The adipose transcriptional response to insulin is determined by obesity, not insulin sensitivity

Rydén, Mikael; Hrydziuszko, Olga; Mileti, Enrichetta; Raman, Amitha; Lange, Jette Bornholdt; Boyd, Mette; Toft, Eva; Qvist, Veronica; Näslund, Erik; Thorell, Anders; Andersson, Daniel P.; Dahlman, Ingrid; Gao, Hui; Sandelin, Albin Gustav; Daub, Carsten O.; Arner, Peter

Published in:
Cell Reports

DOI:
10.1016/j.celrep.2016.07.070

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
The Adipose Transcriptional Response to Insulin Is Determined by Obesity, Not Insulin Sensitivity

Graphical Abstract

Highlights

- Adipose gene expression is determined in non-obese and obese subjects
- Acute hyperinsulinemia induces a significant overall transcriptional response
- The transcriptional response in obese subjects differs from that in non-obese subjects
- The transcriptional response in obese subjects does not depend on insulin sensitivity

Authors

Mikael Rydén, Olga Hrydziuszko, Enrichetta Mileti, ..., Albin Sandelin, Carsten O. Daub, Peter Arner

Correspondence
carsten.daub@ki.se (C.O.D.), peter.arner@ki.se (P.A.)

In Brief

Ryden et al. performed transcriptomic profiling in adipose tissue from non-obese and obese subjects discordant in insulin sensitivity. The transcriptional response to hyperinsulinemia was similar among obese subjects and differed from that in non-obese subjects. The two obese groups differed only in a limited set of genes, thereby challenging the notion of healthy obesity.
The Adipose Transcriptional Response to Insulin Is Determined by Obesity, Not Insulin Sensitivity

Mikael Rydén, Olga Hrydziuszko, Enrichetta Mileti, Amitha Raman, Jette Bornholdt, Mette Boyd, Eva Toft, 
Veronica Qvist, Erik Näsflund, Anders Thorell, Daniel P. Andersson, Ingrid Dahlman, Hui Gao, Albin Sandelin, 
Carsten O. Daub, and Peter Arner

INTRODUCTION

Up to 30% of obese subjects display normal fasting plasma glucose/lipid levels and normotension, a phenotype referred to as “metabolically healthy obesity,” which implies that a significant proportion of obese individuals may need less vigorous interventions to avoid metabolic/cardiovascular complications (Blüher, 2010; Karelis, 2008; Primeau et al., 2011; Samocha-Bonet et al., 2012; Sims, 2001). A hallmark characteristic among these individuals is high insulin sensitivity. Several studies have shown that insulin-sensitive obese (ISO) subjects have lower visceral fat accumulation, less ectopic fat and arterial atherosclerosis, higher plasma adiponectin levels, and a more favorable inflammation profile than insulin-resistant obese (IRO) individuals (Blüher, 2010; Karelis, 2008; Primeau et al., 2011; Samocha-Bonet et al., 2012; Xu et al., 2013). It is also well established that the two obesity phenotypes differ in the subcutaneous white adipose tissue (sWAT) itself (Xu et al., 2013). ISO individuals have smaller fat cells and less pronounced inflammation than IRO individuals, which is also reflected at the gene expression level (Elbein et al., 2011; Qatanani et al., 2013). However, the transcriptional profiles of sWAT have been investigated in the fasting state, when insulin levels are low (Elbein et al., 2011; Qatanani et al., 2013). As insulin is expected to induce profound alterations in gene expression, it is not clear how such changes relate to insulin sensitivity and clinical profiles. This has prompted some investigators to determine the transcriptional response to insulin in sWAT collected before and during hyperinsulinemic euglycemic clamp. ISO and IRO subjects displayed a clear but globally similar transcriptional response to insulin, which differed from the small effects observed in NO subjects. In the obese, 231 genes were altered; 71 were enriched in ISO subjects (e.g., phosphorylation processes), and 52 were enriched in IRO subjects (e.g., cellular stimuli). Common cardio-metabolic risk factors and gender do not influence these findings. This study demonstrates that differences in the acute transcriptional response to insulin are primarily driven by obesity per se, challenging the notion of healthy obese adipose tissue, at least in severe obesity.
### RESULTS

#### Cohort Description

Clinical data are summarized in Table 1. As expected, there were major differences in the clinical profile between the NO group and the obese groups, in particular for the IRO group. Compared with the ISO group, the IRO group displayed significantly higher values for waist-to-hip ratio, systolic blood pressure, fasting plasma glucose, insulin, and triglycerides, as well as lower HDL (high-density lipoprotein)-cholesterol. Mean plasma insulin levels during clamp were also slightly higher in the IRO group. M values in the NO group were, on average, ~40% higher than those in the ISO group, but there was a considerable overlap (Figure 1A).

#### Gene Expression Profiles Are Altered after Insulin Stimulation for 2 hr in All Subjects

Taking into account the expression data from all the subjects put together, there was a clear overall expression response to hyperinsulinemia, including several genes involved in insulin signaling. Using a false discovery rate (FDR) of <0.05, we found 786 CAGE tag clusters to be altered during the clamp (expressed as fasting divided by hyperinsulinemia; f/hi) (Figure 1B). Out of these, 493 corresponded to annotated genes, and there was a more pronounced transcriptional upregulation (641 out of 786 tag clusters; 82%) upon hyperinsulinemia (i.e., the f/hi quotient was decreased). Eight insulin responding tag clusters (Figure 1B) corresponded to eight genes in the insulin signaling pathway and included PIK3R3, PIK3R1, and IRS2 (Figure 1C). A subset of these genes (SREBF1, PIK3R3, and IRS2) was validated by qPCR in the

---

### Table 1. Clinical Characteristics of the Study Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>ISO (n = 21; M/F, 0/21)</th>
<th>IRO (n = 30; M/F, 6/24)</th>
<th>NO (n = 17; M/F, 3/14)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menopause, yes/no</td>
<td>6/15</td>
<td>11/13</td>
<td>4/10</td>
<td>0.40</td>
</tr>
<tr>
<td>Nicotine use, yes/no</td>
<td>2/19</td>
<td>4/26</td>
<td>1/16</td>
<td>0.70</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 ± 12</td>
<td>45 ± 11</td>
<td>42 ± 13</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39 ± 3</td>
<td>39 ± 5</td>
<td>24 ± 3</td>
<td>0.81</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.07</td>
<td>1.00 ± 0.07</td>
<td>0.88 ± 0.05</td>
<td>0.0007</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>55 ± 7</td>
<td>53 ± 9</td>
<td>22 ± 8</td>
<td>0.37</td>
</tr>
<tr>
<td>fP-Glucose (mmol/l)</td>
<td>5.1 ± 0.4</td>
<td>5.9 ± 2.0</td>
<td>5.0 ± 0.4</td>
<td>0.041</td>
</tr>
<tr>
<td>fP-Inulin (mU/l)</td>
<td>9.7 ± 5.1</td>
<td>18.9 ± 9.5</td>
<td>6.0 ± 3.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>fP-Cholesterol (mmol/l)</td>
<td>4.9 ± 0.7</td>
<td>5.2 ± 1.2</td>
<td>4.5 ± 0.9</td>
<td>0.19</td>
</tr>
<tr>
<td>fP-HDL cholesterol (mmol/l)</td>
<td>1.4 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>0.044</td>
</tr>
<tr>
<td>fP-Triglycerides (mmol/l)</td>
<td>1.1 ± 0.5</td>
<td>1.5 ± 0.8</td>
<td>0.9 ± 0.3</td>
<td>0.010</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>134 ± 14</td>
<td>142 ± 15</td>
<td>120 ± 13</td>
<td>0.044</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 ± 15</td>
<td>83 ± 11</td>
<td>76 ± 10</td>
<td>0.76</td>
</tr>
<tr>
<td>Resting pulse rate (beats per minute)</td>
<td>68 ± 12</td>
<td>74 ± 13</td>
<td>62 ± 11</td>
<td>0.074</td>
</tr>
<tr>
<td>M value (mg/kg · min)</td>
<td>6.2 ± 0.9</td>
<td>3.1 ± 0.9</td>
<td>8.6 ± 2.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean fP-insulin level during clamp (60–120 min, mU/l)</td>
<td>223 ± 43</td>
<td>258 ± 56</td>
<td>202 ± 32</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Values are given as actual numbers or means ± SD. They were compared by Student’s t test or chi-square test. ISO, insulin-sensitive obese; IRO, insulin-resistant obese; M, males; F, females; fP, fasting plasma.
Global Gene Expression Profiles in NO, ISO, and IRO Subjects in the Fasting and Hyperinsulinemic States

In the present dataset, it is possible to compare the groups in several different ways, as outlined in Figures S1A–S1C. Principal-component analysis (PCA) for all differentially expressed tag clusters between groups and conditions, corresponding to the comparisons in Figure S1B, showed a clear distinction between the NO group and both obese groups (Figures S2A and S2B). As expected, the ISO and IRO groups in the fasting state displayed a higher expression of genes in pro-inflammatory pathways compared with the NO group (data not shown). When focusing on the actual insulin response, i.e., the changes from fasting to hyperinsulinemia (f/hi; see comparison in Figure S1C), the majority of the differentially expressed tag clusters between the ISO group (246 out of 295; 83%) and the IRO group (190 out of 246; 77%) were upregulated (f/hi; Figure 2A). Surprisingly, after correction for multiple testing, insulin altered only the expression of four tag clusters in the NO group, two of which were not annotated, while two represented the genes FRMD6-AS2 and NPC1 (Figure 2A). The effect on NPC1 expression was confirmed by qPCR (Figure S2C). PCA of data from the fasting and hyperinsulinemic states showed that the two obese groups were indistinguishable but that both were clearly separated from the NO group (Figure 2B). Principal component (PC) 1 primarily reflected the insulin effect (Figure 2C). In contrast, in the fasting state, PC2 separated the NO group from the obese groups but not the IRO group from the ISO group (Figure 2D). Upon hyperinsulinemia, there was no further change in the NO group, while the ISO and IRO groups were altered but remained different from the NO group (Figure 2D).

Analysis of Insulin-Induced Genes in ISO and IRO Subjects

As indicated for insulin responses in Figure 2 and further supported by the global comparisons in Figure S2, the predominant difference between the controls and the IRO and ISO groups appeared to be obesity per se. This made it difficult to assess the possible differences between the ISO and IRO groups when including all three groups. To further evaluate what drives the differences in insulin-induced transcriptional response in obesity, we performed a multiple regression analysis of the data from the obese groups from Figure 2A in relation to individual insulin sensitivity (i.e., M value) and other, possibly, contributing factors. This demonstrated that the M value and BMI contributed to the variations. However, the results were not influenced by gender or common risk factors such as waist-to-hip ratio, fasting lipid/insulin levels, pulse rate, or blood pressure (Table 2), indicating that obesity and insulin sensitivity, rather than associated cardio-metabolic risk factors, explains the differences in gene expression.
expression upon hyperinsulinemia. A comparison of the two obese groups showed that 380 tag clusters (FDR < 0.05), corresponding to 231 genes, were altered in hyperinsulinemia (Figure 3A; Table S1). Almost half were responding in both the ISO and IRO groups (161 tag clusters, 42%; 108 genes, 47%), and among these, the degree of upregulation was more pronounced in the ISO group (116 out of the 133 upregulated tag clusters, 87%; Figure 3B). The 231 genes mapped to KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways involved in, e.g., metabolic function, insulin signaling/resistance, MAPK signaling, circadian rhythm, and cancer (Figure 3C). Genes in these pathways were found among common as well as ISO- and IRO-group-associated genes. In order to gain some further insight into the biological meaning of the findings, a gene ontology (GO) analysis focusing on biological processes was also performed. This revealed that the top five most significantly enriched GO terms (p < 0.05) were response to lipid, cellular response to lipid, response to organic cyclic compound, response to steroid hormone, and fat cell differentiation (Table S2). Because, in the present article, we focused on the effects of obesity and insulin resistance, we used these two MeSH (Medical Subject Headings) terms in a PubMatrix (http://pubmatrix.irp.nia.nih.gov/) search of the genes listed in Table S1. This revealed 34 ISO- and 27 IRO-enriched genes, as well as 48 common genes (Figure 3D). The genes (down- or upregulated) displaying the most pronounced differences between the

---

**Figure 2. Overall Differences in Insulin-Induced Gene Expression among the NO, ISO, and IRO Groups**

(A) The number of differentially expressed CAGE tag clusters altered by fasting/hyperinsulinemia (f/hi) according to the way of comparison in Figure S1C. (B) Principal-component (PC) analysis plot summarizing the high-dimensional transcriptional data from (A) for NO, ISO, and IRO groups along PC1 and PC2, explaining the most variance in the data. Circles represent 95% confidence intervals. (C and D) Statistical analyses (t test) of group differences in (B) along PC1 (C) and PC2 (D). **p < 0.01; ***p < 0.001; ns, not significant. See also Figure S1C.
ISO and IRO groups are displayed in Figures 3E and 3F. Among those most prominent in ISO individuals were RORC, RPGR, KLF9, IRS2, and DDIT4. Those in the IRO group included PPARG1 and TAGAP. Expression changes of these seven genes were confirmed by qPCR (Figure S3). The five most significant and biologically relevant GO processes for the genes identified in Figure 3D are summarized in Table 3. The common ones belonged to biological pathways involved in lipid metabolism and cell differentiation (e.g., NPC1, PPARG1B, and CEBPD). The ISO-enriched genes (e.g., IRS2, TRIB1, and MIDN) were primarily involved in phosphorylation processes, while the genes significantly altered in the IRO group (e.g., PIK3R1, FOS, and PPARG1A) were associated with fat cell differentiation and cellular stimuli such as responses to endogenous/exogenous factors.

Influence of Gender
Most of the included subjects were female, but there were men in the NO and IRO groups. Exclusion of male subjects did not impact our findings in a major way, as exemplified by PCA plots (Figures S2D and S2E).

DISCUSSION

Herein, we report findings on global transcriptional profiles in the sWAT of NO and obese subjects, using a sequencing method that allows for broader analyses than conventional expression arrays (Kawaji et al., 2014). We found a clear transcriptional response to insulin in our global analysis. However, after correction for multiple testing, this was only significant among the obese individuals except for four tag clusters in the NO group. Thus, NO individuals may regulate their short-term insulin response in a different way, possibly via post-transcriptional mechanisms. The small effects in NO also exclude the possibility that we used an insulin infusion rate during clamp that was too high, potentially masking differences between the ISO and IRO groups. Because of the strong influence of obesity per se, it was necessary to omit the NO group in the analyses in order to allow identification of the differences between the ISO and IRO groups. Around half of the genes were common for the ISO and IRO groups, although the magnitude of the insulin response was more pronounced for the upregulated genes in the ISO group, most probably reflecting these subjects’ higher level of insulin sensitivity.

Our analyses identified 108 insulin-responding genes that were common and 123 that were enriched in either the ISO or the IRO group. This shows that there are small but quantifiable differences between the ISO and IRO groups in the adipose tissue response to insulin at the gene expression level. Notably, the obesity/insulin resistance-linked genes (i.e., with at least one publication in the literature) constituted less than half (109 out of 231) of all the insulin-responsive genes in obesity (individual genes exemplified in Results). The remaining 122 genes may be of interest in future studies of the pathophysiological mechanisms of insulin action in human WAT. Nevertheless, it should be stressed that, although statistically significant, the differences between the ISO and IRO groups were small and primarily of quantitative nature, supporting the conclusion that the ISO and IRO groups display a strong similarity, at least in the short-term response to insulin.

The observation that the two obese phenotypes displayed very similar insulin responses could provide a clue for why ISO subjects display an increased morbidity and cardiovascular risk, compared with NO individuals (Fan et al., 2013; Kramer et al., 2013; Roberson et al., 2014). The sWAT in ISO individuals may simply not be as metabolically normal as previously believed. Our present findings are in line with results following weight reduction induced by low-calorie diet (Viguier et al., 2012). This study showed similar improvements in sWAT gene expression in obese subjects with or without the metabolic syndrome. Thus, the transcriptional control upon either hyperinsulinemia or weight loss appears to be very similar in ISO and IRO subjects.

We categorized the obese into ISO or IRO based solely on insulin sensitivity. Currently, there is no consensus on how to define a “metabolically healthy obese,” phenotype and various scoring strategies have been used, as reviewed (Blüher, 2010; Karelis, 2008; Primeau et al., 2011; Samocha-Bonet et al., 2012; Xu et al., 2013). Nevertheless, as mentioned earlier, the cardio-metabolic risk profile had no influence on our findings.

The obese subjects were subdivided based on the 25th upper percentile of insulin sensitivity (Hofstedt et al., 2010). However, this has no important bearing on the results, as we obtained similar findings using individual insulin sensitivity values as a continuous variable (in multiple regression analyses). There is no consensus on how to express clamp data. We used the most common way, i.e., M value/body weight. However, correcting M values for lean body mass yielded virtually the same results. Insulin levels during clamp were slightly higher in the IRO group than in the ISO group (Table 1). These small differences can hardly have influenced our findings on gene expression. Furthermore, as mentioned earlier, fasting insulin levels had no bearing on the results.

In the present work, we obtained sequencing-based data on 136 samples from 68 individuals. This can be regarded as very
Figure 3. Identification of Genes Altered by Hyperinsulinemia in ISO and IRO Groups
(A) Venn diagram of tag clusters and genes significantly altered by hyperinsulinemia in the ISO or IRO group.
(B) Comparison between expression fold change (fasting/hyperinsulinemia; f/hi) of tag clusters common in ISO and IRO groups.

(legend continued on next page)
large numbers, using a genome-wide sequencing technique by today’s standards. Moreover, based on results from a smaller study on 40 subjects using CAGE (Persson et al., 2015), our present cohort was sufficiently large to detect gene expression differences between the three groups. Despite this, we did not have sufficient statistical power to allow a subgroup analysis of, e.g., the influence of different age groups.

There are some caveats with the present study. Because the obese subjects were scheduled to undergo bariatric surgery, our results may only pertain to individuals with severe obesity. We investigated solely subcutaneous sWAT as it is, for obvious ethical reasons, virtually impossible to obtain visceral WAT biopsies during clamp. Still, there is no obvious reason to believe that regional differences in gene expression are important for the present findings. In fact, previous studies of subcutaneous and visceral WAT have found that the impact of obesity and metabolic status is very similar in the two regions in the fasting state (Klimcákova et al., 2011). Moreover, studies of visceral WAT would require intraoperative fat biopsies during general anesthesia. It has been convincingly demonstrated that this procedure induces an acute insulin-resistant state immediately following the incision of the intra-abdominal wall (Felländer et al., 1994). This makes valid assessments of acute insulin responses in any target tissue during general surgery uncertain. Another aspect is the possibility that the gene expression response during fasting and hyperinsulinaemia may differ between subcutaneous and tissues such as skeletal muscle and liver. However, previous comparisons have shown that differences in gene expression between BMI-matched individuals discordant in insulin sensitivity are more pronounced in subcutaneous than in skeletal muscle (Elbein et al., 2011). Furthermore,

Table 3. Gene Ontology Analysis of Insulin-Induced Genes in ISO and IRO Groups

<table>
<thead>
<tr>
<th>GO Term</th>
<th>Description</th>
<th>p Value</th>
<th>Genes</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO:0071396</td>
<td>cellular response to lipid</td>
<td>1.8E-2</td>
<td>HDAC5, KLF9, RORC, SGK1, SSTR2, TRIB1, VDR</td>
<td>ISO</td>
</tr>
<tr>
<td>GO:0071383</td>
<td>cellular response to steroid hormone stimulus</td>
<td>1.8E-2</td>
<td>KLF9, RORC, SGK1, SSTR2, VDR</td>
<td>ISO</td>
</tr>
<tr>
<td>GO:0033673</td>
<td>negative regulation of kinase activity</td>
<td>1.7E-2</td>
<td>DRD1, DUSP6, IRS2, LRP5, MIDN, TRIB1</td>
<td>ISO</td>
</tr>
<tr>
<td>GO:0045963</td>
<td>negative regulation of phosphate metabolic process</td>
<td>1.5E-3</td>
<td>APOC1, DDIT4, DRD1, DUSP6, IRS2, LRP5, MIDN, MYO1D,TRIB1</td>
<td>ISO</td>
</tr>
<tr>
<td>GO:0010563</td>
<td>negative regulation of phosphorus metabolic process</td>
<td>1.5E-3</td>
<td>APOC1, DDIT4, DRD1, DUSP6, IRS2, LRP5, MIDN, MYO1D,TRIB1</td>
<td>ISO</td>
</tr>
<tr>
<td>GO:0010035</td>
<td>response to inorganic substance</td>
<td>7.0E-4</td>
<td>FABP4, FOS, NR4A1, PPARG1A, SLC1A3, SLC30A1, TNFAIP3</td>
<td>IRO</td>
</tr>
<tr>
<td>GO:0042493</td>
<td>response to drug</td>
<td>4.0E-3</td>
<td>EGR1, FOS, HSD11B2, PPARG1A, SLC1A3, SNCA, SREBF1</td>
<td>IRO</td>
</tr>
<tr>
<td>GO:005159</td>
<td>response to cAMP</td>
<td>3.0E-2</td>
<td>EGR1, FOS, PIK3R1, SREBF1</td>
<td>IRO</td>
</tr>
<tr>
<td>GO:0071869</td>
<td>response to catecholamine</td>
<td>3.0E-2</td>
<td>EGR1, SNCA, PPARG1A</td>
<td>IRO</td>
</tr>
<tr>
<td>GO:0045444</td>
<td>fat cell differentiation</td>
<td>1.0E-3</td>
<td>DDIT3, ENPP1, FABP4, NR4A1, SREBF1, PPARG1A</td>
<td>IRO</td>
</tr>
<tr>
<td>GO:0045444</td>
<td>fat cell differentiation</td>
<td>8.0E-6</td>
<td>AACS, CEBPD, CREB5, LGALS12, NR1D1, PER2, SNAI2, ZBTB16, ZC3H12A</td>
<td>common</td>
</tr>
<tr>
<td>GO:0032330</td>
<td>regulation of chondrocyte differentiation</td>
<td>3.0E-4</td>
<td>CTGF, RARG, SNAI2, SOX9, ZBTB16</td>
<td>common</td>
</tr>
<tr>
<td>GO:0048545</td>
<td>response to steroid hormone</td>
<td>3.0E-3</td>
<td>AACS, ABC2C, CDKN1A, CTGF, ERRfi1, NPC1, NR1D1, PPARG1B, RARG</td>
<td>common</td>
</tr>
<tr>
<td>GO:0071396</td>
<td>cellular response to lipid</td>
<td>1.0E-6</td>
<td>AACS, ADAMTS1, ERRfi1, HMGCS1, NPC1, NR1D1, PDK4, PLAU, RARG, SNAI2, SOX9, ZC3H12A</td>
<td>common</td>
</tr>
<tr>
<td>GO:0001503</td>
<td>ossification</td>
<td>1.0E-3</td>
<td>BCOR, CEBPD, COL5A2, CTGF, PPARG1B, SKI, SNAI2, SOX9, ZBTB16</td>
<td>common</td>
</tr>
</tbody>
</table>

The top five most significant and biologically relevant Gene Ontology (GO):Biological processes are listed for the genes identified in Figure 3D. The p values are Bonferroni corrected in the GO analysis. ISO, insulin-sensitive obese; IRO, insulin-resistant obese.
with regard to influence of gender, the number of men was too small to allow a detailed comparison in this respect. Again, for lack of statistical power, we had to keep the male subjects in the analyses. Nevertheless, gender distribution did not influence our correlation analysis, and PCA of insulin gene responses in women was similar to that in all subjects (Figures S2D and S2E). Therefore, we do not believe that sex impacts on our results to any significant degree. Finally, because both pre- and postmenopausal women were included, we cannot exclude that menopausal state may influence our results. Nevertheless, as evident from Table 1, the three groups were well matched for this factor.

In summary, obese subjects with preserved insulin sensitivity have globally almost the same WAT gene expression response to insulin as BMI- and body-fat-matched insulin-resistant individuals. This is independent of cardio-metabolic risk factors, thereby questioning the notion of a “healthy obese state,” at least in the sWAT of women with severe obesity. A comparison with NO subjects confirms that the major factor explaining the differences in short-term insulin response is obesity per se. Nevertheless, small but clear quantitative differences in the transcriptional response to insulin in the ISO and IRO groups are observed that are linked to specific biological pathways involving, e.g., phosphorylation processes, cellular stimuli, and fat cell differentiation. The clinical and pathophysiological relevance of these differences will be addressed in additional studies, which we encourage by making the full tag cluster dataset publically available. The dataset can also be used for future studies addressing other gene regulatory mechanisms, e.g., those facilitated by specific gene promoters, enhancer usage, and long non-coding RNAs.

EXPERIMENTAL PROCEDURES

Subjects

The 51 obese subjects were enrolled in a clinical trial studying the outcome of gastric bypass surgery (ClinicalTrials.gov ID NCT01727245). From one obese patient, sWAT RNA was of insufficient quality, preventing accurate analyses. Hence, only CAGE data from the remaining 50 subjects are reported herein. We also recruited 17 healthy never-obese subjects. In this group, RNA quality was adequate in samples from 15 subjects. Investigations were performed in the morning after an overnight fast. Height, weight, and waist circumference were measured in duplicate. Abdominal sWAT biopsies were obtained and analyzed by The Karolinska University Hospital’s accredited routine clinical chemistry laboratory. Abdominal sWAT biopsies were obtained from the paraumbilical region by needle aspiration under local anesthesia. Thereafter, a hyperinsulinemic, euglycemic clamp was performed as described previously (Hagström-Toft et al., 2001). An intravenous bolus dose of insulin (1.6 U/m² body surface area; Actrapid, Novo Nordisk) was given, followed by intravenous infusion of insulin (0.12 U/m² min) for 120 min. Plasma glucose values were measured in duplicate every fifth minute (HemoCue). Euglycemia was maintained between 4.5 and 5.5 mmol/l (81 and 99 mg/dl) by a variable intravenous infusion of glucose (200 mg/ml). The mean glucose infusion rate (glucose disposal) between 60 and 120 min was determined (M value, milligrams of glucose uptake per kilogram of body weight per minute). Mean plasma insulin at 60 and 120 min of clamp was calculated.

The second sWAT biopsy was obtained from the contralateral paraumbilical side during the last 5 min of the clamp.

ISO was predefined based on data from a previously published study of obese women as the 25th upper percentile of the M value (Hofstetter et al., 2010). This gave a cutoff value of 4.65 mg/kg/min. Based on this figure, the 50 subjects from which CAGE data could be obtained were divided into two subgroups: 21 as ISO and 29 as IRO. Expressing M values per lean body mass instead of total body weight resulted in an identical subdivision, except that one subject was reclassified from IRO to ISO. Three ISO and nine IRO patients were on pharmacotherapy against hypertension. One IRO patient had diet-/lifestyle-treated type 2 diabetes. The study was approved by the regional ethics board in Stockholm. Informed written consent was obtained before enrolment.

eqPCR

RNA isolation, cDNA synthesis, real-time qPCR, and analysis of gene expression (using the \( \Delta Ct \) method) were performed as described previously (Gao et al., 2014). Details on TaqMan probes (Applied Biosystems) and SyBR primers (some of which were designed by us but provided by Sigma-Aldrich) are available upon request. Gene expression was normalized to 18S rRNA or LRP10.

5 Cap Analysis of Gene Expression Profiling and Data Processing

Total adipose RNA was extracted using the RNasy Lipid Tissue Mini Kit (QiAGEN), followed by RNA up-concentration, which was measured using a Nanodrop ND-1000 (Thermo Fisher Scientific). RNA quality was determined using the bioanalyzer RNA 6000 Pico Kit (Agilent Technologies). CAGE libraries were prepared as described previously (Takahashi et al., 2012), with an input of 1,000 ng total RNA. Samples were run individually. Four CAGE libraries with different barcodes were pooled prior to sequencing and applied to the same sequencing lane. Libraries were prepared in a random order to avoid systematic errors, as described previously (Takahashi et al., 2012), and sequenced using Illumina Hi-Seq 2500 or 2000. Sequenced reads were mapped to the human genome using Bowtie (Langmead et al., 2009). Nearby mapped reads on the same strand were merged into 35,693 tag clusters (genomic regions) using Paraclu (Frith et al., 2008). Tag per million (TPM) normalized read counts in these clusters from the 136 RNA samples formed the expression data matrix of interest. Tag clusters with an expression \( \geq 0.5 \) TPM in at least 20% of the samples were maintained, resulting in 30,331 tag clusters. ENSEMBL genome annotation (Kersey et al., 2014) was used to assign the tag clusters to a total of 15,518 genes. All raw data are uploaded at https://export.uppmax.uu.se/b2013047/CelReportsTables/.

Bioinformatic Analyses

Pathway and gene ontology analyses were performed using standard webtools, including KEGG Mapper (http://www.genome.jp/kegg/tool/map_pathway1.htm) and ToppFun (https://toppgene.cchmc.org).

Statistical Methods

Unless otherwise stated, values are means ± SD and compared by an unpaired two-sided t test assuming unequal variances. One-sided tests were used in qPCR validation experiments comparing ISO and IRO subjects. Nominal parameters were compared by chi-square test. Specific for CAGE data, the PCA scores were tested using an unpaired t test assuming unequal variance. Multiple regression and differential expression analyses for CAGE data were performed using global linear models (GLMs) implemented in edgeR (Robinson et al., 2010), and significance was determined by Benjamini-Hochberg-corrected FDR.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.070.

AUTHOR CONTRIBUTIONS

all co-authors; Collection of Data, D.P.A., J.B., M.B., I.D., H.G., E.M., E.N.,

ACKNOWLEDGMENTS

We thank nurses Yvonne Widlund, Britt-Marie Leijonhufvud, and Katariina Her-
tel, as well as laboratory engineers Eva Sjölin and Elisabeth Dunger for excel-
lent technical assistance. The computations were performed on resources
provided by SNIC through Uppsala Multidisciplinary Center for Advanced
Computational Science (UPPMAX) under Project b2013047. We thank Sci-
ence for Life Laboratory in Stockholm as well as the National High-throughput
DNA Sequencing Centre in Copenhagen for help with CAGE sequencing.

The work in Sweden was supported by two grants from the Novo Nordisk
Foundation (including the Tripartite Immuno-metabolism Consortium [TrIC],
grant NNF15CC0018486), the Swedish Research Council, the EASD/Lilly
Foundation, the Stockholm County Council, the Swedish Diabetes Associa-
tion, the Erling Persson Family Foundation, CIMEDE, and the Diabetes
Research Program at Karolinska Institutet. The work in Denmark was sup-
ported by the Lundbeck and Novo Nordisk Foundations.

Received: October 19, 2015
Revised: April 22, 2016
Accepted: July 26, 2016
Published: August 18, 2016

REFERENCES

Arner, P., Bäckdahl, J., Hemmingsson, P., Stenvinkel, P., Eriksson-Hogling,
D., Näslund, E., Thorell, A., Andersson, D.P., Caidahl, K., and Rydén, M.
(2015). Regional variations in the relationship between arterial stiffness and
Blüher, M. (2010). The distinction of metabolically ‘healthy’ from ‘unhealthy’
obese individuals. Curr. Opin. Lipidol. 21, 38–43.
Caminci, P., Sandelin, A., Lenhard, B., Katayama, S., Shimokawa, K., Pon-
(2006). Genome-wide analysis of mammalian promoter architecture and evo-
Elbein, S.C., Kern, P.A., Rasouli, N., Yao-Borengasser, A., Sharma, N.K., and
muscle from glucose-tolerant, insulin-sensitive, and insulin-resistant indi-
mates matched for BMI. Diabetes 60, 1019–1029.
obesity and cardio-metabolic abnormality on the risk of cardiovascular dis-
ase: a meta-analysis of prospective cohort studies. Int. J. Cardiol. 168,
4761–4768.
Flint, A.J., Hu, F.B., Glynn, R.J., Caspard, H., Manson, J.E., Willett, W.C., and
Rimm, E.B. (2010). Excess weight and the risk of incident coronary heart dis-
ase among men and women. Obesity (Silver Spring) 18, 377–383.
Forrest, A.R., Kawaji, H., Rehli, M., Baille, J.K., de Hoon, M.J., Haberle, V.,
Lassmann, T., Kulakovskiy, I.V., Lizio, M., Itoh, M., et al.; FANTOM Consor-
tium and the RIKEN PMI and CLST (DGT) (2014). A promoter-level mammalian
Frith, M.C., Valen, E., Krogh, A., Hayashizaki, Y., Caminci, P., and Sandelin,
Res. 18, 1–12.
Gao, H., Meijhart, N., Fretz, J.A., Arner, E., Lorente-Cebrián, S., Ehrlund, A.,
Dahlman-Wright, K., Gong, X., Strömblad, S., Douagi, I., et al. (2014). Early
B cell factor 1 regulates adipocyte morphology and lipolysis in white adipose
tissue. Cell Metab. 19, 981–992.
Hagström-Toft, E., Thörne, A., Reynisdottir, S., Moberg, E., Rössner, S., Bolin-
lipolysis in the regulation of lipid oxidation during caloric restriction in vivo.
Diabetes 50, 1604–1611.
Hoffstedt, J., Arner, E., Wahrenberg, H., Andersson, D.P., Ovist, V., Lön, P.,
adipose tissue morphology on the metabolic profile in morbid obesity. Dia-
betologia 53, 2496–2503.
372, 1281–1283.
Kawai, H., Lizio, M., Itoh, M., Kanaori-Katayama, M., Kaino, A., Nishiyori-Su-
FANTOM Consortium (2014). Comparison of CAGE and RNA-seq transcrip-
tome profiling using clonally amplified and single-molecule next-generation
sequencing. Genome Res. 24, 708–717.
Kersey, P.J., Allen, J.E., Christensen, M., Davis, P., Falin, L.J., Grabmueler, C.,
Genomes 2013: scaling up access to genome-wide data. Nucleic Acids Res.
42, D546–D552.
Klimáková, E., Roussel, B., Marquez-Quiliones, A., Kováčová, Z., Kováč-
várová, M., Combes, M., Siklova-Vlková, M., Hejnová, J., Šramková, P., Bou-
loumé, A., et al. (2011). Worsening of obesity and metabolic status yields
similar molecular adaptations in human subcutaneous and visceral adipose
tissue: decreased metabolism and increased immune response. J. Clin. Endo-
crinol. Metab. 96, E73–E82.
Kramer, C.K., Zinman, B., and Retnakaran, R. (2013). Are metabolically health-
ly overweight and obesity benign conditions?: A systematic review and meta-
memory-efficient alignment of short DNA sequences to the human genome.
Genome Biol. 10, R25.
Lennard, J., Leijonhufvud, K., Gong, X., Stromblad, S., Douagi, I., et al.;
FANTOM Consortium (2014). Comparison of CAGE and RNA-seq transcrip-
tome profiling using clonally amplified and single-molecule next-generation
sequencing. Genome Res. 24, 708–717.
Ortega, F.B., Lee, D.C., Katmarzyk, P.T., Ruiz, J.R., Sui, X., Church, T.S., and
Blair, S.N. (2013). The intriguing metabolically healthy but obese phenotype:
Persson, H., Kwon, A.T., Ramilowski, J.A., Silberberg, G., Söderhäll, C., Ors-
mark-Pietras, C., Nordlund, B., Konradi, J.R., de Hoon, M.J., Melén, E.,
et al. (2015). Transcriptome analysis of controlled and therapy-resistant child-
hood asthma reveals distinct gene expression profiles. J. Allergy Clin. Immu-
nol. 136, 639–648.
Primeau, V., Codere, L., Karelius, A.D., Brochu, M., Lavoie, M.E., Messier, V.,
patients who are metabolically healthy. Int. J. Obes. 35, 971–981.
Qatanani, M., Tan, Y., Dobrin, R., Greenewalt, D.M., Hu, G., Zhao, W., Olefsky,
inflammation and mitochondrial function in adipose tissue defines extreme in-
Roberson, L.L., Aneni, E.C., Mazik, W., Agatston, A., Feldman, T., Rousse-
, M., Tran, T., Blaha, M.J., Santos, R.D., Sposato, A., et al. (2014). Beyond
BMI: The “metabolically healthy obese” phenotype & its association with clin-
ical/subclinical cardiovascular disease and all-cause mortality – a systematic
review. BMC Public Health 14, 14.
conductor package for differential expression analysis of digital gene expres-
sion data. Bioinformatics 26, 139–140.
Samocha-Bonet, D., Chisholm, D.J., Tonks, K., Campbell, L.V., and Green-
field, J.R. (2012). Insulin-sensitive obesity in humans – a “favorable fat” pheno-
Sims, E.A. (2001). Are there persons who are obese, but metabolically healthy?
Metabolism 50, 1499–1504.
Song, Y., Manson, J.E., Meigs, J.B., Ridker, P.M., Buring, J.E., and Liu, S.
(2007). Comparison of usefulness of body mass index versus metabolic risk
factors in predicting 10-year risk of cardiovascular events in women. Am. J.
Cardiol. 100, 1654–1658.


