

Genetic variation near *IRS1* associates with reduced adiposity and an impaired metabolic profile

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Genome-wide association studies have identified 32 loci influencing body mass index, but this measure does not distinguish lean from fat mass. To identify adiposity loci, we meta-analyzed associations between ~2.5 million SNPs and body fat percentage from 36,626 individuals and followed up the 14 most significant ($P < 10^{-6}$) independent loci in 39,576 individuals. We confirmed a previously established adiposity locus in *FTO* ($P = 3 \times 10^{-26}$) and identified two new loci associated with body fat percentage, one near *IRS1* ($P = 4 \times 10^{-11}$) and one near *SPRY2* ($P = 3 \times 10^{-8}$). Both loci contain genes with potential links to adipocyte physiology. Notably, the body-fat-decreasing allele near *IRS1* is associated with decreased *IRS1* expression and with an impaired metabolic profile, including an increased visceral to subcutaneous fat ratio, insulin resistance, dyslipidemia, risk of diabetes and coronary artery disease and decreased adiponectin levels. Our findings provide new insights into adiposity and insulin resistance.

Adiposity is a key risk factor for a number of common metabolic disorders, such as type 2 diabetes and cardiovascular disease¹. Although the recent global increase in adiposity has been driven by lifestyle changes, family and twin studies suggest that there is also a substantial genetic component contributing to inter-individual variation in adiposity². The specific loci accounting for this variation are largely unknown.

Recent genome-wide association studies (GWAS) have identified 32 common loci associated with body mass index (BMI)³⁻⁸, the

most commonly used index of adiposity and the diagnostic criterion for obesity¹. These loci, however, account only for a small fraction of the variation in BMI⁸. Although BMI is generally a good indicator of adiposity and disease risk, it does not distinguish between lean and fat body mass. Using body fat percentage, a more accurate measure of body composition, may identify new loci more directly associated with adiposity. Therefore, we conducted a meta-analysis of 15 GWAS of body fat percentage, including altogether 36,626

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Table 1 Stage 1 and 2 results of SNPs near *IRS1* and *SPRY2* and in *FTO* that were associated with body fat percentage at genome-wide significant levels

Locus	Meta-analysis	Frequency effect allele	Per allele change in body fat % β^a	Explained variance (%)	Stage 1		Stage 2		Stage 1+2	
					<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>	<i>n</i>	<i>P</i>
rs2943650 (near <i>IRS1</i>) Chr. 2: 226,814,165 bp Effect allele: T	All individuals	0.64	-0.16	0.03	36,574	7.9×10^{-9}	39,576	1.9×10^{-4}	76,150	3.8×10^{-11}
	Europeans	0.64	-0.16	0.03	29,017	3.1×10^{-6}	39,576	1.9×10^{-4}	68,593	6.0×10^{-9}
	Indian Asians	0.69	NA	NA	7,557	2.7×10^{-4}	NA	NA	NA	NA
	Men	0.64	-0.20	0.06	19,751	4.1×10^{-8}	24,047	3.4×10^{-5}	43,798	2.9×10^{-11}
	European men	0.63	-0.20	0.06	13,216	4.1×10^{-6}	24,047	3.4×10^{-5}	37,263	1.8×10^{-9}
	Women	0.64	-0.06	0.00	16,823	0.0027	15,529	0.47	32,352	9.0×10^{-3}
rs534870 (near <i>SPRY2</i>) Chr. 13: 79,857,208 bp Effect allele: A	All individuals	0.68	-0.14	0.02	36,488	3.2×10^{-6}	34,342	2.6×10^{-3}	70,831	6.5×10^{-8}
	Europeans	0.70	-0.14	0.02	28,931	7.9×10^{-7}	34,342	2.6×10^{-3}	63,273	3.2×10^{-8}
	Indian Asians	0.68	NA	NA	7,557	0.52	NA	NA	NA	NA
	Men	0.68	-0.18	0.04	19,726	0.0016	20,537	2.1×10^{-3}	40,263	1.1×10^{-5}
	European men	0.69	-0.18	0.04	13,190	8.6×10^{-5}	20,537	2.1×10^{-3}	33,727	1.6×10^{-6}
	Women	0.69	-0.06	0.01	16,763	9.0×10^{-4}	13,805	0.34	30,568	2.2×10^{-3}
rs8050136 (<i>FTO</i>) Chr. 16: 52,373,776 bp Effect allele: C	All individuals	0.60	-0.33	0.11	36,537	3.9×10^{-17}	34,105	4.4×10^{-11}	70,642	2.7×10^{-26}
	Europeans	0.59	-0.33	0.11	28,980	4.6×10^{-16}	34,105	4.4×10^{-11}	63,085	5.6×10^{-25}
	Indian Asians	0.68	NA	NA	7,557	0.011	NA	NA	NA	NA
	Men	0.60	-0.29	0.10	19,739	2.5×10^{-8}	20,624	6.0×10^{-7}	40,363	1.3×10^{-13}
	European men	0.59	-0.29	0.10	13,204	2.1×10^{-7}	20,624	6.0×10^{-7}	33,828	1.7×10^{-12}
	Women	0.60	-0.39	0.13	16,798	1.2×10^{-8}	13,481	1.6×10^{-5}	30,279	1.1×10^{-12}
European women	0.59	-0.39	0.13	15,776	2.2×10^{-8}	13,481	1.6×10^{-5}	29,257	2.7×10^{-12}	

We defined genome-wide significance as $P < 5 \times 10^{-8}$. The effect allele for each locus is the body fat percentage decreasing (major) allele. Chromosomal positions are indicated according to build 36 and allele coding based on the positive strand. Chr., chromosome; NA, no individuals available for analysis.

^aEffect sizes in percentages obtained from stage 2 studies only, which included only individuals of European descent.

individuals of European ($n = 29,069$) and Indian-Asian ($n = 7,557$) descent and followed up the most significant findings in up to 39,576 European individuals.

RESULTS

Stage 1 GWAS meta-analysis of body fat percentage

We first performed a meta-analysis for associations of body fat percentage with ~2.5 million genotyped or imputed SNPs from 15 studies, including up to 36,626 individuals of European ($n = 29,069$) and Indian-Asian ($n = 7,557$) descent (Online Methods and **Supplementary Fig. 1**). To identify genetic loci that may associate with body fat percentage in European individuals only, we performed an additional meta-analysis of individuals of European descent. We also performed meta-analyses in men ($n_{\text{European}} = 13,280$, $n_{\text{Indian-Asian}} = 6,535$) and women ($n_{\text{European}} = 15,789$, $n_{\text{Indian-Asian}} = 1,022$) separately to identify sex-specific associations with body fat percentage. Genetic variants in *FTO*, the fat mass and obesity-associated gene, and near *IRS1*, the insulin receptor substrate 1 gene, showed genome-wide significance ($P < 5 \times 10^{-8}$) at this stage (**Table 1** and **Fig. 1**). To confirm the loci near *IRS1* and in *FTO* and to identify more adiposity loci, we took forward 14 SNPs representing the 14 most significant and independent loci for which association with body fat percentage reached $P < 10^{-6}$ in all individuals combined, in Europeans, in men or in women (**Supplementary Table 1** and **Supplementary Fig. 2**). We considered loci to be independent when they were in low linkage disequilibrium (LD) ($r^2 < 0.3$) or were >1 Mb apart.

Stage 2 analyses identify three body fat percentage loci

We examined the associations of the 14 SNPs with body fat percentage in up to 39,576 additional individuals of European descent from 11 studies (stage 2) (Online Methods, **Supplementary Table 2** and **Supplementary Fig. 1**). In a joint meta-analysis of stage 1 and

stage 2 results, 3 of the 14 SNPs reached genome-wide significance ($P < 5 \times 10^{-8}$) for association with body fat percentage (**Table 1** and **Supplementary Table 3**). We confirmed associations for the SNP in *FTO* (chr16: rs8050136; $P_{\text{all}} = 3 \times 10^{-26}$) and for the SNP near *IRS1* (chr2: rs2943650; $P_{\text{all}} = 4 \times 10^{-11}$), which both reached genome-wide significance in stage 1, and identified a third locus near *SPRY2*, the sprouty homolog 2 gene (chr 13: rs534870; $P_{\text{Europeans}} = 3 \times 10^{-8}$). The locus near *SPRY2* showed association with body fat percentage only in Europeans and not in Indian Asians, whereas the effect sizes for the loci near *IRS1* and in *FTO* were similar in meta-analyses of Europeans only compared to Europeans and Indian Asians combined (**Table 1** and **Supplementary Table 4**). The association of the SNP near *IRS1* with body fat percentage was significantly ($P_{\text{sex-difference}} = 0.02$) more pronounced in men ($P = 3 \times 10^{-11}$) than in women ($P = 9 \times 10^{-3}$) (**Table 1** and **Supplementary Table 3**). Whereas *FTO* is a well-established adiposity gene^{3,5}, the loci near *IRS1* and *SPRY2* have not been previously implicated in adiposity. Therefore, we focused our follow-up analyses on the loci near *IRS1* and *SPRY2* to estimate their impact on related metabolic traits and to gain insight into the potential functional roles of these two new adiposity loci.

Follow up of the locus near *IRS1*

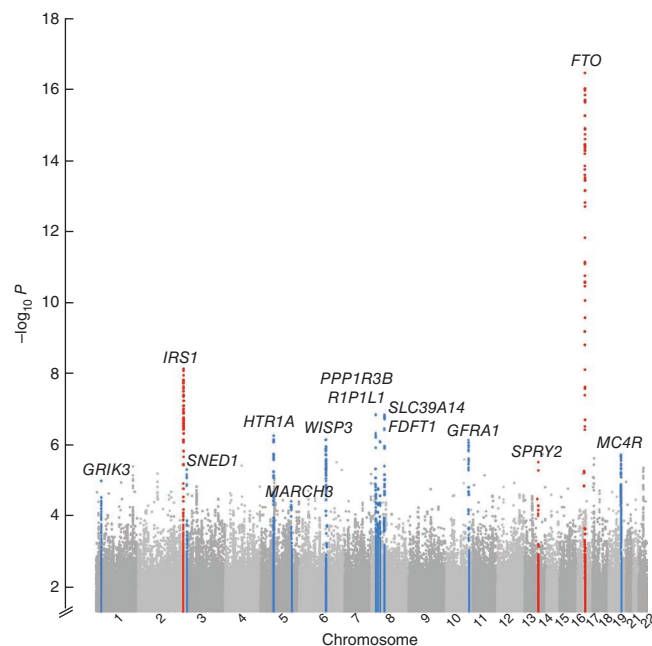
rs2943650 near *IRS1* was associated with a 0.16% lower body fat percentage per copy of the major allele. The effect was stronger in men than in women ($\beta = 0.20\%$ and $\beta = 0.06\%$ per allele, respectively). Notably, despite the highly significant associations with body fat percentage, we found no convincing evidence of association between the SNP near *IRS1* and BMI ($P_{\text{all}} = 0.32$, $P_{\text{men}} = 0.16$ and $P_{\text{women}} = 0.79$) or other obesity-related traits (**Supplementary Table 5**). As BMI represents both fat and lean mass, whereas body fat percentage is a measure of the relative proportion of these two tissues, our observation suggests that the locus near *IRS1* specifically influences

Figure 1 Manhattan plot showing the significance of association with body fat percentage for SNPs in the stage 1 meta-analysis of all individuals ($n = 36,626$). SNPs are plotted on the x axis according to their position on each chromosome against association with body fat percentage on the y axis (shown as $-\log_{10} P$). The loci highlighted in blue are the 11 loci that reached an association $P < 10^{-6}$ in the stage 1 meta-analysis of all individuals, Europeans, men or women and were taken forward for follow-up analyses but did not achieve genome-wide significance ($P < 5 \times 10^{-8}$) in the meta-analyses combining GWAS and follow-up data. The three loci colored in red are those that reached genome-wide significant association ($P < 5 \times 10^{-8}$) in the meta-analyses combining GWAS and follow-up data.

adiposity, or alternatively, influences fat mass and lean body mass in opposite directions.

rs2943650 is located 500 kb upstream of *IRS1*, an important mediator of insulin and insulin-like growth factor-1 (IGF-1) signaling (Fig. 2). Previous GWAS have identified SNPs near *IRS1*, which are in high LD with rs2943650 ($r^2 > 0.8$ in the HapMap European CEU population), to be associated with various metabolic traits^{9–11}. Notably, although we observed the major allele of rs2943650 to be associated with lower body fat percentage, prior work suggests that the major allele of rs2972146 ($r^2 = 0.95$ with rs2943650) is associated with higher triglycerides and lower high-density lipoprotein (HDL) cholesterol⁹, that the major allele of rs2943641 ($r^2 = 1.00$ with rs2943650) is associated with increased insulin resistance and risk of type 2 diabetes¹⁰ and that the major allele of rs2943634 ($r^2 = 0.83$ with rs2943650) is associated with increased risk of coronary artery disease¹¹.

To better understand how genetic variation in the locus near *IRS1* is associated with both lower body fat percentage and a more adverse metabolic profile, we performed a series of focused follow-up analyses on the association of rs2943650 with lipid profiles, indices of insulin sensitivity, fat distribution and circulating levels of leptin and adiponectin in the stage 2 studies that had all or some of these traits measured (Online Methods and Supplementary Fig. 1). These analyses confirmed that the body-fat-percentage-decreasing allele of rs2943650 is associated with higher triglycerides and lower HDL cholesterol levels and with increased insulin resistance, as indicated by the increased ratio of insulin area under the curve (AUC) to glucose AUC and decreased Matsuda¹² and Gutt¹³ insulin sensitivity indexes (Fig. 3 and Supplementary Table 6). Consistent with the sex difference observed for the association of



rs2943650 with body fat percentage, the associations with HDL cholesterol and triglyceride levels were more pronounced in men ($n = 9,937$ and $n = 10,659$, respectively) than in women ($n = 10,659$ and $n = 10,848$, respectively) ($P_{\text{sex-difference}} = 0.027$ and $P = 0.025$, respectively) (Fig. 3 and Supplementary Table 6), whereas associations with indices of insulin resistance were similar in both sexes.

To examine whether the association of the locus near *IRS1* with body fat percentage is mediated through association with insulin sensitivity, we performed an analysis for body fat percentage adjusted for insulin sensitivity among 6,489 men of the METSIM (Metabolic Syndrome in Men) study (Supplementary Fig. 3). Similarly, to examine whether the association of *IRS1* with body fat percentage could explain association with insulin sensitivity, we carried out an analysis for insulin sensitivity adjusted for body fat percentage. Although the effect size for the association of the rs2943650 (near *IRS1*) major allele with reduced body fat percentage did not significantly ($P_{\text{difference}} = 0.38$) change after

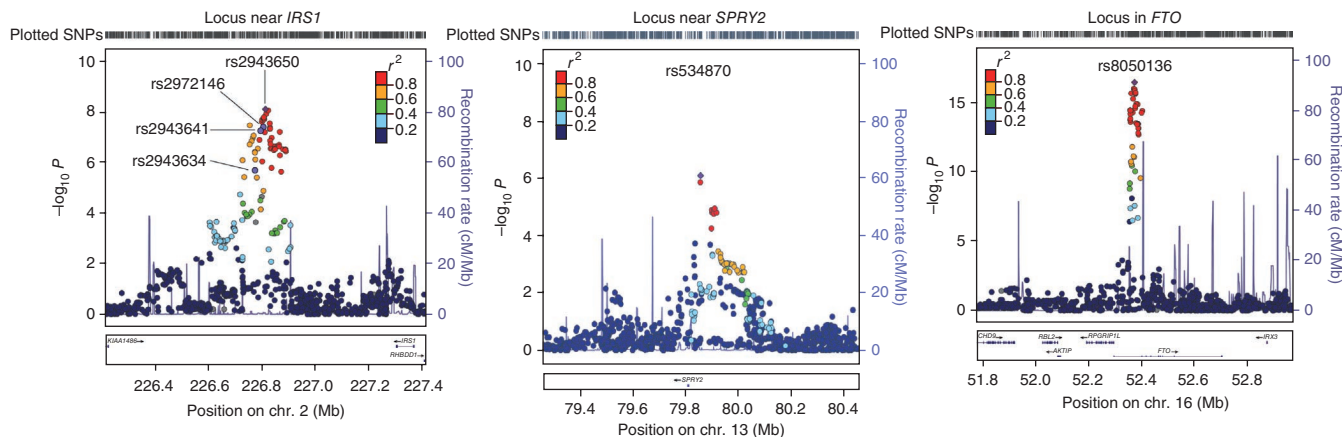
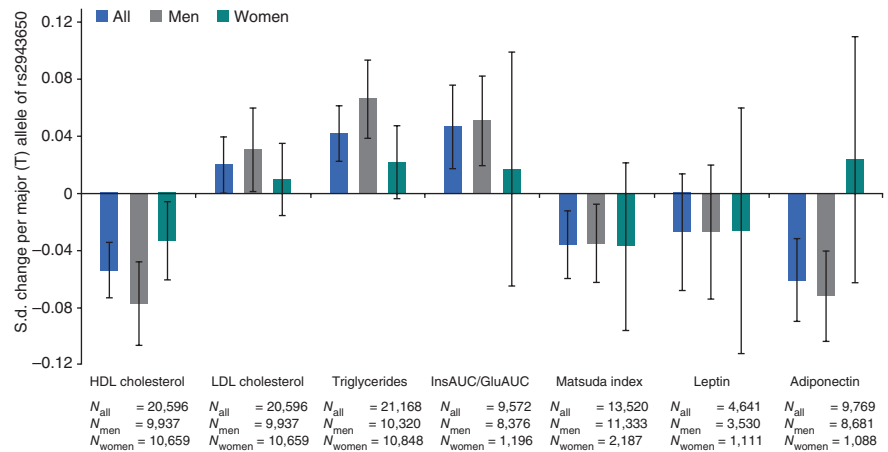


Figure 2 Regional plot of the loci near *IRS1*, near *SPRY2* and in *FTO* that reached genome-wide significant evidence for association with body fat percentage. The plotted data for the locus near *SPRY2* are from the meta-analysis of individuals of European descent only, and the data for the loci near *IRS1* and in *FTO* are from the meta-analysis of all individuals. The rs2943650 (near *IRS1*), rs534870 (near *SPRY2*) and rs8050136 (*FTO*) SNPs that showed the strongest association with body fat percentage are indicated. For the locus near *IRS1*, rs2972146, rs2943641 and rs2943634, which have been associated with blood levels of HDL cholesterol and triglycerides⁹, risk of type 2 diabetes¹⁰ and risk of coronary artery disease¹¹, respectively, in GWAS meta-analyses, are also indicated. The plot was generated using LocusZoom⁴⁴ (see URLs).

Figure 3 Association of the body-fat-percentage-decreasing (T) allele of rs2943650 near *IRS1* with blood lipids, insulin sensitivity traits, leptin and adiponectin. The error bars indicate 95% confidence intervals. All traits were inverse normally transformed to approximate normality (mean = 0, s.d. = 1) in men and women separately. All models were adjusted for age and age squared. The numeric values for the associations are presented in **Supplementary Table 6**. We found a significant difference between men and women for the levels of HDL cholesterol ($P = 0.027$), triglycerides ($P = 0.025$) and adiponectin ($P = 0.040$). InsAUC/GluAUC, insulin area under the curve (AUC) to glucose AUC ratio.



adjusting for insulin sensitivity, the association with reduced insulin sensitivity became significantly stronger when we adjusted for body fat percentage ($P_{\text{difference}} = 0.035$). These observations suggest that the locus near *IRS1* may have a primary effect on body fat percentage and that the association with decreased insulin sensitivity is partly mediated by changes in body fat percentage, at least in men.

We next examined whether the concurrent association of the locus near *IRS1* with lower body fat percentage and an adverse metabolic profile could be caused by joint associations with body fat distribution. Therefore, we determined associations of rs2943650 with abdominal visceral and subcutaneous fat obtained by computerized tomography in the GWAS meta-analysis data of 10,557 individuals (C.S.F., I.B.B., Y.L. & T.B.H., data not shown). We found that the locus near *IRS1* was associated with an adverse distribution of body fat in men, meaning the body-fat-percentage-decreasing allele reduced subcutaneous fat in men ($P = 1.8 \times 10^{-3}$, $n = 4,997$) but not in women ($P = 0.063$, $n = 5,560$), whereas we observed no association with visceral fat in either men ($P = 0.95$) or in women ($P = 0.63$). Most evidently, the body-fat-decreasing allele of the locus near *IRS1* was associated with a higher ratio of visceral adipose tissue to subcutaneous adipose tissue in men ($P = 6.1 \times 10^{-6}$) but not in women ($P = 0.31$). Our data thus suggest that the locus near *IRS1* may associate with a reduced storage of subcutaneous fat in men, which could contribute to the associations of this locus with insulin resistance and dyslipidemia by leading to an ectopic deposition of lipids¹³.

Having shown association of the locus near *IRS1* with the quantity and distribution of body fat and with related metabolic traits, we aimed to determine whether this locus is associated with measures of adipocyte function. Leptin and adiponectin are two hormones (adipokines) produced exclusively in adipose tissue that respond in a reciprocal manner to changes in fat mass and insulin resistance. Higher levels of leptin and lower levels of adiponectin correlate with increased body fat and insulin resistance¹⁴. Leptin data was available for 4,641 individuals, and adiponectin data was available for 9,769 individuals participating in our stage 2 meta-analysis (Online Methods and **Supplementary Fig. 1**). Notably, the body-fat-percentage-decreasing allele was associated with lower adiponectin levels in men ($P = 6.1 \times 10^{-6}$, $n = 8,681$), which is in contrast to what we expected given the inverse correlation between body fat percentage and adiponectin levels. We observed no association with lower adiponectin levels in women ($n = 1,088$), which was significantly different from the association in men ($P_{\text{sex-difference}} = 0.040$) (**Fig. 3** and **Supplementary Table 6**). The association between the locus near *IRS1* and leptin levels was not significant, which could be because of low statistical power, as the size of the sample available was small

(**Fig. 3** and **Supplementary Table 6**). Notably, recent studies in leptin-deficient (*ob/ob*) mice have shown that transgenic overexpression of adiponectin permits metabolically healthy expansion of subcutaneous adipose tissue, preventing accumulation of lipids in liver and retaining insulin sensitivity¹⁵. Vice versa, we speculate that the lower adiponectin levels, seen in men with the fat-percentage-decreasing allele of the locus near *IRS1*, might be associated with the lack of ability to expand subcutaneous adipose tissue, leading to a flux of lipids into liver and increased insulin resistance through a lipotoxic mechanism¹⁶.

To examine whether rs2943650 modifies the function of *IRS1*, we studied gene expression profiles within subcutaneous adipose tissue and blood from 604 Icelandic individuals (the deCODE cohort)^{6,17}, within liver ($n = 567$), subcutaneous ($n = 610$) and omental ($n = 742$) adipose tissue from individuals who underwent bariatric surgery¹⁸, and within normal cortical brain samples of 193 individuals of European descent¹⁹ (Online Methods). We found that the body-fat-percentage-decreasing allele is associated with lower *IRS1* expression in subcutaneous and omental adipose tissue but not in liver, brain or blood (**Supplementary Table 7** and **Supplementary Fig. 4**). The association with reduced expression in subcutaneous and omental adipose tissues seemed more pronounced in men than in women. Previous studies have shown that the body-fat-percentage-decreasing allele of rs2943641 in the same locus ($r^2 = 1.0$ with rs2943650) is associated with reduced expression of IRS-1 protein and reduced insulin-induced phosphatidylinositol 3-OH kinase activity in skeletal muscle¹⁰.

Finally, to determine if there is a sex difference in the adipose tissue expression of *IRS1*, we analyzed gene expression in isolated adipocytes from male and female mice. The data showed that adipocytes from females express higher levels of *Irs1* than adipocytes from males in both visceral and subcutaneous fat depots (**Supplementary Fig. 5**). We followed up this finding in human adipose tissue and found that the expression of *IRS1* was significantly greater in visceral adipose tissue from women ($n = 75$) than in visceral fat from men ($n = 26$), whereas we saw no sex difference in *IRS1* expression in subcutaneous adipose tissue (**Supplementary Fig. 6**). The higher basal levels of *IRS1* in female adipose tissue could, at least in theory, buffer women against the modest impairment of *IRS1* expression associated with genetic variation near *IRS1*.

Follow up of the locus near *SPRY2*

rs534870, which reached $P = 3 \times 10^{-8}$ in our meta-analysis of European individuals only, is located 54 kb downstream of *SPRY2* with no other genes nearby (**Fig. 2**). The body-fat-decreasing (major) allele of rs534870 was associated with a 0.14% decrease in body fat

percentage. Unlike for the locus near *IRS1*, the association was similar in men and women ($P_{\text{sex-difference}} = 0.62$), and we observed no association in Indian Asians (Table 1). We found a modest association for rs534870 with BMI, body weight and risk of obesity in a meta-analysis of all stage 2 studies (Supplementary Table 8). There was no association between the locus near *SPRY2* and blood lipids, but we found a nominally significant association between the body-fat-percentage-decreasing allele and increased insulin sensitivity measured with the Gutt insulin sensitivity index¹³ (Supplementary Table 8). The association with Gutt index was not significant after adjustment for body fat percentage ($P = 0.2$). The body-fat-percentage-decreasing allele of rs534870 was modestly associated with decreased *SPRY2* expression in whole blood. In contrast with the locus near *IRS1*, there was no association between rs534870 and *SPRY2* expression in adipose tissue, brain or liver (Supplementary Table 7 and Supplementary Fig. 4).

SPRY2 encodes a negative feedback regulator of the Ras/mitogen-activated protein kinase pathway²⁰. At the cellular level, overexpression of *SPRY2* inhibits migration and proliferation of a variety of cell types in response to serum and growth factors^{21–23}. Recent studies have identified *Spry1*, a homolog of *Spry2*, as a critical regulator of adipose tissue differentiation in mice²⁴. The loss of *Spry1* function resulted in a low bone mass and high body fat phenotype.

Established obesity loci and body fat percentage

Previous GWAS have examined BMI as an index of adiposity^{3–8}, and the recent meta-analysis by the GIANT (Genetic Investigation of Anthropometric Traits) Consortium increased the total number of established BMI susceptibility loci to 32 (ref. 8). The associations of the 32 confirmed BMI loci with body fat percentage were all directionally consistent with the previously established BMI associations (binomial sign test $P < 0.0001$), and associations for 17 of these loci reached nominal statistical significance (Supplementary Table 9). Our stage 1 sample size of 36,626 individuals was small compared to the GIANT stage 1 meta-analysis of BMI, which included 123,865 individuals, and we thus had insufficient power to confirm all 32 loci as body-fat-percentage loci. Furthermore, as BMI is a composite trait of fat and lean mass, BMI loci may associate with BMI by increasing fat mass, lean mass or both. Disentangling whether the established BMI loci associate with body fat percentage *per se* or with body mass overall will require larger sample sizes.

Other GWAS have identified three loci associated with waist circumference^{25,26} and five loci associated with extreme obesity^{27,28}. Similar to BMI loci, the associations of these eight loci with body fat percentage were directionally consistent with the previously established associations (binomial sign test $P = 0.008$), and associations for one waist circumference locus and two extreme obesity loci reached nominal significance (Supplementary Table 9).

Apart from GWAS that examined traits related to overall adiposity, recent GWAS studies have identified 14 loci associated with waist-to-hip ratio adjusted for BMI^{26,29}, a measure of body fat distribution. We found no association between these waist-to-hip loci and increased body fat percentage (Supplementary Table 9), which is consistent with the observation that these 14 loci are not or only very weakly associated with BMI and likely due to the fact that these loci were identified after accounting for BMI in the analyses.

DISCUSSION

Using a two-stage genome-wide association meta-analysis including up to 76,150 individuals, we identified three loci convincingly associated with body fat percentage. Although *FTO* was previously established as an obesity susceptibility locus^{3,5}, the loci near *IRS1* and near

SPRY2 have not previously been identified in the large-scale GWAS for BMI⁸, waist circumference^{25,26}, waist-to-hip ratio^{26,29} or extreme obesity^{27,28}, suggesting that these loci have a specific association with body fat percentage.

The locus near *IRS1* is associated with lower body fat percentage in men, and more specifically with proportionally less subcutaneous compared to visceral fat. Of particular interest is the pattern of association with other metabolic traits, which was opposite to what would be expected based on the known association between lower body fat percentage and improved metabolic profile. In effect, the fat-percentage-decreasing allele of the locus near *IRS1* was associated with higher levels of insulin resistance, an adverse lipid profile and lower levels of adiponectin in men. Furthermore, the fat-percentage-decreasing alleles of SNPs in the locus near *IRS1* have previously been associated with increased risk of type 2 diabetes¹⁰ and coronary artery disease¹¹.

We, and others¹⁰, showed that genetic variation near *IRS1* is associated with reduced *IRS1* expression in major insulin target tissues, including adipose tissue and muscle, which may explain the association of this locus with increased whole-body insulin resistance and risk of type 2 diabetes. The locus near *IRS1* is one of the few loci thought to increase risk of type 2 diabetes through an effect on insulin resistance, whereas other diabetes loci predominantly associate with measures of impaired beta-cell function^{10,30}. However, the mechanisms linking the locus near *IRS1* with type 2 diabetes may be more complex than previously thought. Our data suggest that genetic variation near *IRS1* may be associated with a reduced ability to store subcutaneous fat, at least in men, which may partly explain the association with whole-body insulin resistance and dyslipidemia. Adipose-tissue insulin sensitivity itself has little impact on whole-body insulin sensitivity, which is largely determined by the liver and muscle³¹. However, impaired ability of subcutaneous adipose tissue to expand may disrupt insulin signaling in liver and muscle by leading to ectopic deposition of lipids³². Such an indirect mechanism could exacerbate the intrinsic impairment of IRS-1 signaling in muscle.

The association of the locus near *IRS1* with body fat percentage and with many of the metabolic traits was more pronounced in men than in women. The mechanistic basis for this sexual dimorphism is yet unclear but may be related to the powerful drive to subcutaneous adipogenesis in women compared to men, which may overcome a defect in IRS-1 function. Men tend to deposit less subcutaneous and more visceral fat than women³³, and IRS-1 may thus have a stronger role in the regulation of subcutaneous fat in men. The association of the locus near *IRS1* with the expression of *IRS1* in subcutaneous adipose tissue was more pronounced in men, indicating that there may be sex differences in the effects of the locus near *IRS1* on gene function itself. We also showed a sex difference in both mouse and human adipose tissue expression of *IRS1*, with adipose tissue from females showing greater *IRS1* expression.

IRS-1 function has been described in animal models. Knockout of *Irs1* in mice leads to hyperinsulinemia and mild-to-moderate insulin resistance despite a lean phenotype^{34,35}. IRS-2 (ref. 36) and IRS-3 (refs. 37,38) partly compensate for the lack of IRS-1. Knockout of *Irs1* and *Irs3* together leads to severe early onset lipoatrophy with marked hyperglycemia, hyperinsulinemia and insulin resistance³⁹. The gene encoding IRS-3 is lacking in humans, which may make humans more dependent on IRS-1. Data from cell lines of *Irs1* knockout animals suggest that *Irs1* is involved in adipocyte differentiation^{40,41}. In *Irs1* knockout mice, the ability of embryonic fibroblast cells to differentiate into adipocytes is reduced by 60%⁴⁰. Cells of knockout mice for both

Irs1 and *Irs2* are completely unable to differentiate into adipocytes and show a severe reduction in white adipose tissue soon after birth⁴⁰.

Our second new locus for body fat percentage, near *SPRY2*, showed association only in Europeans and not in Indian Asians. Different from the locus near *IRS1*, the association between the body-fat-percentage-decreasing allele of the locus near *SPRY2* and insulin resistance was in the expected direction, meaning this allele associated with higher insulin sensitivity, and adjustment for body fat percentage attenuated the association. Similar to the locus near *IRS1* (refs. 40,41), the locus near *SPRY2* may play a role in regulating adipose tissue differentiation²⁴. Different from the GWAS of BMI, which have mainly established loci mechanistically linked with central nervous system control of appetite and energy expenditure⁸, our meta-analysis of body fat percentage indicates that loci harboring genes with potential links with adipocyte physiology may also play important roles in the regulation of body adiposity.

Our stage 1 meta-analyses included individuals of European and of Indian Asian descent. The Indian Asian individuals were mainly of north Indian descent ('ancestral north Indians', a western Eurasian population) and thus more closely related to Europeans, and, to a lesser extent, to Asians ('ancestral south Indians')⁴². Furthermore, the overall body fat percentage of the Indian Asian sample did not differ from that of individuals of European descent in our study. However, despite some similarities, genetic differences between European and Indian-Asian populations remain, and as differences in body composition between both ethnicities have been documented⁴³, we also performed a stage 1 GWAS in Europeans only. Exclusion of the Indian Asians did not affect the associations observed for the locus near *IRS1*, but it did for the locus near *SPRY2*. More specifically, the locus near *IRS1* was associated with body fat percentage in individuals of European and of Indian-Asian descent at stage 1. The association for the locus near *SPRY2*, however, was only seen in Europeans, whereas we saw no association in Indian Asians. These observations illustrate the value of including the Indian-Asian sample, as stratified analyses allowed us to infer the ethnic specificity of the identified loci.

In summary, we identified a locus near *IRS1* that is associated with reduced body fat percentage and adipose tissue *IRS1* expression in men but also with a combination of adverse metabolic and disease risk traits, including lower levels of subcutaneous fat, increased insulin resistance, dyslipidemia, decreased circulating levels of adiponectin and increased risk of diabetes and coronary artery disease. Furthermore, genetic variation in a locus near *SPRY2* associates with body fat percentage in individuals of European descent. Our findings provide new insights into adiposity and insulin resistance.

URLs. LocusZoom⁴⁴, <http://csg.sph.umich.edu/locuszoom/>; METAL, <http://www.sph.umich.edu/csg/abecasis/Metal/>.

METHODS

Methods and any associated references are available in the online version of the paper at <http://www.nature.com/naturegenetics/>.

Note: Supplementary information is available on the Nature Genetics website.

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A full list of author contributions appears in the **Supplementary Note**.

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ONLINE METHODS

Study design. We designed a multi-stage study (**Supplementary Fig. 1**) comprising a GWAS meta-analysis for body fat percentage (stage 1) of data of up to 36,626 individuals of European ($n = 29,069$) or Indian-Asian ($n = 7,557$) descent from 15 studies and selected 14 SNPs with $P < 1 \times 10^{-6}$ for follow up in stage 2. Stage 2 comprised up to 34,556 additional European individuals from 11 studies. Body fat percentage in stage 1 and stage 2 cohorts was measured either with bioimpedance analysis (BIA) or dual energy X-ray absorptiometry (DEXA), as described in the **Supplementary Note**. To explore the comparability of the methods, we studied the correlation between body fat percentage obtained by DEXA and BIA in the Fenland study ($n = 2,535$), in which both measurements were taken at the same time point. The Fenland study is a population-based cohort of European men and women between the ages of 30 and 60 years (**Supplementary Note**). The Pearson correlation coefficient showed that the DEXA and BIA measurements of body fat percentage are highly correlated ($r = 0.92$). The correlation between body fat percentage with BMI was moderate ($r = 0.62$ measured by DEXA and $r = 0.58$ measured by BIA, respectively).

Meta-analysis of stage 1 and 2 summary statistics identified three loci that reached genome-wide significance ($P < 5 \times 10^{-8}$) for association with body fat percentage. Two of these loci (near *IRS1* and near *SPRY2*) had not been previously identified in genome-wide association studies for BMI⁸, waist circumference^{25,26}, waist-to-hip ratio^{26,29} or extreme obesity^{27,28}. Subsequently, we performed a series of focused follow-up analyses (stage 3) to estimate the impact of these two new established body fat percentage loci and to explore their potential functional roles.

Stage 1 genome-wide association meta-analysis of body fat percentage.

Genotyping. The 15 studies included in the stage 1 meta-analysis were genotyped using Affymetrix, Illumina and Perlegen whole-genome genotyping arrays (**Supplementary Note**). To allow for meta-analysis across different marker sets, imputation of polymorphic HapMap European CEU SNPs was performed using MACH⁴⁵, IMPUTE⁴⁶ or BAMBAM⁴⁷ (**Supplementary Note**). Indian-Asian genotype data in the LOLIPOP study was imputed using pooled haplotypes from all three HapMap populations (CEU, YRI and JPT+CHB). Imputation scores for the successful SNPs were slightly lower for Indian Asians than for Europeans. The Indian-Asian GWAS of men genotyped with the Illumina 610K array had an average r^2 -hat of 0.942, whereas the GWAS from the GOOD study, genotyped with the same chip, had an average r^2 -hat of 0.956. In the Indian-Asian GWAS genotyped with the Perlegen array, the average r^2 -hat was 0.751, whereas it was 0.851 in the European GWAS sample from the same study, genotyped with the same chip. In previously published data⁴⁸, comparisons of imputed and experimentally derived genotypes in Indian Asians yielded an estimated imputation error rate of 2.86% per allele and an estimated average r^2 -hat of 89.8%

Association analysis with body fat percentage. Each study performed single marker association analyses with body fat percentage using an additive genetic model implemented in MACH⁴⁵, Merlin⁴⁹, SNPTEST⁴⁶ or PLINK⁵⁰. Body fat percentage was adjusted for age and age squared and inverse normally transformed to a mean of 0 and an s.d. of 1. Analyses were stratified by sex. To allow for relatedness in the Framingham Heart, Amish HAPI Heart, Family Heart and Erasmus Rucphen studies, regression coefficients were estimated in the context of a variance component model that modeled relatedness in men and women combined with sex as a covariate. In the Twins UK study, only one twin was randomly selected for the analyses from monozygotic twin pairs, whereas from dizygotic twin pairs, both twins were used for the analyses. Association analyses accounted for the relatedness between dizygotic twin pairs.

Before performing meta-analyses on the genome-wide association data for the 15 studies, SNPs with poor imputation quality scores (r^2 -hat < 0.3 in MACH, $\text{proper_info} < 0.4$ in IMPUTE or the ratio of observed to expected dosage variance < 0.3 in BAMBAM) were excluded for each study. All individual GWAS were genomic control corrected before meta-analysis. Individual study-specific genomic control values ranged from 0.979 to 1.052 (**Supplementary Note**).

Meta-analysis of stage 1 association results. Next, we performed the stage 1 meta-analyses using the inverse variance method, which is based on β coefficients and standard errors from each individual GWAS. The meta-analyses were

performed for all individuals combined, for European individuals, for men and for women using METAL (see URLs). The genomic control values for the meta-analyzed results were 1.074, 1.065, 1.052 and 1.040 in all individuals, European individuals, men and women, respectively (**Supplementary Fig. 7**).

Selection of SNPs for follow up. Fourteen SNPs, representing the 14 most significant ($P < 1 \times 10^{-6}$) independent loci in all individuals, Europeans, men or women (**Table 1** and **Supplementary Fig. 2**) were selected for replication analyses (stage 2). Loci were considered independent when they were in low LD ($r^2 < 0.3$) or were >1 Mb apart. SNPs which had been genotyped in less than 50% of the samples and/or that had a minor allele frequency $< 0.5\%$ were excluded. For some loci, the SNP with the strongest association could not be genotyped for technical reasons and was substituted by a proxy SNP that was in high LD with it ($r^2 > 0.8$) according to the HapMap CEU data. We tested the association of these 14 SNPs in four *de novo* and seven *in silico* replication studies in stage 2.

Test for sex difference. The differences between the effect sizes in men and women for the strongest signals were assessed with a *t* test with additional correction for the correlation between β coefficients in men and women in the GWAS data as follows:

$$t = (\beta_{\text{men}} - \beta_{\text{women}}) / \sqrt{(se_{\text{men}}^2 + se_{\text{women}}^2 - 2\text{corr}(\beta_{\text{men}}, \beta_{\text{women}})se_{\text{men}}se_{\text{women}})}$$

Stage 2 follow up of 14 most significant loci. Samples and genotyping. Directly genotyped data for the 14 SNPs was available from up to 22,485 adults of European ancestry from four studies (**Supplementary Note**). Samples and SNPs that did not meet the quality control criteria defined by each individual study were excluded. Minimum genotyping quality control criteria were defined as Hardy-Weinberg equilibrium $P > 10^{-6}$, call rate $>90\%$ and concordance $>99\%$ in duplicate samples in each of the follow-up studies. Association results were also obtained for the 14 most significant SNPs from 10,713 individuals of European ancestry from seven GWAS that had not been included in the stage 1 analyses (**Supplementary Note**). Studies included between 719 and 3,132 individuals and were genotyped using Affymetrix and Illumina genome-wide genotyping arrays. Autosomal HapMap SNPs were imputed using either MACH⁴⁵ or IMPUTE⁴⁶. SNPs with poor imputation quality scores from the *in silico* studies (r^2 -hat < 0.3 in MACH or $\text{proper_info} < 0.4$ in IMPUTE) were excluded.

Association analyses and meta-analysis. We tested the association between the 14 SNPs and body fat percentage in each *in silico* and *de novo* stage 2 study separately as described for the stage 1 studies. We subsequently performed a meta-analysis of β coefficients and standard errors from the stage 2 studies using the inverse variance method. Data was available for at least 31,705 individuals for 11 SNPs, except for three SNPs (rs17149412 in *FDFT1*, rs7736910 near *HTR1A* and rs7738021 near *MARCH3*), for which data was only available for ~24,400 individuals because of technical challenges relating to the genotyping and imputation of these SNPs. Next, we performed a meta-analysis of the summary statistics of the stage 1 and stage 2 meta-analyses using the inverse variance method in METAL. Differences in effect sizes between men and women were assessed as in stage 1.

Stage 3 follow up of the loci near *IRS1* and *SPRY2*. **Associations with anthropometric traits and obesity measures.** The associations of the loci near *IRS1* and *SPRY2* with BMI, body weight, height, risk of being obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) and risk of being overweight ($\text{BMI} \geq 25 \text{ kg/m}^2$) were tested in all stage 2 studies (**Supplementary Fig. 1** and **Supplementary Note**). Additional analyses for waist circumference, hip circumference and waist-to-hip ratio were performed in three of the stage 2 studies (EPIC-Norfolk, Fenland and MRC Ely) (**Supplementary Fig. 1**). The associations of the SNPs with quantitative secondary traits (BMI, body weight, height, waist circumference, hip circumference and waist-to-hip ratio) were tested with linear regression. The associations with overweight or obese status were assessed using logistic regression. All quantitative traits were analyzed as non-transformed data. All tests assumed an additive genetic model and were stratified by sex, adjusting for age and age squared. Summary statistics (β coefficients and standard errors) were meta-analyzed using the inverse variance method of METAL (see URLs).

Evidence for association of the locus near *IRS1* with subcutaneous and visceral fat, obtained by computerized tomography from 10,557 individuals,

was extracted from another GWAS meta-analysis (C.S.F., I.B.B., Y.L. & T.B.H., data not shown).

Associations with blood lipids. Associations of the loci near *IRS1* and *SPRY2* with blood levels of HDL cholesterol, triglycerides and low-density lipoprotein (LDL) cholesterol were examined in three of the stage 2 studies, including EPIC-Norfolk, Fenland and MRC Ely (**Supplementary Fig. 1**). In the EPIC-Norfolk Study, HDL cholesterol and triglycerides were measured from non-fasting blood, whereas fasting blood samples were available in the MRC Ely and Fenland studies. The concentration of LDL cholesterol was calculated using the Friedewald formula in all three studies. The associations of the SNPs with lipid levels were tested with linear regression in men and women separately, assuming an additive genetic model and adjusting for age, age squared and the use of lipid lowering medication. An inverse normal transformation for lipid levels was performed in men and women separately before the analyses. Summary statistics (β coefficients and standard errors) were pooled using the inverse variance meta-analysis method of METAL (see URLs).

Associations with insulin sensitivity traits. The associations of the locus near *IRS1* with insulin sensitivity traits (M/I ratio, with glucose infused (M) derived by the circulating insulin concentration (I); insulin area under the curve (AUC) to glucose AUC ratio; Matsuda insulin sensitivity index¹²; and Gutt insulin sensitivity index¹³) were examined in five studies (Metabolic Syndrome in Men (METSIM), MRC Ely, Relationship between insulin sensitivity and cardiovascular disease risk (RISC), Uppsala Longitudinal Study of Men (ULSAM) and Whitehall II), of which three (RISC, ULSAM and Whitehall II) were not part of our stage 2 meta-analysis of body fat percentage (**Supplementary Fig. 1**). The RISC and ULSAM studies had data from both euglycemic-hyperinsulinemic clamps and oral glucose tolerance tests (OGTT), whereas the METSIM, MRC Ely and Whitehall II studies had OGTT data only. The M/I ratios were thus only available from the RISC and ULSAM studies, whereas the three other insulin sensitivity traits were available from all five cohorts. The samples and assays used for the measurement of circulating levels of glucose and insulin are shown in the **Supplementary Note**. The insulin AUC to glucose AUC ratio and Matsuda and Gutt insulin sensitivity indexes were calculated using data from all available measurement time points. As the Whitehall II cohort had only two measurement time points available, the results from Whitehall II were not included in our meta-analysis of the association between the locus near *IRS1* and the insulin AUC to glucose AUC ratio.

Individuals were excluded from the analyses on insulin sensitivity traits if they had self-reported or physician-diagnosed diabetes or were using oral antidiabetic drugs or insulin. The associations of the SNPs near *IRS1* with insulin sensitivity traits were tested with linear regression in men and women separately, assuming an additive genetic model and adjusting for age and age squared. All insulin sensitivity traits were inverse normally transformed in men and women separately before the analyses. Summary statistics (β coefficients and standard errors) were pooled using the inverse variance meta-analysis method of METAL (see URLs).

Associations with leptin and adiponectin levels. The associations of the locus near *IRS1* with circulating levels of adiponectin were examined in three studies participating in our stage 2 meta-analysis (METSIM, MRC Ely and Osteoporotic Fractures in Men (MrOS) Sweden) and in the RISC study (**Supplementary Note**). The samples and assays used for the measurement of leptin and adiponectin are listed in the **Supplementary Note**. The associations were tested using linear regression in men and women separately, assuming an additive genetic model and adjusting for age and age squared. Analyses of leptin levels in the MrOS Study were additionally adjusted for study center. Leptin and adiponectin levels were inverse normal transformed in men and women separately before the analyses. Summary statistics (β coefficients and standard errors) were pooled with the inverse variance meta-analysis method of METAL (see URLs).

Expression quantitative trait loci analyses. The gene expression analyses for the loci near *IRS1* and *SPRY2* in subcutaneous adipose tissue and in whole blood from Icelandic individuals were carried out as described in detail previously (GEO database: GSE7965 and GPL3991)^{6,17}. In brief, 603 individuals with adipose tissue samples and 745 blood samples were genotyped with the Illumina 317K or 370K chip. The RNA samples were hybridized to a single custom made human array containing 23,720 unique oligonucleotide probes. Association was tested between the SNPs and the mean logarithm (\log_{10}) expression ratio (MLR) adjusting for age, sex and age \times sex, as well as for

differential cell count in the blood analyses, assuming an additive genetic model and accounting for familial relatedness.

The gene expression analyses for the loci near *IRS1* and *SPRY2* in liver, subcutaneous and omental fat tissue from American subjects who underwent bariatric surgery have been described in detail previously¹⁸. In brief, liver ($n = 567$), subcutaneous ($n = 610$) and omental ($n = 742$) fat tissue were obtained. RNA was extracted and hybridized to a custom Agilent 44,000-feature microarray composed of 39,280 oligonucleotide probes targeting transcripts representing 34,266 known and predicted genes. All subjects were genotyped on the Illumina 650Y SNP genotyping arrays. We tested *cis* associations between each SNP and the adjusted gene expression data using linear regression adjusting for age, race, gender and surgery year.

The expression quantitative trait loci analyses in cortical tissue have also been described in detail previously (GEO database: GSE8919)¹⁹. In brief, DNA and RNA of neuropathologically normal cortical brain samples of 193 individuals (mean age 81 years and range 65–100 years) of European descent were isolated. DNA was genotyped using the Affymetrix Gene-Chip Human Mapping 500K Array Set, and genotypes were imputed using the data from the Phase II HapMap CEU population. RNA expression was assessed with the Illumina Human RefSeq-8 Expression BeadChip system. *Cis* association analyses assumed an additive model and were adjusted for sex and age at death.

Analyses of *Irs1* expression in mouse adipocytes. Mice were housed in a temperature-controlled environment in groups of two to five at 22–24 °C using a 12 h light, 12 h dark cycle. Some cohorts were singly housed to measure food intake. The mice were fed standard chow (#2916, Harlan-Teklad) and water was provided *ad libitum*. Care of all animals and procedures were approved by the University of Texas Southwestern Medical Center.

The fractionation of stromal-vascular cells and adipocytes was performed as described previously⁵¹ with slight modifications. Briefly, stromal-vascular cells were isolated from pooled white adipose depots (inguinal, perigonadal, retroperitoneal and interscapular (the white adipose tissue juxtaposed to the interscapular brown adipose tissue)) that were explanted and minced into fine pieces (2–5 mm²). The adipose pieces were then digested in adipocyte isolation buffer (100 mM HEPES pH 7.4, 120 mM NaCl, 50 mM KCl, 5 mM glucose, 1 mM CaCl₂, 1.5% BSA) containing 1 mg/ml collagenase at 37 °C with constant slow shaking (~120 rpm) for 2 h. During the digestion period, the suspension was triturated several times through a pipet to dissociate the clumps. The suspension was then passed through an 80 mm mesh to remove undigested clumps and debris. The effluent was centrifuged at 500 g for 10 min, and the pellet was washed once in 5 ml PBS. To isolate floating adipocytes, the collagenase-treated mixture was passed through a 210 mm mesh, centrifuged at 500 g for 10 min, and floating adipocytes were collected from the top. Adipocyte gene expression was analyzed by quantitative PCR.

Analyses of *IRS1* expression in human adipose tissue. We analyzed 108 (from 29 men and 79 women) subcutaneous and 105 visceral (from 29 men and 76 women) adipose tissue samples from participants with a BMI between 20 kg/m² and 68 kg/m² who were recruited at the Endocrinology Service of the Hospital Universitari Dr. Josep Trueta (Girona, Spain). All subjects were of European origin and reported that their body weight had been stable for at least three months before the study. Individuals with liver and renal diseases were excluded. All subjects gave written informed consent. Adipose tissue samples were obtained from subcutaneous and visceral fat depots during elective surgical procedures (cholecystectomy, surgery of abdominal hernia and gastric bypass surgery). All samples were washed, fragmented and immediately flash frozen in liquid nitrogen before storing at –80 °C.

RNA was prepared from the adipose tissue samples using RNeasy Lipid Tissue Mini Kit (QIAGEN). The integrity of each RNA sample was checked by Agilent Bioanalyzer (Agilent Technologies). Total RNA was quantified by a spectrophotometer (GeneQuant, GE Health Care) and reverse transcribed to complementary DNA (cDNA) using a High Capacity cDNA Archive Kit (Applied Biosystems Inc.) according to the manufacturer's instructions.

Gene expression was assessed by real time PCR using an ABI Prism 7000 Sequence Detection System (Applied Biosystems Inc.) and TaqMan. The following commercially available and pre-validated TaqMan primer and probe sets were used: endogenous control PPIA (4333763, cyclophilin A, Applied Biosystems Inc.) and target gene Insulin Receptor Substrate 1 (*IRS1*, Hs00178563_m1, Applied Biosystems Inc.). The RT-PCR TaqMan reaction was

performed in a final volume of 25 μ l. The cycle program consisted of an initial denaturing of 10 min at 95 $^{\circ}$ C, 40 cycles of 15 s denaturing phase at 95 $^{\circ}$ C, and 1 min annealing and extension phase at 60 $^{\circ}$ C. A threshold cycle (Ct value) was obtained for each amplification curve, and a Δ Ct value was first calculated by subtracting the Ct value for human cyclophilin A (*PPIA*) RNA from the Ct value for each sample. Fold changes relative to the endogenous control were then determined by calculating $2^{-\Delta\text{Ct}}$. The gene expression results are thus expressed as an expression ratio relative to *PPIA* gene expression according to the manufacturers' guidelines.

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