Cytokine 161 (2023) 156080

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Interleukin-33 inhibits glucose uptake in human adipocytes and its expression in adipose tissue is elevated in insulin resistance and type 2 diabetes

Maria J Pereira [a,*], Ayesha Azim [a], Susanne Hetty [a], Bipasha Nandi Jui [a], Joel Kullberg [b,c], Martin H Lundqvist [a], Jan W Eriksson [a]

[a] Department of Medical Sciences, Clinical Diabetes and Metabolism, Uppsala University, Uppsala, Sweden
[b] Department of Surgical Sciences, Section of Radiology, Uppsala University, Uppsala, Sweden
[c] Antaros Medical, Mölndal, Sweden

ARTICLE INFO

Keywords:
IL-33
Type 2 diabetes
Adipose tissue
Glucose uptake
Inflammation

ABSTRACT

Objective: Interleukin-33 (IL-33) is associated with obesity-related inflammation. We aim to investigate IL-33 expression in subcutaneous adipose tissue (SAT) in type 2 diabetes (T2D) subjects and its effects on human adipocyte glucose uptake.

Methods: Expression of IL-33 was analysed in SAT from cohort studies including subjects with and without obesity and T2D and correlated with insulin resistance and obesity markers. Magnetic resonance imaging (MRI) of tissue fat volumes was performed. We investigated the effects of IL-33 treatment on *ex vivo* adipocyte glucose uptake.

Results: T2D subjects had higher IL-33 gene and protein expression in SAT than the control subjects. IL-33 mRNA expression was positively correlated with markers of dysglycemia (e.g. HbA1c), insulin resistance (e.g. HOMA-IR) and adiposity (BMI, visceral adipose tissue volume, liver and pancreas fat %). In multiple linear regression analyses, insulin resistance and T2D status were the strongest predictors of IL-33, independent of BMI. IL-33 mRNA expression was negatively correlated with expression of genes regulating adipocyte glucose uptake, lipid storage, and adipogenesis (e.g.glucose transporter 1 and 4 (*GLUT1/4*), fatty acid binding protein 4 (*FABP4*), and *PPARG*). Additionally, incubation of SAT with IL-33 reduced adipocyte glucose uptake and GLUT4 gene and protein expression.

Conclusions: Our findings suggest that T2D subjects have higher IL-33 gene and protein expression in SAT than control subjects, which is associated with insulin resistance and reduced gene expression of lipid storage and adipogenesis markers. IL-33 may reduce adipocyte glucose uptake. This opens up a potential pharmacological route for reversing insulin resistance in T2D and prediabetes.

1. Introduction

Obesity promotes a state of low-grade systemic inflammation that contributes to metabolic disorders such as cardiovascular diseases and type 2 diabetes (T2D) [1]. Althought several studies have demonstrated that the immune cells and cytokines have an important role on metabolic regulation [2–3], the mechanisms underlying obesity-induced inflammation and subsequent metabolic dysregulation are not completely understood.

Interleukin-33 (IL-33) is a member of the IL-1 cytokine family that controls innate and adaptive immune cell activity in adipose tissue. In adipose tissue, IL-33 is predominantly produced by resident mesenchyme-derived stromal cells [4–5], including epithelial cells, endothelial cells, and adipose progenitor cells [6–7]. IL-33 is released upon cell injury or tissue damage and acts as an "alarmin" by targeting resident immune cells that express the IL-33 receptor, called ST2 (serum stimulation-2, encoded by *IL1RL1* gene) [8]. IL-33 acts in many different cell types, such as T_{regs}, T_H2 lymphocytes, mast cells, and type-2 innate

* Corresponding author at: Department of Medical Sciences, Uppsala University - Rudbecklaboratoriet hus R3, våning 2 Dag Hammarskjölds väg 20 - 751 85 Uppsala, Sweden.
E-mail address: maria.pereira@medsci.uu.se (M.J. Pereira).

https://doi.org/10.1016/j.cyto.2022.156080
Received 8 June 2022; Received in revised form 5 October 2022; Accepted 18 October 2022
Available online 8 November 2022
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lymphoid cells (ILC2) (reviewed [9]) and upregulates the expression of type 2 cytokines (e.g. IL-4, IL-5, IL-13) [10] which contributes to the maintenance of adipose tissue homeostasis. IL-33 signal transduction also depends on the expression of ST2 [11]. ST2 has two alternatively spliced isoforms; the membrane-bound ST2 receptor and the circulating or soluble form of ST2 (sST2). The latter contains the extracellular domain responsible for IL-33 binding but lacks the transmembrane and intracellular domain. The interaction of sST2 with IL-33 blocks the IL-33/ST2 transduction pathway and counteracts the effect of IL-33, thus acting as a decoy receptor (31). In addition to its receptor-dependent effects, IL-33 has a nuclear role in the transcriptional regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) [12]. The IL-33/ST2 axis has also been implicated in the pathogenesis of chronic inflammatory diseases, such as rheumatoid arthritis [13,14], as well as having anti-inflammatory effects in obesity and atherosclerosis [15,16].

In recent years, IL-33 has been suggested to have a role in adipose tissue homeostasis. Mice lacking IL-33 or its receptor ST2 develop increased adiposity and worsened metabolic profiles following high-fat diet feeding [17,18]. IL-33 treatment of obese mice reduces visceral adipose tissue and adipocyte size, attenuates adipose tissue inflammation, and ameliorates insulin resistance [17–19]. Because IL-33 mediates its effects through type 2 immune cells, it would be reasonable to expect that its expression is reduced in obesity. However, results from human studies have given inconsistent results. Some studies have shown that IL-33 expression in adipose tissue and circulating levels are elevated in metabolically unhealthy overweight/obese individuals [7,20] compared to metabolically healthy overweight/obese individuals. In contrast, others have shown that IL-33 is negatively correlated with BMI and body weight in lean/overweight, but not in obese, individuals [21]. The divergent results could also indicate that other potential mechanisms, such as IL-33 neutralisation by the soluble form of its receptors, or its inactivation by caspases (reviewed in [22]), which in turn, may explain the impaired action of IL-33 in obesity.

Limited research has been performed concerning the role of IL-33 in adipose tissue metabolism. Hasan A et al. [23] found that IL-33 expression in adipose tissue is inversely associated with glycemia in subjects with T2D but not in subjects with prediabetes. However, in that study, T2D subjects were on any type of glucose-lowering medication, which may have altered the outcome. Additionally, the direct effects of IL-33 on adipocyte glucose metabolism have not been explored. In the current study, we aim to investigate the expression levels of IL-33 in human subcutaneous adipose tissue (SAT) in subjects with or without T2D and obesity and its association with genes involved in adipose tissue metabolism. In addition, we investigated the effects of IL-33 treatment on human adipocyte glucose uptake.

2. Methods

2.1. Subjects

A **first cohort** (*cohort 1*) included 20 control and 20 T2D subjects matched for sex, age and BMI. The T2D subjects were on a stable dose of metformin as their only diabetes medication. Blood biochemical analysis, SAT biopsies, oral glucose tolerance test (OGTT), and assessment of body volume composition using magnetic resonance imaging (MRI) were performed, as previously described [24]. This cohort was used to explore IL-33 mRNA expression in SAT and associations with insulin resistance and obesity markers.

A **second cohort** (*cohort 2*) included 9 control and 11 T2D subjects and was used to validate associations from the first cohort.

A **third cohort** (*cohort 3*), included 11 healthy subjects (7 men and 4 women, age 25 (21-31) years (median (interquantile range, IR)), BMI 25.9 (22.6–30.7) Kg/m^2, and was used to study the direct effect of IL-33 treatment of adipose tissue on gene expression of key factors related to adipose tissue function and on adipocyte glucose uptake.

Anthropometric and clinical characteristics of subjects from *cohort 1* and *2* are presented in Table 1. Anthropometric measurements, fasting blood samples and SAT biopsies were collected at the Department of Medical Sciences, Clinical Diabetology and Metabolism, Uppsala University, Sweden. Blood biochemical analyses were performed at the Department of Clinical Chemistry, Uppsala University Hospital. SAT samples were obtained by needle aspiration of the lower part of the abdomen after local dermal anaesthesia with lidocaine (Xylocaine, AstraZeneca, Södertälje, Sweden). One part of the adipose tissue biopsy (*cohort 1–2*) was snap-frozen in liquid nitrogen and used for RNA-seq and western-blot. Another part of the tissue was used to isolate adipocytes for glucose uptake and lipolysis assays, and adipocyte size, as previously reported [24,25].

Subjects with type 1 diabetes, endocrine disorders, cancer, or other major illnesses were excluded, as were those having ongoing medication with systemic glucocorticoids, beta-blockers, and immune-modulating therapies. The study was approved by the Regional Ethics Review Board in Uppsala (Dnr2013/330, Dnr2013-183/494, Dnr2018/385), and the reported investigations have been carried out following principles endorsed by the Declaration of Helsinki. All participants gave their written informed consent.

2.2. MRI

In *cohort 1*, MRI was used to assess volumes of abdominal SAT, visceral adipose tissue (VAT), and liver and pancreas fat content. VAT and SAT volumes were normalized to body weight (kg) to adjust for body size. Methods have previously been reported [24].

2.3. Adipose tissue incubation with IL-33

SAT was incubated in DMEM containing 6 mM glucose (Sigma, St Louise MO, USA), 10 % bovine serum albumin (Gibco, Life Technologies, Paisley, UK) and 1 % penicillin–streptomycin (PEST, Gibco, Life Technologies) without (control) or with two concentrations of recombinant IL-33 (200 & 1000 pg/ml) (Sigma, St. Louise, MO, USA) for 24 h at 37 °C, 5 % CO_2. Physiological serum IL-33 concentrations have been shown to range from 25 pg/mL in healthy control subjects to 225 pg/mL in obese subjects [20]; therefore, to reflect clinical condition and achieve a maximal effect, both physiological (200 pg/ml) and supraphysiological (1000 pg/ml) concentrations were used. Following incubation with IL-33, part of the adipose tissue was used to assess gene and protein expression levels and part was digested with collagenase as described below, and isolated adipocytes were used for glucose uptake assay.

2.4. Western-blot

Adipose tissue from control and T2D subjects (n = 3, both), or incubated with and without IL-33 for 24 h (n = 4), was homogenised in ice-cold lysate buffer (25 mM Tris-HCl; 0.5 mM EGTA; 25 mM NaCl; 1 % Nonidet P-40; 1 mM Na3VO4; 10 mM NaF (all from Sigma), 100 nM okadaic acid (Alexis Biochemicals, Lausen, Switzerland), 1X Complete protease inhibitor cocktail (Roche, Indianapolis, IN, USA), and pH 7.4) at 4 °C for 2 h. The supernatant, a fat-free extract, was obtained by centrifugation at 12 000 g, 15 min, 4 °C, and was collected and saved at − 80 °C. Protein content was measured with the BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Total lysate (15 µg/lane) were loaded into Mini-PROTEAN TGX Stain-free precast gels (Bio-Rad, Richmond, CA) and subjected to SDS-PAGE, transferred to a nitrocellulose membrane and immunoblotted with anti-IL-33 (1:1000, MA5-15773, Invitrogen by Thermo Scientific Fisher, Rockford, IL, USA) and anti-GLUT4 (1:1000, MA1-83191, Invitrogen) overnight at 4 °C. Membranes were then incubated with secondary antibody linked to horseradish peroxidase-conjugated anti-mouse (1:1000, Cell Signaling Technologies) for 1 h at room temperature. Anti-GAPDH (1:1000,

Table 1
Anthropometry and blood chemistry of adipose tissue donors.

	Cohort 1			*Cohort 2*		
	Control	T2D	P-value	Control	T2D	P-value
Men/Women (number)	(10/10)	(10/10)		(3/6)	(2/9)	
Age (years)	60 (52–68)	60 (52–65)	0.695	59 (51–63)	54 (43–59)	0.102
Plasma glucose (mmol/L)	5.9 (5.5–6.7)	8.1 (7.1–9.2)	< 0.001	6.0 (5.7–6.2)	8.5 (7.3–8.8)	< 0.001
Serum insulin (mU/L)	10.4 (8.0–12.8)	15.3 (9.9–19.4)	0.040	7.3 (6.2–11.8)	28.0 (22.0–38.0)	< 0.001
Serum C-Peptide (nmol/L)	0.90 (0.65–1.09)	1.0 (0.85–1.28)	0.155	0.74 (0.51–0.83)	1.61 (1.30–1.78)	< 0.001
HbA1c, IFCC (mmol/mol)	37 (34–40)	47 (42–55)	< 0.001	36 (31–39)	55 (47–58)	< 0.001
HbA1c, %	5.54 (5.29–5.79)	6.41 (6.02–7.14)	< 0.001	5.45 (4.99–5.72)	7.18 (6.45–7.46)	< 0.001
HOMA-IR	2.83 (1.87–3.65)	4.31 (3.32–7.63)	0.004	1.80 (1.60–3.20)	9.85 (7.33–13.10)	< 0.001
Matsuda	3.69 (2.51–5.72)	2.53 (1.45–3.29)	0.020	5.63 (2.88–6.78)	1.38 (1.02–1.59)	< 0.001
Plasma HDL-cholesterol (mmol/L)	1.25 (1.20–1.48)	1.15 (0.98–1.28)	0.043	1.40 (1.15–1.60)	0.96 (0.88–1.00)	0.003
OGTT AUC Glucose (min*mmol/L)	1333 (1163–1616)	2453 (2058–2787)	< 0.001	1244 (1187–1842)	2388 (2194–2938)	< 0.001
OGTT AUC Insulin (min*mU/L)	9103 (6947–11765)	7254 (5037–11014)	0.160	7046 (5289–13436)	14,074 (8704–23049)	0.102
OGTT AUC FFA (min*μmol/L)	22,289 (19381–28183)	29,998 (24796–38154)	0.005	13,750 (6228–14056)	24,185 (21955–30812)	0.002
BMI (kg/m^2)	30.7 (28.2–34.6)	30.06 (26.86–34.32)	0.808	28.6 (25.1–30.8)	37.3 (32.5–39.2)	< 0.001
Waist-hip-ratio	0.98 (0.89–1.01)	0.99 (0.95–1.03)	0.199	0.92 (0.88–0.93)	0.97 (0.94–1.00)	0.014
SAT volume/body weight (mL/kg)	50.9 (40.3–64.1)	46.1 (30.8–63.8)	0.545	na	na	
VAT volume/body weight (mL/kg)	31.6 (27.0–40.6)	42.1 (31.5–45.9)	0.088	na	na	
Liver fat (%)	7.8 (2.6–15.5)	13.7 (5.1–20.2)	0.220	na	na	
Pancreas fat (%)	4.1 (1.5–10.4)	7.3 (3.6–14.3)	0.220	na	na	
Adipocyte diameter (μm)	108 (100–117)	108 (97–114)	0.507	111 (91–124)	115 (98–120)	0.621
Body fat (%)	na	na	na	33 (25–39)	44 (40–48)	0.011

Data are median (IQR).
HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model of insulin resistance; HDL, high density lipoprotein; OGTT AUC, oral-glucose-tolerance test, area under the curve; FFA, free-fatty acids; BMI, body mass index; SAT, subcutaneous adipose tissue volume; VAT, visceral adipose tissue volume; na = not available.

ABS16, Millipore, Temecula, CA) or total protein (stain-free technology, Bio-Rad) were used as the loading control. Detection was made with chemiluminescence reagent (ECL, Amersham Biosciences GE Healthcare) using a ChemiDocTM MP System (Bio-Rad).

2.5. Gene expression

Transcriptomics. SAT from cohort 1 and cohort 2 was used for RNAseq at Exiqon A/S [26], and Novogene [27], respectively. The data was used to determine IL-33 expression in control and T2D subjects and correlations between IL-33 mRNA and markers of hyperglycemia, insulin resistance, and adiposity and expression of adipose tissue function genes and inflammatory markers.

Real-time quantitative PCR. Total RNA was extracted from whole adipose tissue following incubation with or without IL-33 with the phenol–chloroform extraction method [28,29]. In brief, adipose tissue was first homogenised with Trizol (Sigma), and chloroform (Sigma) was used for phase separation. Isopropanol and GlycoBlue (Invitrogen, ThermoFisher Scientific) were added to the aqueous phase in a new tube and incubated overnight at −20 °C. On the second day, the pellet was washed 3 times with 70 % ethanol, left to air dry, and diluted in nuclease-free water. The concentration and purity of total RNA were measured with the Nanodrop (Thermo Scientific). cDNA was synthesised using a High-Capacity cDNA Reverse Transcriptase kit (Applied Biosystems) according to the manufacturer's guidelines. mRNA expression was determined using TaqMan gene expression assays (Thermo Fisher) for peroxisome proliferator-activated receptor gamma (*PPARG*, Hs01115513), fatty acid synthase (*FASN*, Hs01005622), adipose triglyceride lipase (*ATGL*, Hs00982042), and glucose transporter 4 (*SLC2A4*, Hs00168966). Gene expression was detected using the QuantStudio 3 sequence detection system (Applied Biosystem) and calculated using a $2^{-\Delta\Delta CT}$. The gene expression levels were normalised to the housekeeping gene *GUSB* (Hs00939627), and all samples were run in duplicates.

2.6. Adipocyte isolation and glucose uptake assay

Adipocytes were isolated and used for glucose uptake, as previously reported [30,31]. Briefly, adipose tissue was digested with collagenase type II (Roche, Manheim, Germany) in Hank's medium 199 (Hank's salts, L-Glutamine, 25 mM HEPES, L-amino acids) (Invitrogen Corporation, Paisley, UK) containing 5.6 mM glucose, 4 % bovine serum albumin (BSA) (Sigma, MO, USA), 150 nM adenosine (Sigma, MO, USA), and pH 7.4 (adjusted with NaOH) for 60 min at 37 °C at 105 rpm in a shaking water-bath. Digested adipose tissues were filtered using 250 μm nylon mesh, and then the cells were washed 4 times with glucose-free Krebs-Ringer bicarbonate medium (KRH) at 5 min intervals. After collagenase digestion, isolated adipocytes were diluted 1:10 in KRH media and incubated at 37 °C with or without human insulin (25 and 1000 μU/ml) for 15 min, followed by an additional incubation period of 45 min with D-[U-14C] glucose (0.26 mCi/ml, 0.86 μM) (Amersham Biosciences, GE Healthcare, Buckinghamshire, UK). Cells were separated from the medium by centrifugation with 1 ml of silicone fluid 100 cS (VWR Chemicals, Leuven, Belgium). Thereafter, the cell pellets were transferred into vials containing 4 ml of scintillation cocktail (Ultima Gold LSC-cocktail from PerkinElmer, Waltham, MA) and glucose uptake rate was calculated as cellular clearance of medium glucose = (c.p.m. cells × volume)/(c.p.m. medium × cell number × time). The vials were put in a Liquid Scintillation Analyser (Perkin Elmer, MA, USA), and cell-associated radioactivity was measured. Cell size (μm in diameter) of 100 adipocytes from the same subject was measured. Experiments were performed in triplicates. Glucose uptake was normalised per cell number.

2.7. Statistical analysis

Data are represented as median and IQR (25th percentile – 75th percentile) unless stated otherwise. Data were checked for normality using the Shapiro-Wilk test and by analysing histograms. Spearman's correlation test and multiple linear regressions were performed between IL-33 mRNA expression and markers of insulin resistance and obesity. Differences between control and T2D subjects were analysed with Mann-Whitney *t*-test. Glucose uptake data were analysed with repeated measures ANOVA, and multiple comparisons were corrected for the false discovery rate using the original Benjamini-Hochberg method. All statistical analyses were performed using IBM SPSS Statistics version 28 and GraphPad Prism 9 software. P-value < 0.05 was considered statistically significant.

3. Results

3.1. IL-33 gene expression in control and T2D subjects

T2D subjects had 1.7-fold higher gene expression of IL-33 ($P < 0.001$) compared to control subjects (Fig. 1A). Furthermore, similar results were observed for *cohort 2* (mean ± SEM; Control: 10.0 ± 1.0 FPKM and T2D: 16.5 ± 1.4 FPKM, $P = 0.018$, data not shown). The IL-33 protein expression showed results consistent with the gene expression (Fig. 1B-C). The expression of the IL-33 receptor, the *IL1RL1* gene, did not differ between the control and T2D subjects (Fig. 1D).

3.2. Association between IL-33 mRNA in adipose tissue and markers of hyperglycemia, insulin resistance, and adiposity

Associations between IL-33 mRNA levels and markers of hyperglycemia, insulin resistance and obesity were explorative and are shown in Table 2 and Fig. 1E-F. When pooling control and T2D together from *cohort 1*, IL-33 mRNA expression in adipose tissue was positively correlated with markers of hyperglycemia and insulin resistance, including fasting glucose, glucose AUC during OGTT, HbA1c, homeostasis model assessment for insulin resistance (HOMA-IR) and free-fatty acids (FFA) AUC during OGTT (all $P < 0.01$), and markers of central adiposity (VAT/body weight and liver and pancreas fat %, all $P < 0.05$), whereas it was negatively correlated with the Matsuda insulin sensitivity index ($P < 0.001$). When subdividing the subjects by diabetes status ($n = 20$/group), the correlation coefficient followed the same direction, but only HOMA-IR and VAT volume/body weight positively correlated with IL-33 mRNA in T2D subjects ($P < 0.05$). In the control group, IL-33 mRNA positively correlated with several markers of insulin resistance including HOMA-IR, fasting insulin and C peptide, and insulin AUC during OGTT ($p < 0.05$), and negatively to Matsuda index ($P < 0.05$). There were no associations between IL-33 mRNA and BMI, waist-hip-ratio, and subcutaneous adipocyte size in *cohort 1*. The possible associations were repeated in *cohort 2* (Table 2), which included subjects with a wider range of BMI (23.2–40.1 kg/m^2) and HOMA-IR (0.50–19.20). In *cohort 2*, IL-33 mRNA also correlated with several markers of hyperglycemia (HbA1c, fasting glucose, OGTT AUC glucose, all $P < 0.05$) and insulin resistance HOMA-IR ($p = 0.006$). In addition, IL-33 mRNA correlated positively with BMI ($P = 0.013$). Expression of the IL-33 receptor, ST2 (*IL1RL1* gene), did not correlate with any of the glycemic or insulin resistance parameters (data not shown) but was positively associated with BMI ($P = 0.009$). In the multivariate regression analyses, Matsuda index (insulin sensitivity) and T2D status were the strongest predictors of IL-33 mRNA expression in cohort 1 and 2. This effect was independent of BMI, WHR, HbA1c, age and sex (Table 3). This model was repeated pooling *cohort 1* and *2* (total 60 subjects), and only T2D status (standardized beta coefficient: 0.57, 95 % CI 3.67, 13.71; $p < 0.01$) predicted IL-33 gene expression in human SAT (r^2 model: 0.37, $p < 0.01$).

3.3. Association between IL-33 expression in adipose tissue and glucose uptake in adipocytes and gene expression of insulin signalling factors

IL-33 mRNA expression in adipose tissue negatively correlate with insulin-stimulated glucose uptake in isolated adipocytes ($P < 0.05$, Fig. 2B) and there was a negative trend with basal glucose uptake ($P = 0.059$, Fig. 2A). Furthermore, IL-33 mRNA expression negatively correlated with gene expression of different insulin signalling factors, i.e. serine-threonine kinase 1 (*AKT1*, $P < 0.01$), *SLC2A1* gene encoded for

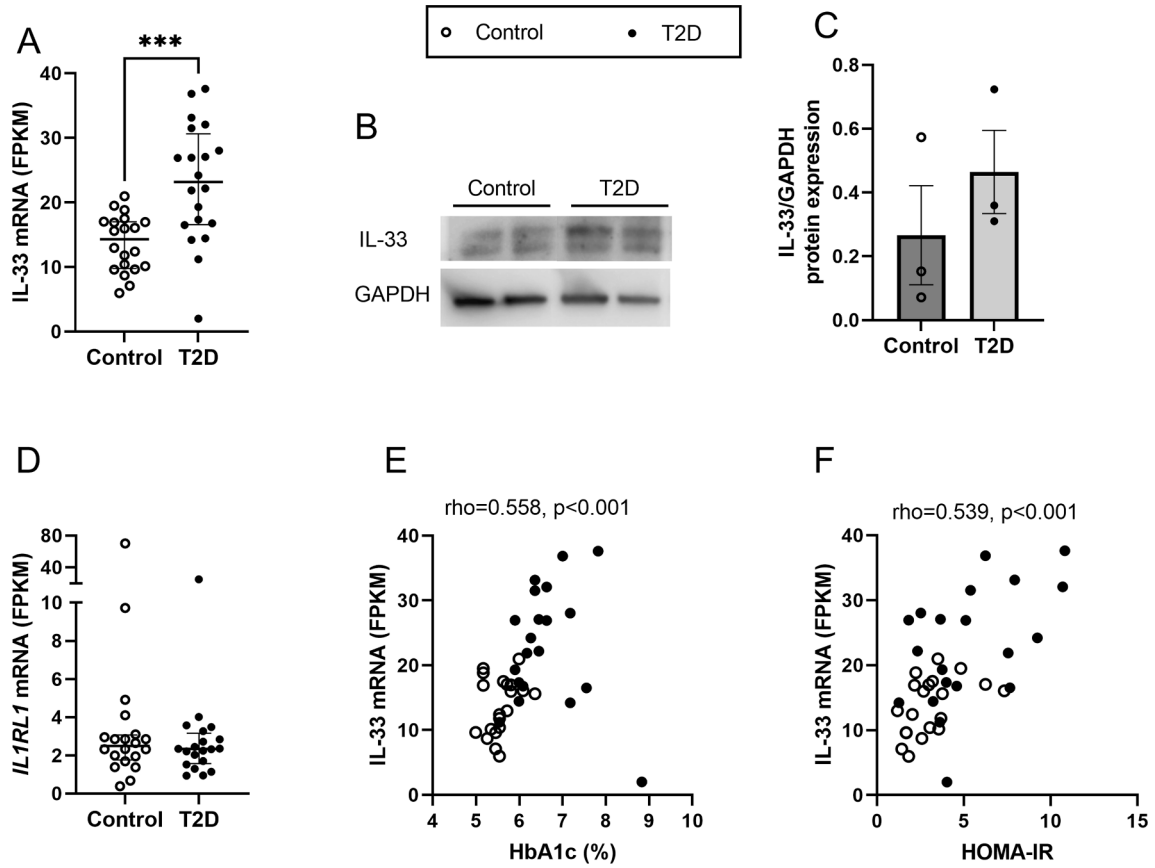

Fig. 1. IL-33 expression in SAT is increased in T2D subjects and associated with hyperglycemia and insulin resistance markers.Expression of IL-33 mRNA (A) and protein (B-C), and its receptor ST2 (*IL1RL1* mRNA) (D) in SAT from controls and T2D subjects in *cohort 1* matched for sex, age and BMI (mRNA, $n = 20$/group; protein, $n = 3$/group). Correlation between IL-33 mRNA levels in SAT and HbA1C (E) and the homeostasis model of insulin resistance HOMA-IR (F). ***$P < 0.001$.

Table 2
Associations between IL-33 gene expression in SAT and markers of hyperglycemia, insulin resistance and adiposity.

	IL-33 mRNA Cohort 1						IL-33 mRNA Cohort 2	
	All		*Control*		*T2D*		*All*	
	Rho	P-value	Rho	P-value	Rho	P-value	Rho	P-value
Hyperglycemia and insulin resistance markers								
HbA1c	**0.558**	**< 0.001**	0.275	0.240	0.224	0.343	**0.489**	**0.029**
HOMA-IR	**0.539**	**< 0.001**	**0.525**	**0.018**	**0.462**	**0.040**	**0.589**	**0.006**
Fasting glucose	**0.558**	**< 0.001**	0.414	0.070	0.402	0.079	**0.486**	**0.030**
Fasting insulin	**0.525**	**0.001**	**0.463**	**0.040**	0.334	0.150	**0.564**	**0.010**
Fating C-peptide	**0.443**	**0.004**	**0.565**	**0.009**	0.300	0.199	**0.533**	**0.015**
HDL-cholesterol	**−0.326**	**0.040**	−0.161	0.497	−0.094	0.693	−0.443	0.051
OGTT AUC glucose	**0.522**	**< 0.001**	0.319	0.171	0.174	0.462	**0.472**	**0.036**
OGTT AUC insulin	0.123	0.451	**0.537**	**0.015**	0.021	0.930	0.269	0.251
OGTT AUC FFA	**0.398**	**0.011**	0.063	0.791	0.370	0.108	0.420	0.066
Matsuda	**−0.539**	**< 0.001**	**−0.519**	**0.018**	−0.391	0.088	−0.364	0.115
Adiposity								
BMI	0.034	0.834	0.275	0.240	−0.131	0.582	**0.544**	**0.013**
Waist-hip-ratio	0.199	0.217	−0.023	0.223	0.402	0.079	0.332	0.152
VAT volume/body weight	**0.371**	**0.028**	0.153	0.572	**0.470**	**0.042**	na	
SAT volume/body weight	−0.150	0.389	−0.209	0.438	−0.188	0.442	na	
VAT/SAT volume	0.269	0.119	0.053	0.846	0.389	0.099	na	
Liver fat %	**0.350**	**0.039**	0.260	0.330	0.437	0.061	na	
Pancreas fat %	**0.341**	**0.045**	0.076	0.778	0.234	0.334	na	
Subcutaneous adipocyte size	0.052	0.751	0.009	0.970	0.179	0.450	−0.200	0.398
Adipocyte glucose uptake ex vivo								
Basal	−0.305	0.059	−0.245	0.298	−0.093	0.705	**−0.574**	**0.008**
Insulin-stimulated (1000 μU/mL)	**−0.394**	**0.013**	−0.075	0.753	−0.398	0.091	**−0.639**	**0.002**
Adipocyte lipolysis ex vivo								
Basal	−0.141	0.386	−0.098	0.682	−0.320	0.169	0.101	0.689
Isoprenaline-stimulated (0.5 μM)	−0.251	0.118	−0.259	0.271	−0.307	0.188	−0.075	0.769

Table presents Spearman's rho correlation coefficient.
Significant Spearman rank correlation values are shown in bold: $P < 0.05$.
HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model of insulin resistance; HDL, high density lipoprotein; OGTT AUC, oral-glucose-tolerance test, area under the curve; FFA, free-fatty acids; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; na = not available.

Table 3
Multilple linear regression of IL-33 mRNA in adipose tissue from subjects with and without T2D *vs* insulin sensitivity and obesity.

	Standardised beta coefficient	SE	95 % CI	p-value	R^2
Cohort 1 (n = 40)					
Matsuda	**−0.35**	0.70	[-3.00, −0.15]	**0.032**	
BMI	−0.17	0.29	[-0.90, 0.29]	0.309	
T2D	**0.43**	3.20	[0.76, 13.81]	**0.030**	
HbA1C	−0.10	2.04	[-5.17, 3.16]	0.625	
WHR	0.19	25.06	[-26.36, 75.73	0.332	
					0.44**
Cohort 2 (n = 20)					
BMI	−0.17	0.32	[-0.84, 0.53]	0.635	
T2D	**0.87**	3.58	[0.075, 16.00]	**0.033**	
					0.45*

Models adjusted for sex and age.
SE, coefficients standard error; CI, confidence interval (lower bound, upper bound); BMI, body mass index; T2D, type 2 diabetes; HbA1c, glycosylated haemoglobin; WHR, waist hip-ratio.
Bold values indicate statistical significance. *$P < 0.05$, **$P < 0.01$.
Data are pooled from all participants, *cohort 1*: 20 control and 20 T2D subjects and *cohort 2*: 9 control and 11 T2D subjects.

glucose transporter 1 (GLUT1, $P < 0.01$) and *SLC2A4* gene encoded for glucose transporter 4 (GLUT4) ($P < 0.01$) (Fig. 2C-E).

3.4. Association between IL-33 gene expression in adipose tissue and gene markers of adipogenesis and lipid storage

Correlations between IL-33 mRNA expression and genes related to adipogenesis, lipid storage and cytokines were performed in *cohort 1*. We found a significant negative association between IL-33 mRNA level and master adipogenesis regulators *PPARG, CEBPA and CEBPB* ($P < 0.01$, all) (Table 4). Similar significant negative associations were found between IL-33 mRNA and several genes involved in lipid storage, including fatty acid binding protein 4 (*FABP4,* $P < 0.05$), 1-acylglycerol-3-phosphate o-acyltransferase 1, 2 and 3 (*AGPAT1/2/3*, $P < 0.01$), and diacylglycerol o-acyltransferase 1 and 2 (*DGAT1/2,* $P < 0.06$). Furthermore, IL-33 mRNA negatively correlated with genes regulating adipocyte lipolysis (e.g. adipose triglyceride lipase (*ATGL*) and monoglyceride lipase (*MGLL*), $P < 0.01$) (Table 4). We selected some of the key genes that are involved in adipogenesis and lipid storage that were found to be significantly associated with IL-33 expression, and we measured their expression in human adipose tissue following treatment with IL-33 for 24 h. Our results show that IL-33 treatment reduces the expression of the master adipogenesis regulator *PPARG* (Fig. 3A) and the lipid storage mediator *FASN* (Fig. 3B). However, IL-33 did not affect *ATGL* expression (Fig. 3C), which was consistent with the lack of association between IL-33 mRNA expression and *ex vivo* adipocyte lipolysis (Table 2).

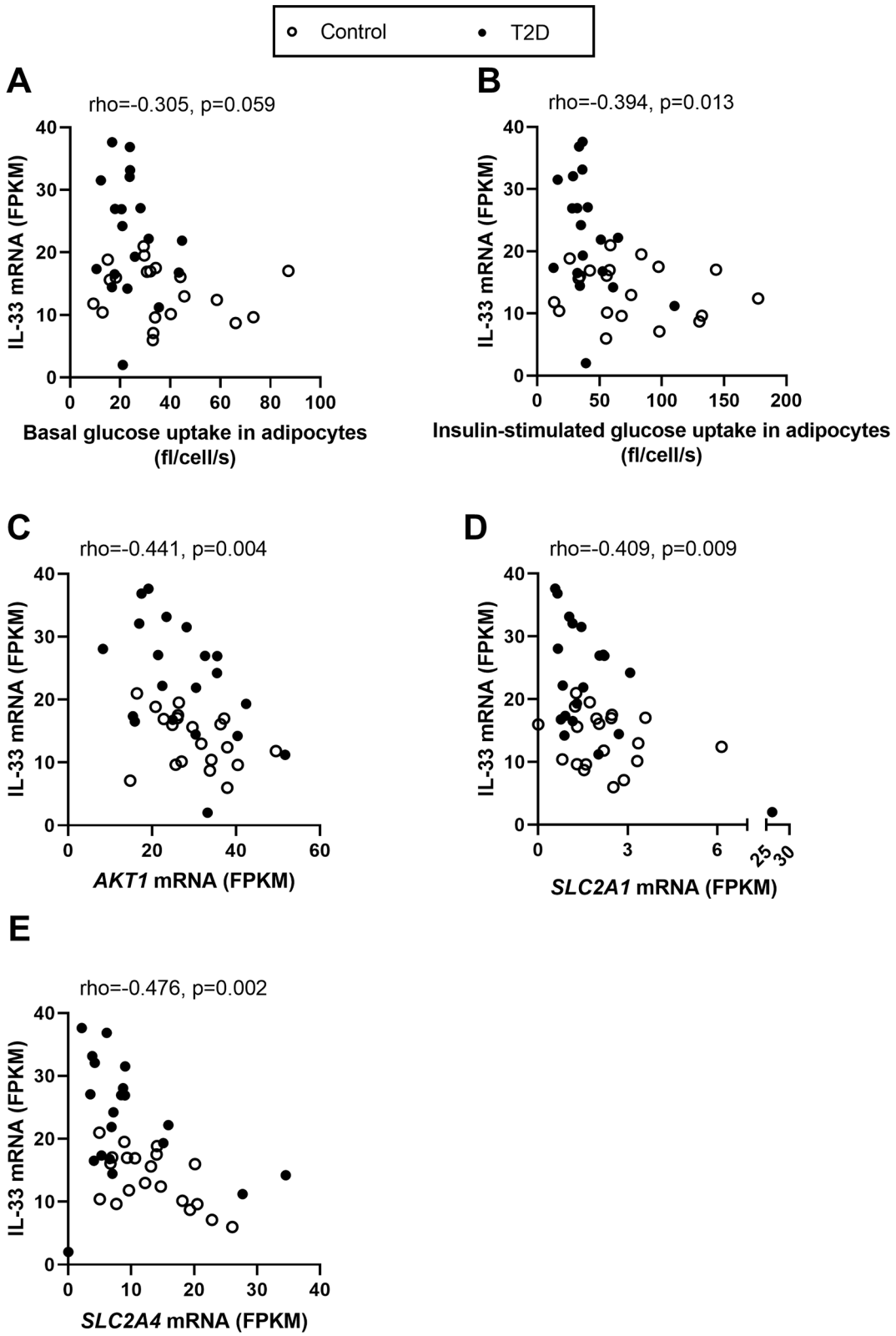

Fig. 2. Association between IL-33 mRNA expression in SAT and adipocyte glucose uptake. IL-33 mRNA expression in SAT negatively correlated with (A) basal and (B) insulin-stimulated (1000 μU/mL) glucose uptake in isolated adipocytes and with the mRNA expression of (C) serine-threonine protein kinase 1 (*AKT1)* and the (D) glucose transporter, GLUT1 (*SLC2A1*) and (E) GLUT4 (*SLC2A4*).

Table 4
Correlation between IL-33 gene expression in SAT and the gene expression of adipose tissue function genes and inflammatory markers.

	Cohort 1	
	Rho	P-value
Adipokines		
ADIPOQ, Adiponectin	0.210	0.194
LEP, leptin	−0.226	0.161
Adipogenesis and lipid storage		
PPARG, Peroxisome Proliferator Activated Receptor Gamma	**−0.422**	**0.007**
CEBPA, CCAAT Enhancer Binding Protein Alpha	**−0.504**	**0.001**
CEBPB, CCAAT Enhancer Binding Protein Beta	**−0.508**	**0.001**
FASN, Fatty Acid Synthase	**−0.447**	**0.004**
LPL, Lipoprotein Lipase	0.046	0.776
FABP4, Fatty Acid Binding Protein 4	**−0.335**	**0.034**
AGPAT1, 1-Acylglycerol-3-Phosphate O-Acyltransferase 1	**−0.549**	**< 0.001**
AGPAT2, 1-Acylglycerol-3-Phosphate O-Acyltransferase 1	**−0.495**	**< 0.001**
AGPAT3, 1-Acylglycerol-3-Phosphate O-Acyltransferase 1	**−0.522**	**0.001**
DGAT1, Diacylglycerol O-Acyltransferase 1	**−0.436**	**0.005**
DGAT2, Diacylglycerol O-Acyltransferase 2	−0.305	0.055
Lipolysis		
ATGL, Adipose triglyceride lipase	**−0.450**	**0.004**
LIPE, Lipase E, Hormone Sensitive Type	−0.278	0.083
MGLL, Monoglyceride Lipase	**−0.462**	**0.003**
ADRA2B, Adrenoceptor Alpha 2B	−0.162	0.318
ADRB2, Adrenoceptor Beta 2	−0.037	0.819
IL-1 family of cytokines		
IL1A, Interleukin-1 alpha	−0.179	0.270
IL1B, Interleukin 1 beta	−0.099	0.541
IL1RN, Interleukin-1 receptor antagonist	0.095	0.559
IL18, Interleukin-18	0.086	0.599
IL36B, Interleukin-36 beta	0.285	0.075
IL36G, Interleukin-36 gamma	−0.146	0.370
IL37, Interleukin-37	−0.282	0.078
Th2 cytokines		
IL4, Interleukin-4	−0.065	0.688
IL5, Interleukin-5	−0.026	0.873
IL13, Interleukin-13	−0.024	0.881

Table presents Spearman's rho correlation coefficient. Bold values indicate statistical significance ($P < 0.05$).

3.5. Association between IL-33 mRNA expression with gene expression of inflammatory cytokines

IL-33 mRNA did not significantly associate with mRNA expression of other IL-1 family cytokines (IL-1A, IL-18, IL-36A, IL-36B, IL-37, and IL-38) (Table 4). In addition, no significant associations were found with other Th2 cytokines (IL-4, IL-5, and IL-13) (Table 4) or with the IL-33 receptor gene *IL1RL1* (data not shown).

3.6. Effect of IL-33 treatment on adipocyte glucose uptake

We next determined whether IL-33 treatment directly affects the expression of insulin signalling factors and adipocyte glucose uptake. Treatment of human adipose tissue with IL-33 reduced gene and protein levels of the glucose transporter GLUT4 (Fig. 3D-F). No changes were observed in mRNA or protein levels of IRS1 and GLUT1, and neither total or phosphorylated AKT following insulin exposure (data not shown). Furthermore, we performed glucose uptake in isolated adipocytes following an *ex vivo* treatment of adipose tissue without (control) or with IL-33. Following a 24 h incubation of SAT with IL-33 (1000 pg/ml), both basal and insulin-stimulated glucose uptake were significantly reduced in isolated adipocytes by about 25–30 % ($P < 0.05$) compared to the control (Fig. 3G).

4. Discussion

The purpose of this study was to investigate the involvement of IL-33 in adipose tissue metabolism. We demonstrated that IL-33 gene and protein expression in human SAT is increased in T2D subjects and associated with insulin resistance. Furthermore, *ex vivo* treatment of SAT with IL-33 reduced glucose uptake in isolated adipocytes.

Our analyses of IL-33 expression with markers of insulin resistance and obesity suggest that IL-33 is positively associated with insulin resistance and increased in subjects with T2D and obesity. These findings are in line with previous publications showing that expression of IL-33 in adipose tissue and circulating levels are increased in subjects with obesity and metabolic disorders [5,16]. As far as we are aware, only one study has previously explored IL-33 mRNA expression in SAT from subjects with or without T2D [23], but they did not report group differences. The reason for this discrepancy is unknown, however, the T2D subjects included in the previous study were described to be on any glucose-lowering medications, while the T2D subjects included in our study were on metformin only. Therefore, it is plausible to consider that other T2D medications, like peroxisome proliferator-activated receptor-gamma (PPARγ) agonists or glucagon-like peptide 1 receptor agonists, could have reduced the gene expression of IL-33 in adipose tissue from these subjects [32,33]. Recently, we have published that circulating IL-33 is elevated in patients with obesity without T2D, but not in patients with obesity and T2D, compared to healthy controls [34]. However, circulating and adipose tissue levels of IL-33 are not necessarily coherent as IL-33 is constitutively produced from the structural and lining cells including fibroblasts, endothelial cells, and epithelial cells of normal human tissues including blood vessels, gastrointestinal tract, liver and lungs [35].

Our gene expression analyses show that *IL-33* mRNA expression in adipose tissue is negatively associated with many genes involved in adipogenesis and lipogenesis, which may imply that increased IL-33 in T2D subjects limit adipose tissue lipid storage and its capacity to expand, as seen in T2D subjects [24]. This negative association was consistent with the downregulation of the expression of the master adipogenesis regulator *PPARG* and the lipid storage mediator *FASN* in human adipose tissue following treatment with IL-33, and with publications showing

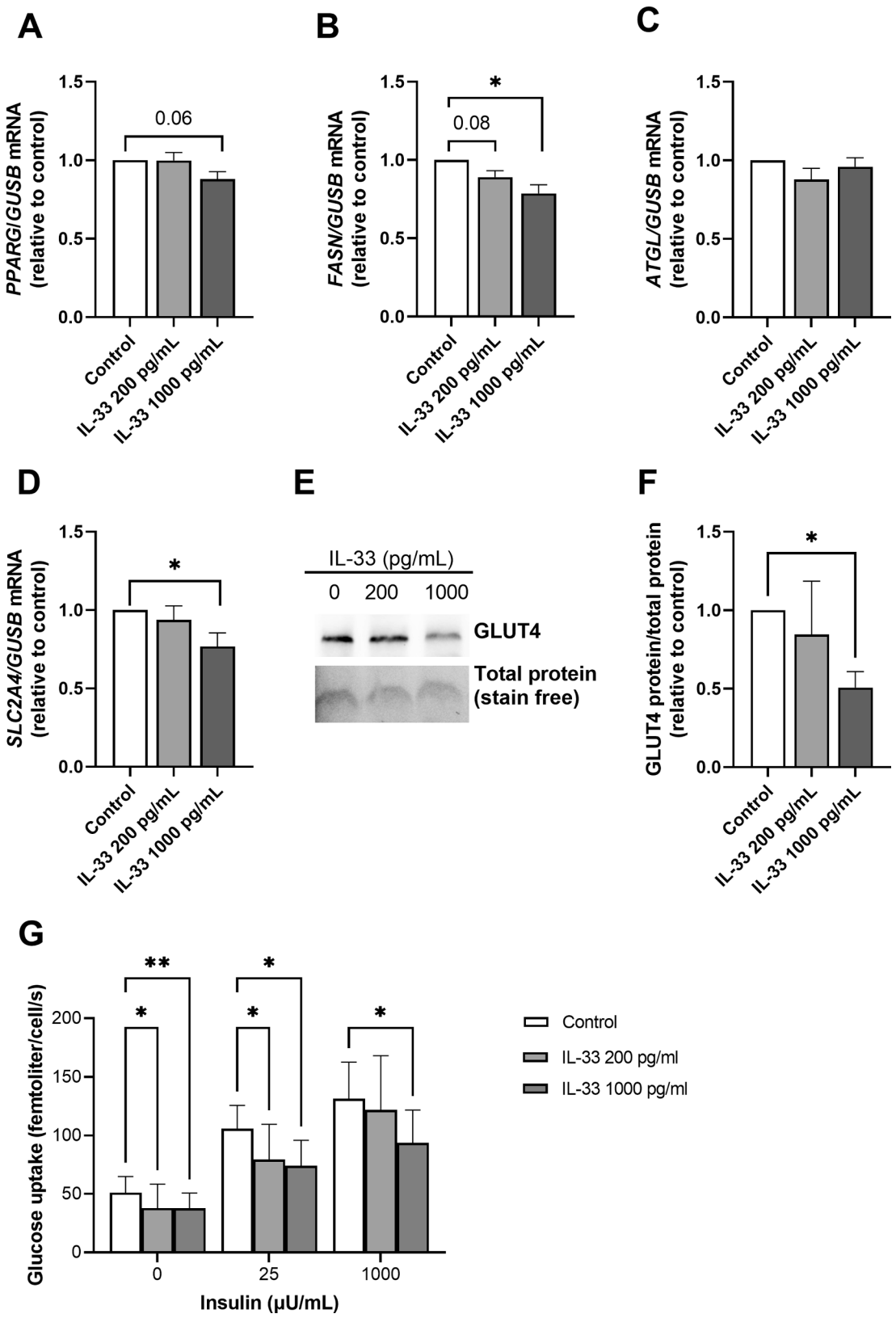

Fig. 3. IL-33 treatment reduces adipogenesis and lipid storage markers and glucose uptake in adipocytes. SAT was incubated without (control) or with IL-33 (200 and 1000 pg/mL) for 24 h and following incubation: mRNA was extracted to measure gene expression of (A) *PPARG*, (B) *FASN*, (C) *ATGL*, and (D) *SLC2A4* (n = 5); (E-F) total lysates were analysed by western-blot for GLUT4 (n = 4); (G) glucose uptake was measured in isolated adipocytes without or with insulin (25 and 1000 μU/mL) (n = 6). mRNA expression was normalised for the housekeeping gene *GUSB* and calculated relative to control (set to 1). Western-blot for GLUT4 was normalised for total protein content (stain-free) and calculated relative to control. *P < 0.05, **P < 0.01, compare to control.

that IL-33 treatment inhibits differentiation and lipid storage in pre-adipocytes isolated from mice and in 3T3-L1 cells [36,37]. Thus, future studies should evaluate the role of IL-33, and its receptor ST2, in human pre-adipocyte differentiation. Given that IL-33 can regulate metabolic inflammation by engaging ILC2s and activating downstream type 2 immune responses [6], we investigated the association between IL-33 mRNA and the expression of Th2 cytokines, IL-4/5/13. However, no significant associations were found between IL-33 and Th2 cytokines or other mediators involved in immune regulation, such as TNF, IL-6 and IL-16. This is also in agreement with a previous study [23]. In addition, no associations were found with other cytokines of the IL-1 family (e.g. IL-18 and IL-1B). IL-33 is mainly expressed in adipose tissue-resident mesenchyme-derived stromal cells [7]. However, we need to consider that this study measured the expression of cytokines in whole adipose tissue, which contains several cell populations, including adipocytes, pre-adipocytes, different immune cell populations, endothelial cells and fibroblasts [38]. Therefore, the expression of IL-33 and other cytokines in the whole adipose tissue does not necessarily reflect the expression in the different adipose tissue cellular subtypes and the dynamics induced by obesity and insulin resistance.

In agreement with the significant positive associations between IL-33 expression in adipose tissue with T2D phenotype and insulin resistance, we observed negative correlations between IL-33 gene expression with adipocyte glucose uptake and expression of key factors involved in insulin signalling (e.g. *AKT1*, *GLUT1* and *GLUT4*). However, since correlations alone do not show causality, we further analysed the effects of IL-33 on adipocyte glucose uptake. IL-33 reduced glucose uptake in both basal and insulin-stimulated conditions. The inhibitory effect was observed with physiological levels of IL-33 (200 pg/mL) seen in overweight/obese subjects [20]. Additionaly, incubation of human adipose tissue with IL-33 reduced the expression of the main glucose transporter GLUT4, without affecting total or phosphorylated AKT following insulin exposure. Therefore these data suggest that the IL-33 inhibitory effects on glucose uptake may be due to reduced expression of the main glucose transporter GLUT4 without affecting insulin signalling. This is the first study demonstrating a regulatory role of IL-33 in human adipocyte glucose uptake. Based on the above data, our results did not support a protective role of IL-33 in adipose tissue glucose metabolism, as previously suggested in mice studies [17,18,19,36]. On the contrary, the current study suggests that the gene expression of IL-33 is upregulated with obesity and insulin resistance and may inhibit glucose uptake in adipocytes. However, these *ex vivo* results might not reflect the *in vivo* pathways, and caution is required in interpreting the results. Also, the expression of the decoy sST2 is increased in subjects with obesity [7] and diabetes [39]. In this study, we did not measure sST2 levels, but *ST2* expression was positively correlated with BMI. Therefore, an increase in the sST2 levels could abrogate the effects of IL-33 in subjects with obesity.

Different therapeutic strategies targeting IL-33/ST2 signalling have been purposed for the treatment of inflammatory diseases, including anti-IL-33 monoclonal antibodies, soluble IL-33 receptors, and anti-ST2 monoclonal antibodies (reviewed in [40]). However, most of these studies relate to beneficial and pathological effects of IL-33/ST2 signalling in the context of allergic inflammation and chronic diseases such as asthma, and allergic rhinitis. Future *in vivo* studies should evaluate whether IL-33 signalling regulate adipose tissue metabolism.

This study has several limitations. *First*, the number of samples in this study were limited. For that reason, we validated key findings in a second cohort. *Second*, the associations shown do not allow any causal inference. *Third*, the *ex vivo* inhibitory effect of IL-33 in adipocyte glucose uptake does not necessarily reflect the *in vivo* situation.

Our findings suggest that T2D patients have higher IL-33 gene and protein expression in SAT compared to healthy controls, and this is associated with insulin resistance, and reduced gene expression of lipid storage and adipogenesis markers. IL-33 may reduce adipocyte glucose uptake. Thus, future functional *in vivo* studies to evaluate the role of IL-33 in adipose tissue metabolism are warranted.

CRediT authorship contribution statement

Maria J Pereira: Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Ayesha Azim:** Investigation, Writing – review & editing. **Susanne Hetty:** Investigation, Writing – review & editing. **Bipasha Nandi Jui:** Investigation, Writing – review & editing. **Joel Kullberg:** Investigation, Resources, Writing – review & editing. **Martin H Lundqvist:** Resources, Writing – review & editing. **Jan W Eriksson:** Conceptualization, Resources, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We gratefully acknowledge the valuable technical, administrative, and analytical contributions and expert advice from co-workers at Clinical Diabetes and Metabolism, Uppsala University and Uppsala University Hospital. We are also grateful to co-workers at AstraZeneca R&D for excellent scientific support.

Funding

This work was supported by research grants from the Excellence of Diabetes Research in Sweden (EXODIAB), the Agnes and Mac Rudberg Foundation, European Commission via the Marie Sklodowska Curie Innovative Training Network TREATMENT (H2020-MSCA-ITN-721236), AstraZeneca R&D, the Uppsala University Hospital ALF grants, the Swedish Diabetes Foundation, the Stiftelsen Familjen Ernfors Foundation, the P. O. Zetterlings Foundation, the Swedish Society for Medical Research, and the NovoNordisk Foundation.

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