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

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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
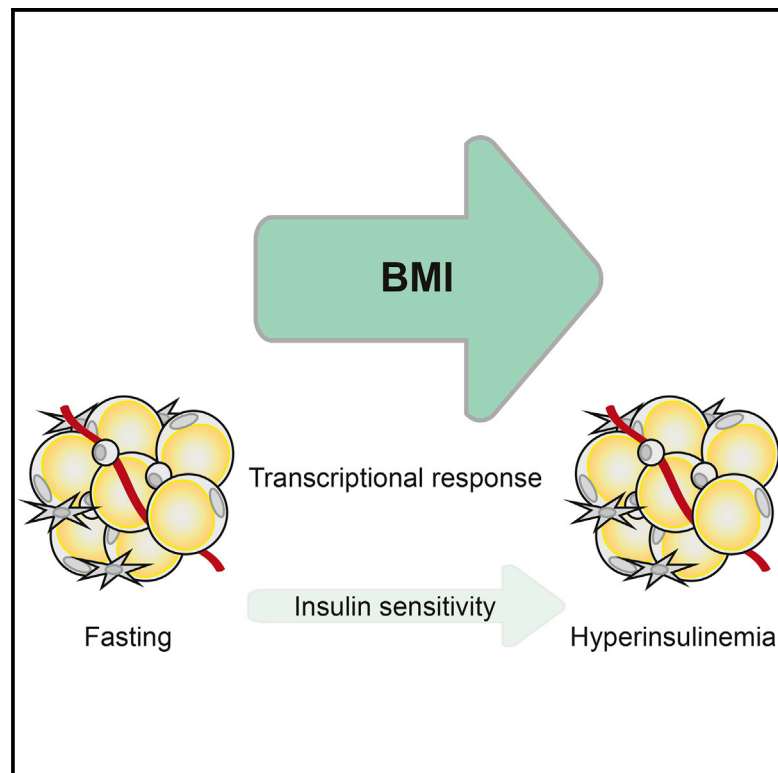
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In Brief

Rydén 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

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SUMMARY

Metabolically healthy obese subjects display preserved insulin sensitivity and a beneficial white adipose tissue gene expression pattern. However, this observation stems from fasting studies when insulin levels are low. We investigated adipose gene expression by 5′Cap-mRNA sequencing in 17 healthy non-obese (NO), 21 insulin-sensitive severely obese (ISO), and 30 insulin-resistant severely obese (IRO) subjects, before and 2 hr into a 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.

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 for 6 hr. Comparisons in limited numbers of lean ISO and IRO subjects have reported some between-group differences (Soronen et al., 2012; Westerbacka et al., 2006). Although relevant, these studies were not designed to address the transcriptional response to insulin in subjects matched for BMI. Thus, in order to fully evaluate the idea of a healthy obese state, insulin responses need to be determined in obese subjects discordant in insulin sensitivity and ideally compared with those in healthy non-obese (NO) subjects.

Although ISO and IRO individuals display different clinical phenotypes, it has been a matter of debate whether they also confer different risks for cardiovascular morbidity and/or mortality (Flint et al., 2010; Lu et al., 2014; Ortega et al., 2013; Song et al., 2007). In fact, several recent meta-analyses have refuted the notion that "healthy obesity" or preserved insulin sensitivity protects against cardiometabolic complications (Fan et al., 2013; Kramer et al., 2013; Roberson et al., 2014). These controversies prompted us

Table 1. Clinical Characteristics of the Study Groups

Variable	ISO (n = 21; M/F, 0/21)	IRO (n = 30; M/F, 6/24)	NO (n = 17; M/F, 3/14)	p Value Chi-Square Test	ISO versus IRO	NO versus ISO	NO versus IRO
Menopause, yes/no	6/15	11/13	4/10	0.40	–	–	–
Nicotine use, yes/no	2/19	4/26	1/16	0.70	–	–	–
Age (years)	41 ± 12	45 ± 11	42 ± 13		0.22	0.75	0.42
BMI (kg/m^2)	39 ± 3	39 ± 5	24 ± 3		0.81	<0.0001	<0.0001
Waist-to-hip ratio	0.93 ± 0.07	1.00 ± 0.07	0.88 ± 0.05		0.0007	0.01	<0.0001
Total fat (kg)	55 ± 7	53 ± 9	22 ± 8		0.37	<0.0001	<0.0001
fP-Glucose (mmol/l)	5.1 ± 0.4	5.9 ± 2.0	5.0 ± 0.4		0.041	0.85	0.035
fP-Insulin (mU/l)	9.7 ± 5.1	18.9 ± 9.5	6.0 ± 3.3		<0.0001	0.13	<0.0001
fP-Cholesterol (mmol/l)	4.9 ± 0.7	5.2 ± 1.2	4.5 ± 0.9		0.19	0.35	0.027
fP-HDL cholesterol (mmol/l)	1.4 ± 0.3	1.2 ± 0.3	1.5 ± 0.3		0.044	0.12	0.0006
fP-Triglycerides (mmol/l)	1.1 ± 0.5	1.5 ± 0.8	0.9 ± 0.3		0.010	0.24	0.0004
Systolic blood pressure (mmHg)	134 ± 14	142 ± 15	120 ± 13		0.044	0.0035	<0.0001
Diastolic blood pressure (mmHg)	82 ± 15	83 ± 11	76 ± 10		0.76	0.17	0.083
Resting pulse rate (beats per minute)	68 ± 12	74 ± 13	62 ± 11		0.074	0.14	0.0016
M value (mg/kg · min)	6.2 ± 0.9	3.1 ± 0.9	8.6 ± 2.0		<0.0001	<0.0001	<0.0001
Mean fP-insulin level during clamp (60–120 min, mU/l)	223 ± 43	258 ± 56	202 ± 32		0.011	0.18	0.0003

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.

to investigate how gene expression in the sWAT of ISO individuals responds to insulin stimulation (hyperinsulinemia). Assuming that the ISO group displays a more "beneficial" gene expression profile in the fasting state compared to the IRO group, we hypothesized that the differences in transcriptional response profiles between the ISO and IRO groups would be more pronounced upon hyperinsulinemia and that the ISO group would be more similar to the NO group than the IRO group. Therefore, we assessed global transcriptional profiles in sWAT from healthy NO subjects and from obese subjects subdivided into ISO and IRO groups according to hyperinsulinemic euglycemic clamp measures. Subcutaneous WAT biopsies were taken before and at the end of the 2-hr clamp. We chose this short period of hyperinsulinemia in order to evaluate direct transcriptional effects of insulin, assuming that longer duration of stimulation may cause secondary effects on gene transcription. Samples were analyzed using global transcriptional profiling with the 5′cap analysis of gene expression (CAGE) (Takahashi et al., 2012). CAGE is based on sequencing the 5′ end of mRNA, thereby assessing the transcriptional start sites (TSS) and their usage (expression) with high resolution and reproducibility (Kawaji et al., 2014). Adjacent TSSs for presumably the same transcripts are collapsed into "tag clusters" corresponding to gene promoters (Frith et al., 2008). Individual genes typically have several tag clusters depending on tissue type (Carninci et al., 2006; Forrest et al., 2014). CAGE provides less biased results than conventional expression arrays, allows for more in-depth analysis, and also detects uncharacterized novel gene transcripts (Suzuki et al., 2009). However, in order to obtain biologically interpretable insights, the present work was focused on analyses of tag clusters corresponding to annotated genes.

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 *PPP1R3B*, *PPP1R3C*, *PIK3R*, and *IRS2* (Figure 1C). A subset of these genes (*SREBF1*, *PIK3R3*, and *IRS2*) was validated by qPCR in the
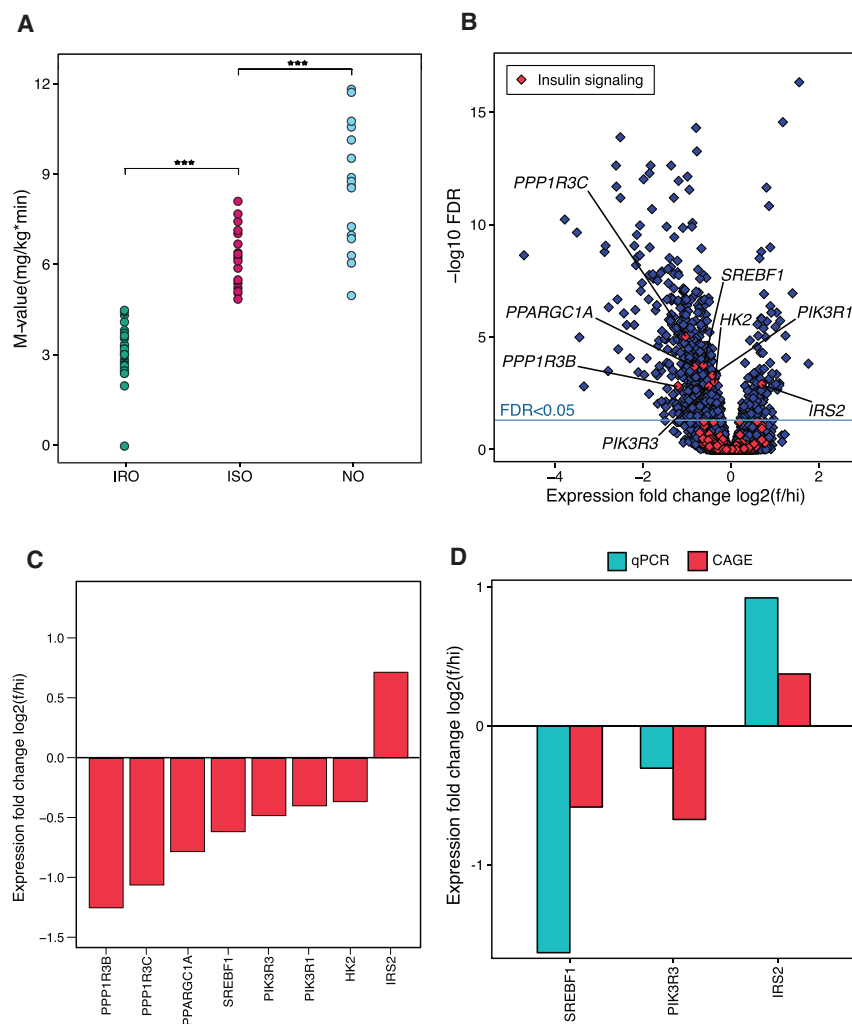
Figure 1. Clamp Validation
(A) Individual M values are detailed for the NO, ISO, and IRO groups. ***p < 0.001.
(B) Volcano plot of the 30,331 detected CAGE tag clusters, corresponding to 15,518 genes, expressed as log fold change fasting/hyperinsulinemia (f/hi) along the x axis and false discovery rate (FDR) on the y axis. In blue: 29,964 tag clusters (15,398 genes) responding to the clamp. In red: 367 tag clusters (122 genes) involved in the insulin signaling pathway. The eight tag clusters corresponding to eight genes involved in the insulin signaling pathway are highlighted. The horizontal line shows an FDR < 0.05.
(C) Expression fold changes (fasting/hyperinsulinemia; f/hi) of the eight insulin signaling pathway genes shown in (B).
(D) qPCR validation of selected genes from (C) in the NO group expressed as f/hi. Paired two-sided t tests gave p values between <0.0001 and 0.015.

NO group and confirmed the findings obtained by CAGE (Figure 1D).

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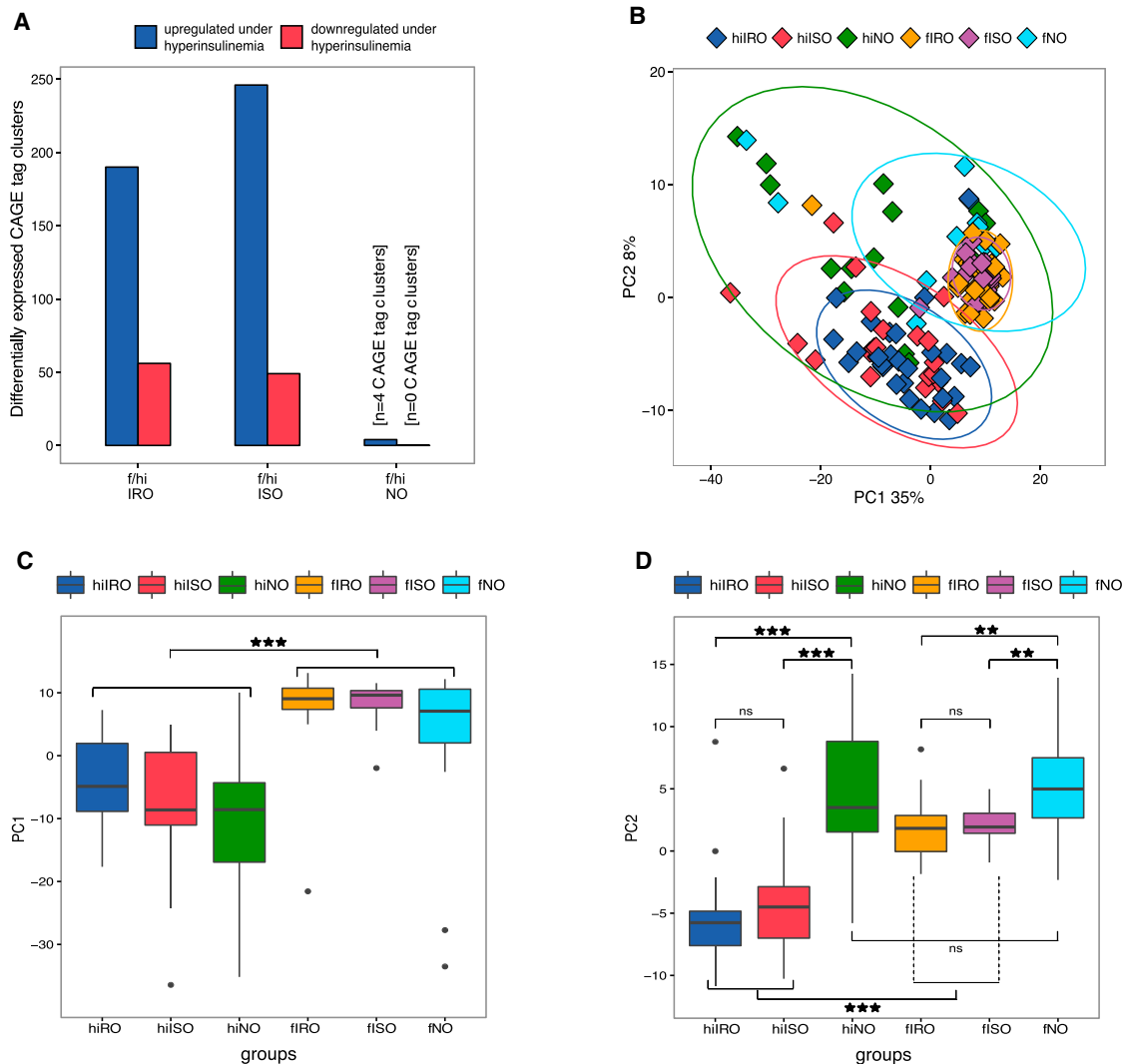 further change in the NO group, while the ISO and IRO groups were altered but remained different from the NO group (Figure 2D).

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

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.

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

Table 2. Correlations between Clinical Parameters and Individual Changes in Overall Gene Expression during Hyperinsulinemia, PC1, in Obese Subjects

Variable	Estimate	SE	t Value	p Value
Intercept	−37.51	19.23	1.95	0.0583
M value	−3.01	1.22	−2.47	0.0181
BMI	1.33	0.46	2.91	0.0060
fP-Cholesterol	1.15	1.73	0.66	0.5122
fP-HDL cholesterol	6.24	5.60	1.11	0.2720
fP-Triglycerides	−3.48	2.50	−1.39	0.1722
fP-Insulin	0.03	0.28	0.10	0.9196
Systolic blood pressure	0.04	0.13	0.31	0.7609
Diastolic blood pressure	−0.16	0.11	−1.46	0.1516
Pulse rate	−0.03	0.13	−0.22	0.8263
Gender	−4.13	5.43	−0.76	0.45

Multiple regression was used. For the whole model, r^2 = 0.45 and p = 0.0045. PC1, principal component 1; fP, fasting plasma; HDL, high-density lipoprotein.

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 *PPARGC1* 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*, *PPARGC1B*, 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 *PPARGC1A*) 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 WAT 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 grooup. This shows that there are small but quantifiable differences between the ISO and IRO groups in the adipose 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 (Viguerie 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 (Hoffstedt 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
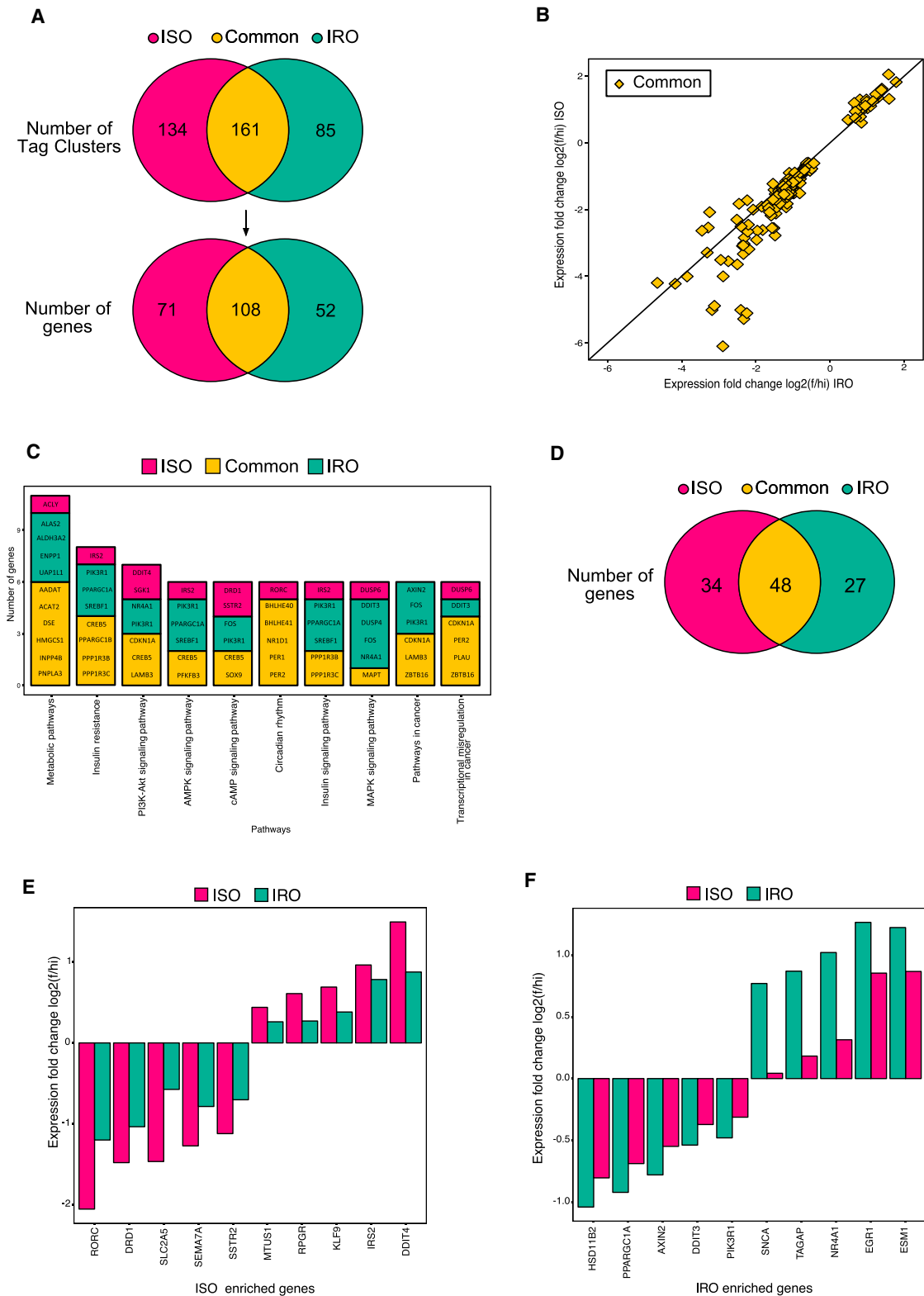
Figure 3. Identification of Genes Altered by Hyperinsulinemia in ISO and IRO Groups

(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)

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

GO Term	Description	p Value	Genes	Type
GO:0071396	cellular response to lipid	1.8E-2	HDAC5, KLF9, RORC, SGK1, SSTR2, TRIB1, VDR	ISO
GO:0071383	cellular response to steroid hormone stimulus	1.8E-2	KLF9, RORC, SGK1, SSTR2, VDR	ISO
GO:0033673	negative regulation of kinase activity	1.7E-2	DRD1, DUSP6, IRS2, LRP5, MIDN, TRIB1	ISO
GO:0045963	negative regulation of phosphate metabolic process	1.5E-3	APOC1, DDIT4, DRD1, DUSP6, IRS2, LRP5, MIDN, MYO1D,TRIB1	ISO
GO:0010563	negative regulation of phosphorus metabolic process	1.5E-3	APOC1, DDIT4, DRD1, DUSP6, IRS2, LRP5, MIDN, MYO1D,TRIB1	ISO
GO:0010035	response to inorganic substance	7.0E-4	FABP4, FOS, NR4A1, PPARGC1A, SLC1A3, SLC30A1, TNFAIP3	IRO
GO:0042493	response to drug	4.0E-3	EGR1, FOS, HSD11B2, PPARGC1A, SLC1A3, SNCA, SREBF1	IRO
GO:005159	response to cAMP	3.0E-2	EGR1, FOS, PIK3R1, SREBF1	IRO
GO:0071869	response to catecholamine	3.0E-2	EGR1, SNCA, PPARGC1A	IRO
GO:0045444	fat cell differentiation	1.0E-3	DDIT3, ENPP1, FABP4, NR4A1, SREBF1, PPARGC1A	IRO
GO:0045444	fat cell differentiation	8.0E-6	AACS, CEBPD, CREB5, LGALS12, NR1D1, PER2, SNAI2, ZBTB16, ZC3H12A	common
GO:0032330	regulation of chondrocyte differentiation	3.0E-4	CTGF, RARG, SNAI2, SOX9, ZBTB16	common
GO:0048545	response to steroid hormone	3.0E-3	AACS, ABCC2, CDKN1A, CTGF, ERRFI1, NPC1, NR1D1, PPARGC1B, RARG	common
GO:0071396	cellular response to lipid	1.0E-6	AACS, ADAMTS1, ERRFI1, HMGCS1, NPC1, NR1D1, PDK4, PLAU, RARG, SNAI2, SOX9, ZC3H12A	common
GO:0001503	ossification	1.0E-3	BCOR, CEBPD, COL5A2, CTGF, PPARGC1B, SKI, SNAI2, SOX9, ZBTB16	common

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.

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 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 (Klimcáková 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 hyperinsulinemia may differ between sWAT 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 sWAT than in skeletal muscle (Elbein et al., 2011). Furthermore,

(C) KEGG pathways to which insulin-responding genes from (A) are mapping.
(D) Number of genes corresponding to genes in (A) after the PubMatrix filtering step described in Results.
(E and F) Top differentially altered genes in the ISO (D) and IRO (E) groups. The y axis shows the expression fold change induced by insulin expressed as f/hi.

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, hip and waist circumference, resting pulse rate, blood pressure, and total body fat content by dual X-ray absorptiometry (DEXA) were determined (Arner et al., 2015). Venous blood was 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/m^2 body surface area; Actrapid, Novo Nordisk) was given, followed by intravenous infusion of insulin (0.12 U/m^2 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 kilograms 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 (Hoffstedt 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 enrollment.

qPCR

RNA isolation, cDNA synthesis, real-time qPCR, and analysis of gene expression (using the ΔΔCt 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 RNeasy 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 35,639 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 ≥0.5 TPM in at least 20% of the samples were maintained, resulting in 30,331 tag clusters. ENSMBL 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/CellReportsTables/.

Bioinformatic Analyses

Pathway and gene ontology analyses were performed using standard webtools, including KEGG Mapper (http://www.genome.jp/kegg/tool/map_pathway1.html) 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

Conception and Design, P.A., C.O.D., and M.R.; Analysis and Interpretation of the Data, P.A., C.O.D., O.H., E.M., M.R., and A.S.; Drafting of the Article, P.A., C.O.D., O.H., E.M., and M.R.; Critical Revision of the Article for Important Intellectual Content, P.A., C.O.D., E.M., and M.R.; Final Approval of the Article,

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