SUPPLEMENTARY DATA

***In-vitro Lipogenesis test***

Fifty milligrams of adipose tissue were incubated in 0.5ml of Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) containing 5% BSA and 2mM glucose, at 37°C for 1h in the presence of 0.5mCi (18.5kBq) of [U-14C] glucose (Amersham Biosciences) with or without 10nM insulin. After transferring the fat sample into 1ml PBS the reaction was stopped by adding 4ml liquid scintillation fluid (Betafluor, National Diagnostics USA, Atlanta, GA). Test tube was incubated overnight at RT with vigorously shaking. A 3ml aliquot of the upper phase was transferred to a scintillation vial and the radioactivity was measured using a Packard liquid scintillation counter (1-3). Lipogenic activity was expressed as nmol Glucose incorporated per h per mg of adipose tissue. The difference between basal and stimulated condition was calculated and expressed as percent increase in lipogenesis.

***Quantitative real-time PCR***

Two microliter of cDNA was brought to a final volume of 20µl in a 96-well plate containing FAST SYBR-Green PCR Master Mix (Applied Biosystems, Foster City, CA), and primers. PCR was performed with 20 seconds of initial denaturation at 95°C, followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. For each run, samples were run in duplicates for both the gene of interest and 18S or beta-Actin. Quantitative analysis was determined by Δ/Δ*CT* method normalized to both a control and 18S message or beta-Actin. Primers were purchased from Integrated DNA Technologies (Coralville, IA, USA).

Primer sequences for the target genes:

- carbohydrate response element-binding protein (ChREBP), forward 5'-GAGACAAGATCCGCCTGAAC-3', reverse 5'-GTCACGAAGCCACACACG-3'
- carbohydrate response element-binding protein alpha (ChREBP-α), forward 5'-AGTGCTTGAGCCTGGCCTAC-3', reverse 5'-TTGTTCAGGCGGATCTT GTC-3'
- carbohydrate response element-binding protein beta (ChREBP-β), forward 5'-AGCGGATTCCAGGTGAGG-3', reverse 5'-TTGTTCAGGCGGATCTTGTC-3'
- patatin-like phospholipase domain-containing protein 3 (PNPLA3), forward 5'-GCCTCCCGGCCAATGT-3', reverse 5'-ACTCTGGTAAGAGAGATGCCTATT TTG-3'
- sterol receptor element binding protein 1c (SREBP1c), forward 5'-CGGAACCATCTTGGCAACA-3', reverse 5'-GCCGGTTGATAGGCAGCTT-3'
- fatty acid synthase (FASN), forward 5'-CGCTCGGCATGGCTATCT-3', reverse 5'-CTCGTTGAAGAACGCATCCA-3'
- acetyl-CoA carboxylase (ACC), forward 5'-GGATGGTGTTCACTCGGTAAT AGA-3', reverse 5'-GGGTGATATGTGCTGCGTCAT-3'
- glucose transporter type 4 (GLUT4), forward 5'-CGTGGGCGGCATGATT-3', reverse 5'-CCAGCATGGCCCTTTTCC-3'
- thioredoxin-interacting protein (TXNIP), forward 5'-AGCCTTGCC CACTGTGACTT-3', reverse 5'-ACAGGGTTCGGCATCTTGAT -3'

and the reference genes:

- 18S ribosomal RNA, forward 5'-CGAACGTCTGCCCTATCAACTT-3', reverse 5'-ACCCGTGGTCACCATGGTA-3'
- β-Actin, forward 5'-TGGATCAGCAAGCAGGAGTATG-3', reverse 5'-GCA TTTGCGGTGGACGAT-3'

SUPPLEMENTARY DATA

RNA expression was studied in 35 subjects (26 group#1 and 9 group#3) using Affymetrix Human Gene 1.0 ST arrays. Total RNA was isolated using TRIzol reagent and was further purified using an RNeasy kit (Qiagen, Valencia, CA). The quality of total RNA was evaluated by the A260/A280 ratio and by electrophoresis on an Agilent Bioanalyzer. Afterwards total RNA was submitted to the Keck Microarray Resource for sample processing and chip hybridization according to the manufacturer's instructions.

*PCA*. First, the expression data matrix of 28,882 probe sets from the 35 GeneChips was subjected to Principal Component Analysis (PCA) in Partek Genomics Suite (Partek Inc., St. Louis, Missouri, USA) to view the overall trend of the data set. PCA allows the identification of key variables in a multidimensional data set, which explain the differences between the experiments in the best way. When assuming $m$ experiments, each with $n$ genes, the aim of the PCA is to reduce the dimensionality of the data matrix by the identification of $r \leq n$ new variables. These $r$ principal components explain the variance of the original $n$ variables as well as possible, while they are uncorrelated and orthogonal. This reduction of dimensionality allows an improved data visualization and analysis.

***Data preprocessing.*** Preprocessing of the array data (background correction, normalization and summarization) was done using the Robust Multi-array Analysis (RMA) algorithm in Partek Genomics Suite (Partek Inc., St. Louis, Missouri, USA). Afterwards a multi-way Analysis of Covariance (ANCOVA) was used to test for differences in means between different groups (glucose tolerance) accounting for confounding nuisance variables such as gender, ethnicity and array batch. Since gender and array batches contributed a considerable variation in the ANCOVA model the data were adjusted for gender and array batches in the following analyses.

***Application of the Westfall-Young strategy to gene sets.*** Since it has been shown that combinations of genes more clearly reveal the effects of a disease than single genes we analyzed 61 gene sets based on NetAFFX annotations (GenMAPP collection) in our data set. The procedure by Westfall and Young for multiple testing was applied to gene sets as described previously. In order to avoid a high rate of false positive results in this multitude of tests for the different genes and gene sets, we added so-called multiple test procedures. We computed adjusted $P$ values for each gene/gene set according to the Westfall-Young procedure, which imbeds univariate F tests into a permutation procedure. This procedure keeps the "family-wise error rate" $\alpha$ in the strong sense, i.e., the lot of all selected genes/gene sets may contain a false positive gene/gene set with the probability $\alpha$, at most. Calculations were done in Bioconductor/R.

SUPPLEMENTARY DATA

**Supplementary Table 1.** Affymetrix gene chip analysis
A. Pathway analysis in subcutaneous adipose tissue of 35 obese adolescents.

| # | Maps | p-value | Ratio |
|---|---|---|---|
| 1 | Cell cycle_Chromosome condensation in prometaphase | 0.001 | 3\|21 |
| 2 | Development_Delta- and kappa-type opioid receptors signaling via beta-arrestin | 0.002 | 3\|23 |
| 3 | Regulation of lipid metabolism_RXR-dependent regulation of lipid metabolism via PPAR, RAR and VDR | 0.004 | 3\|30 |
| 4 | Development_Gastrin in differentiation of the gastric mucosa | 0.008 | 3\|38 |
| 5 | Development_VEGF-family signaling | 0.009 | 3\|41 |
| 6 | Transcription_CREB pathway | 0.011 | 3\|44 |
| 7 | Mitochondrial unsaturated fatty acid beta-oxidation | 0.012 | 3\|45 |
| 8 | Neurophysiological process_ACM regulation of nerve impulse | 0.013 | 3\|46 |
| 9 | Development_TGF-beta-dependent induction of EMT via MAPK | 0.014 | 3\|47 |
| 10 | Development_HGF signaling pathway | 0.014 | 3\|47 |
| 11 | Pyruvate metabolism | 0.015 | 3\|49 |
| 12 | Neurophysiological process_GABAergic neurotransmission | 0.016 | 3\|50 |
| 13 | Development_TGF-beta receptor signaling | 0.016 | 3\|50 |
| 14 | Development_Beta-adrenergic receptors signaling via cAMP | 0.018 | 3\|52 |
| 15 | Atherosclerosis_Role of ZNF202 in regulation of expression of genes involved in Atherosclerosis | 0.021 | 2\|21 |
| **16** | **Transcription_ChREBP regulation pathway** | **0.021** | **2\|21** |
| 17 | Cell cycle_Sister chromatid cohesion | 0.023 | 2\|22 |
| 18 | Cell cycle_Initiation of mitosis | 0.029 | 2\|25 |
| 19 | Development_Regulation of epithelial-to-mesenchymal transition (EMT) | 0.031 | 3\|64 |
| 20 | Neurophysiological process_Dopamine D2 receptor transactivation of PDGFR in CNS | 0.031 | 2\|26 |

SUPPLEMENTARY DATA

B. Gene analysis in subcutaneous adipose tissue of 35 obese adolescents.

| Gene Symbol | p-value | Fold-Change (3 vs. 1) | gene_assignment |
|---|---|---|---|
| TRERF1 | 0.0005 | -1.36 | NM_033502 |
| AMY1A | 0.0009 | -1.45 | NM_004038 |
| AMY2A | 0.0010 | -1.47 | NM_000699 |
| HADH | 0.0019 | -1.30 | NM_005327 |
| CDHR3 | 0.0022 | -1.33 | NM_152750 |
| COQ3 | 0.0023 | -1.31 | NM_017421 |
| SCML1 | 0.0024 | -1.31 | NM_001037540 |
| GLUL | 0.0028 | -1.31 | NM_002065 |
| GIMAP7 | 0.0031 | -1.35 | NM_153236 |
| KLHL4 | 0.0033 | -1.81 | NM_019117 |
| GPR133 | 0.0033 | -1.59 | NM_198827 |
| GSTT2 | 0.0039 | -1.78 | NM_000854 |
| GULP1 | 0.0040 | -1.35 | NM_016315 |
| STBD1 | 0.0045 | -1.37 | NM_003943 |
| KAL1 | 0.0059 | -1.43 | NM_000216 |
| SCML2 | 0.0061 | -1.33 | NM_006089 |
| BRMS1L | 0.0072 | -1.30 | NM_032352 |
| **MLXIPL** | **0.0102** | **-1.31** | **NM_032951** |
| NAP1L3 | 0.0106 | -1.34 | NM_004538 |
| HAS2 | 0.0108 | -1.39 | NM_005328 |
| SULF1 | 0.0109 | -1.45 | NM_001128205 |
| C1QTNF3 | 0.0109 | -2.12 | NM_181435 |
| MRC2 | 0.0121 | -1.34 | NM_006039 |
| ANGPTL5 | 0.0121 | -1.56 | NM_178127 |
| RAB7A | 0.0125 | -1.37 | NM_004637 |
| FNDC1 | 0.0135 | -1.95 | NM_032532 |
| PECR | 0.0154 | -1.36 | NM_018441 |
| FGF16 | 0.0155 | -1.42 | NM_003868 |
| STOX1 | 0.0157 | -1.86 | NM_152709 |
| GPR64 | 0.0157 | -1.45 | NM_001079858 |
| ITGA11 | 0.0188 | -1.38 | NM_001004439 |
| PIGF | 0.0198 | -1.35 | NM_173074 |
| CD55 | 0.0199 | -1.44 | NM_001114752 |
| HMGN3 | 0.0202 | -1.32 | NM_004242 |
| VIT | 0.0217 | -1.66 | NM_053276 |
| C17orf58 | 0.0221 | -1.45 | NM_181656 |
| PDE10A | 0.0237 | -1.32 | NM_006661 |
| GLB1L | 0.0259 | -1.32 | NM_024506 |
| SLC18A2 | 0.0259 | -1.45 | NM_003054 |
| MS4A2 | 0.0269 | -1.55 | NM_000139 |
| PDZRN4 | 0.0269 | -1.50 | NM_013377 |

| | | | |
|---|---|---|---|
| TSGA10 | 0.0273 | -1.54 | NM_025244 |
| IMMP1L | 0.0273 | -1.44 | NM_144981 |
| AMY2B | 0.0286 | -1.34 | NM_020978 |
| F10 | 0.0291 | -1.32 | NM_000504 |
| THBS4 | 0.0301 | -1.74 | NM_003248 |
| PYGM | 0.0314 | -1.33 | NM_005609 |
| UTS2D | 0.0335 | -1.37 | NM_198152 |
| PI16 | 0.0346 | -1.69 | NM_153370 |
| GALNT12 | 0.0351 | -1.31 | NM_024642 |
| MARCH3 | 0.0354 | -1.34 | NM_178450 |
| ADH1A | 0.0375 | -1.34 | NM_000667 |
| GPR126 | 0.0377 | -1.31 | NM_020455 |
| DBF4 | 0.0390 | -1.30 | NM_006716 |
| QPCT | 0.0397 | -1.30 | NM_012413 |
| LRRC17 | 0.0399 | -1.52 | NM_005824 |
| GPD1L | 0.0422 | -1.40 | NM_015141 |
| **PNPLA3** | **0.0432** | **-1.71** | **NM_025225** |
| CASQ2 | 0.0433 | -2.05 | NM_001232 |
| C1D | 0.0447 | -1.34 | NM_006333 |
| RNF157 | 0.0449 | -1.40 | NM_052916 |
| FOXP2 | 0.0452 | -1.30 | NM_148898 |
| FIGF | 0.0454 | -1.61 | NM_004469 |
| ADRB3 | 0.0460 | -1.41 | NM_000025 |

SUPPLEMENTARY DATA

**Supplementary Table 2.** Metabolic characteristics of the obese adolescents undergoing repeated fat biopsy (n=9).

| | **Converter (n=4)** | | **Non-Converter (n=5)** | |
|---|---|---|---|---|
| | **Biopsy I** | **Biopsy II** | **Biopsy I** | **Biopsy II** |
| Glucose tolerance | IGT | NGT | NGT | NGT |
| Gender (female/male) | 1/3 | | 4/1 | |
| Ethnicity (C / H / AA) | 1/2/1 | | 2/1/2 | |
| Age (years) | 16 ± 3 | 18 ± 2 | 16 ± 3 | 19 ± 2 |
| Tanner Stage | 4 ± 1 | 5 ± 1 | 4 ± 1 | 5 ± 0 |
| BMI-z | 2.2 ± 0.1 | 2.3 ± 0.2 | 2.1 ± 0.7 | 2.2 ± 0.4 |
| BMI | 34.2 ± 4.5 | 36.3 ± 2.2 | 36.1 ± 7.7 | 38.2 ± 7.9 |
| %fat | 38.2 ± 6.4 | 37.2 ± 7.5 | 41.4 ± 9.3 | 44.7 ± 7.9 |
| Fasting Glucose (mg/dl) | 102 ± 17 | 91 ± 5 | 91 ± 3 | 93 ± 6 |
| 2h Glucose (mg/dl) | 150 ± 10 | 126 ± 10 | 125 ± 8 | 123 ± 11 |
| HOMA-IR | 9.6 ± 1.8 | 8.6 ± 3.6 | 7.1 ± 4.7 | 5.4 ± 3.2 |
| Adiponectin (□g/ml) | 2.8 ± 0.9 | 6.3 ± 2.2 | 11.0 ± 8.1 | 9.9 ± 5.1 |
| VAT/SAT ratio | 0.14 ± 0.04 | 0.09 ± 0.03 | 0.09 ± 0.03 | 0.08 ± 0.01 |

C= Caucasians; H= Hispanics; AA= African Americans; BMI-z= Body Mass Index-z score

**Supplementary Table 3.** Metabolic characteristics of the obese adolescents undergoing liver biopsy (n=8).

| | **2h glucose <120 (n=3)** | **2h glucose >140 (n=5)** | **p-value** |
|---|---|---|---|
| Age (years) | 11.0 ± 1.0 | 15.2 ± 1.5 | **0.003** |
| Gender (female/male) | 1/2 | 4/1 | 0.187* |
| Ethnicity (C / H / AA) | 0/3/0 | 2/3/0 | 0.206* |
| BMI-z | 2.34 ± 0.11 | 2.45 ± 0.34 | 0.528 |
| Fasting Glucose (mg/dl) | 91.5 ± 7.3 | 108.9 ± 27.5 | 0.239 |
| 2h Glucose (mg/dl) | 109.7 ± 10.0 | 184.0 ± 35.4 | **0.007** |
| ALT (U/l) | 138.3 ± 91.3 | 162.2 ± 139.0 | 0.780 |
| AST (U/l) | 86.0 ± 41.2 | 102.4 ± 89.3 | 0.737 |

*= chi square; C= Caucasians; H= Hispanics; AA= African Americans; BMI-z= Body Mass Index-z score; ALT=Alanine aminotransferase ; AST=Aspartate aminotransferase

SUPPLEMENTARY DATA

## References

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