GENE EXPRESSION CHANGES OF ENERGY METABOLISM REGULATORS IN ADIPOSE TISSUE OF WOMEN WITH GDM

Kleiblova Petra[1, 2,] • Kleibl Z.[1] • Krejci V.[2] • Ticha I.[1] • Haluzik M.[3]*

Inst. of Biochemistry and Exp. Oncology[1], Dept. of Obstetrics and Gynecology[2], 3rd Dept. of Medicine[3] 1st Faculty of Medicine, Charles University in Prague and General Faculty Hospital, Czech Republic, *pekleje@lf1.cuni.cz

Gestational diabetes mellitus (GDM):

The condition of glucose intolerance appearing in woman usually in the second trimester of pregnancy with prevalence 5-7% of pregnancies worldwide. GDM is considered to be an independent risk factor for subsequent type 2 diabetes mellitus development.

The AIM of our study was to characterize expression profile of genes participating to the energy metabolism regulations in adipose tissue pre-selected by gene expression array.

Tab.1. Characteristics of 27 study subjects (GDM and healthy nondiabetic women).

All subjects	Number of subjects	BMI before pregnancy Mean <range> [kg/m^2]	BMI 3rd day after delivery Mean <range> [kg/m^2]	Age Mean [y]
Control group	13	22.7 <19.0-30.5>	25.5 <20.8-32.3>	33.1
GDM	14	28.9 <18.8-38.2>	31.2 <19.9-42.4>	34.1
- GDM-Insulin	9	30.1 <19.7-38.2>	32.9 <19.9-42.4>	34.6
- GDM-Diet	5	26.8 <18.8-35.4>	28.3 <22.0-34.9>	33.2

Tab.2. Subgroups of normal-weight controls (pre-gestational BMI 20-25) and obese diabetic women (pre-gestational BMI>30).

BMI-selected	Number of subjects	BMI before pregnancy Mean <range> [kg/m^2]	BMI 3rd day after delivery Mean <range> [kg/m^2]	Age Mean [y]
Control group	8	22.4 <20.4-24.8>	25.3 <23.1-28.0>	33.4
GDM	9	33.0 <25.1-38.2>	35.4 <30.9-42.4>	33.2
- GDM-Insulin	7	32.8 <25.1-38.2>	36.1 <30.9-42.4>	33.4
- GDM-Diet	2	33.7 <32.0-35.4>	33.1 <31.2-34.9>	32.5

Fig.1. cRNA array analysis (and corresponding clinical data) of insulin-related signaling pathway in 13 selected subcutaneous and visceral adipose tissue samples of control and GDM women (clustering by MeV software).

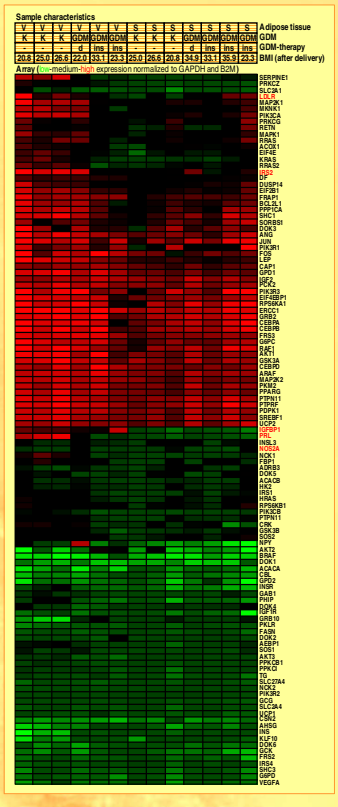
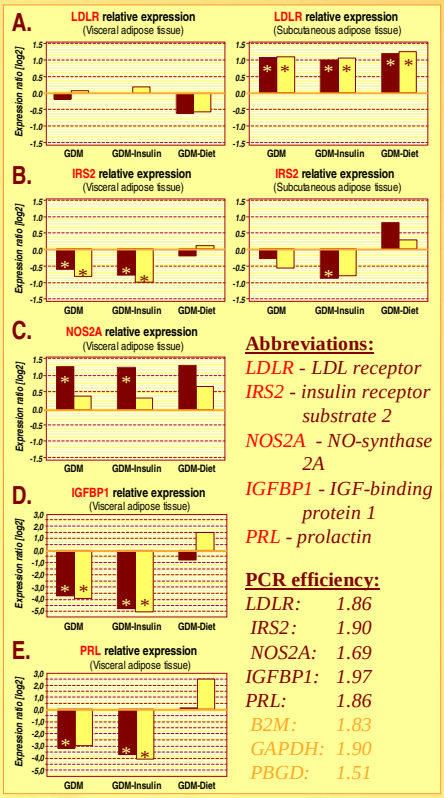


*Fig.2. qPCR assessment of differentially expressed genes in visceral (left) and related subcutaneous (right) adipose tissue in all GDM, GDM-insulin-treated, and GDM-diet-compensated women comparing to healthy woman control group (log2 expression = 0). * = significant changes (P<0.05).*



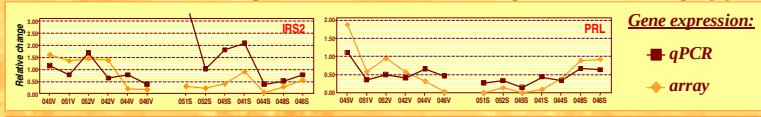
Abbreviations:

LDLR - LDL receptor
IRS2 - insulin receptor substrate 2
NOS2A - NO-synthase 2A
IGFBP1 - IGF-binding protein 1
PRL - prolactin

PCR efficiency:

LDLR:	*1.86*
IRS2:	*1.90*
NOS2A:	*1.69*
IGFBP1:	*1.97*
PRL:	*1.86*
B2M:	*1.83*
GAPDH:	*1.90*
PBGD:	*1.51*

Fig.4. Correlation of IRS2 and PRL genes expression assessed by qPCR and arrays in panel of subcutaneous and visceral adipose tissue assessed (relative expression to housekeeping genes).



METHODS:

The **women with single pregnancy parturient** by Caesarean section were enrolled. All subjects gave the informed consent approved by local ethical committee.

Subcutaneous and visceral (omental) adipose tissue samples were obtained during surgery in **14 women with GDM** (9 treated by s.c. insulin and 5 treated by diet regimes) and **13 healthy lean women** in control group with physiological pregnancy. Mean age and gestation age at the delivery were comparable in all groups (***Tab.1 and 2***).

The tissue samples were preserved in RNAlater (Qiagen) in -20°C until total **RNA isolation** by RNeasy Lipid Tissue Mini Kit (Qiagen) according to manufacturer.

Genes with differential gene expression were identified using **gene expression array** targeted to 128 genes involving to insulin receptor pathway (Human Insulin Signaling Pathway Microarray; Superarray; ***Fig. 1***) in a group of 5 patients with GDM (3 treated by insulin, 2 by diet regimes) and 3 healthy controls.

Expressions of selected genes (***Fig. 2***) were analyzed **by qPCR using SybrGreen** (LightCycler 2.0; Roche) and quantified by **REST-384-beta** software or **qGENE** software.

qPCR technical notes:

- *All RNA samples were treated by DNase.*
- *SuperScript III and random hexanucleotides were used for cDNA synthesis.*
- *qPCR for all genes were optimized for Mg^{2+}, annealing temperatures; efficiency (and test sensitivity) were assessed (**Fig.3**).*
- *Amplicons specificities were evaluated in each reaction by agarose gel electrophoresis. PCR amplicons using in-house primer sets were sequenced.*
- *Samples were analyzed in doublets.*
- *Negative controls were implemented in each run.*
- *Normalization by REST-384 was performed to 3 house-keepings (B2M, GAPDH and PBGD).*

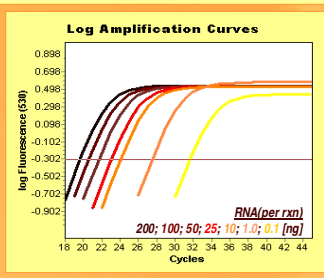


Fig.3. PCR efficiency of all tested genes were estimated based on RNA dilution curves evaluated in REST.

RESULTS:

The most apparent changes in **visceral adipose tissue** of pregnant women with GDM involved up-regulation of NOS2A mRNA (2.5x; p=0.005) and down-regulation of PRL (10.2x; p=0.024), IRS2 (1.5x; p=0.030), and IGFBP1 (12.6x; p=0.021) comparing to healthy cohort. In the subgroup of pregnant women with GDM treated by insulin down-regulation of PRL was 15x (p=0.008) and IGFBP1 29.7x (p=0.006). Moreover, in subgroup of obese pregnant women with GDM treated by insulin the decrease of PRL (19.3x; p=0.024) and IGFBP1 (33.5x; p=0.018) expression, together with decreased IRS2 (1.9x; p=0.021) expression was found (comparing to healthy controls).

In **subcutaneous adipose tissue** of GDM patients the 2.1-fold increase of LDLR (p=0.004) was found. Additionally, in a subgroup of pregnant women with GDM treated by insulin the down-regulation of IRS2 (1.8x; p=0.018) was detected.

CONCLUSIONS:

1. **This pilot results indicate substantial alteration of gene expression of insulin pathway regulators in GDM patients.** Currently running prospective study will score the ability of gene expression profiling for the early diagnostics of the type 2 DM risk development in GDM patients.
2. Using insulin-related pathways arrays another 16 genes differentially expressed in adipose/visceral are currently evaluated (except these reported here). Using a qPCR approach, larger cohort of analyzed individuals was evaluated. The comparison of array and qPCR demonstrates a good correlation of expression analysis results (*Fig. 4*).
3. **Pathogenesis of GDM is influenced by a differential involvement of subcutaneous/visceral adipose tissues.**

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