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A polygenic risk score of lipolysis-increasing alleles determines visceral fat mass and proinsulin conversion

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Context: Primary dysregulation of adipose tissue lipolysis due to genetic variation and independent of insulin resistance could explain unhealthy body fat distribution and its metabolic consequences.
Objective: To analyze common single nucleotide polymorphisms (SNPs) in 48 lipolysis-, but not insulin-signaling-related genes, to form polygenic risk scores of lipolysis-associated SNPs and to investigate their effects on body fat distribution, glycemia, insulin sensitivity, insulin secretion and proinsulin conversion.
Study Design, Participants and Methods: SNP array, anthropometric and metabolic data were available from up to 2789 non-diabetic participants of the Tübingen Family (TÜF) study of type-2 diabetes characterized by oral glucose tolerance tests (OGTTs). In a subgroup (N=942), magnetic resonance measurements of body fat stores were available.
Results: We identified insulin-sensitivity-independent nominal associations ($p<0.05$) of SNPs in ten genes with plasma FFAs, in seven genes with plasma glycerol and in six genes with both, plasma FFAs and glycerol. A score formed of the latter SNPs (in *ADCY4*, *CIDEA*, *GNAS*, *PDE8B*, *PRKAA1*, *PRKAG2*) was associated with plasma FFA and glycerol measurements ($1.4*10^{-9} \leq p \leq 1.2*10^{-5}$), visceral adipose tissue mass ($p=0.0326$) and proinsulin conversion ($p \leq 0.0272$). The more lipolysis-increasing alleles a subject had, the lower was his visceral fat mass and the lower his proinsulin conversion.
Conclusions: We found evidence for a genetic basis of adipose tissue lipolysis due to common SNPs in *CIDEA*, AMP-activated protein kinase subunits and cAMP signaling

components. A genetic score of lipolysis-increasing alleles determined lower visceral fat mass and lower proinsulin conversion.

A score of seven common lipolysis-increasing alleles in ADCY4, CIDEA, GNAS, PDE8B, PRKAA1 and PRKAG2 associates with lower visceral fat mass and lower proinsulin conversion.

Introduction

Chronically elevated plasma free fatty acids (FFAs) exert multiple detrimental effects of metabolic relevance summarized under the term lipotoxicity: FFAs provoke tissue inflammation and insulin resistance [1] as well as pancreatic β-cell dysfunction [2]; furthermore, FFAs induce vascular inflammation, endothelial dysfunction and promote atherosclerotic events [3]. Therefore, high FFA concentrations are thought to contribute to the metabolic syndrome and type-2 diabetes.

A major reason of elevated plasma FFA concentrations is, apart from chronically high fat intake, increased adipose tissue lipolysis [4]. Adipose tissue lipolysis is determined, e.g., by the type of adipocyte with visceral adipocytes having higher lipolytic activity than subcutaneous adipocytes [5], by the adipocyte diameter with large adipocytes having higher lipolytic activity than small adipocytes [6], and by circulating hormones. Insulin and catecholamines are the most important hormonal regulators of lipolysis: catecholamines stimulate, whereas insulin suppresses adipose tissue lipolysis. Since insulin resistance is very closely associated with elevated lipolytic rates and vice versa [7,8], data on primary causes of increased lipolysis independent of insulin resistance are hitherto not well described.

In the last decade, the era of genome-wide association studies (GWAS) has provided valuable insights into the genetic architecture of many diseases (NHGRI-EBI GWAS Catalog [https://www.ebi.ac.uk/gwas/]). With respect to type-2 diabetes, GWAS did not only reveal common genetic variants in more than 100 genes associated with the disease but also collected first evidence for their involvement in disease-related pathophysiological events, such as disproportionate body fat distribution, insulin resistance and insulin secretion failure [9]. Due to a lack of larger studies with *in-vivo*-measures of lipolysis, however, GWAS addressing primary defects in lipolysis, i.e., insulin-resistance-independent dysregulation of lipolysis, were not performed yet.

Having now available in the Tübingen Family (TÜF) study for type-2 diabetes genome-wide genotyping data, FFA and glycerol measurements as well as estimates of insulin sensitivity derived from a 5-point oral glucose tolerance test (OGTT), we asked whether an insulin-sensitivity-independent genetic basis of adipose tissue lipolysis exists. To this end, we analyzed 316 common single nucleotide polymorphisms (SNPs) in 48 lipolysis-, but not insulin-signaling-related genes for insulin-sensitivity-independent association with plasma FFA and glycerol concentrations, formed polygenic risk scores of FFA- and/or glycerol-associated SNPs and investigated their effects on body fat distribution, glycemia, insulin sensitivity, insulin secretion and proinsulin conversion.

Materials and Methods

Ethics statement.

The study adhered to the ethical guidelines laid down in the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Eberhard Karls University Tübingen. All study participants gave their informed written consent to the study.

Subjects.

An overall study population of 2789 genotyped non-diabetic German subjects with anthropometric and metabolic phenotype data was recruited from the ongoing TÜF study [10]. TÜF currently comprises more than 3,500 non-related individuals at increased risk for

type-2 diabetes characterized by a family history of type-2 diabetes, a BMI ≥27 kg/m^2, impaired fasting glycemia, and/or previous gestational diabetes. TÜF participants undergo assessment of medical history, smoking status and alcohol consumption habits, physical examination, routine blood tests and OGTTs. In the genotyped study population, anthropometric data (gender, age, BMI) and glucose measurements were available from all 2789 participants, magnetic-resonance-imaging-derived visceral and total adipose tissue (VAT, TAT) and -spectroscopy-derived intrahepatic lipid data, insulin sensitivity and insulin secretion measurements, proinsulin conversion, plasma FFA and plasma glycerol data were available from differently sized subgroups (Table 1). The study population did not include participants on any medication known to influence glucose tolerance, insulin sensitivity or insulin secretion.

OGTT.
A standardized 75-g OGTT with venous blood sampling at time-points 0, 30, 60, 90 and 120 min was performed following a 10-h overnight fast as described earlier [10]. Blood was sampled to determine blood glucose, plasma concentrations of FFAs and glycerol and serum concentrations of insulin, proinsulin and C-peptide.

Quantification of body fat and body fat compartments.
Body mass index (BMI; in kg/m²) was calculated as weight divided by squared height. Body fat content (in %) was determined by bioelectrical impedance (BIA-101, RJL systems, Detroit, MI, USA). To exactly quantify TAT and VAT contents (in % body weight, both), whole-body magnetic resonance imaging was performed on a 1.5-T whole-body imager (Magnetom Sonata, Siemens Healthineers, Erlangen, Germany) as described [11]. Localized stimulated echo acquisition mode ^{1}H-magnetic resonance spectroscopy was used to determine the content of intrahepatic lipids as previously described [12].

Clinical chemistry.
Plasma glucose concentrations (in mmol/L) were measured with a bedside glucose analyzer (glucose oxidase method, Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma FFA and glycerol concentrations (in µmol/L, both) were quantified using enzymatic assays from WAKO Chemicals (Neuss, Germany) and Sigma-Aldrich (Munich, Germany), respectively. Serum insulin, proinsulin and C-peptide concentrations (in pmol/L, all) were determined by commercial chemiluminescence assays for ADVIA Centaur (Siemens Medical Solutions, Fernwald, Germany).

Calculations.
Homeostasis model assessment of insulin resistance (HOMA-IR; in 10^{-6} mol*U*L^{-2}) was calculated as $c(glucose_0)*c(insulin_0)/22.5$ with c=concentration and insulin concentrations converted from pmol/L to µU/mL [13]. The OGTT-derived insulin sensitivity index (ISI; in 10^{19} L^2*mol^{-2}) was estimated as proposed by Matsuda and DeFronzo [14]: $10{,}000/[c(glucose_0)*c(insulin_0)*c(glucose_{mean})*c(insulin_{mean})]^{½}$. Insulin secretion was estimated from the OGTT using two recently reported indices [15]: area under the curve (AUC) of insulin from 0 to 30 min divided by AUC of glucose from 0 to 30 min ($insulin_{AUC0\text{-}30}/glucose_{AUC0\text{-}30}$) and AUC of C-peptide divided by AUC of glucose both in the same time interval ($C\text{-}peptide_{AUC0\text{-}30}/glucose_{AUC0\text{-}30}$; in 10^{-9}, both indices). Both indices were calculated as $[c(insulin_0 \text{ or } C\text{-}peptide_0)+c(insulin_{30} \text{ or } C\text{-}peptide_{30})]/[c(glucose_0)+c(glucose_{30})]$. AUCs from 0 to 120 min of analytes with increasing concentrations during the OGTT (glucose in mmol/L*h, insulin and proinsulin in pmol/L*h) were calculated with the trapezoid method: $0.5*(0.5*c_0+c_{30}+c_{60}+c_{90}+0.5*c_{120})$. For the decline of FFA and glycerol concentrations during the entire 120 min, inverse AUCs (iAUCs; in µmol/L*h, both) were calculated according to the formula previously reported [16]: $0.5*[0.5*(|c_0-c_{30}|)]+0.5*[0.5*(|c_{30}-c_{60}|)+c_0-c_{30}]+0.5*[0.5*(|c_{60}-c_{90}|)+c_0-c_{60}]+0.5*[0.5*(|c_{90}-c_{120}|)+c_0-c_{90}]$. Proinsulin conversion

(dimensionless) was estimated at 0 min, 30 min and during the entire 120 min as $c(proinsulin_X)/c(insulin_X)$ with X=0 min, 30 min or AUC from 0 to 120 min.

Selection of lipolysis-related genes and of common SNPs therein.
Based on (i) information provided by articles that were identified via a stringent PubMed [https://www.ncbi.nlm.nih.gov/pubmed] search using the combination of search terms "regulation", "adipocyte" and "lipolysis[Title]" and (ii) subsequent validation of the identified candidate genes by interrogation of OMIM [https://www.omim.org/] and UniProt [https://www.uniprot.org/], we established a list of 58 genes with strong evidence for involvement in the regulation of lipolysis and at least moderate expression in adipose tissue according to GTEx RNA-Seq data (≥15 transcripts per kilobase million in subcutaneous and/or visceral adipose tissue) [https://www.gtexportal.org/]. The list comprised genes encoding lipases and lipase cofactors, lipid vesicle components (perilipins, cell death-inducing DFFA-like effectors), fatty-acid-binding proteins, cAMP signaling components (adrenergic receptors, calcitonin receptor-like receptor and RAMP coreceptors, neuropeptide Y receptor Y1, prostaglandin E receptors, heterotrimeric G-proteins, adenylate cyclases, subunits of protein kinase A (PKA), cAMP phosphodiesterases), cGMP signaling components (NO synthases, atrial natriuretic peptide receptors, guanylate cyclases, cGMP phosphodiesterases), subunits of AMP-activated kinase (AMPK), tumor necrosis factor receptors and mitogen-activated protein kinases (Supplemental Table 1 in [17]). As it was our intention to identify primary genetic defects in lipolysis independent of insulin sensitivity, genes constituting the proximal insulin signaling pathway, such as the insulin receptor, insulin receptor substrates, phosphatidylinositol 3-kinase, protein kinase B, were *a priori* excluded. In the genomic loci (gene regions plus 2 kb 5'-flanking regions) of these 58 genes, we looked for common [minor allele frequency (MAF) ≥0.05], bi-allelic and non-linked (r^2<0.8) SNPs with genotyping success rates ≥75 % and genotypes in Hardy-Weinberg equilibrium (p≥0.05) available from our recently collected genome-wide genotyping data using the 700-K Infinium Global Screening Array from Illumina (SanDiego, CA, USA) [18]. Based on these criteria, we selected 311 SNPs in 48 genes. In addition, information provided by the GTEx portal revealed the existence of 373 so-called *cis*-eSNPs, i.e., SNPs acting in *cis* on the expression of the selected lipolysis-related genes in subcutaneous and/or omental adipose tissue. Because many of these SNPs were in high linkage disequilibrium (r^2≥0.8), only twenty-five SNPs were depicted on the Global Screening Array and subjected to the above mentioned selection and quality criteria. Five *cis*-eSNPs neither identical nor linked to any of the 311 SNPs selected before survived this procedure and were included in the association analysis. Thus, a total of 316 SNPs was finally selected (Supplemental Table 2 in [17]).

Statistical analysis and generation of polygenic scores.
Hardy-Weinberg equilibrium of genotype distribution was tested by χ^2-test with one degree of freedom. Prior to association analysis, all continuous variables were inverse-normal transformed to ensure normal distribution of data. Linear regression analysis was performed with the standard least squares method choosing the trait of interest (FFA concentration, glycerol concentration, other metabolic traits) as outcome variable, the SNP genotype or the genetic score (in the additive inheritance model, both) as independent variable and gender, age, BMI and ISI (or insulin concentration) as confounding variables as indicated. When testing 316 SNPs in parallel, a Bonferroni-corrected p-value <0.000163 was considered statistically significant. Unweighted polygenic scores were generated by summing up all nominally (p<0.05) lipolysis-increasing alleles. In the analysis testing associations of the polygenic scores, the significance threshold was set at p<0.05. We did not correct for the metabolic traits tested in parallel since these were not independent. In the multiple linear

regression models, the study was sufficiently powered (1-β≥0.8, two-sided p<0.05) to detect SNP effects on fasting FFA concentrations ≥2.5 % in the overall population (N=2725) and on fasting glycerol concentrations ≥6.5 % in the subgroup with glycerol measurements (N=768) taking into account the chosen MAF threshold ≥0.05. For all analysis, the statistical software JMP 13.0.0 (SAS Institute, Cary, NC, USA) was used.

Results

The study population's phenotypes assessed in this study and their sample sizes are presented in Table 1.

Single SNP analysis.
To find insulin-sensitivity-independent associations of SNPs with lipolysis, we analyzed 316 Global-Screening-Array-derived SNPs (including five *cis*-eSNPs) in 48 hypothesis-driven candidate genes for association with fasting FFAs, FFAs at time-point 120 min of the OGTT, iAUC of FFAs during the entire OGTT, fasting glycerol, glycerol at time-point 120 min and iAUC of glycerol during the OGTT. Gender, age, BMI and ISI (or, instead of ISI, the insulin concentration at the respective time-point/-interval) were included in the multiple linear regression models as confounding variables. Applying Bonferroni correction for multiple testing, we identified 62 SNPs with nominal associations (0.000163≤p<0.05), but no SNP with significant association (p<0.000163; Table 2 and Supplemental Table 2 in [17]). Twenty-eight SNPs in ten genes (*GNAI1*, *GNAI3*, *GUCY1B3*, *MAPK6*, *MGLL*, *NOS3*, *PDE5A*, *PRKAR2B*, *PTGER3*, *PTGER4*) were nominally associated with plasma FFAs, 27 SNPs in seven genes (*ADCY3*, *ADCY5*, *PDE3B*, *PDE8A*, *PLIN4*, *PRKAR1A*, *PRKAR2A*) with plasma glycerol, and seven SNPs in six genes (*ADCY4*, *CIDEA*, *GNAS*, *PDE8B*, *PRKAA1*, *PRKAG2*) with both, plasma FFAs and glycerol (Table 2, Figures 1a and b). Screening the GWAS collection of the Type-2 Diabetes Knowledge Portal of the Accelerating Medicines Partnership[http://www.type2diabetesgenetics.org/], we found suggestive genome-wide evidence ($p<10^{-6}$) for association with anthropometric/metabolic traits for six of these SNPs: five SNPs (rs1541984, rs11676272, rs12891732, rs8014112, rs6026584) were associated with body height, two with BMI and childhood obesity (rs1541984, rs11676272), one with hip and waist circumference (rs11676272) and one with total cholesterol and low-density-lipoprotein cholesterol (rs518076; Table 2).

Polygenic scores and their associations with FFAs and glycerol.
Based on the single SNP analysis, we generated four different polygenic scores by summing up the nominally lipolysis-increasing alleles. The FFA-Only Score contained all alleles exclusively associated with increased FFA, but not with glycerol concentrations. Analogously, the Glycerol-Only Score comprised only alleles associated with increased glycerol, but not with FFA concentrations. The FFA&Glycerol Score contained only alleles associated with both, increased FFA and increased glycerol concentrations, and the Overall Score comprised all FFA- and/or glycerol-increasing alleles. Supplemental Figures 1a-d in [17] provide the allele distributions of these scores. Refraining from correction for multiple testing, all scores were significantly associated with at least four of six FFA and glycerol measurements (FFA_0, FFA_{120}, $FFA_{iAUC0-120}$, $glycerol_0$, $glycerol_{120}$, $glycerol_{iAUC0-120}$; $9.3*10^{-11}\leq p\leq 0.0324$) (Supplemental Table 2 and Supplemental Figures 2 and 3 in [17]). The increase in fasting FFAs ranged from +3.3 µmol/L (Glycerol-Only Score) to +12.5 µmol/L (FFA&Glycerol Score) per risk allele, the increase in fasting glycerol ranged from +1.3 µmol/L (FFA-Only Score) to +5.5 µmol/L (FFA&Glycerol Score) per risk allele (Supplemental Figures 2 and 3 in [17]).

Associations of polygenic scores with body fat distribution, glycemia, insulin sensitivity, insulin secretion and proinsulin conversion.

After appropriate adjustment, neither the Overall Score nor the FFA-Only Score nor the Glycerol-Only Score were associated with body fat distribution (BMI, bioelectrical impedance-derived body fat content, TAT, VAT, intrahepatic lipids), glycemia ($glucose_0$, $glucose_{120}$, $glucose_{AUC0-120}$), insulin sensitivity (HOMA-IR, ISI), insulin secretion ($insulin_{AUC0-30}/glucose_{AUC0-30}$, $C\text{-}peptide_{AUC0-30}/glucose_{AUC0-30}$) or proinsulin conversion ($proinsulin_0/insulin_0$, $proinsulin_{30}/insulin_{30}$, $proinsulin_{AUC0-120}/insulin_{AUC0-120}$; Table 3). By contrast, the FFA&Glycerol Score was significantly associated with VAT content (p=0.0326; Figure 2) and proinsulin conversion ($proinsulin_{30}/insulin_{30}$ p=0.0272, $proinsulin_{AUC0-120}/insulin_{AUC0-120}$ p=0.0174; Table 3). The more lipolysis-increasing alleles a subject had, the lower was his visceral fat mass and the lower his proinsulin conversion. The effect size of the score on VAT content was -0.06 % body weight per risk allele (Figure 2).

Discussion

The primary aim of this study was to investigate whether common SNPs exist in humans that determine, independently of the individual's insulin sensitivity, variation in lipolysis rates and, if so, whether they affect body fat distribution, glycemia, insulin sensitivity, insulin secretion or proinsulin conversion. To avoid influences of insulin sensitivity, we excluded genes from the lipolysis-regulating gene list that are involved in proximal insulin signaling (insulin receptor, insulin receptor substrates, phosphatidylinositol 3-kinase, protein kinase B) and adjusted all association analysis for ISI or, alternatively, the insulin concentration at the respective time-point of the OGTT.

No single SNP among the 316 SNPs tested showed insulin-sensitivity-independent effects on lipolysis strong enough to become significant upon Bonferroni correction for multiple comparisons. This would point to, if at all, rather modest effect sizes of the SNPs. However, combining the 62 nominally lipolysis-associated SNPs to different polygenic scores resulted in associations with FFA and/or glycerol concentrations with individual p-values even passing the genome-wide significance level ($p<5*10^{-8}$). This finding reveals the existence of a primary impact of common genetic variation on lipolysis and demonstrates additivity of effects among at least some of these SNPs. One of the best effects sizes reached was an increase in ~12.5 µmol/L fasting FFAs per risk allele (with the FFA&Glycerol Score) meaning that subjects with 10 risk alleles (~9 % of the study population) have on average 50 µmol/L higher fasting FFAs than subjects with 6 risk alleles (~14 % of the study population; Supplemental Figure 1d in [17]). Based on the mean fasting FFA concentration of the study population, i.e., 595 µmol/L, a difference of 50 µmol/L appears clinically meaningful.

We observed that there were SNPs/genes showing exclusive association with FFA, but not with glycerol concentrations and, vice versa, SNPs/genes showing association with glycerol, but not with FFA concentrations (Table 2, Figures 1a and b). This unexpected finding is probably due to power limitations of our study, e.g., given by the different sample sizes of our FFA (N=~2700) and glycerol (N=~770) measurements and the unequal coverage of genes by SNPs (e.g., one *PRKAA1*-SNP, 77 *PRKAG2*-SNPs; Supplemental Table 1 in [17]).

In this context, the fact that we also detected SNPs/genes associated with both, FFA and glycerol concentrations (Table 2, Figures 1a and b) led us to assume that these SNPs/genes, summed up to the FFA&Glycerol Score, were the ones most robustly associated with lipolysis. These genes were *ADCY4*, *CIDEA*, *GNAS*, *PDE8B*, *PRKAA1* and *PRKAG2*. Some of them are involved in cAMP signaling: *GNAS* encodes the α-subunit of the heterotrimeric stimulatory G-protein ($G\alpha_S$) that activates adenylate cyclases; *ADCY4* encodes one of the adipocyte adenylate cyclase isoforms that, upon $G\alpha_S$ binding, synthesize cAMP [19]; and *PDE8B* encodes a high-affinity cAMP-specific phosphodiesterase isoform with rather restricted tissue expression that hydrolyses cAMP [20]. The cAMP/PKA pathway is well

known to act downstream of G-protein-coupled receptors, such as β-adrenergic receptors and prostaglandin E receptors, and to stimulate lipolysis via HSL activation. *PRKAA1* and *PRKAG2* encode two subunits of AMPK, a sensor of cellular energy load that is activated when cellular ATP levels drop and *vice versa* AMP levels rise and that acts as a central metabolic switch activating energy-providing catabolic pathways (including lipolysis [21]) while inhibiting energy-consuming anabolic pathways. Finally, *CIDEA* encodes the cell death-inducing DFFA-like effector A, a protein strongly expressed in adipose and mammary tissue that was described to bind to lipid droplets and to regulate their fusion thus favoring lipid deposition and counteracting lipolysis [22].

With respect to metabolic endpoints, we identified VAT-reducing and proinsulin-conversion-reducing effects of the FFA&Glycerol Score. Whereas reduction in VAT mass due to genetically increased lipolysis is obvious and plausible, the mechanistic underpinnings of the score's association with proinsulin conversion are currently unclear. FFAs released from adipose tissue could directly provoke pancreatic β-cell dysfunction including proinsulin conversion failure. This could for instance result from FFAs' well-known chronic effects on β-cell viability (lipotoxicity) [23]. The finding however that insulin secretion was not impaired by the lipolysis-increasing alleles (Table 3) argues against this possibility. On the other hand, the association of the FFA&Glycerol Score with proinsulin conversion could be independent of lipolysis and just reflect a specific role of genes included in this score, such as those encoding cAMP signaling components, in proinsulin conversion. In line with this suggestion, incretin-stimulated cAMP signaling in β-cells was reported to affect proinsulin processing [24]. The observation that we did not see any associations of the other three scores on metabolic traits despite their good associations with FFA and glycerol measurements could be due to the exclusion of the most robust lipolysis-regulating SNPs contained in the FFA&Glycerol Score, as in the case of the FFA-Only and the Glycerol-Only Scores, or due the dilution of these SNPs, as in the case of the Overall Score.

As major limitations of our study, we acknowledge the limited sample size of our glycerol measurements and the lack of replication. The latter is certainly due to the lack of sufficiently sized studies with FFA and glycerol measurements during a 5-point OGTT. Such studies are urgently needed to identify and verify SNP effects on lipolysis like those reported in our study here.

In conclusion, we found evidence for a genetic basis of adipose tissue lipolysis due to common SNPs in *CIDEA*, in AMPK subunits and in components of the cAMP/PKA signaling pathway and independent of insulin sensitivity. Moreover, a genetic score of lipolysis-increasing alleles determined lower visceral fat mass and lower proinsulin conversion.

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The authors have nothing to disclose

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Figure 1. Number of SNPs (a) and of SNP-containing genes (b) nominally associated with measures of lipolysis. FFA – free fatty acids; glyc – glycerol; SNP – single nucleotide polymorphism

Figure 2. Association of the FFA&Glycerol Score with visceral adipose tissue (VAT) mass. The score was formed by summing up FFA- and glycerol-increasing alleles (= risk alleles) as described in the Results section. VAT was quantified by magnetic resonance imaging as described in the Methods section. The effect size given is the absolute effect size per risk allele in % of body weight. BW – body weight; FFA – free fatty acids

Table 1. Traits assessed in the study population and sample sizes (64 % women, 36 % men)

Trait	Mean	SD	N
Age (y)	43	14	2789
BMI (kg/m²)	31.0	9.3	2789

Body fat (%)	34.4	13.0	2656
TAT (% BW)	33.7	9.6	940
VAT (% BW)	4.04	2.04	942
IHL (%)	6.66	6.68	927
$Glucose_0$ (mmol/L)	5.24	0.56	2789
$Glucose_{120}$ (mmol/L)	6.41	1.61	2789
$Glucose_{AUC0-120}$ (mmol/L*h)	14.98	3.14	2778
$Insulin_0$ (pmol/L)	90	72	2774
$Insulin_{120}$ (pmol/L)	572	576	2754
$Insulin_{AUC0-120}$ (pmol/L*h)	1225	913	2735
FFA_0 (µmol/L)	595	249	2725
FFA_{120} (µmol/L)	95	120	2672
$FFA_{iAUC0-120}$ (µmol/L*h)	704	394	2632
$Glycerol_0$ (µmol/L)	105	68	768
$Glycerol_{120}$ (µmol/L)	49	35	766
$Glycerol_{iAUC0-120}$ (µmol/L*h)	88	93	761
HOMA-IR (10^{-6} mol*U*L^{-2})	3.58	3.11	2774
ISI (10^{19} L^2*mol^{-2})	12.04	8.73	2733
$Insulin_{AUC0-30}/glucose_{AUC0-30}$ (10^{-9})	53	36	2756
$C\text{-}peptide_{AUC0-30}/glucose_{AUC0-30}$ (10^{-9})	192	74	2706
$Proinsulin_0/insulin_0$	0.074	0.100	2634
$Proinsulin_{30}/insulin_{30}$	0.021	0.023	2645
$Proinsulin_{AUC0-120}/insulin_{AUC0-120}$	0.033	0.032	2539

Subscript numbers indicate time-points of the oral glucose tolerance test with 0 = fasting state. (i)AUC – (inverse) area under the curve; BMI – body mass index; BW – body weight; FFA – free fatty acids; HOMA-IR – homeostasis model assessment of insulin resistance; IHL – intrahepatic lipids; ISI – insulin sensitivity index; N – number (sample size); SD – standard deviation; TAT – total adipose tissue; VAT – visceral adipose tissue

Table 2. SNPs nominally associated with measures of lipolysis

Gene	**Chr**	**SNP**	**FFA**	**Glyc**	**GWAS phenotypes** ($p<10^{-6}$)[1]
ADCY3	2	rs1541984		x	height, BMI, childhood obesity
ADCY3	2	rs11676272		x	height, BMI, hip, waist, childhood obesity
ADCY4	14	rs12891732	x	x	height
ADCY4	14	rs3212254	x		-
ADCY4 (*cis*-eSNP)	14	rs8014112	x		height
ADCY5	3	rs11927367		x	-
CIDEA (*cis*-eSNP)	18	rs7504200	x	x	-
GNAI1	7	rs7776571	x		-
GNAI1	7	rs916905	x		-
GNAI3	1	rs518076	x		LDL cholesterol, cholesterol
GNAS	20	rs8125112	x		-
GNAS	20	rs6026567	x		-
GNAS	20	rs6026584	x	x	height
GNAS	20	rs919197		x	-
GUCY1B3	4	rs3796575	x		-
MAPK6	15	rs4447367	x		-
MAPK6	15	rs10851507	x		-
MGLL	3	rs11705710	x		-
MGLL	3	rs664910	x		-
NOS3	7	rs7830	x		-
PDE3B	11	rs7114131		x	-
PDE3B	11	rs7109368		x	-
PDE5A	4	rs10012485	x		-
PDE5A	4	rs6534146	x		*no data available*
PDE8A	15	rs11854452		x	-
PDE8A	15	rs62019481		x	-
PDE8B	5	rs12652928		x	-
PDE8B	5	rs34802194	x		-
PDE8B	5	rs12515498		x	-
PDE8B	5	rs17683162		x	-
PDE8B	5	rs10942819	x		-
PDE8B	5	rs90684		x	*no data available*
PLIN4	19	rs1609717		x	-
PRKAA1	5	rs249429	x	x	*no data available*
PRKAG2	7	rs5017429		x	-
PRKAG2	7	rs17173197		x	-

PRKAG2	7	rs2538039		x	-
PRKAG2	7	rs2374229		x	*no data available*
PRKAG2	7	rs4726070		x	-
PRKAG2	7	rs11771330	x	x	-
PRKAG2	7	rs11773668		x	-
PRKAG2	7	rs79215320		x	-
PRKAG2	7	rs6965926	x		-
PRKAG2	7	rs73728288		x	-
PRKAG2	7	rs56037571	x		-
PRKAG2	7	rs9648724	x		-
PRKAG2	7	rs75379928	x	x	-
PRKAG2	7	rs13225852	x	x	-
PRKAG2	7	rs13233608	x		-
PRKAG2	7	rs6950343		x	-
PRKAG2	7	rs11971588		x	-
PRKAG2	7	rs12669153		x	-
PRKAG2	7	rs4725435		x	-
PRKAR1A	17	rs2286562		x	-
PRKAR2A	3	rs7647812		x	-
PRKAR2B	7	rs975935	x		*no data available*
PTGER3	1	rs1536537	x		-
PTGER3	1	rs622721	x		-
PTGER3	1	rs726764	x		-
PTGER3	1	rs2206343	x		-
PTGER3	1	rs8179390	x		-
PTGER4	5	rs4133101	x		-

BMI – body mass index; Chr – chromosome; FFA – free fatty acids; Glyc – glycerol; GWAS – genome-wide association study; LDL – low-density lipoprotein; SNP – single nucleotide polymorphism. [1]Significance level for suggestive evidence of association with anthropometric and/or metabolic traits in genome-wide association studies.

Table 3. Associations of genetic scores with body fat distribution, glycemia, insulin sensitivity, insulin secretion, and proinsulin conversion

	Overall Score			**FFA-Only Score**			**Glycerol-Only Score**			**FFA&Glycerol Score**			
Trait	stand. beta	p_{add}	N	stand. beta	p_{add}	N	stand. beta	p_{add}	N	stand. beta	p_{add}	N	**Adjustment**
BMI (kg/m²)	-0.0131	0.05	2400	-0.0049	0.8	2532	-0.0098	0.6	2604	-0.0285	0.1	2756	gender, age
Body fat (%)	-0.0131	0.4	2290	0.0078	0.6	2411	-0.0239	0.1	2484	-0.0128	0.4	2623	gender, age
TAT (% BW)	-0.0064	0.8	817	0.0046	0.9	854	-0.0066	0.8	886	-0.0077	0.8	932	gender, age
VAT (% BW)	-0.0172	0.5	819	0.0067	0.8	856	-0.0036	0.9	888	-0.0495	**0.0326**	934	gender, age
IHL (%)	-0.0364	0.3	805	-0.0247	0.5	842	-0.0111	0.7	873	-0.0571	0.067	919	gender, age
$Glucose_0$ (mmol/L)	-0.0044	0.8	2400	-8.5E-05	1.0	2532	0.0031	0.9	2604	-0.0172	0.3	2756	gender, age, BMI
$Glucose_{120}$ (mmol/L)	0.0077	0.7	2400	0.0136	0.5	2532	-0.0126	0.5	2604	0.0085	0.6	2756	gender, age, BMI
$Glucose_{AUC0-120}$ (mmol/L*h)	0.0014	0.9	2391	-0.0009	1.0	2522	0.0038	0.8	2594	-0.0025	0.9	2745	gender, age, BMI
HOMA-IR (10^{-6} mol*U*L^{-2})	-0.0024	0.9	2385	0.0026	0.9	2517	0.0069	0.6	2589	-0.0200	0.2	2741	gender, age, BMI
ISI (10^{19} L^2*mol^{-2})	0.0131	0.4	2351	-0.0009	1.0	2482	0.0043	0.8	2551	0.0235	0.098	2702	gender, age, BMI
$Insulin_{AUC0-30}$/$glucose_{AUC0-30}$ (10^{-9})	-0.0020	0.9	2351	0.0079	0.6	2482	-0.0062	0.6	2551	-0.0035	0.8	2700	gender, age, BMI, ISI
C-$peptide_{AUC0-30}$/$glucose_{AUC0-30}$ (10^{-9})	0.0035	0.8	2297	0.0131	0.4	2424	-0.0055	0.7	2493	-0.0121	0.4	2639	gender, age, BMI, ISI
$Proinsulin_0$/$insulin_0$	0.0228	0.3	2259	0.0140	0.5	2386	0.0031	0.9	2456	0.0206	0.3	2604	gender, age, BMI
$Proinsulin_{30}$/$insulin_{30}$	0.0314	0.1	2272	0.0129	0.5	2398	0.0078	0.7	2468	0.0418	**0.0272**	2614	gender, age, BMI
$Proinsulin_{AUC0-120}$/$insulin_{AUC0-120}$	0.0325	0.1	2180	0.0159	0.4	2305	0.0025	0.9	2367	0.0451	**0.0174**	2510	gender, age, BMI

The effect sizes given are the standardized beta coefficients (stand. beta) derived from the multiple linear regression models. BMI – body mass index; BW – body weight; FFA – free fatty acids; HOMA-IR – homeostasis model assessment of insulin resistance; IHL – intrahepatic lipids; ISI – insulin sensitivity index; p_{add} – p-value of the additive inheritance model; N – number (sample size); TAT – total adipose tissue; VAT – visceral adipose tissue

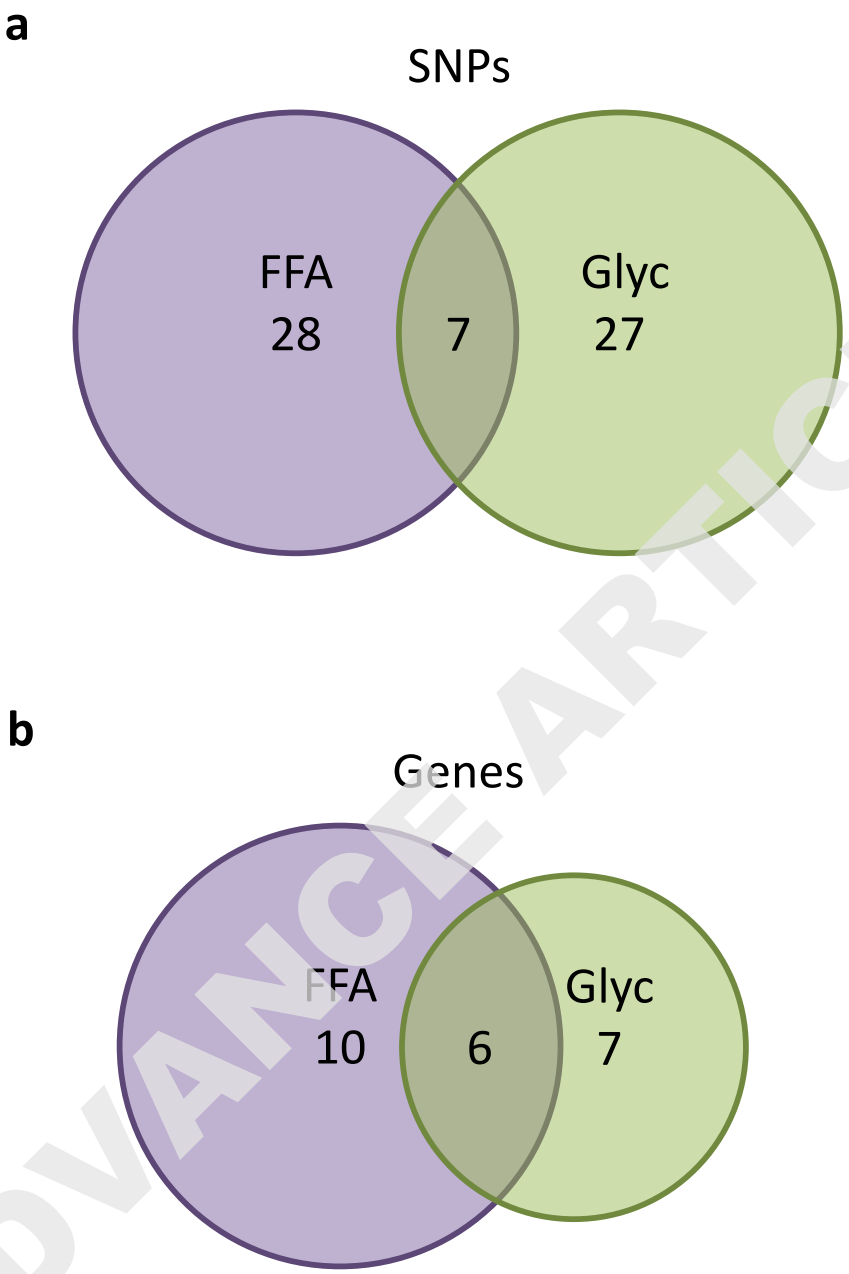

Figure 1

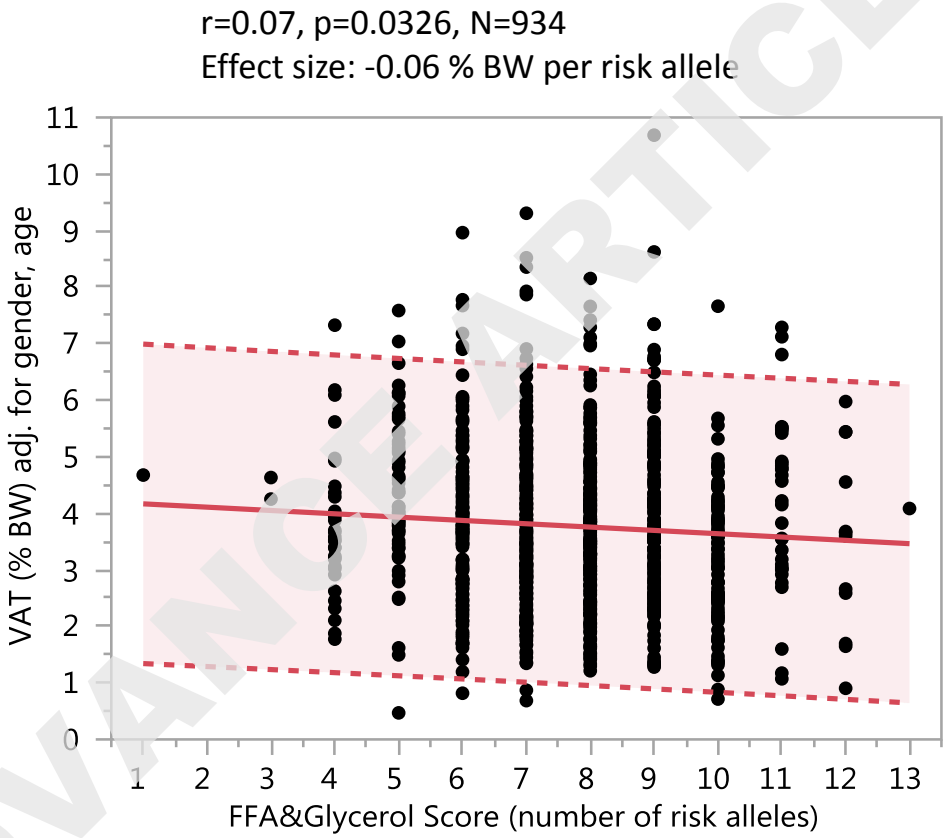

Figure 2