Pancreatic fat cells of humans with type 2 diabetes display reduced adipogenic and lipolytic activity

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Abbreviations: A, adipocytes; ANP, atrial natriuretic peptide; AT, adipose tissue; DEG, differentially expressed genes; FCS, fetal calf serum; FFA, free fatty acids; GO, gene ontology; HGF, hepatic growth factor; IL-6, interleukin 6; IQR, interquartile range; KRH, Krebs-Ringer-HEPES buffer; MCP-1, monocyte chemoattractant protein 1; MUFA, monounsaturated fatty acid; NGT, normal glucose tolerance; P, preadipocytes; PC, principal component; PD, prediabetic, comprising “impaired glucose tolerant and/or impaired fasting blood glucose”; PPP, partial pancreatectomized patients; SC, subcutaneous; T2D, type 2 diabetes; TG, triglyceride; VEGF, vascular endothelial growth factor.

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Abstract

Obesity, especially visceral fat accumulation, increases the risk of type 2 diabetes (T2D). The purpose of this study was to investigate the impact of T2D on the pancreatic fat depot. Pancreatic fat pads from 17 partial pancreatectomized patients (PPP) were collected, pancreatic preadipocytes isolated and *in vitro* differentiated. Patients were grouped using HbA1c into normal glucose tolerant (NGT), prediabetic (PD) and T2D. Transcriptome profiles of preadipocytes and adipocytes were assessed by RNAseq. Insulin sensitivity was estimated by quantifying AKT phosphorylation on western blots. Lipogenic capacity was assessed with oil red O staining, lipolytic activity via fatty acid release. Secreted factors were measured using ELISA. Comparative transcriptome analysis of preadipocytes and adipocytes indicates defective upregulation of genes governing adipogenesis (*NR1H3*), lipogenesis (*FASN*, *SCD*, *ELOVL6*, *FADS1*) and lipolysis (*LIPE*) during differentiation of cells from T2D-PPP. In addition, the ratio of leptin/adiponectin mRNA was higher in T2D than in NGT-PPP. Preadipocytes and adipocytes of NGT-PPP were more insulin sensitive than T2D-PPP cells in regard to AKT phosphorylation. Triglyceride accumulation was similar in NGT and T2D adipocytes. Despite a high expression of the receptors *NPR1* and *NPR2* in NGT and T2D adipocytes, lipolysis was stimulated by ANP 1.74-fold in NGT cells only. This stimulation was further increased by the PDE5 inhibitor dipyridamole (3.09-fold). Dipyridamole and forskolin increased lipolysis receptor-independently 1.88-fold and 1.48-fold, respectively, solely in NGT cells. In conclusion, the metabolic status persistently affects differentiation and lipolysis of pancreatic adipocytes. These alterations could aggravate the development of T2D.

INTRODUCTION

Obesity is a major risk factor for the development of type 2 diabetes (T2D) (1). While the role of liver steatosis and non-alcoholic fatty liver disease (NAFLD) in the development of insulin resistance, metabolic syndrome and T2D is well accepted, the role of excessive lipid accumulation in the pancreas is controversially discussed (2, 3). Using non-invasive imaging methods for the quantification of tissue fat content clinical association studies do not give a uniform picture (2, 4-8). A previous clinical study of our group suggests that increased pancreatic fat is associated with impaired insulin secretion in patients with prediabetes (PD, comprising “impaired glucose tolerant and/or impaired fasting blood glucose”) but not in humans with normal glucose tolerance (NGT) (4). In a following study, a negative association of pancreatic fat content with insulin secretion was also found in nondiabetic humans with a high genetic risk score for insulin resistance and diabetes (9). These observations suggest that pancreatic fat may take part in the progressive deterioration of islet function in a subtype of humans only.

A more detailed, histological analysis of the human pancreatic tissue revealed a substantial infiltration of adipocytes which contributes to the higher percentage of pancreatic fat content (7). Adipocytes store fuel in a large intracellular lipid droplet, but they are particularly secretory cells which release cytokines, chemokines and adipokines in addition to metabolites e.g. fatty acids (10-12). Consequently, secreted factors of infiltrating adipocytes may exert paracrine effects on neighboring cells. Previously, we described that pancreatic preadipocytes and adipocytes produce and secrete chemokines and cytokines when challenged with palmitate and fetuin-A, a hepatokine increasingly secreted by fatty liver (7). These results could at least partially explain the divergent outcome of human association studies and suggest

that stimulation of local adipocytes by factors released from fatty liver alter the adipocyte secretome and consequently the paracrine impact of local fat cells on islet function.

To unravel the specific pancreatic fat cell functions, we characterized preadipocytes and *in vitro* differentiated adipocytes obtained from resections of partial pancreatectomized patients (PPP). This *in vitro* approach by using standardized protocols enables a comparative analysis of purified cell populations and their differentiation capacity. The highly variable genetic and environmental background among the patients, together with the variable anatomical location and the heterogeneous composition of the resected tissue, including fibrosis and inflammation, disables a comparative transcriptome analysis. Therefore, preadipocyte populations were prepared from fat pads using differential centrifugation. The absence of immune cells (CD68 positive) and endothelial cells (CD31 positive) was confirmed by fluorescence-activated cell sorting (FACS). The preadipocyte cell population retains proliferative capacity which permits the expansion of the cells (13). This expansion increases the material of a defined cell population obtained from small surgical leftovers. The assessment of the metabolic status of the tissue donors allows a correlation of preadipocyte and adipocyte characteristics with metabolic/diabetic traits.

Here, we present a first analysis of human pancreatic fat cells which suggests that a diabetic environment persistently alters the functional characteristics of adipocytes.

MATERIALS AND METHODS

Ethical approval

The study protocols were approved by the Ethics Commission of the Medical Faculty and the University Hospital of the University of Tübingen in accordance with the declaration of Helsinki for pancreatic (no. 697/2011BO1 and 563/2019BO2) and subcutaneous (no. 446/2016BO2) tissue sampling. Written informed consent was given from all patients.

Patient recruitment and human pancreatic resections

Peripancreatic fat pads were obtained from 17 PPP (15 males, 2 females, age 48-82 years, BMI 22-40 kg/m²). Subject characteristics are given in Table 1 and Supplementary Table 1 (14). PPP underwent surgery in consequence of pancreatic adenocarcinoma (n=9), neuroendocrine tumor (n=2), intraductal papillary mucinous neoplasia (n=2), duodenal carcinoma (n=1), chronic pancreatitis (n=1) or other (n=2). The patients were defined as normal glucose tolerant (NGT) with HbA1c<5.7%, prediabetic (PD) with HbA1c between 5.7% and 6.4% and diabetic (T2D) with HbA1c≥6.5%. The three groups did not differ in regard to sex, age and BMI. T2D-PPP were treated with insulin (n=2), metformin (n=3), SGLT inhibitor (n=1) or DPP4 inhibitors (n=2). Subcutaneous (SC) fat pads were obtained from 6 morbidly obese, non-diabetic patients (3 males, 3 females, age 27-67 years, BMI>40 kg/m²; Supplementary Table 1 and 2 (14)) undergoing gastric sleeve surgery. These patients did not present other severe diseases apart from morbid obesity. They were not treated with medication which may interfere with glucose tolerance.

Measurement of insulin secretion and sensitivity

Blood samples were collected from patients prior to surgery. Insulin secretion and sensitivity were calculated using the HOMA2 method (15). Insulin secretion (HOMA2%B) was computed from fasting glucose and C-peptide levels. Insulin sensitivity (HOMA2%S) was assessed using fasting glucose and specific insulin levels. HOMA2 disposition index, a measure of beta-cell function adjusted for insulin sensitivity, was calculated using the following formula: HOMA2%B × HOMA%S/10000. No correlation was found between the parameters of insulin secretion and sex, age, BMI and VAT (data not shown).

Abdominal fat quantification using CT

Patients (n=12) underwent an abdominal computed tomography (CT) examination prior to surgery. Quantification of total, visceral and subcutaneous fat area was performed from a single slice at the umbilical level, which has been shown to be a reliable representative slice for total abdominal fat volumes (16). The fat areas were manually segmented by measuring pixels with densities between -30 and -190 Hounsfield units to delineate total, visceral, and subcutaneous compartments and to compute the cross-sectional area of each in cm^2.

Preadipocyte isolation and in vitro differentiation

Pancreatic and subcutaneous preadipocytes were isolated from fat biopsies as previously described (17, 18). Isolated preadipocytes were expanded in DMEM/Ham's mixture F12 (1:1) containing: 2 mmol/L glutamine, 20% (v/v) fetal calf serum (FCS), 1% (v/v) chicken embryo extract (Sera Laboratories International, Horsted Keynes, UK), 1% (v/v) antibiotics, and 0.5 mg/ml amphotericin B. Adipocyte differentiation was achieved after 14 days cultivation in DMEM/Ham's mixture F12

(1:1) containing (in mmol/L): 2 glutamine, 17 pantothenate, 1 biotin, 10 troglitazone, 0.001 insulin; (in mg/ml): 2 apotransferin, 0.5 amphotericin; 5% (v/v) FCS and 1% (v/v) antibiotics. During the first 7 days of differentiation, medium was supplemented with (in mmol/L): 0.5 3-isobutyl-1-methyl-xanthine (IBMX), 0.001 cortisol and 0.05 indomethacin. During the second week of differentiation adipocytes were cultured in DMEM/Ham's mixture F12 without supplements. Adipocytes differentiated in medium supplemented with monounsaturated fatty acids (MUFA) received a fatty acid mixture containing linoleic acid and oleic acid (1:1, both at 0.1 mmol/L) for 14 days.

Transcriptome analysis (RNAseq)

Aliquots of preadipocytes and adipocytes were used for RNA extraction (NucleoSpin® RNA, Macherey-Nagel, Düren, Germany). RNA integrity (RIN ≥ 9) was measured with Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed as described previously at the Center for Molecular and Cellular Bioengineering (CMCB, Technical University Dresden, Germany). In short, mRNA was isolated from 1 µg RNA by poly-dT enrichment using the NEBNext Poly(A) mRNA Magnetic Isolation Module. After fragmentation, the samples were subjected to the workflow for strand-specific RNAseq library preparation (Ultra Directional RNA Library Prep II, NEB) and 75 bp single read sequencing was performed on Illumina NextSeq500. After sequencing, FastQC was used to perform quality control. Differential expression between preadipocytes and adipocytes was tested with the R package DESeq2 v2.7.R (19). Selected genes have been published previously (20). The complete analysis is now presented and available under GEO code (waiting for code number).

Gene ontology (GO) enrichment analysis was performed with the Metascape database (http://metascape.org) using a $p<0.01$ as a cut-off criterion (21). Fold

change of ≥2 and ≤-2 and Benjamini-Hochberg adjusted p-values of ≤0.05 were used as criteria to select differentially expressed genes (DEG).

Oil red O staining

Mature adipocytes were fixed with 4% paraformaldehyde for 30 min at RT and subsequently incubated with oil red O for further 30 min. Excess of oil red O was washed with PBS and lipid droplets were examined using a light microscope (EVOS M5000, Thermo Fischer Scientific Invitrogen, Karlsruhe, Germany). Oil red O-positive area was analyzed using ImageJ.

Lipolysis measurements

Adipocytes were preincubated in DMEM/Ham's nutrient mixture F12 (1:1) without any additive for 3 hours at 37°C. Subsequently, cells were incubated for 3 hours at 37°C with Krebs-Ringer-HEPES (KRH) buffer containing (in mmol/L): 135 NaCl, 4.8 KCl, 2.6 $CaCl_2$, 1.17 KH_2PO_4, 1.18 $MgSO_4$, 5 $NaHCO_3$, 10 HEPES, 5 glucose and 0.5% (w/v) fatty acid-free BSA (pH 7.4). Release of free fatty acids (FFA) was determined using a commercial kit (Sigma-Aldrich, Munich, Germany). The concentrations of cellular proteins were measured by Bradford assay (BioRad Laboratories, Munich, Germany). Basal lipolysis was set to 100% due to the high variation between patients. Human atrial natriuretic factor (1-28) trifluoroacetate salt, i.e. atrial natriuretic peptide (ANP), was purchased from Bachem (Bubendorf, Switzerland). Forskolin, IBMX, isoproterenol and dipyridamole were purchased from Sigma-Aldrich.

Western blotting

Adipocytes were insulin and FCS-starved for 18 h. Preadipocytes and adipocytes were preincubated for 1 h at 37°C in KRH buffer. Thereafter, the cells were exposed to different concentrations of insulin for 15 min and protein extraction immediately

started by lysing the cells in RIPA buffer (pH 7.5) containing (in mmol/L): 150 NaCl, 25 Tris/HCl, 2 EDTA, 10 NaF, 1 Na_3VO_4 and (in % (v/v)): 10 glycerol, 1 nonidet-P40, 0.1 SDS, 0.1 $C_{24}H_{39}NaO_4$, 1 protease inhibitor cocktail (Sigma-Aldrich). Proteins were separated by SDS-PAGE on 8% gels and blotted onto nitrocellulose membranes. Membranes were incubated overnight with primary antibodies in TBS containing 0.1% (v/v) Tween-20 and 5% (w/v) non-fat dry milk, followed by washing steps and an incubation of 1 h with horse radish peroxidase-coupled secondary antibody (1:2000; GE Healthcare, Munich, Germany; Cat# NA934, RRID:AB_772206). The used antibodies were: anti-P-Ser473-AKT (1:1000; Cell Signaling Technology, Danvers, MA, USA; Cat# 9271, RRID:AB_329825); anti-AKT (1:1000, Cell Signaling Technology; Cat# 9272, RRID:AB_329827); anti-tubulin (1:1000; Cell Signaling Technology; Cat# 2148, RRID:AB_2288042). P-AKT and AKT were validated previously (22). The protein and phosphoprotein bands, visualized on parallel blots, were quantified relative to the house keeping protein band on the respective blot using Image Lab Software (BioRad Laboratories).

Secretome analysis

Growth factors, cytokines and adipokines secreted into the medium were quantified using the Bio-Plex Pro Human Cytokine, Chemokine and Growth Factor Assay (BioRad Laboratories). Secreted adiponectin was measured using the Bio-Plex Pro Human Diabetes Assay (BioRad Laboratories).

Statistics

Statistical analysis was performed using GraphPad Prism (GraphPad Software Inc, La Jolla, CA, USA). Differences between two groups were assessed by Student's t-test. ANOVA with Tukey post-testing was used when more than two groups were

compared. In addition, categorical variables were analyzed using JMP (JMP software, SAS Institute Inc., Cary, NC, USA). Person chi-square test was used to show the relationship between two categorical variables. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Diabetogenic environment impairs pancreatic adipocyte differentiation

To understand whether chronic hyperglycemia impacts on function and differentiation of pancreatic fat cells, preadipocytes were isolated from fat pads collected from PPP and differentiated *in vitro* into adipocytes. The patients were stratified into 3 groups, NGT, PD and T2D according to HbA1c values (Table 1). HOMA2%B and HOMA2 disposition index were lower in T2D compared to NGT, indicative for impaired insulin secretion in T2D. Comparative transcriptome analysis was performed with preadipocytes and *in vitro* differentiated adipocytes (Fig. 1). Principal component analysis (PCA) displayed two distinct clusters (PC1) separating the preadipocyte and adipocyte populations. This indicates efficient *in vitro* differentiation (Fig. 1*A*). The variation observed among the preadipocytes of different donors (PC2) was less pronounced than the variation among the respective adipocytes. The differentiation evoked an upregulation of 398 and a downregulation of 625 genes in the transcriptome of NGT-preadipocytes (Fig. 1*B*). Particularly, genes typical for differentiated fat cells were upregulated, such as transcription factors *PPARG*, *CEBPA*, *CEBPB* and *NR1H3*, ensuing upregulation of adiponectin mRNA levels (*ADIPOQ,* Fig. 1*C-E*). Unexpectedly, leptin mRNA levels (*LEP*) were lower after differentiation (Fig. 1*D*). Furthermore, enzymes involved in triglyceride storage (e.g. *FASN*, *ELOVL6*, *SCD*, *PLIN1* and *PLIN4*) as well as lipolytic enzymes and proteins enabling fatty acid secretion were upregulated upon differentiation (e.g. *PNPLA2*, *LIPE*, *FABP4*; Fig. 1*F-I*).

In an attempt to detect differences between adipocytes from PPP with NGT, PD and T2D, the transcriptomes of these three groups were compared (Fig. 2). Despite a substantial overlap of 255 upregulated and 397 downregulated genes (Fig. 2*A*, *B*),

several upregulated genes were lower expressed in adipocytes of T2D- than of NGT-PPP among them the induced genes *GPD1*, *PLIN1* and *CIDEC* and a large variety of genes involved in lipogenesis, lipid storage, lipid uptake and lipolysis (Fig. 2*C*-*N*). These results indicate T2D-dependent impaired lipid metabolism and reduced differentiation capacity of human pancreatic adipocytes. The mRNA levels of *SLC2A4* (GLUT4), an important differentiation marker of adipocytes was low but increased in cells of NGT-PPP upon differentiation (Fig. 2*O*). In contrast, *SLC2A1* (GLUT1) as well as *SREBF1* mRNA levels were high in all cells (Fig. 2*P*, *Q*).

The successful *in vitro* differentiation of preadipocytes into adipocytes was further supported by the GO enrichment analysis (Fig. 2*R*). Most of the significantly enriched GO terms are attributed to fatty acid and lipid metabolism (Fig. 2*R*, Supplementary Table 3 and Supplementary Fig. 1 (14)). The comparison between NGT, PD and T2D samples supports the assumption that T2D negatively interferes with adipocyte differentiation. Thus, GO terms enriched to a lesser extend or not enriched in T2D compared to NGT were linked to triglyceride (TG) synthesis, lipolysis and adipokine secretion, e.g. PPAR signaling pathway, fat cell differentiation, fatty acid metabolism, protein kinase B signaling and glycerol-3-phosphate metabolic process (Supplementary Table 3 (14)). Furthermore, the downregulated DEG were enriched in GO terms related to angiogenesis, extracellular matrix remodeling, cytoskeleton organization and cell motility (Supplementary Fig. 2 (14) and ref. (20)). In addition, evaluation of transcription factor interaction with upregulated genes inferred by TRRUST (transcriptional regulatory relationships unraveled by sentence-based text-mining) shows that adipocyte-specific factors (*CEBPB*, *PPARG* and *SREBF1*) are more active in NGT than in T2D (Fig. 2*S*, marked in red).

In conclusion, *in vitro* differentiation of preadipocytes into adipocytes is impaired in T2D-PPP compared to NGT-PPP.

Impaired insulin signaling in pancreatic adipocytes of human with T2D

Since transcriptome analysis suggested impaired adipogenesis in adipocytes derived from PPP with T2D, and insulin is the most prominent factor promoting adipocyte differentiation, we hypothesize that preadipocytes of PPP with T2D are insulin resistant and retained this phenotype in cell culture, following expansion and *in vitro* differentiation.

In order to examine insulin sensitivity, phosphorylation of AKT was stimulated with increasing concentrations of insulin (1, 10, 100 nmol/L; Fig. 3). Stimulation of AKT phosphorylation by the highest supramaximal concentration of insulin (100 nmol/L) was used as reference and set to 100% for the respective cell preparations. Subcutaneous adipocytes of insulin sensitive humans were used as control. Insulin, at 10 nmol/L, elicited a comparable AKT phosphorylation in pancreatic preadipocytes and adipocytes of NGT-PPP (37±9.3% and 38±8.2% (n=5), respectively) as well as in subcutaneous preadipocytes and adipocytes (45±12.5% and 54±18.4% (n=5-6), respectively). However, AKT phosphorylation induced by 10 nmol/L insulin was significantly lower in preadipocytes and adipocytes of T2D-PPP (19±3.5% and 20±6% (n=5), respectively; $p<0.01$ vs SC; Fig. 3*A*-*F*). These data support the results of the expression profiles and show that, also functionally, preadipocytes from T2D-PPP retained the insulin resistant phenotype even after *in vitro* expansion and differentiation.

Impaired lipolysis of differentiated pancreatic adipocytes with T2D

Next the adipocyte secretome was examined, since putative paracrine effects of adipocytes are exerted via locally secreted factors. Fatty acids, which are components of the adipocyte secretome, are important cofactors of glucose-induced insulin secretion ensuring proper stimulation of secretion (7). Sympathetic nerve activation as well as glucagon, especially during fasting and exercise, stimulate fatty acid secretion from adipocytes. Therefore, we examined the lipolytic activity of differentiated pancreatic adipocytes in response to isoproterenol, a specific beta-adrenergic receptors agonist, and glucagon. TG accumulation was visible in pancreatic adipocytes after differentiation (Fig. 4*A*). Since lipid accumulation of pancreatic adipocytes was much lower than that of subcutaneous adipocytes, pancreatic adipocytes were cultured in MUFA-enriched medium which increased cellular lipid accumulation to levels detected in subcutaneous adipocytes (Fig. 4*A*, *B*). No differences of lipid accumulation between pancreatic adipocytes from PPP with NGT and T2D were observed.

Basal lipolysis of pancreatic adipocytes was similar among the NGT and T2D groups (80.14±17.78 nmol/L×mg protein^{-1}×h^{-1} and 80.70±51.82 nmol/L×mg protein^{-1}×h^{-1} (n=5), respectively; n.s.; t-test). Lipolysis was stimulated neither by isoproterenol nor by glucagon (Fig. 4*C*). In adipocytes from PPP with NGT only forskolin, 5 µmol/L, which activates adenylyl cyclase by bypassing receptors, increased lipolysis 1.48-fold (Fig. 4*C*). Addition of the phosphodiesterase inhibitor IBMX had no further stimulatory effect. The lipolytic rate of pancreatic adipocytes was not improved when cells were cultured in the presence of MUFAs (Supplementary Figure 3 (14)). In adipocytes of PPP with T2D none of the stimuli elicited a lipolytic response (Fig. 4*C* and Supplementary Figure 3 (14)). In contrast, fatty acid release of subcutaneous

adipocytes was stimulated 12.1-fold by forskolin (basal lipolysis 39.73±23.29 nmol/L×mg protein^{-1}×h^{-1} (n=5)), significantly augmented by isoproterenol (2.7-fold) and inhibited by insulin (Fig 4*D*). In pancreatic fat cells mRNA levels of the glucagon receptor and beta-adrenergic receptors were low in comparison to the high amount of insulin receptor mRNA (Fig. 4*E*, *F*). This low expression of the respective receptor could explain the absence of detectable effects of glucagon and isoproterenol on lipolysis.

In contrast to the adrenoceptors, pancreatic adipocytes contained high mRNA levels of the natriuretic peptide receptors 1 and 2 (*NPR1* and *NPR2*; Fig. 4*G*). Furthermore, expression of *PDE5A*, a selective cGMP-hydrolyzing molecule, was much higher than *PDE3B*, which preferentially hydrolyzes cAMP (Fig 4*H*). Receptor and phosphodiesterase expression profile could suggest that the cGMP pathway is more important in eliciting lipolysis than the cAMP pathway. Indeed, exposure of NGT adipocytes to ANP stimulated lipolysis 1.74-fold, inhibition of PDE5 by dipyridamole 1.88-fold and both together increased lipolysis 3.09-fold (Fig. 4*I*). Insulin, an activator of PDE3B, did not counteract the stimulatory effect of ANP on lipolysis. In T2D-PPP adipocytes lipolysis was not stimulated by ANP. These results suggest that in isolated *in vitro* differentiated human pancreatic adipocytes lipolysis is preferentially stimulated by ANP and persistently impaired in T2D.

Secretome of pancreatic preadipocytes and adipocytes

Additional components of the adipocyte secretome which may interfere with insulin secretion were evaluated via the transcriptome and via measurement of the respective product released into the supernatant. The mRNA levels have been presented previously (20). Of note, differentiation was accompanied by a 13-fold induction of *ADIPOQ* mRNA levels (Fig. 1*D*; ref. (20)). In contrast, leptin mRNA levels

were already high in preadipocytes and declined during differentiation (Fig. 1*D*; ref. (20)). The ratio of leptin/adiponectin mRNA correlated positively with HbA1c (Fig. 5*A*). In agreement with adiponectin mRNA levels, secreted adiponectin was detected in the supernatant of adipocytes only (Fig. 5*B*). Leptin secretion was detected in the supernatants of preadipocytes of PD and T2D and of adipocytes of NGT, PD and T2D (Fig. 5*C*). T2D fat cells secreted more leptin than NGT cells. Growth factors (VEGF and HGF) were secreted from preadipocytes and adipocytes, whereas chemokines (IL-6 and MCP-1) were mainly produced by preadipocytes (Fig. 5*D*-*G*). In comparison, the secretome of subcutaneous fat cells changed in a similar direction during differentiation as the NGT-PPP secretome: Leptin and adiponectin secretion increased while VEGF and IL-6 secretion decreased (Fig. 5*B*-*G*). These results suggest that T2D pancreatic fat cells (preadipocytes and adipocytes) secrete more leptin than NGT, while the secretion of other factors including adiponectin, growth factors and chemokines/cytokines were not different.

In conclusion, diabetes, i.e. chronic hyperglycemic episodes reflected by increased HbA1c, is associated with changes of the transcriptome and function of pancreatic fat cells, which may elicit an altered fat cell – islet crosstalk and further accelerate disease development.

DISCUSSION

The development of T2D is associated with systemic insulin resistance (23). Our data show that both pancreatic preadipocytes and *in vitro* differentiated adipocytes of T2D-PPP display insulin resistance. In accordance, insulin-dependent AKT phosphorylation was reduced in pancreatic fat cells from T2D-PPP compared to NGT-PPP (Fig. 3). Furthermore, during adipogenesis, target genes of the insulin-sensitive transcription factor SREBP1c, such as *FASN* (FAS), *ELOVL6*, *SCD* and *FADS1,* were not upregulated in cells of T2D-PPP, contrary to NGT-PPP (Fig. 2*K*-*N*). The reduced insulin sensitivity is not a consequence of reduced expression of IR, in agreement with a previous observation in insulin resistant subcutaneous adipocytes (Fig. 4*E;* ref (24)). It is likely that the lower insulin sensitivity observed in T2D-PPP is related to alterations downstream of the insulin receptor (24-26).

This insulin resistance did not translate to a reduced capacity of triglyceride storage, indicating that insulin resistance might reduce the rate of lipogenesis rather than the storage capacity. Although *SLC2A4* mRNA level increased in NGT-PPP upon differentiation, GLUT4 expression remained most likely too low for a sufficient activation of *de novo* lipogenesis in *in vitro* differentiated pancreatic adipocytes. This feature seems to represent an intrinsic defect of pancreatic preadipocytes as subcutaneous preadipocytes retain a much higher *de novo* lipogenic activity *in vitro* than the pancreatic preadipocytes. Supplementation of culture medium with MUFAs increased triglyceride storage in pancreatic adipocytes but the formation of a central lipid droplet was still incomplete.

Despite insulin resistance, the basal lipolytic activity of adipocytes from T2D-PPP was similar to that of NGT-PPP, and refractory to stimuli. This feature is distinct to the lipolytic behavior of visceral adipocytes, which increase their lipolysis along with

insulin resistance (27). Fatty acid release was stimulated 1.5-fold by forskolin, exclusively in NGT-PPP, while isoproterenol and glucagon were ineffective. The direct stimulation of adenylyl cyclase by forskolin bypasses receptor activation which in pancreatic adipocytes is most likely non-functional given the very low expression of beta-adrenergic and the absence of glucagon receptors (Fig. 4*E*, *F*; ref (28-30)). Since IBMX, a phosphodiesterase inhibitor, had no significant stimulatory effect on lipolysis, increased phosphodiesterase activity seems not to contribute to the low responsiveness of the cells. The reduced lipogenic capacity was also not the reason for the low lipolytic response. Thus, pancreatic adipocytes differentiated in MUFA-supplemented medium did not secrete more fatty acids in spite of increased lipid storage. In comparison to the subcutaneous adipocytes, where forskolin stimulated lipolysis 12-fold, the cAMP-dependent stimulation of lipolysis is less efficient in human pancreatic adipocytes (Fig. 4*D*). In agreement, a recent study in mice reported lower RNA expression of lipogenic and lipolytic markers in peripancreatic adipose tissue in comparison to subcutaneous and other visceral fat depots (31).

Compatible with the high expression of *NPR1* and *NPR2*, ANP stimulated fatty acid release of NGT pancreatic adipocytes. The expression of *NPR1* and *NPR2* in other human fat depots was first described in the late 90's (32). Lipolysis was stimulated by natriuretic peptides in isolated adipocytes as well as in human *in vivo* studies using ANP infusion (33, 34). The effect of ANP is exerted through the activation of guanylate cyclase, an increase of intracellular cGMP and subsequent activation of PKG (35, 36). Thus, the effect of ANP is not counteracted by insulin, which interferes with the cAMP pathway (33, 34, 37). Insulin activates PDE3B, while cGMP is mainly hydrolyzed by PDE5 (38-40). Adipocytes normally have a higher expression of PDE3B than of PDE5, and inhibition of PDE5 did not increase ANP stimulation in

445 subcutaneous and visceral human adipocytes (35, 41-43). In pancreatic adipocytes,
446 we found more *PDE5A* than *PDE3B* mRNA levels (Fig. 4*H*). In addition, dipyridamole
447 increased lipolysis in the presence and absence of ANP. However, T2D adipocytes
448 did not respond to ANP and dipyridamole despite the high mRNA levels of *NPR1*,
449 *NPR2* and *PDE5A* (Fig. 4*G*, *H*). These observations suggest a metabolic/humoral,
450 i.e. through cGMP, rather than a neuronal regulation, i.e. through cAMP, of fatty acid
451 release from isolated, *in vitro* differentiated pancreatic adipocytes.

452 A role of locally secreted fatty acids in insulin hypersecretion has been proposed
453 using a mouse model (44). The failure of sympathetic innervation and of glucagon,
454 stress factors which increase during fasting and exercise, to stimulate fatty acid
455 release from pancreatic adipocytes, could prevent insulin hypersecretion under
456 hypoglycemic situations. However, whether, in humans, pancreatic adipocytes exert
457 paracrine effects on insulin secretion via fatty acid release needs further
458 experimental evidence. In this study, we report functional differences between
459 adipocytes of NGT- and T2D-PPP as lipolysis of T2D-PPP adipocytes was refractory
460 to any stimuli.

461 Beside metabolites, adipocytes secrete also adipokines (20). Multiple observations
462 suggest beneficial effect of adiponectin on beta-cell function and survival and
463 inhibitory effects of leptin on insulin secretion (45). Adiponectin receptors are
464 expressed in human beta-cells (46). Leptin acts as an anorexogenic hormone mainly
465 on NPY/AGRP and POMC neurons in the hypothalamus (47). Whether leptin acts
466 directly on insulin secretion, in view of a low abundance of leptin receptor mRNA in
467 human beta-cells is a yet unresolved question (46). Leptin, however, affects insulin
468 receptor signaling which aggravates insulin resistance (25). Here, we found an
469 increasing mRNA leptin/adiponectin ratio positively associated with HbA1c. Leptin

secretion was higher in preadipocytes and adipocytes of T2D-PPP than of NGT-PPP. Increases in leptin/adiponectin plasma levels is observed in patients with T2D and related to impaired insulin sensitivity (48-50). Of note, secreted adiponectin levels were more than 1000-times higher than of leptin suggesting that a beneficial paracrine effect of adiponectin would be more likely than an inhibitory action of leptin on insulin secretion (51).

Additional factors secreted by preadipocytes and adipocytes are cytokines and chemokines of which IL-6 and MCP-1 production was higher in the undifferentiated fat cells. Moreover, IL-6 secretion in PD and T2D adipocytes is higher than in NGT adipocytes, which could be an additional factor contributing to impaired adipogenesis. Increased adipose IL-6 secretion is observed in obese patients with insulin resistance and T2D and correlates with decreased adipogenic capacity (52). Previously we found that fetuin-A and palmitate specifically increased the production of IL-6 and MCP-1 through TLR4 in pancreatic fat cells, which might lead to increased islet macrophage infiltration (7). Macrophages in turn can further impair preadipocyte differentiation, and exacerbate adipocyte IL-6 and MCP-1 secretion while decreasing adiponectin release (53). Thus exposure to diabetogenic factors triggers a proinflammatory state which can ultimately contribute to beta-cell damage.

The observation that DEG downregulated during adipogenesis are enriched in GO terms specific for angiogenesis, extracellular matrix remodeling, cytoskeleton organization and cell motility, and that T2D-PPP display reduced adipogenesis, suggest that preadipocytes play an important role in matrix remodeling including fibrosis in T2D-PPP (54, 55). How local changes in matrix remodeling impact on beta-cell function remains to be tested.

In conclusion, the metabolic status impacts on the secretome of local pancreatic fat cells and this may influence islet function via paracrine actions.

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DISCLOSURES

The authors have no conflicts of interest that are directly relevant to the contents of this study.

References

1. **Verma S, Hussain ME**. Obesity and diabetes: An update. *Diabetes Metab Syndr* 11(1):73-79, 2017.

2. **Begovatz P, Koliaki C, Weber K, Strassburger K, Nowotny B, Nowotny P, Müssig K, Bunke J, Pacini G, Szendrodi J, Roden M**. Pancreatic adipose tissue infiltration, parenchymal steatosis and beta cell function in humans. *Diabetologia* 58(7):1646-1655, 2015.

3. **Guglielmi V, Sbraccia P**. Type 2 diabetes: Does pancreatic fat really matter? *Diabetes Metab Res Rev* 34(2), 2018.

4. **Heni M, Machann J, Staiger H, Schwenzer NF, Peter A, Schick F, Claussen CD, Stefan N, Häring HU, Fritsche A**. Pancreatic fat is negatively associated with insulin secretion in individuals with impaired fasting glucose and/or impaired glucose tolerance: a nuclear magnetic resonance study. *Diabetes Metab Res Rev* 26(3):200-205, 2010.

5. **Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R**. Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 54(10):2506-2514, 2011.

6. **van der Zijl NJ, Goossens GH, Moors CC, van Raalte DH, Muskiet MH, Pouwels PJ, Blaak EE, Diamant M**. Ectopic fat storage in the pancreas, liver, and abdominal fat depots: impact on beta-cell function in individuals with impaired glucose metabolism. *J Clin Endocrinol Metab* 96(2):459-467, 2011.

7. **Gerst F, Wagner R, Kaiser G, Panse M, Heni M, Machann J, Bongers MN, Sartorius T, Sipos B, Fend F, Thiel C, Nadalin S, Königsrainer A, Stefan N, Fritsche A, Häring HU, Ullrich S, Siegel-Axel D**. Metabolic crosstalk between fatty pancreas and fatty liver: effects on local inflammation and insulin secretion. *Diabetologia* 60(11):2240-2251, 2017.

8. **Staaf J, Labmayr V, Paulmichl K, Manell H, Cen J, Ciba I, Dahlbom M, Roomp K, Anderwald CH, Meissnitzer M, Schneider R, Forslund A, Widhalm K, Bergquist J, Ahlstrom H, Bergsten P, Weghuber D, Kullberg J**. Pancreatic Fat Is Associated With Metabolic Syndrome and Visceral Fat but Not Beta-Cell Function or Body Mass Index in Pediatric Obesity. *Pancreas* 46(3):358-365, 2017.

9. **Wagner R, Jaghutriz BA, Gerst F, Barroso Oquendo M, Machann J, Schick F, Loffler MW, Nadalin S, Fend F, Königsrainer A, Peter A, Siegel-Axel D, Ullrich S, Häring HU, Fritsche A, Heni M**. Pancreatic Steatosis Associates With Impaired Insulin Secretion in Genetically Predisposed Individuals. *J Clin Endocrinol Metab* 105(11), 2020.

10. **Cao Y**. Angiogenesis modulates adipogenesis and obesity. *J Clin Invest* 117(9):2362-2368, 2007.

11. **Stern JH, Rutkowski JM, Scherer PE**. Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell Metab* 23(5):770-784, 2016.

12. **Kojta I, Chacinska M, Blachnio-Zabielska A**. Obesity, Bioactive Lipids, and Adipose Tissue Inflammation in Insulin Resistance. *Nutrients* 12(5), 2020.

13. **Van Harmelen V, Rohrig K, Hauner H**. Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. *Metabolism* 53(5):632-637, 2004.

14. **Oquendo MB, Gerst F, Lorza-Gil E, Moller A, Wagner R, Fend F, Königsrainer A, Heni M, Siegel-Axel D, Häring HU, Ullrich S, Birkenfeld AL**. Supplementary data from: Pancreatic fat cells of humans with type 2 diabetes display reduced adipogenic and lipolytic activity. Figshare 2020. Deposited 09.12.2020. doi: 10.6084/m9.figshare.13353290. Available from: https://figshare.com/s/70962868c4b943acdcd6.

15. **Levy JC, Matthews DR, Hermans MP**. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 21(12):2191-2192, 1998.

16. **Schwenzer NF, Machann J, Schraml C, Springer F, Ludescher B, Stefan N, Haring H, Fritsche A, Claussen CD, Schick F**. Quantitative analysis of adipose tissue in single transverse slices for estimation of volumes of relevant fat tissue compartments: a study in a large cohort of subjects at risk for type 2 diabetes by MRI with comparison to anthropometric data. *Invest Radiol* 45(12):788-794, 2010.

17. **Böhm A, Halama A, Meile T, Zdichavsky M, Lehmann R, Weigert C, Fritsche A, Stefan N, Königsrainer A, Häring HU, de Angelis MH, Adamski J, Staiger H**. Metabolic signatures of cultured human adipocytes from metabolically healthy versus unhealthy obese individuals. *PLoS One* 9(4):e93148, 2014.

18. **Siegel-Axel DI, Ullrich S, Stefan N, Rittig K, Gerst F, Klingler C, Schmidt U, Schreiner B, Randrianarisoa E, Schaller HE, Stock UA, Weigert C, Königsrainer A, Häring HU**. Fetuin-A influences vascular cell growth and production of proinflammatory and angiogenic proteins by human perivascular fat cells. *Diabetologia* 57(5):1057-1066, 2014.

19. **Love MI, Huber W, Anders S**. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 15(12):550, 2014.

20. **Gerst F, Wagner R, Oquendo MB, Siegel-Axel D, Fritsche A, Heni M, Staiger H, Häring HU, Ullrich S**. What role do fat cells play in pancreatic tissue? *Mol Metab* 25:1-10, 2019.

21. **Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK**. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun* 10(1):1523, 2019.

22. **Kaiser G, Gerst F, Michael D, Berchtold S, Friedrich B, Strutz-Seebohm N, Lang F, Haring HU, Ullrich S**. Regulation of forkhead box O1 (FOXO1) by protein kinase B and glucocorticoids: different mechanisms of induction of beta cell death in vitro. *Diabetologia* 56(7):1587-1595, 2013.

23. **Staiger H, Machicao F, Fritsche A, Haring HU**. Pathomechanisms of type 2 diabetes genes. *Endocr Rev* 30(6):557-585, 2009.

24. **Smith U**. Impaired ('diabetic') insulin signaling and action occur in fat cells long before glucose intolerance--is insulin resistance initiated in the adipose tissue? *Int J Obes Relat Metab Disord* 26(7):897-904, 2002.

25. **Hennige AM, Stefan N, Kapp K, Lehmann R, Weigert C, Beck A, Moeschel K, Mushack J, Schleicher E, Häring HU**. Leptin down-regulates insulin action through phosphorylation of serine-318 in insulin receptor substrate 1. *FASEB J* 20(8):1206-1208, 2006.

26. **Boucher J, Kleinridders A, Kahn CR**. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harb Perspect Biol* 6(1), 2014.

27. **Morigny P, Houssier M, Mouisel E, Langin D**. Adipocyte lipolysis and insulin resistance. *Biochimie* 125:259-266, 2016.

28. **Lafontan M, Barbe P, Galitzky J, Tavernier G, Langin D, Carpene C, Bousquet-Melou A, Berlan M**. Adrenergic regulation of adipocyte metabolism. *Hum Reprod* 12 Suppl 1:6-20, 1997.

29. **Schmidt SL, Bessesen DH, Stotz S, Peelor FF, 3rd, Miller BF, Horton TJ**. Adrenergic control of lipolysis in women compared with men. *J Appl Physiol (1985)* 117(9):1008-1019, 2014.

30. **Pereira MJ, Thombare K, Sarsenbayeva A, Kamble PG, Almby K, Lundqvist M, Eriksson JW**. Direct effects of glucagon on glucose uptake and lipolysis in human adipocytes. *Mol Cell Endocrinol* 503:110696, 2020.

31. **Chanclon B, Wu Y, Vujicic M, Bauza-Thorbrugge M, Banke E, Micallef P, Kanerva J, Wilder B, Rorsman P, Wernstedt Asterholm I**. Peripancreatic adipose tissue protects against high-fat-diet-induced hepatic steatosis and insulin resistance in mice. *Int J Obes (Lond)* 44(11):2323-2334, 2020.

620 32. **Sarzani R, Dessi-Fulgheri P, Paci VM, Espinosa E, Rappelli A**. Expression of
621 natriuretic peptide receptors in human adipose and other tissues. *J Endocrinol Invest*
622 19(9):581-585, 1996.

623 33. **Sengenes C, Berlan M, De Glisezinski I, Lafontan M, Galitzky J**. Natriuretic
624 peptides: a new lipolytic pathway in human adipocytes. *FASEB J* 14(10):1345-1351, 2000.

625 34. **Moro C, Polak J, Richterova B, Sengenes C, Pelikanova T, Galitzky J, Stich V,**
626 **Lafontan M, Berlan M**. Differential regulation of atrial natriuretic peptide- and adrenergic
627 receptor-dependent lipolytic pathways in human adipose tissue. *Metabolism* 54(1):122-131,
628 2005.

629 35. **Moro C, Klimcakova E, Lafontan M, Berlan M, Galitzky J**. Phosphodiesterase-5A
630 and neutral endopeptidase activities in human adipocytes do not control atrial natriuretic
631 peptide-mediated lipolysis. *Br J Pharmacol* 152(7):1102-1110, 2007.

632 36. **Nishikimi T, Iemura-Inaba C, Akimoto K, Ishikawa K, Koshikawa S, Matsuoka H**.
633 Stimulatory and inhibitory regulation of lipolysis by the NPR-A/cGMP/PKG and NPR-C/G(i)
634 pathways in rat cultured adipocytes. *Regul Pept* 153(1-3):56-63, 2009.

635 37. **Moro C, Galitzky J, Sengenes C, Crampes F, Lafontan M, Berlan M**. Functional
636 and pharmacological characterization of the natriuretic peptide-dependent lipolytic pathway
637 in human fat cells. *J Pharmacol Exp Ther* 308(3):984-992, 2004.

638 38. **Degerman E, Landstrom TR, Wijkander J, Holst LS, Ahmad F, Belfrage P,**
639 **Manganiello V**. Phosphorylation and activation of hormone-sensitive adipocyte
640 phosphodiesterase type 3B. *Methods* 14(1):43-53, 1998.

641 39. **Degerman E, Belfrage P, Manganiello VC**. Structure, localization, and regulation of
642 cGMP-inhibited phosphodiesterase (PDE3). *J Biol Chem* 272(11):6823-6826, 1997.

643 40. **Zaccolo M, Movsesian MA**. cAMP and cGMP signaling cross-talk: role of
644 phosphodiesterases and implications for cardiac pathophysiology. *Circ Res* 100(11):1569-
645 1578, 2007.

646 41. **Kraynik SM, Miyaoka RS, Beavo JA**. PDE3 and PDE4 isozyme-selective inhibitors
647 are both required for synergistic activation of brown adipose tissue. *Mol Pharmacol*
648 83(6):1155-1165, 2013.

649 42. **Omar B, Banke E, Ekelund M, Frederiksen S, Degerman E**. Alterations in cyclic
650 nucleotide phosphodiesterase activities in omental and subcutaneous adipose tissues in
651 human obesity. *Nutr Diabetes* 1:e13, 2011.

652 43. **Armani A, Marzolla V, Rosano GM, Fabbri A, Caprio M**. Phosphodiesterase type 5
653 (PDE5) in the adipocyte: a novel player in fat metabolism? *Trends Endocrinol Metab*
654 22(10):404-411, 2011.

655 44. **Quiclet C, Dittberner N, Gassler A, Stadion M, Gerst F, Helms A, Baumeier C,**
656 **Schulz TJ, Schurmann A**. Pancreatic adipocytes mediate hypersecretion of insulin in
657 diabetes-susceptible mice. *Metabolism* 97:9-17, 2019.

658 45. **Zhao YF, Feng DD, Chen C**. Contribution of adipocyte-derived factors to beta-cell
659 dysfunction in diabetes. *Int J Biochem Cell Biol* 38(5-6):804-819, 2006.

660 46. **Segerstolpe A, Palasantza A, Eliasson P, Andersson EM, Andreasson AC, Sun**
661 **X, Picelli S, Sabirsh A, Clausen M, Bjursell MK, Smith DM, Kasper M, Ämmälä C,**
662 **Sandberg R**. Single-Cell Transcriptome Profiling of Human Pancreatic Islets in Health and
663 Type 2 Diabetes. *Cell Metab* 24(4):593-607, 2016.

664 47. **Schwartz MW, Woods SC, Porte D, Jr., Seeley RJ, Baskin DG**. Central nervous
665 system control of food intake. *Nature* 404(6778):661-671, 2000.

666 48. **Oda N, Imamura S, Fujita T, Uchida Y, Inagaki K, Kakizawa H, Hayakawa N,**
667 **Suzuki A, Takeda J, Horikawa Y, Itoh M**. The ratio of leptin to adiponectin can be used as
668 an index of insulin resistance. *Metabolism* 57(2):268-273, 2008.

669 49. **Thorand B, Zierer A, Baumert J, Meisinger C, Herder C, Koenig W**. Associations
670 between leptin and the leptin / adiponectin ratio and incident Type 2 diabetes in middle-aged

671 men and women: results from the MONICA / KORA Augsburg study 1984-2002. *Diabet Med*
672 27(9):1004-1011, 2010.

673 50. **Bravo C, Cataldo LR, Galgani J, Parada J, Santos JL**. Leptin/Adiponectin Ratios
674 Using Either Total Or High-Molecular-Weight Adiponectin as Biomarkers of Systemic Insulin
675 Sensitivity in Normoglycemic Women. *J Diabetes Res* 2017:9031079, 2017.

676 51. **Skurk T, Alberti-Huber C, Herder C, Hauner H**. Relationship between adipocyte
677 size and adipokine expression and secretion. *J Clin Endocrinol Metab* 92(3):1023-1033,
678 2007.

679 52. **Almuraikhy S, Kafienah W, Bashah M, Diboun I, Jaganjac M, Al-Khelaifi F,**
680 **Abdesselem H, Mazloum NA, Alsayrafi M, Mohamed-Ali V, Elrayess MA**. Interleukin-6
681 induces impairment in human subcutaneous adipogenesis in obesity-associated insulin
682 resistance. *Diabetologia* 59(11):2406-2416, 2016.

683 53. **Liu LF, Craig CM, Tolentino LL, Choi O, Morton J, Rivas H, Cushman SW,**
684 **Engleman EG, McLaughlin T**. Adipose tissue macrophages impair preadipocyte
685 differentiation in humans. *PLoS One* 12(2):e0170728, 2017.

686 54. **Mariman EC, Wang P**. Adipocyte extracellular matrix composition, dynamics and role
687 in obesity. *Cell Mol Life Sci* 67(8):1277-1292, 2010.

688 55. **Ruiz-Ojeda FJ, Mendez-Gutierrez A, Aguilera CM, Plaza-Diaz J**. Extracellular
689 Matrix Remodeling of Adipose Tissue in Obesity and Metabolic Diseases. *Int J Mol Sci*
690 20(19):4888, 2019.

691

692

693 **Figure legends**

694

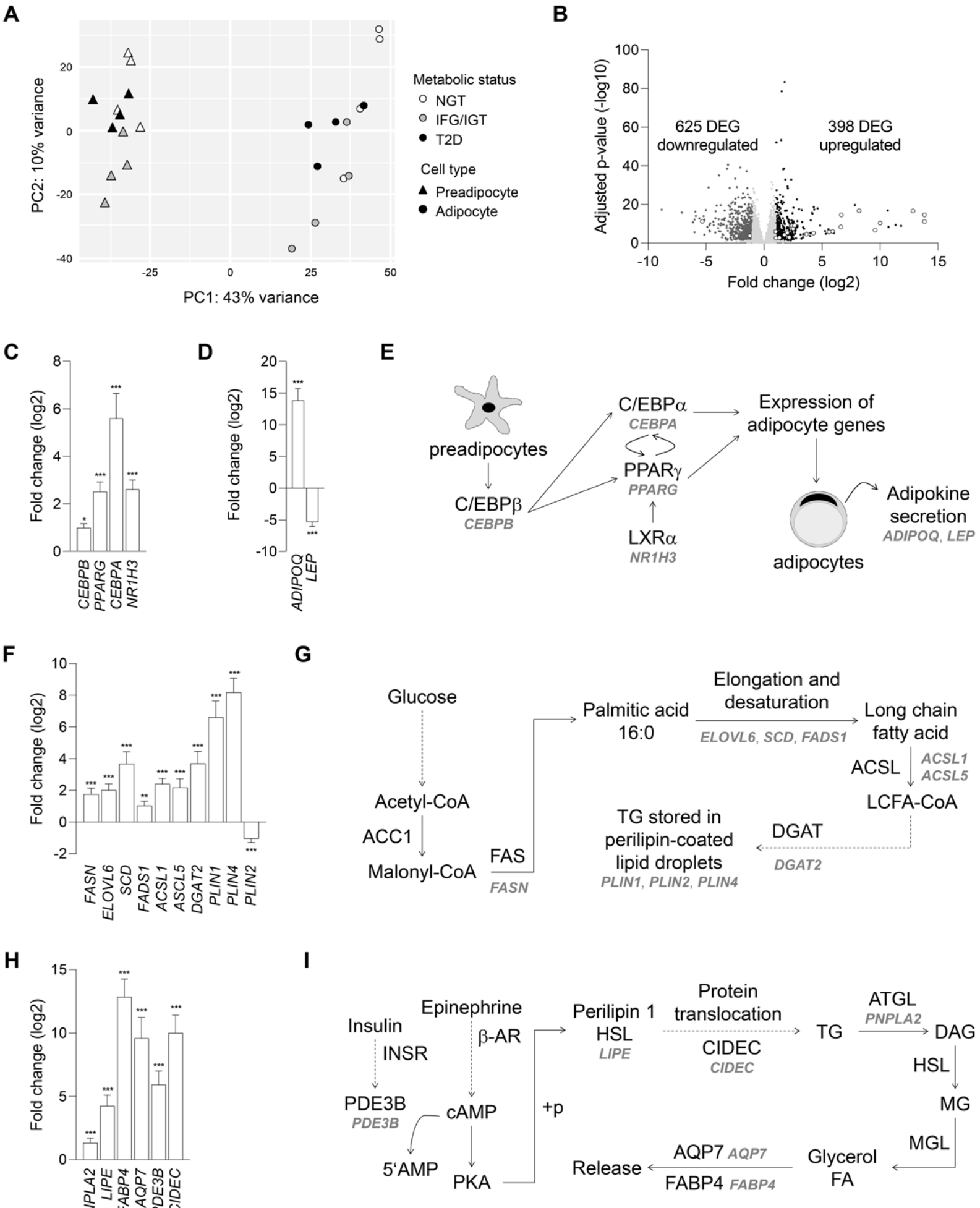
695 Fig. 1. *In vitro* differentiation of pancreatic adipocytes leads to significant changes in
696 transcriptome. *A:* Principal component (PC) analysis of preadipocyte (triangle) and
697 adipocyte (circle) RNAseq data (NGT, white symbols; PD, grey symbols; T2D, black
698 symbols). *B:* Volcano plot depicting upregulated (black) and downregulated (dark
699 grey) DEG after differentiation of NGT. Genes presented in (*C-I*) are marked in white
700 with black border. *C-I:* Transcription factors and pathways associated with (*C-E*)
701 adipogenesis, (*F-G*) genes involved in triglyceride synthesis and storage and (*H-I*)
702 lipolysis. *C, D, F, H:* Fold change in mRNA levels of differentiated adipocytes vs
703 preadipocytes. Shown are mean + SEM, n=4. *p<0.05, **p<0.01, ***p<0.001 vs
704 preadipocyte; Benjamini-Hochberg correction for multiple testing. DEG, differentially
705 expressed genes; NGT, normal glucose tolerance; PD, prediabetes; T2D, type 2
706 diabetes.

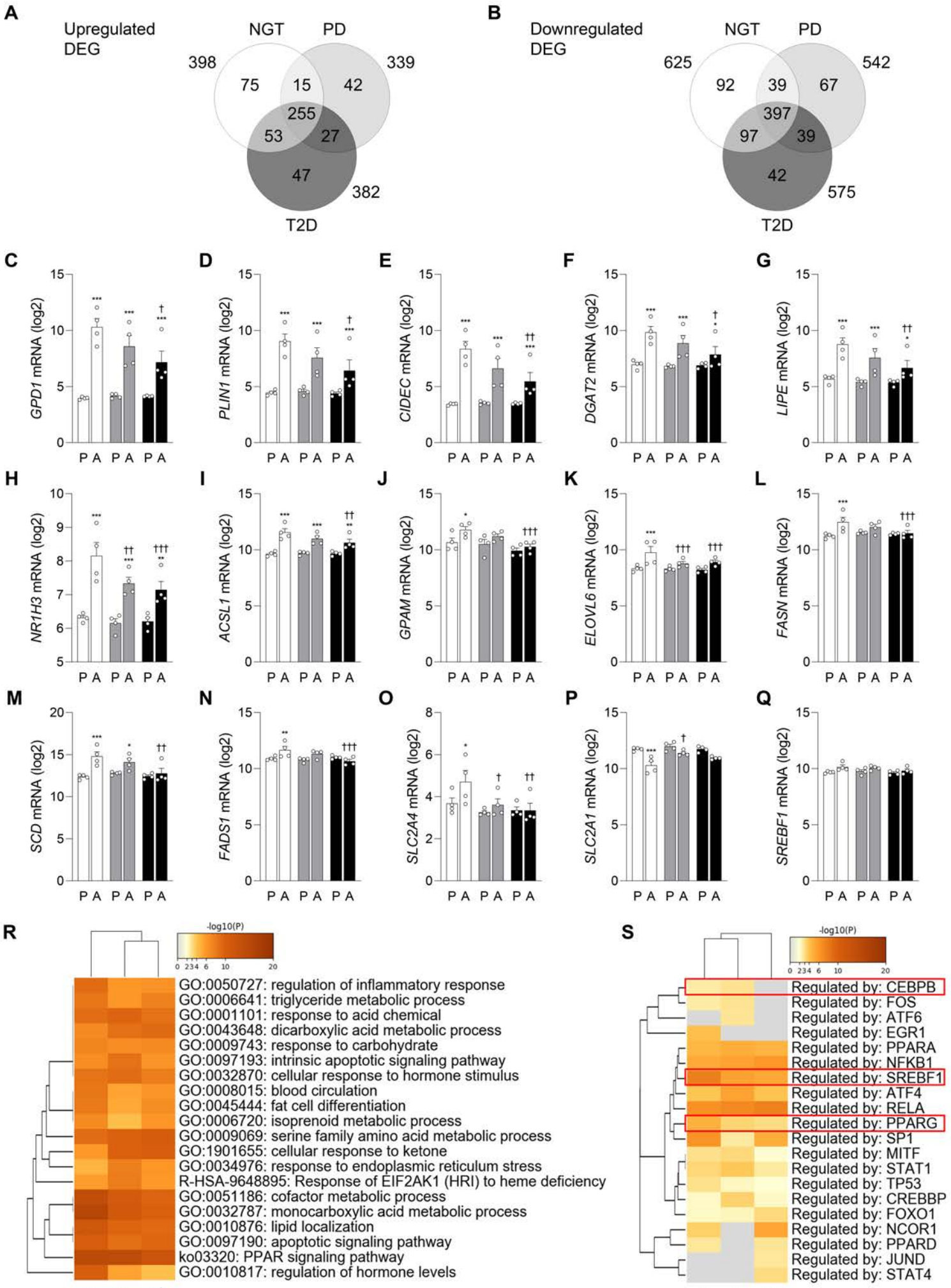
707

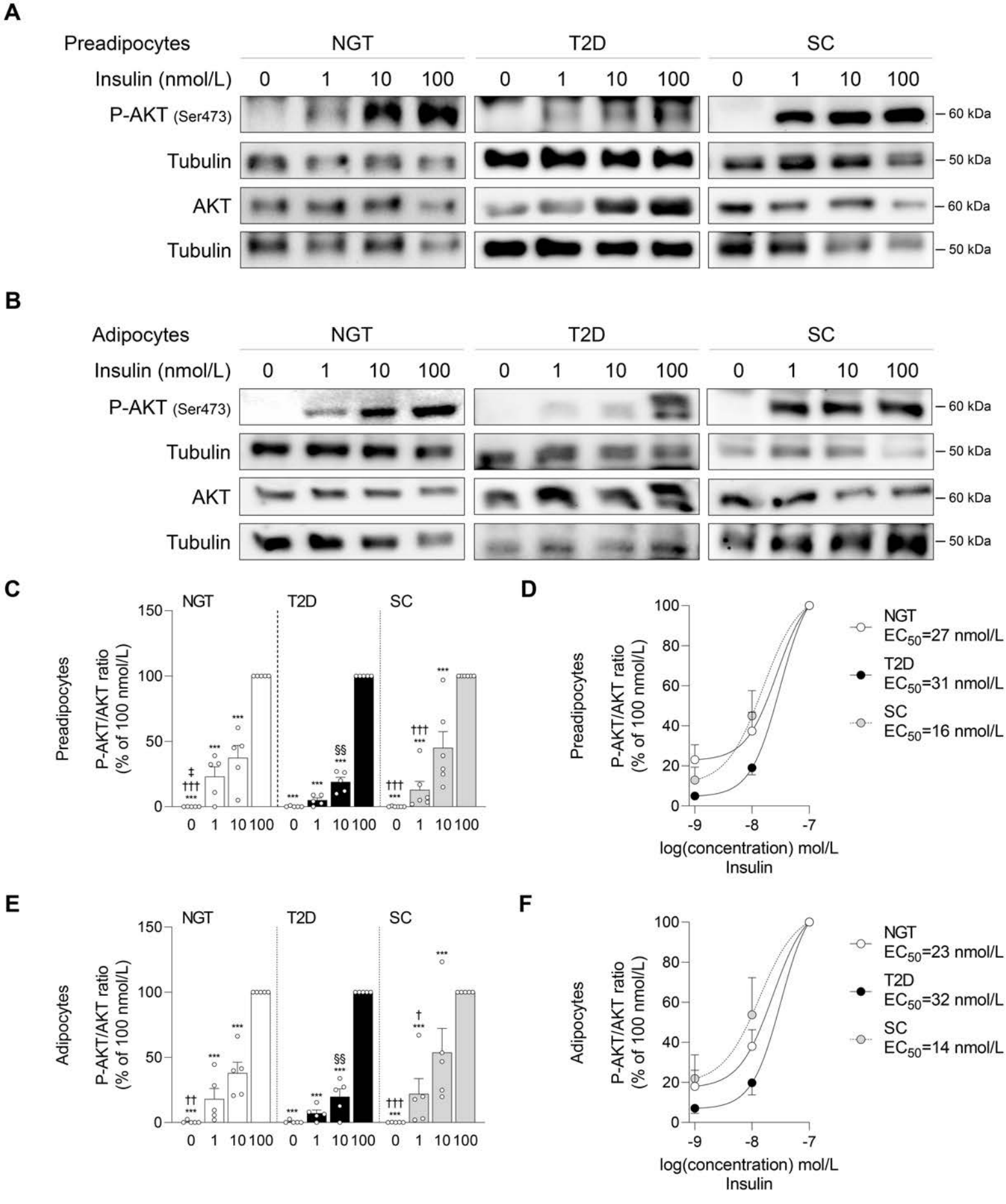
708 Fig. 2. Diabetogenic environment impairs differentiation of pancreatic adipocytes. *A-*
709 *B:* Venn diagrams showing the number of (*A*) upregulated and (*B*) downregulated
710 DEG in adipocytes of NGT (white), PD (grey) and T2D (black) groups and their
711 intersections. *C-Q*: mRNA levels of selected genes depicted from RNAseq data
712 expressed as mean + SEM, n=4 (NGT, white columns; PD, grey columns; T2D, black
713 columns). *R:* Heatmap of top 20 enriched GO terms colored by p-value (-log10) of
714 upregulated genes in differentiated adipocytes. *S:* Heatmap of top 20 enriched
715 transcription factors of TRRUST (transcriptional regulatory relationships unravelled
716 by sentence-based text-mining) category in Metascape colored by p-value (-log10).
717 *p<0.05, **p<0.01, ***p<0.001 vs respective preadipocyte; Benjamini-Hochberg

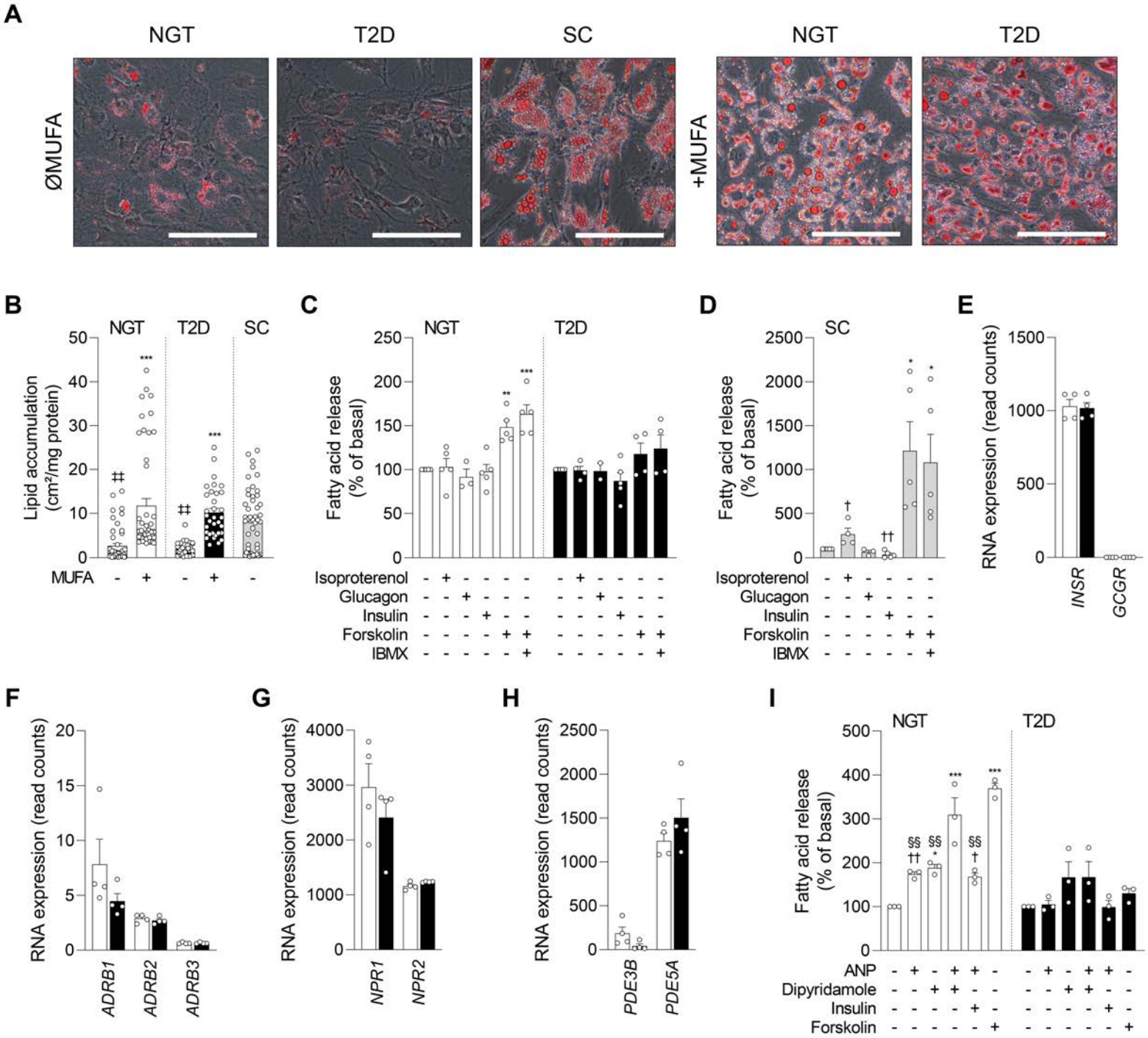
718 correction for multiple testing. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ vs NGT adipocytes;
719 Wald chi-squared test. A, adipocytes; DEG, differentially expressed genes; NGT,
720 normal glucose tolerance; P, preadipocytes; PD, prediabetes; T2D, type 2 diabetes.
721
722 Fig. 3. Reduced insulin sensitivity of T2D adipocytes. *A-B:* Representative Western
723 blots of AKT phosphorylation at serine 473 in pancreatic and subcutaneous (*A*)
724 preadipocytes and (*B*) adipocytes. *C-F:* Quantification of AKT phosphorylation
725 normalized to the effect of 100 nmol/L insulin in (*C, D*) preadipocytes and (*E, F*)
726 adipocytes. *D, F:* Log concentration-response curve and EC_{50} of insulin on AKT
727 phosphorylation in (*D*) preadipocytes and (*F*) adipocytes. Results are expressed as
728 mean + SEM, n=5-6. ***p<0.001 vs respective 100 nmol/L insulin; $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$,
729 $^{\dagger\dagger\dagger}p<0.001$ vs respective 10 nmol/L insulin; $^{\ddagger}p<0.05$ vs respective 1 nmol/L insulin;
730 $^{\S\S}p<0.01$ vs SC 10 nmol/L insulin; two-way ANOVA followed by Tukey post-testing.
731 NGT, normal glucose tolerance (white columns and symbols); SC, subcutaneous
732 adipocytes (light grey columns and symbols); T2D, type 2 diabetes (black columns
733 and symbols).
734
735 Fig. 4. Impaired stimulation of lipolysis in T2D adipocytes. *A:* Intracellular neutral
736 lipids stained with oil red O in pancreatic adipocytes differentiated in standard
737 medium or medium complemented with monounsaturated fatty acids (+MUFA).
738 Subcutaneous adipocytes (SC, light grey columns) differentiated in standard medium
739 were used as control. Scale bar = 150 µm. *B:* Quantification of oil red O stained lipid
740 droplets of 33 to 49 images from 3 independent preparations for each condition using
741 imageJ. *C-D:* cAMP-induced lipolysis measured as fatty acid release normalized to
742 the respective basal lipolysis rate of (*C*) pancreatic adipocytes of NGT-PPP and T2D-

743 PPP and (*D*) SC adipocytes. *E-H:* RNA expression of (*E*) insulin (*INSR*) and
744 glucagon receptors (*GCGR*), (*F*) beta-adrenoceptors (*ADRB1*, *ADBR2*, *ADBR3*), (*G*)
745 natriuretic peptide receptors (*NPR1*, *NPR2*) and phosphodiesterases (*PDE3B*,
746 *PDE5A*) in pancreatic adipocytes. *I:* ANP-induced lipolysis in NGT-PPP and T2D-
747 PPP. Results are expressed as mean + SEM, n=2-5. *p<0.05, **p<0.01, ***p<0.001
748 vs respective control; ‡‡p<0.01 vs SC under the same condition; §§p<0.01 vs ANP +
749 dipyridamole; one-way ANOVA followed by Tukey post-testing. †p<0.05, ††p<0.01 vs
750 respective control; t-test. NGT, normal glucose tolerance (white columns); T2D, type
751 2 diabetes (black columns). Isoproterenol 1 µmol/L, glucagon 10 nmol/L, insulin 10
752 nmol/L, forskolin 5 µmol/L, IBMX 100 µmol/L, ANP (atrial natriuretic peptide) 10
753 nmol/L, dipyridamole 10 µmol/L.
754
755 Fig. 5. Metabolic effect on fat cell secretome. *A:* Positive correlation of *LEP*/*ADIPOQ*
756 mRNA ratio to HbA1c. *B-G:* secreted adipokines, (*B*) adiponectin and (*C*) leptin;
757 angiogenic factors (*D*) VEGF and (*E*) HGF; cytokine (*F*) IL-6; and chemokine (*G*)
758 MCP-1 expressed as mean + SEM, n=3-4. *p<0.05, **p<0.01 vs respective
759 preadipocyte; ‡p<0.05 vs NGT preadipocytes; †p<0.05, ††p<0.01 vs NGT adipocytes;
760 t-test. A, adipocytes; HGF, hepatic growth factor; IL-6, interleukin-6; MCP-1,
761 monocyte chemoattractant protein 1; NGT, normal glucose tolerance (white symbols
762 and columns); P, preadipocytes; PD, prediabetes (grey symbols and columns); SC,
763 subcutaneous (light grey columns); T2D, type 2 diabetes (black symbols and
764 columns); VEGF, vascular endothelial growth factor.









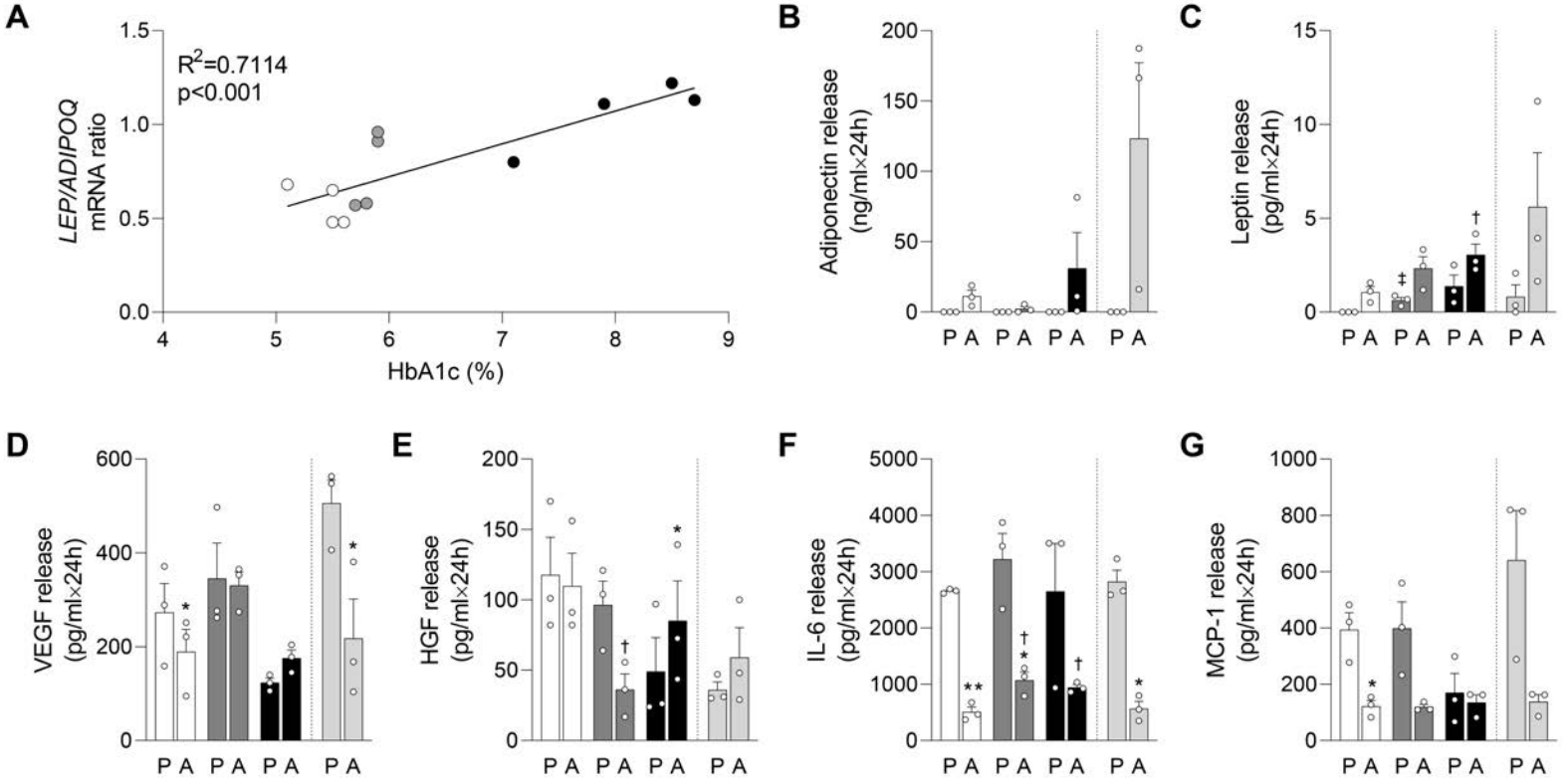


Table 1. Characterization of human pancreatic fat donors.

Trait	NGT	PD	T2D	p[1]
N (% males)	5 (80)	6 (100)	6 (83)	0.643
Age (years)	65.00 (16.00)	67.50 (21.00)	64.50 (13.00)	0.928
BMI (kg/m²)	24.11 (6.15)	27.62 (7.15)	27.58 (14.63)	0.309
Fasting glucose (mmol/l)	4.89 (1.01)	5.81 (1.36)	9.03 (4.02)**,††	0.011
HbA1c (%)	5.50 (0.25)	5.80 (0.25)‡‡	7.90 (1.58)***,†††	0.001
Fasting insulin (pmol/l)	52.00 (36.00)	69.00 (98.80)	63.00 (49.25)	0.878
Proinsulin (pmol/l)	1.00 (0.50)	2.00 (15.00)[b]	1.50 (2.25)	0.469
C-peptide (µg/l)	351.00 (191.00)	420.50 (292.00)	357.00 (603.50)	0.446
HOMA2%B	78.20 (9.85)	68.30 (19.35)	38.05 (45.87)**,††	0.009
HOMA2%S	89.00 (106.50)	80.00 (117.55)	68.50 (63.55)	0.223
HOMA2 disposition index	0.73 (0.86)	0.50 (0.75)	0.27 (0.57)‡	0.037
Triglycerides (mg/dl)	171.00 (131.50)	156.00 (114.50)	167.00 (275.70)	0.371
Total cholesterol (mg/dl)	268.00 (71.00)	215.00 (107.00)	190.00 (212.20)	0.889
HDL cholesterol (mg/dl)	35.00 (23.50)	41.50 (24.75)	27.50 (47.75)	0.967
LDL cholesterol (mg/dl)	184.00 (62.50)	136.50 (83.80)	113.50 (76.80)	0.379
Total AT (cm²)	418.80 (404.40)[a]	461.30 (334.00)[b]	474.70 (320.50)[c]	0.881
Subcutaneous AT (cm²)	233.10 (217.70)[a]	220.10 (193.10)[b]	241.00 (136.40)[c]	0.585
Visceral AT (cm²)	185.10 (216.10)[a]	247.60 (134.80)[b]	189.70 (225.30)[c]	0.910

Results expressed as median (IQR). [a]available from 4 subjects; [b]available from 5 subjects; [c]available from 3 subjects. $^{**}p<0.01$, $^{***}p<0.001$ vs NGT; $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ vs PD; one-way ANOVA followed by Tukey post-testing. $^{\ddagger}p<0.05$, $^{\ddagger\ddagger}p<0.01$ vs NGT; t-test. p[1], p-value between T2D and non-diabetics (NGT+PD). AT, adipose tissue; NGT, normal glucose tolerance; PD, prediabetes; T2D, type 2 diabetes.