and 180 min after ingestion of a mixed meal normalized by body mass (6 kcal/kg). Biopsy samples from *m. vastus lateralis* was taken before and 1 h after a meal to assess gene expression (RNA sequencing) and search for genes correlating with markers of impaired glucose metabolism in the basal and postprandial state.
RESULTS. Strong correlations ($|\rho|>0.7$ and $p<0.001$) between the gene expression and the level of insulin, C-peptide, glucose or glycated hemoglobin in the basal and/or postprandial state was found for 75 genes. Of these, 17 genes had marked differences (>1.5-fold) in expression between healthy people and patients, or differences in expression changes in response to a meal. We can note genes whose role in impaired glucose metabolism has already been shown earlier (*FSTL1*, *SMOC1*, *GPCPD1*), as well as a number of other genes that are promising for further study of the mechanisms of insulin resistance in skeletal muscle.
CONCLUSION. Skeletal muscle expression markers were identified as promising candidates for future targeted studies aimed at studying the mechanisms of insulin resistance and searching for potential therapeutic targets.

KEYWORDS: type 2 diabetes mellitus; gene expression; skeletal muscle; insulin; correlation analysis; obesity.

ЭКСПРЕССИОННЫЕ МАРКЕРЫ СКЕЛЕТНОЙ МЫШЦЫ ЧЕЛОВЕКА, АССОЦИИРОВАННЫЕ С НАРУШЕНИЯМИ ГЛЮКОЗНОГО МЕТАБОЛИЗМА В БАЗАЛЬНОМ СОСТОЯНИИ И ПОСЛЕ ПРИЕМА ПИЩИ

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ВВЕДЕНИЕ. Скелетные мышцы играют ключевую роль в углеводном метаболизме организма. Дисрегуляция инсулин-зависимого захвата глюкозы в скелетной мускулатуре нарушает обмен углеводов в организме и может приводить к развитию ожирения и сахарного диабета 2 типа (СД2).
ЦЕЛЬ. Выявить экспрессионные маркеры (гены) скелетной мышцы человека, ассоциированные с нарушениями глюкозного метаболизма в базальном состоянии и после приема смешанной пищи, нормированной на массу тела.
МАТЕРИАЛЫ И МЕТОДЫ. В исследовании приняли участие три группы по 8 человек: здоровые добровольцы, пациенты с ожирением без и с СД2. Пробы венозной крови брали утром натощак (09:00) и через 30 мин, 60 мин, 90 мин, 120 мин и 180 мин после приема смешанной пищи, нормированной на массу тела (6 ккал/кг). Биопсические пробы из *m. vastus lateralis* брали до и через 1 ч после приема пищи для оценки экспрессии генов (РНК-секвенирование) и поиска генов, коррелирующих с маркерами нарушения глюкозного метаболизма в базальном состоянии и после приема пищи.
РЕЗУЛЬТАТЫ. Для 75 генов была найдена сильная корреляция ($|\rho|>0,7$ и $p<0,001$) между их экспрессией и уровнем инсулина, С-пептида, глюкозы или гликированного гемоглобина в базальном и/или постпрандиальном состоянии. Экспрессия 17 из этих генов имела выраженные отличия (>1,5 раза) между здоровыми людьми и пациентами либо выраженные изменения в ответ на прием пищи. Для них можно отметить такие гены, роль которых в нарушении метаболизма глюкозы уже была показана ранее (*FSTL1*, *SMOC1*, *GPCPD1*), а также другие — которые перспективны для дальнейшего изучения механизмов возникновения инсулиновой резистентности в скелетной мышце.

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ЗАКЛЮЧЕНИЕ. Были выявлены экспрессионные маркеры скелетной мышцы, являющиеся перспективными кандидатами для будущих целевых исследований, направленных на изучение механизмов возникновения инсулинорезистентности и поиск потенциальных терапевтических мишеней.

КЛЮЧЕВЫЕ СЛОВА: сахарный диабет 2 типа; экспрессия генов; скелетная мышца; инсулин; корреляционный анализ; ожирение.

INTRODUCTION

Obesity is one of the main contributors to the development of socially significant chronic metabolic diseases, such as metabolic syndrome and type 2 diabetes mellitus (T2D). In Russia, the number of patients with T2D is growing and today is about 3% of the population [1]. Skeletal muscles play a key role in the carbohydrate metabolism in the body, since even at rest their contribution to insulin-dependent glucose consumption exceeds 80% [2]. This means that dysregulation of insulin-dependent glucose uptake by skeletal muscle impairs carbohydrate metabolism and can lead to obesity and T2D.

The insulin resistance of skeletal muscle is mainly associated with impaired insulin-dependent signaling regulating translocation of glucose transporter GLUT4 and postprandial uptake of glucose from the bloodstream. However, it turned out that in individuals with obesity and T2D, skeletal muscles in a basal state (fasting) have pronounced changes in the phosphorylation of many signaling molecules that are not part of the canonical insulin signaling cascade, as well as a number of transcription factors [3], which is in good agreement with the change in the transcriptional profile of the muscles of patients [4–6]. These changes can be caused by a change in the blood insulin and other hormones and metabolites in the basal state, as well as the sensitivity of skeletal muscles to them. Indeed, a number of studies have found significant correlations of gene expression in fasting skeletal muscle with blood insulin levels and some markers of metabolic disorders. For example, a study of the transcriptome of skeletal muscles in individuals with different body mass index revealed 180 significant correlations of gene expressions with the insulin resistance index (Homeostatic Model Assessment for Insulin Resistance, HOMA IR), including, among other things, some regulators of lipid metabolism and the AKT-mTOR signaling pathway that regulates protein synthesis and proteolysis, gene expression, etc. [7]. In addition, a meta-analysis of transcriptomic data showed that the expression of the insulin receptor gene (*INSR*) and its substrate (*IRS2*) negatively correlate with the level of fasting insulin in skeletal muscle of individuals with different levels of insulin sensitivity [8].

On the other hand, a change in basal expression of skeletal muscle genes can be regulated by a change in the content of insulin and other substances caused by regular meals. Indeed, several studies have shown that an increase in blood insulin during clamp testing (up to 100 mU/L or ~0.6 nM) after 3–4 hours alters the expression of several hundred genes in skeletal muscle of healthy individuals [9,10]. In addition, significant associations of gene expression in muscle tissue and adipose tissue with carbohydrate metabolism during insulin stimulation (clamp test) have already been found [11]. However, it must be noted that in healthy individuals and in obese individuals with or without T2D, there might be a significant difference in the increase and absolute level of insulin during everyday meals from those obtained under the clamp test conditions and between themselves. Therefore, a great interest exists in studies examining changes in glucose metabolism and gene expression in skeletal muscle in reaction to meals, normalized by body weight [12–14]

PURPOSE OF THE STUDY

The purpose of the study is to identify expression markers (genes) of human skeletal muscle associated with impaired glucose metabolism in the basal state and after meals, normalized by body weight.

MATERIALS AND METHODS

Study design

A multicenter interventional prospective comparative study.

Study location and time

The organization of the study was described in our previous publications [15,16]. Studies with patients were carried out at the Endocrinology Research Centre from April 2022 to May 2023. Studies with healthy volunteers were carried out at the Institute of Biomedical Problems, Russian Academy of Sciences, during the period from March 2022 to September 2023.

Populations

The characteristics of the volunteers are shown in Table 1. The study included 8 healthy volunteers («H») (inclusion criteria: BMI<25; absence of diagnosed T2D), 8 obese patients («Ob») (BMI>30; absence of diagnosed T2D) and 8 obese patients with T2D (BMI>30; diagnosed T2D). T2D patients were on glucose-lowering therapy; this therapy was interrupted for 1–2 days (sodium glucose cotransporter-2 inhibitors, dipeptidyl peptidase-4 inhibitors, sulfonylurea derivatives, biguanide [metformin]) and/or for 7 days (glucagon-like peptide-1 receptor agonist [semaglutide]) prior to the mixed meal tolerance test.

Description of intervention

All volunteers completed a short form survey SF-12 [17] to subjectively assess their physical abilities. Venous blood samples from *v. cephalica* were taken in the morning on an empty stomach (09:00) and 30 min, 60 min, 90 min, 120 min and 180 min after ingestion of mixed food product Resource 2.0 (Nestle Health Science, France, 3 ml or 6 kcal/kg b.w.; protein : fat : carbohydrate ratio 1:2.3:2.7 kcal/kg b.w.) (Figure 1A). Needle biopsy was taken from the middle of *m. vastus lateralis* under local anesthesia (2 mL of 2% lidocaine), before and one hour after meals (the second biopsy was taken 10 cm proximal to the first) as described elsewhere [15, 16].

Table 1. Characteristics of volunteers

Male/Female	Reference	H	Ob	T2D
		4/4	**3/5**	**4/4**
Age, years		41.0 (34.5–46.8)	40.0 (25.0–51.0)	**58.0*# (48.0–63.5)**
Body mass index, kg/m^2	18.5–25	22.5 (19.4–24.0)	**43.5*** (37.2–50.1)**	**39.3** (38.2–45.1)**
Glucose, mmol/L	<6.1	4.9 (4.5–5.2)	5.1 (5.0–5.4)	**6.4** (5.8–9.9)**
Insulin, mU/L	2.6–24.9	7.2 (5.7–7.5)	**19.5** (16.3–32.3)**	**24.0** (16.4–35.7)**
C-peptide, ng/mL	1.1–4.4	2.0 (1.7–2.3)	**3.4* (2.6–5.0)**	**4.4** (3.3–6.4)**
HbA_{1c}, %	4.0–6.0	5.3 (5.0–5.6)	5.2 (5.0–5.5)	**6.3*## (5.8–8.3)**

Note: Median and interquartile range are presented; * – difference from control, # – difference from Ob; one, two, and three characters, $p \leq 0,05$, $\leq 0,01$, and $\leq 0,001$, respectively.

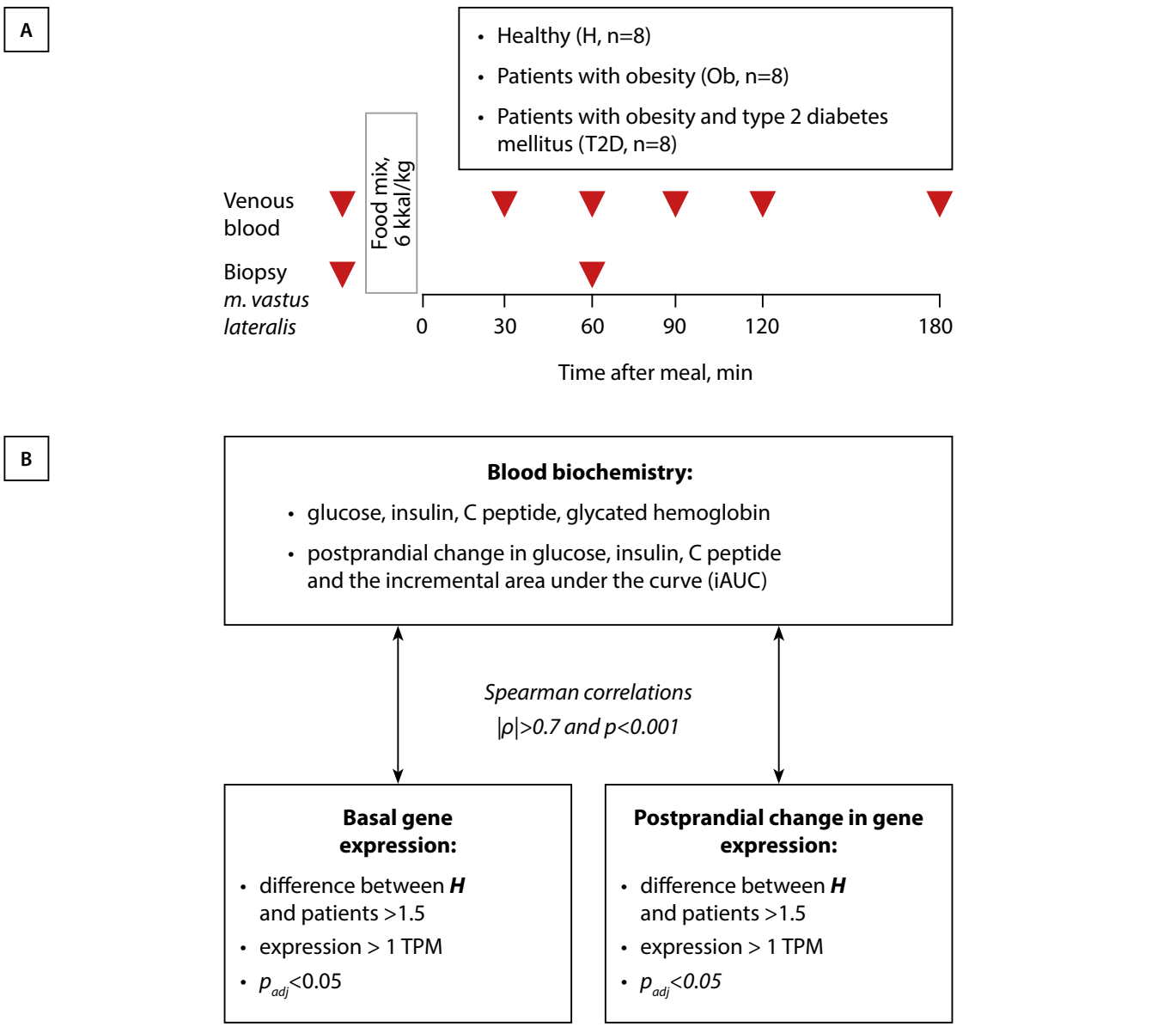

Figure 1. Experimental design (A) and flow chart of search for expression markers associated with glucose metabolism parameters (B).

Laboratory test methods

Glycated hemoglobin (HbA_{1c}) in whole blood was determined by high performance liquid chromatography using D10 analyzer (BioRad, USA), serum glucose was determined using Architect c8000 automatic analyzer (Abbott Diagnostics, USA); immunoreactive insulin and C-peptide were determined in serum using Cobas 6000 electrochemiluminescence analyzer (Roche, Switzerland) as described elsewhere [13,14].

The whole-genome RNA sequencing and bioinformatic data processing were performed as described elsewhere [16]. In brief, total RNA was isolated using spin columns (RNeasy Mini Kit, Qiagen, Germany) and its concentration was measured (Qubit 4 fluorometer and Qubit RNA HS Assay Kit (Thermo Scientific, USA). After RNA integrity assessment (RIN>7; capillary electrophoresis on TapeStation 4150 and High Sensitivity RNA ScreenTape Kit, Agilent, Germany), chain-specific libraries were prepared with the NEBNext Ultra II Directional RNA Library Preparation Kit (New England BioLabs, USA) and then sequenced (75 nucleotides from one end, covering about 55 million reads per sample) on NextSeq 550 analyzer (Illumina, USA).

Bioinformatics and statistical analysis

RNA reads were mapped to the human genome (primary assembly GRCh38.p13) using the Hisat2 v.2.2.1 software. The number of uniquely mapped reads for the exons of each gene was calculated using the Rsubread package (R software environment). Genes that changed expression were determined using the DESeq2 method with correction for Benjamin-Hochberg multiple comparison (p_{adj}<0.05; R environment), expression level was determined using TPM value (Kallisto software, v0.46.2).

After removal of low-expression genes (TPM<1), correlations between protein-coding gene expression and biochemical parameters were determined using Spearman rank correlation coefficient; only strong relationships were used for further analysis: |ρ|>0.7 and p<0.001 (Figures 1B, 3A).

For biological interpretation and finding correlations between basal gene expression and biochemical parameters only those genes were taken whose expression differed by more than 1.5-fold between healthy volunteers and patients (DESeq2 test, p_{adj}<0.05) (Figures 1B, 3B).

For biological interpretation and finding correlations between changes in gene expression after meals and biochemical parameters only those genes were used whose expression changes in response to meals significantly differed by more than 1.5-fold between healthy volunteers and patients (Mann-Whitney test, p<0.05).

Kruskal-Wallis univariate analysis of variance corrected for multiple comparison (Dunn test) at significance level of 0.05 was used to assess differences between samples for biochemical parameters.

Protein classes were determined using the KEGG BRITE database.

Ethical review

The study was carried out in accordance with the Helsinki Declaration and approved by the Biomedical Ethics Committee, Institute of Biomedical Problems, Russian Academy of Sciences (Minutes No. 613 of March 29, 2022) and the Local Ethics Committee of the Endocrinology Research Centre (Minutes No. 4 of February 14, 2022). Written informed consent was obtained from all volunteers.

RESULTS

Characteristics of groups and mixed meal tolerance test

Obese patients (Ob) and obese patients with diabetes mellitus (T2D) differed from healthy patients (H) by elevated basal insulin and C-peptide levels, obese patients with type 2 diabetes mellitus had elevated glucose and glycated hemoglobin levels (Table 1). As we described earlier [15], the dynamics of biochemical parameters after meal differed between groups. In Ob and T2D groups, there was an increased postprandial level and an increase in the area under the curve (iAUC) of C-peptide and insulin (Figures 2A and B). In contrast to the control group, glucose levels in patients were increased up to the second hour after eating (as expected), whereas iAUC was increased only in the T2D group (Figure 2).

Correlations of basal gene expression with parameters of glucose metabolism

In the basal state, 526, 174 and 397 genes were found which changed expression compared to healthy controls in the Ob group, T2D group and pooled group of patients, respectively. However, only 38 of these showed significant and strong correlations (|ρ|> 0.7) with basal insulin, C-peptide, fasting glucose, or glycated hemoglobin. In order to identify potential biologically significant correlations, we selected 11 genes, the expression of which differed between the healthy control and any group of patients more than 1.5-fold (Figure 3A, Table 2, supplementary figure). When analyzing the relationship of basal expression with a change in glucose level in response to meal, significant correlations were found for four genes: positive for *NFIL3* and *VGLL2*, and negative for *CADM1* and *GPCPD1*) (Figure 3A, Table 2, supplementary figure).

Correlations of postprandial gene expression changes with measures of glucose metabolism

Significant correlations between healthy volunteers and patients were found for 37 genes; among them, only two genes showed a significant (by >1.5) change in the amplitude of postprandial expression – *MEOX1* (Mesenchyme Homeobox 1) and *TXNDC12* (Thioredoxin Domain Containing 12) (Figure 3B, supplementary figure).

DISCUSSION

In our study, we examined associations between biochemical parameters of the blood characterizing glucose metabolism abnormalities and gene expression in skeletal muscle in the basal state and after meals in healthy subjects, obese patients without and with T2D. It is important to note that the change in these indicators was studied in nearly physiological conditions, namely after the intake of mixed food normalized by body weight. The study identified 17 genes, the expression of which was associated with metabolic disorders and the level of insulin, C-peptide or glucose (on an empty stomach or in response to a meal) (supplementary figure).

Table 2. Gene functions with expression differences between groups >1.5 and showing significant strong correlations of basal expression with fasting parameters of glucose metabolism and their postprandial changes.

Gene symbol	Gene name	Molecular function (Genecards Database)	Change in expression in patients
CADM1	Cell Adhesion Molecule 1	Intercellular adhesion regulator	↓**T2D, Ob**
CPXM2	Carboxypeptidase X, M14 Family Member 2	Extracellular carboxypeptidase	↑**T2D, Ob**
FSTL1	Follistatin Like 1	Secreted glycoprotein involved in a variety of physiological processes	↑**T2D, Ob**
GPCPD1	Glycerophosphocholine Phosphodiesterase 1	Glycerophospholipid metabolism enzyme	↓**T2D**
HCST	Hematopoietic Cell Signal Transducer	Phosphoinositide 3-kinase adaptor protein	↑**T2D, Ob**
MFAP5	Microfibril Associated Protein 5	Glycoprotein, a component of extracellular matrix microfibrils	↑**T2D, Ob**
NEK10	NIMA Related Kinase 10	Kinase, ERK1/ERK2 signaling activator	↓**T2D**
NFIL3	Nuclear Factor, Interleukin 3 Regulated	Transcription factor	↑**T2D**
SLC16A10	Solute Carrier Family 16 Member 10	Thyroid hormone and aromatic amino acid transporter	↑**T2D, Ob**
SMOC1	SPARC Related Modular Calcium Binding 1	Secreted protein	↓**T2D**
SVEP1	Sushi, Von Willebrand Factor Type A, EGF And Pentraxin Domain Containing 1	Regulator of Ca^{2+} metabolism and vasoconstriction	↑**T2D, Ob**
TRIM50	Tripartite Motif Containing 50	E3 ubiquitin transferase	↓**T2D**
TUBB6	Tubulin Beta 6 Class V	Microtubule cytoskeleton component	↑**T2D, Ob**
UBTD1	Ubiquitin Domain Containing 1	Ubiquitin-like protein, may be involved in cellular aging	↑**T2D, Ob**
VGLL2	Vestigial Like Family Member 2	Transcriptional cofactor	↑**T2D**

Note: Arrows indicate the direction of expression change in obese patients (Ob) and obese patients with type 2 diabetes mellitus (T2D) compared to healthy volunteers.

Fasting insulin and C-peptide expression markers

Insulin is an anabolic hormone that regulates many processes in skeletal muscle. In insulin resistance, chronically elevated insulin levels and/or excessive postprandial increments can lead to alterations in insulin-dependent cell signaling and gene expression in skeletal muscle. We identified several expression markers in skeletal muscle that correlate closely with basal levels or postprandial changes in insulin levels. Among them, genes were found for which a role in the regulation of glucose metabolism and the development of metabolic disorders was already demonstrated.

For example, by analogy with our study, which showed that the basal expression of the adipomyokin gene *FSTL1* positively correlates with fasting levels of C-peptide and insulin (Figure 4A), the authors of [18] found that the level of *FSTL1* in the blood is positively associated with obesity and insulin resistance. On L6 myoblasts and primary dog myoblasts, it was shown that *FSTL1*, regulates AMPK-dependent glucose uptake and expression of GLUT4 [19]. Moreover, infusion of *FSTL1* to dogs with the induced heart failure positively influenced metabolism of fat acids and glucose oxidation [20]. These data and our results suggest that insulin-induced increases in *FSTL1* expression in patients may be one of the compensatory mechanisms aimed at prevention of disorders in glucose uptake in muscles.

The negative correlations of the expression of the *SMOC1* gene with the level of insulin and C-peptide found in our study are consistent with the data on the positive correlation of its protein level in plasma with insulin sensitivity in humans [21]. In mice, intraperitoneal administration of *SMOC1* or increased expression of its gene in the liver improved glucose metabolism and insulin sensitivity by increasing the expression of gluconeogenesis regulatory genes [21]. In humans, the relationship between blood *SMOC1* levels and carbohydrate metabolism disorders is still unclear, however, our study points to the potential role of changes in this gene expression in skeletal muscle in the development of metabolic disorders.

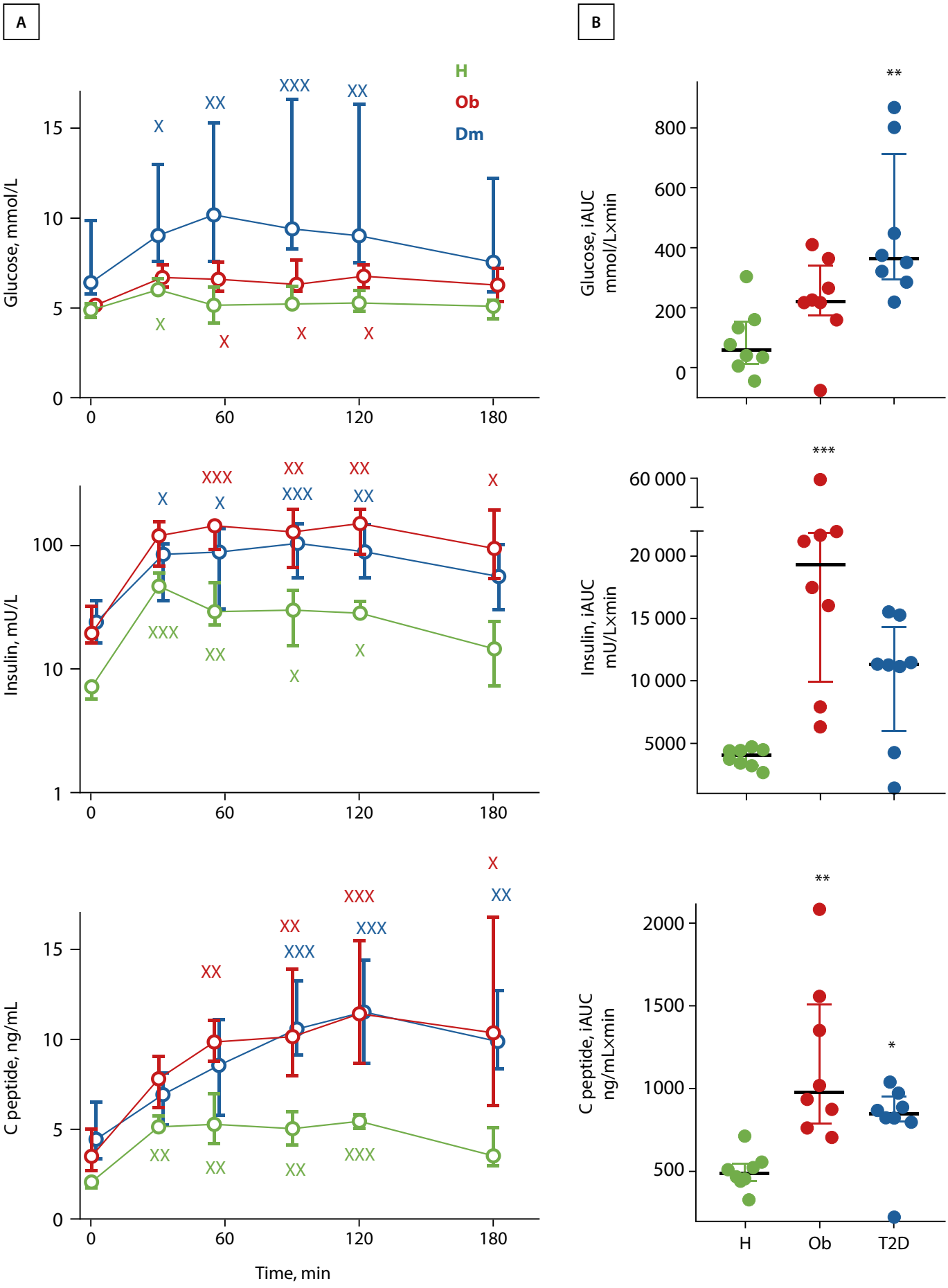

Figure 2. Change in blood glucose, insulin and C-peptide after ingestion of mixed weight-normalized food in healthy subjects (H), obese patients without T2D (Ob) and with T2D (T2D). Trends of these parameters (A) and incremental area under the curve (iAUC) (B) are presented; × – difference from the baseline; * – difference from the control. One, two and three characters – $p \leq 0{,}05$, $\leq 0{,}01$ and $\leq 0{,}001$, respectively.

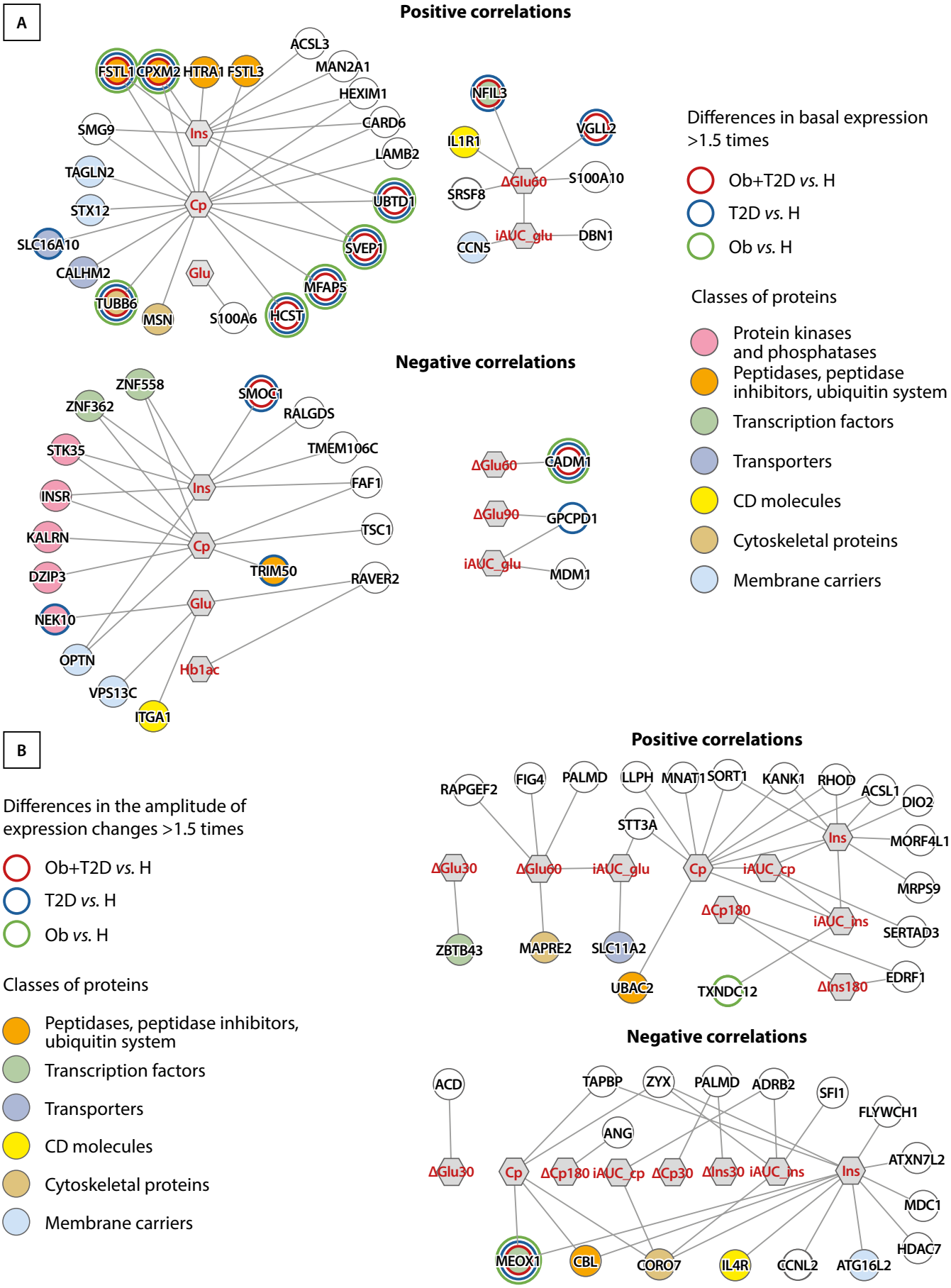

Figure 3. Correlations of basal expression (A) and postprandial skeletal muscle gene expression changes (B) with basal level (morning, fasting) of C-peptide, insulin, and venous blood glucose, and with their postprandial increment. Lines show strong significant correlations (|ρ|>0.7; p<0.001). Colored circles show genes whose basal expression (A) or change in expression (B) in response to food differs between groups by >1.5-fold (green – Ob *vs.* H, blue T2D *vs.* H, red – pooled group Ob+T2D *vs.* H). Circle filling shows the protein class. Ins – insulin, Cp – C-peptide, Glu – glucose, HbA_{1c} – glycated hemoglobin, iAUC – incremental area under the curve of glucose for 180 min after meal, Δ – postprandial changes.

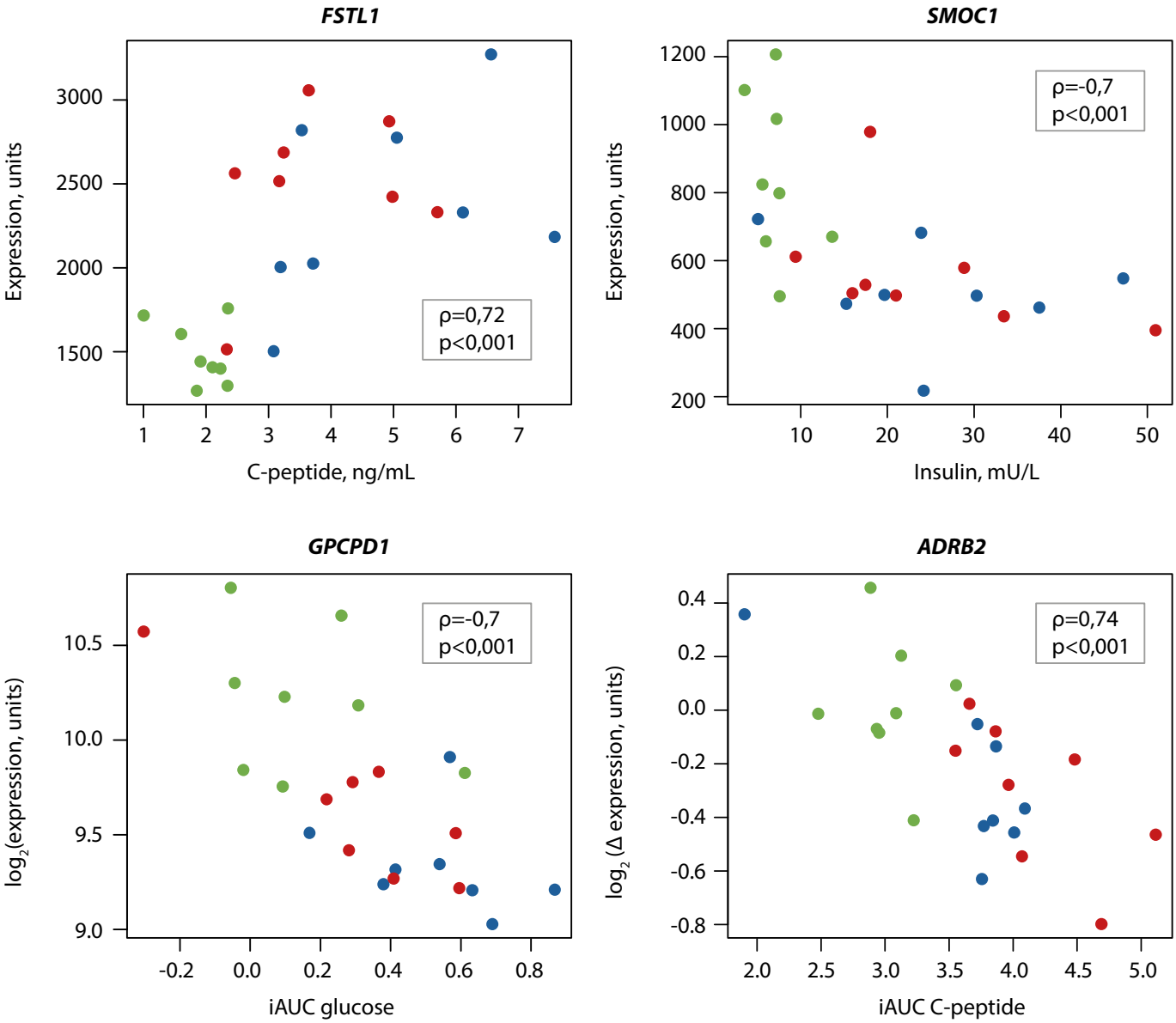

Figure 4. Examples of correlations between potential expression markers of metabolic disorders (Follistatin like 1, SPARC Related Modular Calcium Bindin 1, Glycerophosphocholine Phosphodiesterase, Adrenoceptor Beta 2 genes) and parameters of glucose metabolism. Healthy volunteers (H) – green, obese patients (Ob) – blue, obese patients with type 2 diabetes mellitus (T2D) – red.

The *UBTD1* and *MFAP5* genes had a positive correlation of basal expression with fasting C-peptide levels. This is consistent with other studies where they are described as expression markers positively correlating with fasting glucose level, glucose consumption rate (*UBTD1*), or degree of obesity or fasting insulin level (*MFAP5*) [11,22].

Expression markers associated with fasting glucose level

Hyperglycemia is a key cause of impaired functioning of various tissues, organs and systems, including skeletal muscles; in particular, its relationship with the development of diabetic myopathy was found [23]. However, the molecular mechanisms of the negative effects of hyperglycemia on skeletal muscle are still poorly understood. We found several expression markers correlated with postprandial glucose levels. For example, basal *GPCPD1* expression was negatively correlated with glucose change in response to the mixed meal tolerance test (Figure 4B). The effect of *GPCPD1* on the development of insulin resistance in humans has not been studied, but it has been shown in mice that inactivation of this gene in muscles causes glycerophosphocholine accumulation, hyperglycemia and insulin resistance, which is consistent with data on an increase in glycerophosphocholine in skeletal muscle of elderly people and patients with T2D [24].

The role of other genes whose expression was found to be associated with postprandial glucose alteration, has not been studied in relation to the regulation of glucose metabolism in skeletal muscles. However, in primary pancreatic beta cell culture, *CADM1* gene expression has been shown to be associated with insulin secretion and glucose level [25], and in primary hepatocyte culture, the *NFIL3* gene regulates glucose production and gluconeogenesis [26].

Postprandial gene expression changes associated with insulin and C-peptide levels

Postprandial changes in the expression of only two genes (transcription factor *MEOX1* and endoplasmic reticulum protein *TXNC12*) correlated with different parameters of glucose metabolism; the role of these genes in regulating glucose metabolism is still unknown. It is worth noting that we included in our analysis only genes with marked differences (>1.5-fold) in postprandial response between patients and healthy individuals. Nevertheless, it is interesting to note the negative correlation between the change in the expression of the beta-2 adrenoceptor gene *ADRB*2 (1.3-fold difference in expression change between healthy and patients) and the changes in insulin and C-peptide (Figure 4). *ADRB2* has been repeatedly associated with insulin resistance and has several polymorphisms reliably associated with obesity [27, 28], and its expression positively correlates with fat loss [29].

STUDY LIMITATIONS

The results of our study were obtained on a small sample; therefore, subsequent studies are required to verify the markers we found. In addition, the presence of comorbidities, as well as the use of glucose-lowering drugs by patients (despite their withdrawal the day before the study) could affect the variability of the data obtained. In our study, only two patients took exclusively glucose-lowering therapy, the rest additionally took other drugs, mainly for control of arterial hypertension and hypercholesterolemia.

CONCLUSION

In our study, 17 expression markers (genes) associated with indicators characterizing disorders of glucose metabolism in the basal state and after meals were identified. The validity of our prediction is supported by the fact that for 7 of them, a relationship with metabolic disorders was already demonstrated, and *FSTL1* and *SMOC1* genes were already considered as potential therapeutic targets for blood glucose control. Other expression markers identified in our study are promising candidates for future targeted studies aimed at investigating the mechanisms of insulin resistance development and may be considered as potential therapeutic targets. In addition, it should be noted that we examined early transcriptome response to mixed food intake; investigating this response at later stages (and looking for associations with impaired glucose metabolism) appears to be a promising area for further research.

SUPPLEMENTARY MATERIALS

Supplementary figure is available at https://zenodo.org/records/10998825

FURTHER INFORMATION

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Conflict of interest The authors declare no clear and potential conflicts of interest related to the content of this paper.

Contributions of authors. P.A. Makhnovsky – scientific leadership of the study, bioinformatic and statistical analysis of data and their biological interpretation, writing and editing the manuscript; N.S. Kurochkina – preparation of RNA samples, RNA sequencing, making significant edits to the manuscript; T.F. Vepkhvadze – conducting interventions and their organization, preparation of RNA samples, making significant edits to the manuscript; A.O. Tomilova – work with patients, conducting interventions and their organization, making significant edits to the manuscript; E.M. Lednev – conducting interventions and their organization, statistical analysis of data, making significant edits to the manuscript; M.V. Shestakova – scientific leadership of the study, organization and concept of the study, biological interpretation of the results, making significant edits to the manuscript; D.V. Popov – scientific leadership of the study, organization and concept of the study, biological interpretation of the results, writing the manuscript.

All authors approved the final version of the manuscript before publication, agreed to be responsible for all aspects of the manuscript, ensuring proper investigation and resolution of issues related to the accuracy or fidelity of any part of the manuscript.

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