Влияние гиперкалорийного рациона и кверцетина на полнотранскриптомный профиль ткани печени крыс линии Zucker-*LEPR*^{fa}

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Обоснование. Пептидный гормон лептин (Lep) занимает центральное место в контроле энергетического гомеостаза и массы тела. Удобной моделью для изучения роли нарушенной рецепции Lep являются крысы Zucker-*LEPR*^{fa}, несущие в гомозиготе мутацию гена *LEPR*. В качестве одного из перспективных биологически активных веществ, корригирующих нарушения обмена веществ при ожирении и метаболическом синдроме, в настоящее время рассматривается кверцетин (Q; 3,3',4',5,7-пентагидроксифлавон).
Цель исследования — изучить экспрессию генов в ткани печени крыс с нарушенной рецепцией Lep под влиянием высокожирового и высокоуглеводного рациона (ВУВЖД) и/или добавки к рациону кверцетина.
Материал и методы. Использовали четыре группы крыс самцов линии Zucker-*LEPR*^{fa}. В течение 61-х суток животные 1-й группы (контроль) получали сбалансированный полусинтетический рацион, 2-й группы — такой же рацион с добавкой Q в дозе 50 мг/кг массы тела, 3-й группы — ВУВЖД (30% жиров по массе сухих веществ и 20% фруктозы вместо воды), 4-й группы — такой же рацион и добавку Q. Полнотранскриптомное профилирование ткани печени выполняли на микрочипах из набора Gene Expression Hybridization Kit («Agilent Technologies»). Полимеразную цепную реакцию в «реальном времени», совмещенную с обратной транскрипцией (ОТ-ПЦР), выполняли для содержащихся в ткани печени транскриптов генов *Crot, FTO, NpY, Prdx1, Prom1, Ugt2b37* и *GAPDH*.
Результаты. Потребление Q и/или ВУВЖД крысами Zucker-*LEPR*^{fa} приводит к значимым изменениям в уровне транскрипции 1604 генов в ткани печени, из которых эффект собственно Q проявляется для 1396 генов. Изменения в транскриптоме ткани печени, вызываемые ВУВЖД, более выражены, чем обусловленные добавкой Q на фоне стандартного рациона. Q оказывал влияние на экспрессию генов, отвечающих за процессы детоксикации ксенобиотиков *(UGT2b37)*, редокс-гомеостаз (*Prdx1*), бета-окисления жирных кислот (*Crot*) и центральные механизмы, воздействующие на чувство голода и насыщения (*NpY*), а также потенцировал либо отменял эффекты ВУВЖД в отношении ряда других функционально значимых генов. Биоинформатический анализ выявил влияние потребления ВУВЖД и/или Q на 23 метаболических пути (KEGGS), из которых 7 [метаболизм стероидов, арахидоновой и линолевой кислот, ретиноидов, лекарственных препаратов и ксенобиотиков (под действием цитохрома Р-450), секреции желчи] были затронуты во всех опытных группах.
Заключение. Изменения в транскриптоме печени крыс Zucker-*LEPR*^{fa}, вызванные потреблением ВУВЖД и/или Q, согласовались с экспериментальными данными об изменении показателей краткосрочной памяти, тревожности и минерального обмена у этих животных.

Ключевые слова: транскриптом, печень, крысы, лептин, ожирение, метаболические пути.

The effect of hypercaloric diet and Quercetin on the full-transcriptome liver tissue profile of Zucker-*LEPR*^{fa} rats

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BACKGROUND: The peptidic hormone leptin (Lep) occupies a central place in the control of energy homeostasis and body weight in mammals. A convenient model for studying the role of impaired Lep reception is the Zucker-*LEPR*^{fa} rats, which carry a mutation in the homozygote of the *LEPR* gene. Quercetin (Q; 3.3 ', 4', 5.7-pentahydroxyflavone) is currently being considered as one of the promising biologically active substances, which allows to correct metabolic disorders in obesity and metabolic syndrome.
AIM: to study changes in the expression of genes in liver tissue of rats with impaired receptivity of Lep under the influence of high-fat and high-carbohydrate diet (HFCR) or/and Q supplementation.
MATERIAL AND METHODS. 4 groups of six male Zucker-*LEPR*^{fa} male rats were used in experiment. Within 61 days the animals of the 1st group (control) received a balanced semi-synthetic diet, the second group received the same diet with the addition of quercetin in a dose of 50 mg/kg of body weight, the third group — the HFCR (30% fat by dry weight and 20% fructose instead of water), the 4th group is the same diet and supplement of quercetin. Full transcriptional profiling of liver tissue was performed on microchips from the Gene Expression Hybridization Kit (Agilent Technologies), a real-time polymerase chain reaction combined with reverse transcription (RT-PCR) was performed for liver transcripts of *Crot, FTO, NpY, Prdx1, Prom1, Ugt2b37* and *GAPDH* genes contained in liver tissue.
RESULTS. It was shown that feeding of Zucker-*LEPR*^{fa} rats with Q and/or HFCR led to significant changes in the level of transcription of 1604 genes in liver tissue, from which the effect of quercetin proper was manifested for 1396 genes. Changes were more pronounced in the transcriptome of liver tissue caused by HFCR, than caused by the addition of Q against the background of a standard diet. Q influenced the expression of genes responsible for xenobiotic detoxification processes (*UGT2b37*), redox homeo-

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stasis (*Prdx1*), beta-oxidation of fatty acids (*Crot*), and central mechanisms affecting hunger and satiety (*NpY*), and potentiated, or abolished the effects of HFCR against a number of other functionally important genes. Bioinformatic analysis revealed the influence of HFCR and/or Q on 23 metabolic pathways (KEGGS), of which 7 (the metabolism of steroids, arachidonic and linoleic acids, retinoids, drugs and xenobiotics (due to cytochrome P-450), bile secretion) were affected in all experimental groups.
CONCLUSIONS. Changes in the transcriptome of the liver of Zucker-*LEPR*^{fa} rats, caused by consumption of HFCR and/or Q, were consistent with experimental data on changes in short-term memory, anxiety and mineral metabolism in these animals.

Keywords: transcriptome, liver, rats, leptin, obesity, metabolic networks and pathways.

Background

maintaining energy homeostasis in humans and mammals under diverse diet conditions involves neurohumoral regulation of food intake by the central nervous system (CNS). Two loops of such regulation are considered in the literature. They include the homeostatic one, which is mediated by the response of the hypothalamic arcuate nucleus neurons to hormonal signals from the peripheral tissues, such as adipokines: leptin (Lep) and ghrelin [1, 2]. The second one is hedonic regulation, which takes place in the superior brain regions directly connected with the taste analyzer via cannabinoid and opioid receptors, as well as their endogenous ligands [3]. Impairment of these regulatory mechanisms can cause dysregulation of the feelings of hunger and satiety resulting in alimentary obesity and metabolic syndrome. The interaction of both regulatory loops is complex; in particular, the systemic inflammation can affect it significantly [4].

Lep, which is secreted by the white adipose tissue, is central to the control of energy homeostasis and body weight. The blood-brain barrier (BBB) is partially permeable to Lep, which provides its entry into the hypothalamus and binding to its specific receptor *LEPR* [5] followed by activation of the SOCS3 signaling cascade [6]. The level of circulating Lep increases in fat accumulation, it penetrates the hypothalamus in greater quantities stimulating POMC neurons and inhibiting NPY/AgRP neurons [1]. In healthy mammals, Lep has anorexigenic, catabolic, lipolytic, and hypoglycemic effects, which provide a negative feedback mechanism. In obesity, the action of Lep is impaired due to a decreased transfer through the BBB or binding to the receptor form circulating in the blood [7].

A convenient model for studying the role of impaired Lep reception in obesity is Zucker-*LEPR*^{fa} rats, which carry a homozygous deleterious mutation in *LEPR* making the corresponding receptor non-functional. As a result, on the one hand, there is no impact of fat reserves on the amount of food consumed in these animals, which leads to the rapid development of obesity with the consumption of a standard balanced diet. On the other hand, disruption of the processes of Lep reception and internalization by cells impairs its clearance thus resulting in a sharp increase in the blood level of this hormone and development of atypical immunotropic effects due to a partial homology between Lep and a number of cytokines and chemokines [8, 9]. Expression of a large number of genes responsible for the functioning of various metabolic pathways determining the homeostasis of an organism changes in the organs and tissues of such animals. These changes are largely consistent with those observed in patients with alimentary obesity.

Minor biologically active substances belonging to bioflavonoids, in particular quercetin (Q; 3,3',4',5,7-pentahydroxyflavone), are currently considered as promising agents for the treatment of these disorders. According to some experimental data [10. 11] and clinical observations [12], Q at doses close to its content in food products attenuates manifestations of the metabolic syndrome triggered by the consumption of an excess of easily digestible carbohydrates. The action of Q is assumed to be associated with the activation of transcription factors PPAR-γ, SREBP1, and AMPk [13]. The efficacy of Q in treating obesity and immunotropic side effects in dysregulated Lep reception has not been studied enough yet.

Objective

the aim of the work is to study changes in gene expression in the liver tissue of Zucker-*LEPR*^{fa} rats fed with a high-fat high-carbohydrate diet or/and Q supplementation by whole-transcriptome microarray profiling with the bioinformatics analysis of the data in R and by real-time RT-PCR.

Methods

the studies were conducted on 24 Zucker-*LEPR*^{fa} male rats (age, 8—10 weeks; average initial body weight, 176±7 g) obtained from Charles River laboratories, Italy. Animal studies complied with the Order of the Ministry of Health of the Russian Federation No. 193 "On approval of the principles of good laboratory practice" dated April 1, 2016. The experimental design was reviewed and approved by the Ethics Committee of the Federal Research Center of Nutrition, Biotechnology and Food Safety (Minutes No. 4 dated April 20, 2017). Animals were divided into 4 groups of equal number (N=6). For a period of 61 days, animals of the first (control) group received a balanced diet according to AIN93M, animals of the 2nd group received the same diet with Q supplementation at a dose of 50 mg/kg body weight, the 3rd group was fed with high-fat diet (30% fat of dry weight) and 20% fructose instead of water (HFHCD), and the 4th group consumed the same diet with Q supplementation. Animals had unrestricted free access to food and drinking liquids. Rats were housed in polycarbonate cages in groups of 2 animals per cage in a 12/12-h light/dark environment at 22±1 °C

air temperature. Daily amounts of the consumed food and water were recorded, and the caloric value of the diet was estimated. Body weight was determined on a weekly basis using an electronic scale with an accuracy of ±1 g.

The animals were removed from the experiment by decapitation under ether anesthesia on day 62. Blood was collected into tubes containing 1.0% heparin solution in 0.15 M NaCl (1:10 v/v), plasma was separated by centrifugation, and biochemical parameters were studied on a Konelab 20i analyzer (Finland). The liver was isolated with sterile surgical instruments made of stainless steel, immediately cooled on ice to a temperature of 0—2 °C, weighed with an accuracy of ±0.01 g and stored at -80 °C.

Liver mRNA was isolated using an Agilent Total RNA Isolation Mini Kit (Agilent Technologies, USA). A 20 mg sample of frozen liver tissue was quickly homogenized in a Potter-Elvehjem glass tissue grinder in 500 mm^3 of lysis buffer (with the addition of 5 mm^3 of β-mercaptoethanol per 500 mm^3 of buffer) at a temperature of 0—2 °C. Further procedures were performed according to the standard protocol [14]. The resulting total RNA was treated with *DNAase I* to remove traces of genomic DNA. RNA concentration was determined on a NanoDrop 1000 spectrophotometer. Next, RNA was dissolved in nuclease-free water to a concentration of 200 ng/mm^3, and the fragmentation degree was analyzed on an Agilent Bioanalyzer 2100 with determination of the RIN (RNA Integrity Number). RNA was stored in nuclease-free water or as isopropanol precipitates at -80 °C. Complementary DNA was synthesized from total mRNA using Agilent AffinityScript QPCR cDNA Synthesis Kit (Agilent Technologies, USA) according to the manufacturer's protocol.

Whole-transcriptome profiling of liver tissue was performed using Gene Expression Hybridization Kit microarray chips (Agilent Technologies) according to the Agilent One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling protocol, version 6.8 [15]. Microchips were scanned using a SureScan Microarray Scanner (Agilent Technologies). The expression value is presented as binary logarithm (logFC) of an increase or decrease in fluorescence compared to the control group. In total, data on the differential expression of 30,003 genes presented on the microchip were obtained for each cDNA sample.

Real-time reverse transcription followed by polymerase chain reaction (RT-PCR) for *Crot, FTO, NpY, Prdx1, Prom1, Ugt2b37*, and *GAPDH* liver transcripts was performed on a CFX96 devise (Bio-Rad, USA) in 25 µl reaction mixture containing the following components: 2.5 µl of 10x TaqPol buffer (contains 2 mM $MgCl_2$; Eurogen, Russia), 1 µl of (R+F) primers (10 µM; Dnk-Sintez, Russia), 0.5 µl of probe (10 µM; Dnk-Sintez, Russia), 0.5 µl of dNTPs (5 mM; SibEnzyme, Russia), 18.5 µl of DNase- and RNase-free water (TermoScientific, USA), 0.5 µl of Taq-polymerase (5 U/µl; Eurogen, Russia), and 1 µl of cDNA (400 ng/µl). At the first stage, Taq-polymerase was activated for 3 min at 95°C, then 45 cycles of amplification were performed (95°C - 15 s, 60°C - 1 min). The expression value is presented as binary logarithm normalized to the level of a reference gene mRNA (β-actin).

Statistical processing of the data was performed using non-parametric Mann-Whitney rank test. The significance of changes in differential gene expression was assessed by analyzing the logarithms of fluorescence intensity normalized to internal controls (Spike-In) using the Benjamini–Hochberg multiple testing correction [16]. SPSS® 16.0 statistical software package was used for calculations.

Results

whole-transcriptome profiling showed a more than 1.41-fold change (in absolute value) in differential expression ($|\log_2 FC| \geqslant 0.5$) of 1,604 genes of all genes presented on the microchip (5.35% of the total number) for groups 2, 3 and 4 compared to group 1 caused by Q diet or/and HFHCD. A total of 117 (7.3%) unique (only representative of this group) genes among 1,396 differentially expressed genes were detected in group 2 (control diet supplemented with Q); 393 (24.5%) and 391 (24.4%) genes were established for groups 3 (HFHCD) and 4 (HFHCD and Q), respectively. Common differential expression of 34 (2.1%), 29(1.8%), and 539 (33.6%) genes was shown for three group pairs: 2 and 3, 2 and 4, 3 and 4, respectively **(fig. 1a)**. A total of 101 genes (6.3%) were found to be differentially expressed in all of the three experimental animal groups compared to group 1. **Tables 1 and 2** present a list of short international symbols of top upregulated and top downregulated genes, respectively.

It was also of particular interest to identify genes the differential expression of which is responsible for the consumption of Q in various diets. In order to do this, differential expression of the genes in group 4 was normalized to group 3, and the resulting set was compared to the sets of genes differentially expressed in group 4 and then in group 2 compared with group 1. The total number of such genes equaled 1,396 **(fig. 1b)**. In addition, the number of genes that responded to Q consumption in all cases (i.e., regardless of whether it was HFHCD or control diet) was 15.

Fig. 2 presents volcano plots with the following coordinates: differential gene expression logarithm ($\log_2 FC$); negative logarithm of probability (–lg(p)) of the null hypothesis on the presence of this effect for all studied genes demonstrating qualitatively the impact degree of the experimental regimens on the transcriptome. HFHCD in group 3 and 4 animals resulted in the greatest dispersion (both up- and downregulated differential expression) of the transcriptome, while the effect of Q in a standard diet turned out to be significantly less pronounced.

Analysis of differential gene expression profiles in individual groups of animals in the R environment allowed us to identify a series of metabolic pathways that are targets for the studied food regimens. The distribution of metabolic pathways (Venn diagram) that have altered de-

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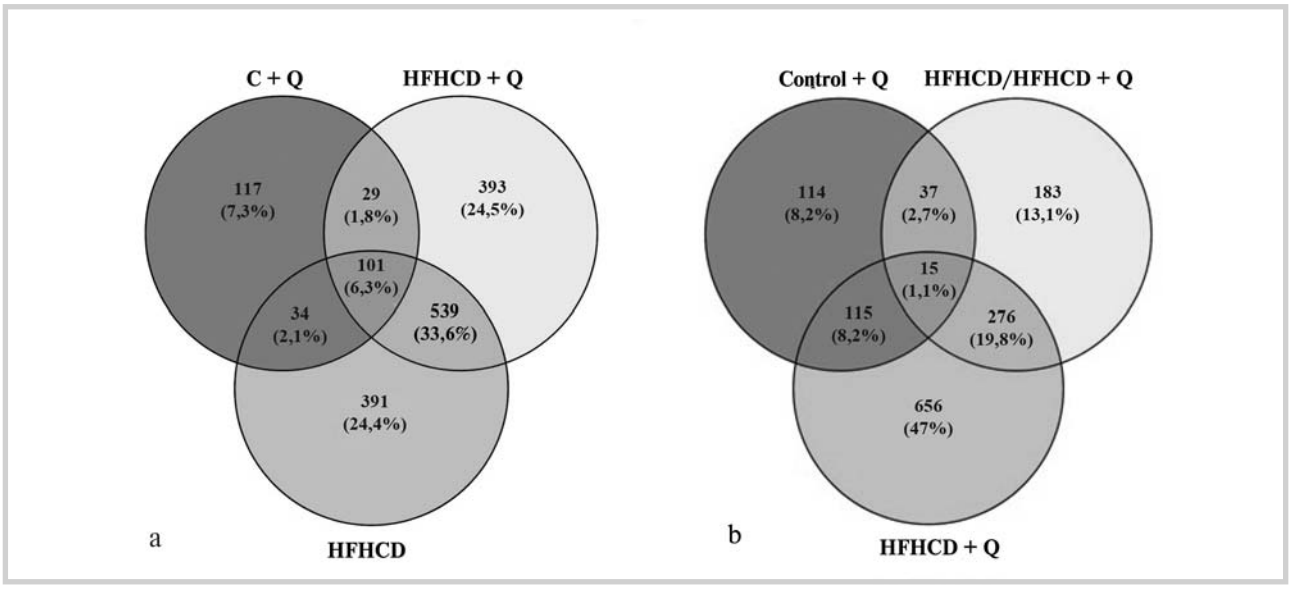

Fig. 1. **Venn diagrams.**

a — a distribution of differentially expressed ($|\log_2 FC| \geqslant 0.5$) genes in test groups 2, 3 and 4 of Zucker-*LEPR*^{fa} rats compared to group 1; distribution of differentially expressed ($|\log_2 FC| \geqslant 0.5$) genes upon quercetin supplementation in rats of the same line in group 4 compared to groups 1 and 3, in group 2 compared to group 1.

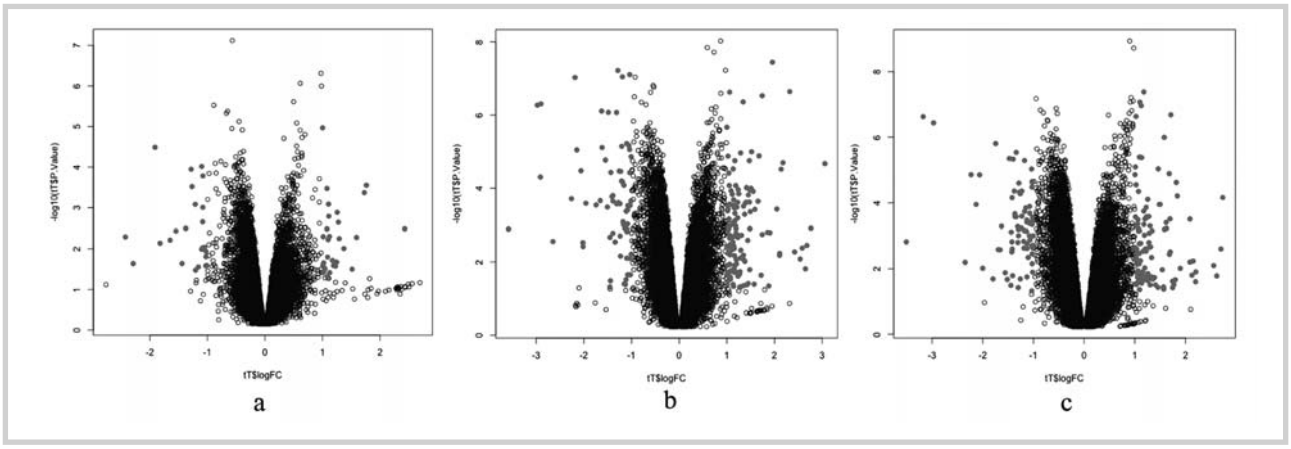

Fig. 2. **«Volcano plots» of differential gene expression in groups 2 (a), 3 (b) and 4 (c) compared to group 1.**

The abscissa axis is the binary logarithm of differential gene expression (FC); the ordinate axis is the negative decimal logarithm of the statistical significance of the effect (p). The genes in quadrants with $|\log_2 FC|>1$; $-\lg(p) >1.301$ ($p<0.05$) are highlighted in color.

pending on the food regimen among the animal groups compared to group 1 is shown in **fig. 3**.

Of the identified genes, a total of 7 metabolic pathways were affected in all three experimental groups of animals. These were the following metabolic pathways:

- rno00830 Retinol metabolism
- rno00140 Steroid hormone biosynthesis
- rno00591 Linoleic acid metabolism
- rno04976 Bile secretion
- rno00982 Drug metabolism - cytochrome P450
- rno00590 Arachidonic acid metabolism
- rno00980 Metabolism of xenobiotics by cytochrome P450.

In addition, there were 10 groups of altered metabolic pathways common for groups 3 and 4, which received HFHCD. There were only one altered pathway noted for group 3 (HFHCD) and 5 pathways for group 4 (HFHCD+Q).

Analysis of changes in 7 metabolic pathways that were common for all food regimens in Zucker-*LEPR*^{fa} rats showed that these changes differ under HFCR and/or Q. An example of such changes is presented for Steroid hormone biosynthesis (rno00140) metabolic pathway in **fig. 4**.

Alteration of Retinol metabolism (rno00830) metabolic pathway included such effects as transcriptional sup-

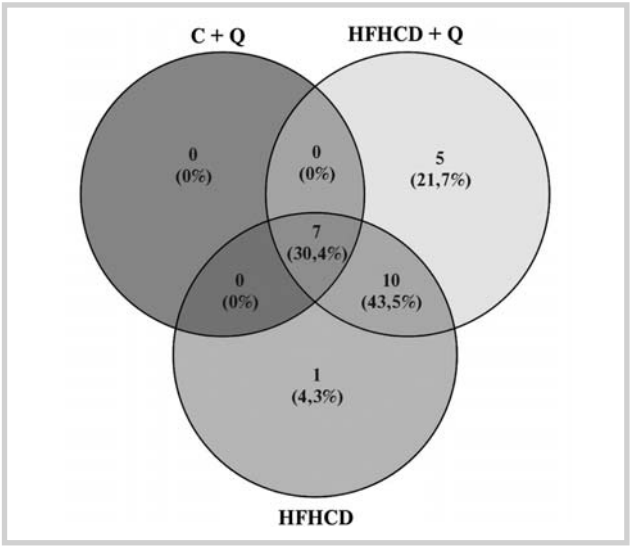

Fig. 3. **Venn diagram of the distribution of metabolic pathways among the groups of Zucker-*LEPR*^{fa} rats used for targeted dietary treatment.**

pression of *DHRS4* (Dehydrogenase/reductase SDR family member 4) and *RDH* (retinol dehydrogenase) upon HFHCD consumption. Q (group 4) partially abolished the first of these effects. Changes in Drug metabolism - cytochrome P450 (rno00982) metabolic pathway under the tested dietary regimens included increase in monoamine oxidase (EC 1.4.3.4) and decrease in glutathione S-transferase (EC 2.5.1.18) expression with Q supplementation, with the effects being mostly opposite to those observed in HFHCD.

It should be also noted that, unlike for group 2, HFHCD in groups 3 and 4 altered PPAR signaling pathway (rno 03320) as compared with group 1. Moreover, supplementation with Q in HFHCD (group 4) abolished changes in differential expression of a series of enzymes comprising these metabolic pathways (EC 2.1.1.245, EC 4.2.3.140, *PGAR, PDK1, faBP, AQP1, MRP4, PKA, ABCG8*) with their expression reaching the basal level.

As presented in **fig. 5**, a significant ($p<0.05$) increase in the plasma level of total calcium and phosphorus was

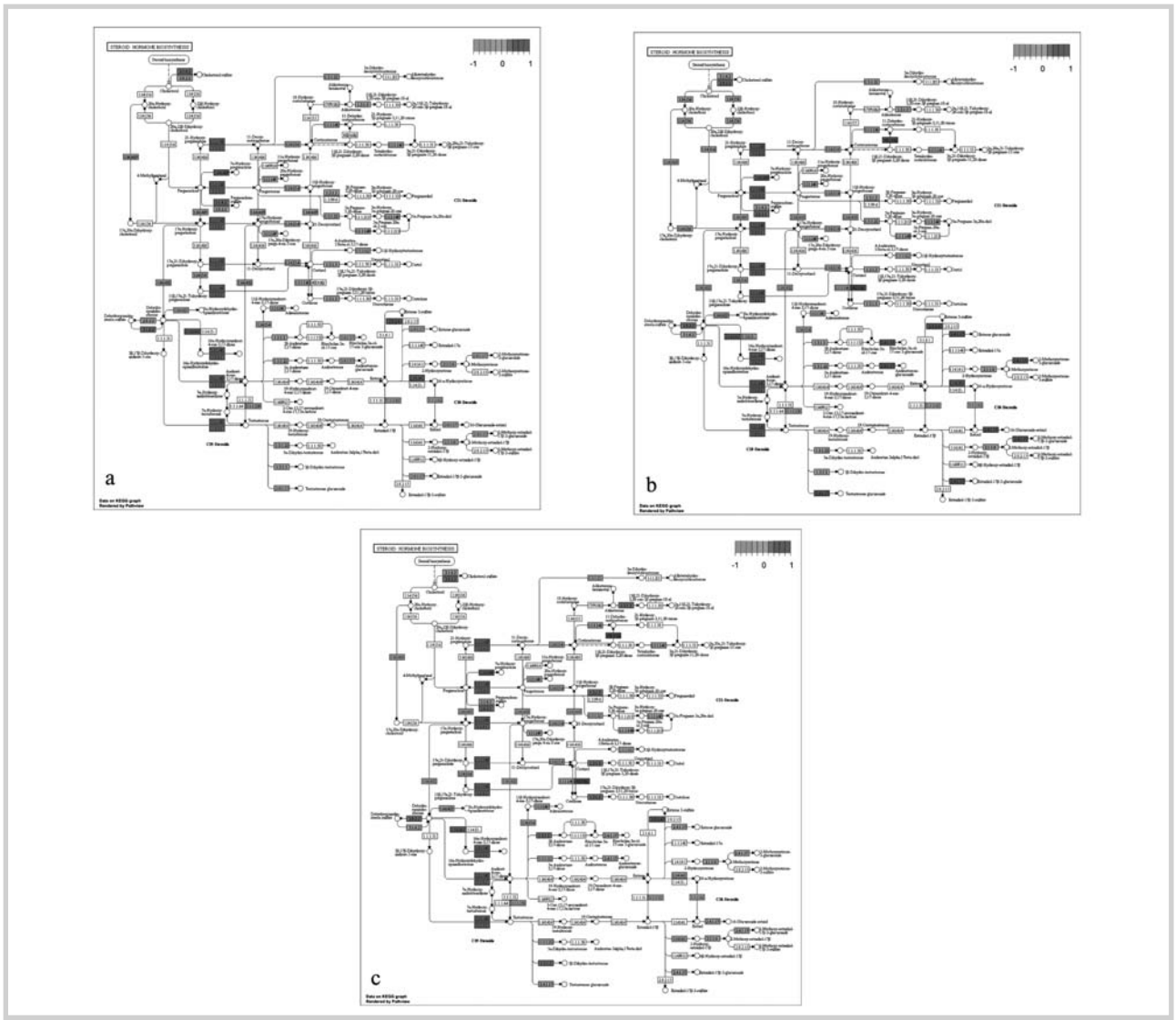

Fig. 4. **Comparative changes in the STEROID HORMONE BIOSYNTHESIS metabolic pathway in groups 2 (a), 3 (b) and 4 (c) of Zucker-*LEPR*^{fa} rats compared to group 1.**

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Table 1. **Top upregulated genes differentially expressed in group 2, 3, 4 rats fed with test diets at p≤0.05 (compared to group 1 receiving a balanced semi-synthetic diet)***

Group	Expression level, $\|\log_2 FC\|$		
	+>1	+0,7–1	+0,5–0,7
2	*LOC102549203*	*Sult1c3*	*Defb17*
	Car3	*LOC102556574*	*Isg15*
	Nat8	*Tsku*	*LOC102549099*
	Cml2	*Socs2*	*Pkib*
	Zswim5	*Rgs3*	*Tac3*
	Slc25a30	*Onecut1*	*LOC103693809*
	LOC102548450	*Per1*	*Crygd*
	Grin3a	*Sult1e1*	*Cry1*
		Kcnip1	*LOC102547221*
		Ctgf	*LOC100909643*
3	*Cyp2c11*	*Ppp1r3b*	*Ltbp2*
	Gfra1	*Ip6k2*	*Rup2*
	LOC100912658	*LOC103690477*	*Cth*
	Slfn4	*Heph*	*Arhgap22*
	Slc6a6	*Nipal2*	*Nmt2*
	Comp	*Myzap*	*Gria3*
	Dcn	*Acsm3*	*Zbtb16*
	LOC102551151	*Cd274*	*LOC102555870*
	Spp1	*Ch25h*	*Hnrnpa2b1*
	Ccdc68	*LOC102552977*	*Cystm1*
4	*Ubd*	*Dcn*	*Itga4*
	Btnl5	*Hmgb1*	*Fam180a*
	Abcb1b	*LOC685067*	*Trim30*
	G6pc	*Cldn23*	*Ube2c*
	RT1-CE5	*Rhoc*	*Tspo2*
	Rbm25	*Zswim5*	*Flvcr2*
	Lpl	*Usp2*	*Slpil3*
	Nol3	*Cox6a2*	*Fam105a*
	Rrm2	*Comp*	*LOC102551574*
	A2m	*Ddit4l2*	*Ppp1r3b*
Upregulated genes common for the groups	2 and 3	*Onecut1, Gck, Defb17, LOC102549099, Cry1, MGC108823, Gcnt4, Apol9a, LOC102552977, Slc6a7*	
	2 and 4	*Pcdh19, Zswim5, Grin3a, Lzts3, A2m, Lhfpl4, Bicc, LOC103690085, Pkib, LOC102547637*	
	3 and 4	*Tsx, Sds, Sdr16c6, Scd2, Stac3, Cyp2c12, LOC100366030, Cyp2c24, Aldh1b1, LOC102549932*	
	2, 3 and 4	*LOC102555663, Cdh17, Oasl, LOC103693974, Cox6a2, Cyp7b1, LOC102552001, Sult1c3, Tsku, Smoc2*	

Note. * Up to 10 most upregulated genes are presented for each of the groups.

noted in Zucker-*LEPR*^{fa} rats receiving HFHCD in comparison with the animals receiving control balanced diet regardless of Q supplementation.

Analysis of relative expression of 7 genes by real-time RT-PCR **(fig. 6)** revealed various effects of the regimens used in the study. Univariate analysis of variance (ANOVA and/or Kruskal–Wallis test) for groups 1—4 demonstrated that increased content of fat and fructose in animal diet significantly ($p<0.05$) affected the level of *Prom1*, *GAPDH* and *FTO* mRNA, while supplementation with Q affected *UGT2b37* and *Prdx1* expression levels. Moreover, the combined effect of HFHCD and Q was found for *NpY* and *Crot*. Overexpression of *Prom1, GAPDH* and *FTO* triggered by HFHCD was observed only in rats that did not receive Q.

Discussion

analysis of changes observed in Steroid hormone biosynthesis (rno00140) metabolic pathway in rats showed that *UGT2B7* (UDP-glucuronosyltransferase, EC 2.4.1.17) is significantly downregulated in group 2 (balanced diet with Q supplementation). This enzyme transfers glucuronic acid residue to steroid hormones (and other hydrophobic metabolites) thus providing their excretion with urine. This may lead to an increase in circulating concentration

Table 2. **Top downregulated genes (in absolute value) differentially expressed in group 2, 3, 4 rats fed with test diets at p≤0.05 (compared to group 1 receiving a balanced semi-synthetic diet)***

Group	Expression level, $\|\log_2 FC\|$		
	<−1	(−1)−(−0,7)	(−0,5)−(−0,7)
2	*Olr292*	*Rgs4*	*Slc7a2*
		Wfdc11	*Lhx8*
	Kcnj15	*Hsd3b5*	*Ppef2*
		Nucb2	*Kcnk5*
	LOC103690608	*LOC103694173*	*LOC100360128*
		LOC102552001	*LOC691485*
	Slc39a5	*Trpm8*	*Ccnb2*
		Olr1353	*Zfp90*
	Upp2	*RGD1309540*	*Tmem179*
		RGD1561517	*Adcy10*
3	*Fmo1*	*Rgs4*	*Pdhb*
	Samd15	*Wfdc11*	*Slc13a5*
	Tmem179	*Hsd3b5*	*LOC102555010*
	Bmp7	*Nucb2*	*Slc16a11*
	Slc2a5	*LOC103694173*	*Agpat3*
	Tas1r1	*Trpm8*	*LOC103692524*
	LOC691485	*Olr1353*	*LOC108348296*
	Rgs12	*LOC102552001*	*Appl2*
	Cacnb4	*RGD1309540*	*Cyp26a1*
		RGD1561517	*LOC103692812*
4	*LOC103692325*	*Dnajc5b*	*Lipe*
	Olr1583	*Bmp7*	*Galt*
	Prtg	*Lamb3*	*Srd5a1*
	Col9a2	*LOC501346*	*Mcpt8*
	Ppef2	*LOC102551647*	*Tp53i13*
	LOC102552469	*RGD1564786*	*Pdzd7*
	Cib2	*LOC102552306*	*Fpr1*
	Mt1	*LOC102551879*	*Taf1d*
	Acot1	*LOC691485*	*Slc25a47*
	LOC10255627	*Cacnb4*	*Retnlg*
Downregulated genes common for the groups	2 and 3	*LOC108349355, Ptprs, LOC100911949, Plekha6, Fnip2, Slc25a29, Derl2, LOC103690779, Lpal2, LOC102547508*	
	2 and 4	*Ino80. LOC100911891, Nr0b2, LOC102550442, LOC103694148, Slc7a2, Shank2, RGD1564786*	
	3 and 4	*Hfe2, Olr1583, Afg1l, LOC102551374, LOC102547136, Lpar2, Rhobtb3, Palm, Sh3d21, Coq10a*	
	2, 3 and 4	*Cpt1a, Fut8, Zfp266, Scgb1a1, Dtx1, Mrnip, Agfg2, Cpeb3, Sdr42e1, Wwox*	

Note. * Up to 10 most downregulated genes are presented for each of the groups.

of testosterone, 2-methoxy- and 2-hydroxyestradiol-17β, estrone, 2-methoxyestrone, and etc. At the same time, *UGT2B7* is significantly upregulated ($\log_2 FC>2$) in group 3 animals, with the same effects apparently being mutually neutralized in group 4. Another gene involved in this metabolic pathway is *CYP17A1* (steroid 17-α-monooxygenase, EC 1.14.14.19). This enzyme belongs to the cytochrome P450 system and performs 17-α-hydroxylation of steroids. As can be seen in **fig. 4**, Q has no significant impact on the expression of this gene in a standard diet. On the contrary, HFHCD suppresses the expression of this enzyme significantly, which cannot be abolished by Q supplementation (group 4). Biological consequences of this effect may include a possible indirect effect on mineral metabolism, in particular, on the level of Ca and P due to the metabolic block of conversion of cortisol and its inactive precursor corticosterone. Indeed, calcium and phosphorus levels were increased in the blood plasma of group 3 and 4 rats as compared to group 1, with Q having no significant effect on the changes in these values **(fig. 5)**. At the same time, group 4 rats receiving HFHCD and Q were characterized by a decreased level of short-term memory and increased anxiety compared to the control group 1 as demonstrated by passive avoidance test [17], which can be

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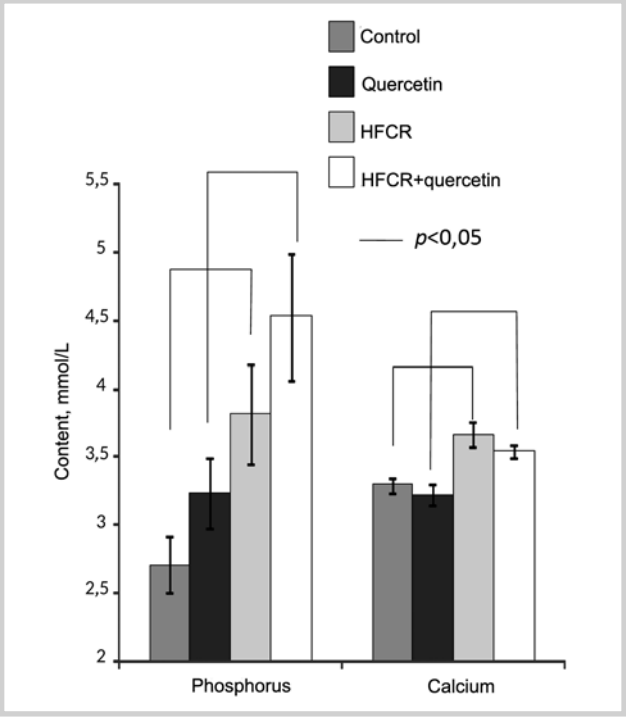

Fig. 5. **Plasma level of total calcium and phosphorus in groups 1–4 of Zucker-*LEPR*^{fa} rats by the end of experiment.**

explained by an elevated stress level in these animals due to impaired cortisol metabolism.

No significant changes were noted in the expression level of corticosteroid 11-β-dehydrogenase isozyme 2 (EC 1.1.1.145) in control diet with Q. HFHCD caused a significant overexpression ($\log_2$FC>1) regardless of Q supplementation. Among the consequences of this effect are metabolic block resulting in accumulation of 11-dehydrocorticosterone and further accumulation of 13α,20α,21α-trihydro-5-β-pregnan-11,20-dione.

Catechol O-methyltransferase (EC 2.1.1.6) is suppressed dramatically by Q ($\log_2$FC<−1), whereas HFCR has no effect. Decreased expression of this multifunctional enzyme responsible for the transfer of the methyl group from S-adenosyl methionine to various substrates (catechol, adrenaline, norepinephrine, steroids) leads to a blockade of 2-methoxyestradiol-17β formation and presumably reduces the level of active catecholamines, which correlates with the previously observed decrease in the mobility index (locomotor activity) for Zucker-*LEPR*^{fa} rats under the action of Q in the elevated plus-maze test [17].

Similar to some other genes of the cytochrome P450 family, *CYP11B1*, which encodes steroid 11-β-monooxygenase (EC 1.4.15.4), was significantly suppressed in HFHCD (which corresponds well with the literature data [18]), while Q (group 4) only partially alleviates this effect. It can enhance the blockade of corticosterone formation with the corresponding effect on mineral metabolism **(see fig. 5)**. It should be also noted that this enzyme is a key factor providing a link between glucocorticoid metabolism (interconversion of cortisol and cortisone) and the levels of cytokines and Lep [19], impaired reception of which is the main feature of the Zucker-*LEPR*^{fa} phenotype. Activity of CYP11B1 in liver negatively correlated with the level of circulating Lep and its soluble receptor [20].

Changes in Retinol metabolism (rno00830) metabolic pathway in the studied rat groups can result in the metabolic block of 9-cis-retinoic acid formation associated with obesity and accompanied by activation of multiple nuclear transcription factors. Change in the expression of *UGT2B7* in relation to the metabolism of retinoids must have led to increased glucuronidation (and clearance) of all-trans retinoic acid. Q and HFHCD had the opposite effect on the expression of lecithin retinol acyltransferase (EC 2.3.1.135), which might lead to an increase in the level of retinol due to the release of its free form in HFHCD, which is partially abolished by Q. In obesity, this may lead to a reduced effect of vitamin A, even in case when the latter is received in normal amounts with food. There is a direct link between obesity, Lep level and metabolic disorders of retinol and its derivatives [21].

Expression of a number of cytochrome P450 enzymes (*CYP3A, CYP4A1*, and etc.) involved in this metabolic pathway also changes, with HFHCD and Q mostly having the opposite effects on these components of the retinoid metabolism. Activation of *CYP3A* observed in Zucker-*LEPR*^{fa} rats receiving HFHCD is consistent with the data on the enhanced activity of this P450 isoform in obesity [22].

Changes in the expression of a number of key carbohydrate energy metabolism genes, xenobiotic detoxification system, and maintenance of the redox homeostasis were found in Zucker-*LEPR*^{fa} rats fed with HFHCD and its combination with Q using RT-PCR targeted at individual nucleotide sequences. These differentially expressed genes included *GAPDH*, which is responsible for the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and NAD [23]. *Prom1* encodes CD133 receptor (prominin), a marker of hematopoietic cells [24]. HFHCD also potentiates the expression of the "obesity gene" *FTO* (α-ketoglutarate-dependent nucleic acid demethylase), which is involved in the epigenetic regulation of metabolic processes [25].

Negative differential expression of the gene *Ugt2b37* (UDP-glucuronosyltransferase isoform) under the action of Q in groups 2 and 4 may indicate a decrease in detoxification of endogenous hydrophobic metabolites, including lipids, the hyperproduction of which can be triggered by fatty liver degeneration [26]. It is important to note that this result is in qualitative agreement with the data of the whole-transcriptome profiling.

Peroxiredoxin, which is encoded by *Prdx1* (thiol-dependent peroxidase, regulator of intracellular Red/Ox homeostasis), participates in the redox regulation of the cell and plays an important role in eliminating peroxides produced during metabolic processes by regulating intracel-

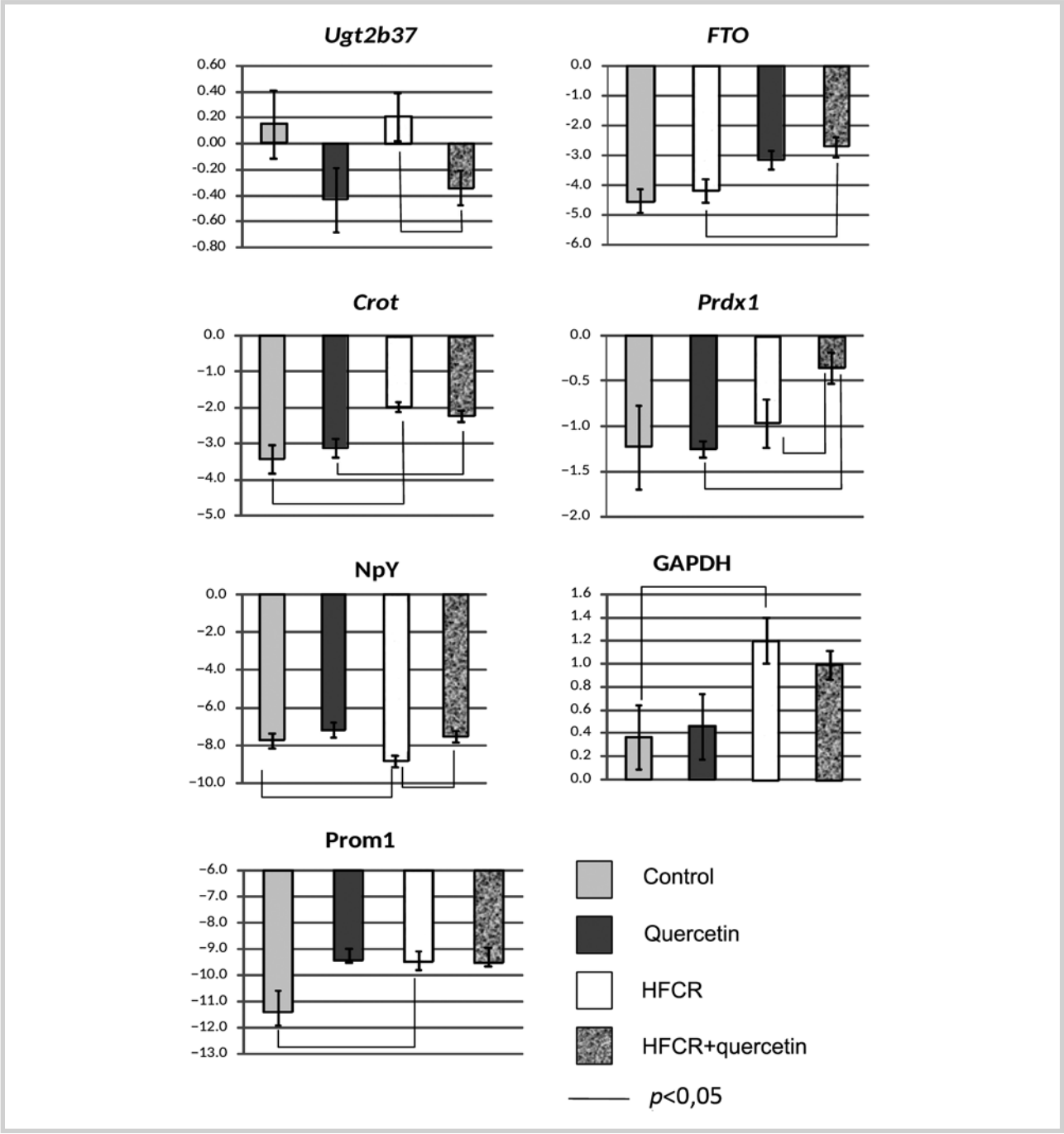

Fig. 6. **Differential expression of *Crot, FTO, NpY, Prdx1, Prom1, Ugt2b37,* and *GAPDH* in the liver of Zucker-*LEPR*^{fa} rats.**

The abscissa axis presents animal groups (see figure legend); the ordinate axis is the binary logarithm of gene expression normalized to the level of β-actin mRNA. Arrows indicate group pairs with statistically significant difference at p<0.05; non-parametric Mann-Whitney U-test.

lular H_2O_2 concentrations. The protein can also break the intramolecular disulfide bond in GDPD5 (glycerophosphodiester phosphodiesterase domain containing 5), which provides the ability of GDPD5 to regulate differentiation of postmitotic motor neurons [27]. The expression level of *Prdx1* in rats receiving HFHCD with Q supplementation was increased 2-fold compared to the control. Thus, one can assume a direct effect of Q on the expression of the gene encoding peroxiredoxin in increased level of peroxides produced during lipid peroxidation upon consumption of lipid-enriched HFHCD.

Orexigenic neuropeptide gene *NpY* responsible for hyperphagia and obesity in rodents [28] is reliably suppressed in HFHCD, while Q, apparently, abolishes this effect. Moreover, unlike, for example, in case of the hypothalamus, such an effect of differential transcription of *NpY* in

liver tissue has been detected for the first time. Expression of *NpY* in rats prone to alimentary obesity is regulated by epigenetic factors [29].

Carnitine octanoyltransferase encoded by *Crot* catalyzes the reversible transfer of the acyl groups of fatty acids between coenzyme A and carnitine thus providing transport of medium- and long-chain acyl-CoA molecules from peroxisomes to the cytosol and mitochondria [30]. Elevated expression of this gene in groups 3 and 4 suggests activation of beta-oxidation of fatty acids in mitochondria associated with the Krebs cycle caused by excessive amounts of exogenous lipids obtained with HFHCD.

Conclusion

We have demonstrated in a model of male Zucker-*LEPR*^{fa} rats genetically prone to obesity due to impaired leptin reception that Q or/and HFHCD significantly alter the transcription level of 1,604 genes in liver tissue, with a total of 1,396 genes of which being susceptible to Q alone. Changes in the liver transcriptome caused by HFHCD are more pronounced than those caused by Q supplementation in a standard diet. Real-time RT-PCR has shown that Q affects the expression of genes responsible for the processes of detoxification of xenobiotics (*UGT2b37*), redox homeostasis (*Prdx1*), beta-oxidation of fatty acids (*Crot*), and key mechanisms affecting hunger and satiety (*NpY*), as well as potentiates or abrogates the effects of HFHCD in relation to some other functionally significant genes. Bioinformatics analysis revealed the effect of HFHCD and/or Q on 23 metabolic pathways (KEGGS), 7 of which [metabolism of steroids, arachidonic and linoleic acids, retinoids, drugs and xenobiotics (mediated by P450), bile secretion] were affected in all the studied groups. Changes in the liver transcriptome of Zucker-*LEPR*^{fa} rats caused by HFHCD and/or Q are in agreement with the experimental data on changes in short-term memory, anxiety and mineral metabolism characteristics in these animals. Key markers of metabolism of steroids, arachidonic and linoleic acids, retinoids, drugs and xenobiotics (mediated by P450), and bile secretion identified by differential gene expression analysis can be used in preclinical trials of pharmacological agents and specialized functional food products developed for the correction of metabolic disorders in obesity.

Дополнительная информация

Дополнительные материалы к статье

Fig. 2. **«Volcano plots» of differential gene expression in groups 2 (a), 3 (b), and 4 (c) compared to group 1.**

The figure is available in color online at https://doi.org/10.14341/probl9936-3229.

Fig. 4. **Comparative changes in STEROID HORMONE BIOSYNTHESIS metabolic pathway in groups 2 (a), 3 (b), and 4 (c) of Zucker-*LEPR*^{fa} rats compared to group 1.**
The figure is available in color online at

a — https://doi.org/10.14341/probl9936-3231

b — https://doi.org/10.14341/probl9936-3421

c — https://doi.org/10.14341/probl9936-3422

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Authors' involvement. Trusov N.V. performed whole-transcriptome microarray analysis, Apryatin S.A. was responsible for experiment design, real-time PCR and text writing («Methods» and «Results» sections); Gorbachev A.Yu. and Naumov V.A. carried out the bioinformatics analysis; Mzhelskaya K.V. conducted experiments with animals, isolated mRNA from liver tissue and performed reverse transcription; Gmoshinski I.V. developed the general research concept, collected and analyzed the literature data, carried out statistical processing and interpretation of the data, participated in text writing («Background», «Discussion» and «Conclusion» sections). All authors made a significant contribution to the research and preparation of the article, read and approved the final version of the article prior to its publication.

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