ARTICLE

Effects of long-term exercise on plasma adipokine levels and inflammation-related gene expression in subcutaneous adipose tissue in sedentary dysglycaemic, overweight men and sedentary normoglycaemic men of healthy weight

Sindre Lee[1,2] · Frode Norheim[1] · Torgrim M. Langleite[1] · Hanne L. Gulseth[2,3] · Kåre I. Birkeland[2,4] · Christian A. Drevon[1]

Abstract

Aims/hypothesis Obesity and insulin resistance may be associated with altered expression and secretion of adipokines. Physical activity can markedly improve insulin sensitivity, but the association with adipokines remains largely unknown. In this study, we examined the effects of physical activity on the subcutaneous white adipose tissue (scWAT) secretome and its relationship to insulin sensitivity.

Methods As reported previously, we enrolled 26 sedentary, middle-aged men (13 dysglycaemic and overweight; 13 normoglycaemic and of healthy weight) into a 12 week, supervised, intensive physical exercise intervention that included two endurance and two resistance sessions each week. Insulin sensitivity was measured as the glucose infusion rate from a euglycaemic–hyperinsulinaemic clamp. In our previous study, we measured maximum oxygen uptake, upper- and lower-body strength and a range of circulating biomarkers, and quantified adipose tissue depots using MRI and magnetic resonance spectroscopy. We have now performed global mRNA sequencing, microarrays and RT-PCR of scWAT and skeletal muscle biopsies, and quantified selected plasma adipokines by ELISA.

Results Insulin sensitivity increased similarly in both dysglycaemic (45%) and normoglycaemic (38%) men after 12 weeks of exercise, as reported previously. mRNA sequencing of scWAT revealed 90 transcripts that responded to exercise in dysglycaemic men, whereas only marginal changes were observed in normoglycaemic men. These results were validated using microarrays and RT-PCR. A total of 62 out of 90 transcripts encoded secreted proteins. Overall, 17 transcripts were upregulated and 73 transcripts were downregulated. Downregulated transcripts included several macrophage markers, and were associated with inflammatory and immune-related pathways. Levels of these immune-related transcripts were enhanced in dysglycaemic men vs normoglycaemic men at baseline, but were normalised after the exercise intervention. Principal component and correlation analyses revealed inverse correlations between levels of these immune-related transcripts and insulin sensitivity at baseline, after the intervention, and for the change between baseline and after the intervention. In addition, levels of these transcripts at baseline could predict exercise-induced improvements in insulin sensitivity. Adipokine levels in scWAT (but not in skeletal muscle) were significantly correlated with corresponding plasma adipokine concentrations, as exemplified by leptin, high-molecular-weight adiponectin and secreted frizzled-related protein 4 (SFRP4). *SFRP4* mRNA was the most exercise-responsive transcript in scWAT from dysglycaemic men, and plasma SFRP4 concentrations were reduced in dysglycaemic men, but not in normoglycaemic men, after 12 weeks of exercise.

✉ Sindre Lee
  sindre.lee@medisin.uio.no

[1] Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Sognsvannsveien 9, 0372 Oslo, Norway

[2] Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, Oslo, Norway

[3] Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health, Oslo, Norway

[4] Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway

Research in context

What is already known about this subject?

- Studies have indicated that lifestyle interventions have beneficial effects on adipokines and insulin resistance, but have only focused on a few adipokines that are mostly related to visceral adipose tissue and weight loss

- Long-term physical exercise has profound effects on the subcutaneous white adipose tissue (scWAT) secretome and insulin sensitivity in mice, but studies in humans are lacking

What is the key question?

- How does 12 weeks of physical exercise influence global expression of secreted factors in scWAT and plasma concentrations of such factors in overweight men with dysglycaemia compared with healthy weight men with normoglycaemia?

What are the new findings?

- Using global mRNA sequencing, we discovered a distinct effect of 12 weeks of exercise on the scWAT secretome specifically for dysglycaemic men, with minimal alterations observed in normoglycaemic men

- These secreted factors were associated with inflammation, and correlated negatively with insulin sensitivity

- Alterations in mRNA expression in scWAT were mirrored in plasma adipokine concentrations, as exemplified by SFRP4

How might this impact on clinical practice in the foreseeable future?

- Our data may aid the discovery of plasma biomarkers of insulin resistance

Conclusions/interpretation This study indicates that scWAT may be an important mediator of exercise-induced improvements in insulin sensitivity, especially in overweight dysglycaemic individuals at increased risk of developing type 2 diabetes.

Keywords Adipokines · Humans · Insulin sensitivity · Long-term exercise · Prediabetes · Subcutaneous adipose tissue

Abbreviations

scWAT	Subcutaneous white adipose tissue
GIR	Glucose infusion rate
MRS	Magnetic resonance spectroscopy
SFRP4	Secreted frizzled-related protein 4
$\dot{V}O_{2max}$	Maximal oxygen uptake

Introduction

Adipose tissue is an active endocrine organ that expresses and secretes multiple metabolically active factors such as leptin, adiponectin, IL-6 and TNF-α [1–3]. These secreted factors are involved in metabolic and inflammatory processes and may act in a paracrine or endocrine way, altering metabolism in the liver, pancreas, skeletal muscle and central nervous system [3, 4]. Dysregulation of these signal molecules is closely related to adipocyte hypertrophy and insulin resistance [5–7], and has been characterised in several studies [3, 8–10].

Insulin resistance is a hallmark of type 2 diabetes mellitus and is closely linked to lifestyle variables such as diet and physical activity [11–14]. Physical activity can substantially increase insulin sensitivity, and exercise-induced alterations in subcutaneous white adipose tissue (scWAT) may affect whole-body metabolic health [4, 15, 16]. Mediators of these effects may involve extensive adaptations in adipokine expression [4, 16]. Numerous studies have focused on the effect of different types of exercise on circulating levels of adipose tissue-derived factors [3, 17–19]. However, the main body of literature on adipokines and exercise is limited to plasma analyses of one or a few targets and the effects of acute exercise [3, 19–22] confounded by weight loss [23] or focused on only visceral adipose tissue [3, 17, 18]. Few studies have addressed long-term exercise-induced alterations in scWAT expression of such factors [22, 24], and these studies have only been performed in insulin-sensitive women [22, 24]. In addition, whereas skeletal muscle has been extensively studied as regards physical exercise [25], significantly less focus has been placed on adipose tissue in this aspect.

In the present study, we investigated the effect of long-term physical exercise on scWAT transcript levels and potential links to insulin sensitivity. We performed global mRNA sequencing on biopsies, together with ELISA measurements of selected

plasma adipokines, and quantified insulin sensitivity using a euglycaemic–hyperinsulinaemic clamp. We hypothesised that 12 weeks of combined endurance and strength training would promote distinct alterations in the scWAT transcriptome among overweight men with dysglycaemia and men of healthy weight with normoglycaemia, and that these changes would correlate with insulin sensitivity.

Methods

Participants and experimental methods standardisation

The MyoGlu study was a controlled clinical exercise intervention trial (ClinicalTrials.gov registration no. NCT01803568) in 26 sedentary white (<1 exercise session/week) men aged 40–65 years of Scandinavian origin performed in 2011–2012 in Oslo, Norway (Fig. 1) [26]. All participants gave written informed consent, and the study was approved by the

Regional Committee for Medical and Health Research Ethics North (Tromsø, Norway; ref. no. 2011/882) and adhered to the Declaration of Helsinki. The study was designed to in-depth phenotypically characterise the effects of an intensive exercise intervention across the glucometabolic spectrum.

We included 13 dysglycaemic, overweight men (fasting glucose ≥5.6 mmol/l, 2 h OGTT ≥7.8 mmol/l or insulin resistance [HOMA-IR >2.0]; BMI 26.8–32.5 kg/m^2) and 13 normoglycaemic men of healthy weight (fasting glucose <5.6 mmol/l and 2 h OGTT <7.8 mmol/l and without a family history of diabetes; BMI 20.9–26.7 kg/m^2). Exclusion criteria included smoking, a family history of diabetes (for control participants only), known hypertension, liver or kidney disease, chronic inflammatory diseases or use of a medication expected to affect glucose metabolism (e.g. lipid-lowering or antihypertensive medications, acetylsalicylic acid, corticosteroids).

Before the intervention, the participants refrained from physical exercise and alcohol for 2 days before testing (Fig. 1). The last session of the 12 week intervention consisted of an endurance session with intervals performed at 85% of
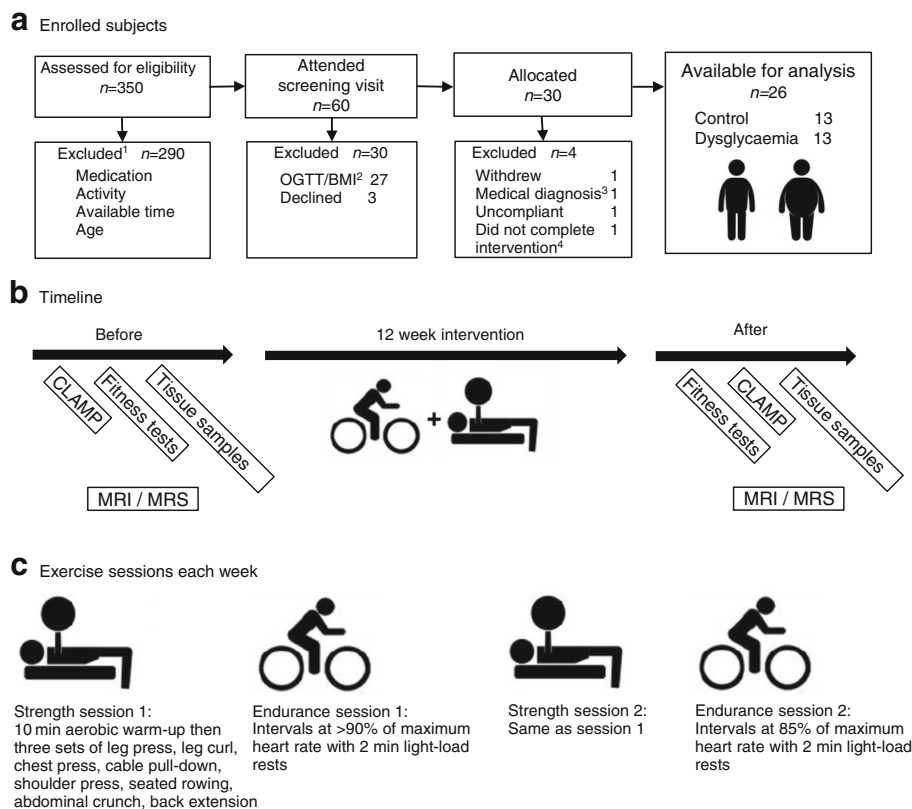
Fig. 1 Study design. (**a**) A total of 26 sedentary men (13 of healthy weight with normoglycaemia [control] and 13 overweight with dysglycaemia) were recruited into two groups. [1]No participants were excluded because of lack of available time; a very small number were excluded because they did not meet the age criterion, ~70% were excluded because of medication or because of family members with diabetes (controls); ~30% were excluded because they had been too active in the past year. [2]OGTT response did not match phenotype, e.g. an obese person

had a normal OGTT response. [3]One participant was diagnosed with acute coronary syndrome. [4]Due to illness. (**b**) The participants donated tissue samples and underwent several tests at baseline, including a euglycaemic–hyperinsulinaemic clamp, before undertaking 12 weeks of an intensive physical exercise intervention followed by re-testing. Three days passed between the last bout of exercise and the euglycaemic–hyperinsulinaemic clamp after the intervention. (**c**) The intervention consisted of four exercise sessions each week

maximum heart rate 3 days prior to repeated testing (Fig. 1). Maximal oxygen uptake ($\dot{V}O_{2max}$) tests and maximum strength tests were performed several days (>3 days) before the euglycaemic–hyperinsulinaemic clamp and tissue sampling (Fig. 1). Tests were performed under similar conditions on separate days both before and after the intervention, with some exceptions for MRI/magnetic resonance spectroscopy (MRS) due to scanner availability.

Diet

The habitual diet of each participant was recorded using a validated food frequency questionnaire [27, 28]. Calculations were performed using the food database AE-10 and the KBS v.7.1 (Kostberegningssystem, Oslo, Norway) food and nutrients calculation system. Alcohol intake was not allowed to exceed 2 units per day. During testing at baseline and after 12 weeks of exercise, the participants consumed a standardised meal after an overnight fast. A carbohydrate-rich meal including bread, apple juice, cheese and jam was adjusted depending on individual energy requirements and provided 23% of the estimated total daily energy expenditure 90–120 min prior to the test. Tests were typically performed in the morning; the standardised meal was the only intake after the overnight fast. Water could be consumed freely.

Table 1 Participant characteristics

| Variable	Baseline		Percentage change after intervention	
	Control participants (n=13)	Dysglycaemic men (n=13)	Control participants (n=13)	Dysglycaemic men (n=13)
Age (years)	50 ± 7	53 ± 6		
Body composition				
Weight (kg)	78.5 ± 8.2	95.4 ± 10.2*	−0.3 ± 2.1	−1.7 ± 2.4[†]
BMI (kg/m^2)	23.5 ± 2.0	29.0 ± 2.4*	0.0 ± 2.0	−1.2 ± 4.5
FFM (kg)[a]	34.9 ± 3.5	37.7 ± 5.0	6.4 ± 3.8[†]	5.3 ± 2.7[†]
scWAT (kg)[a]	10.3 ± 2.7	18.0 ± 4.2*	−6.6 ± 9.2[†]	−7.3 ± 6.0[†]
IAAT (kg)[a]	4.0 ± 2.0	8.8 ± 2.6*	−16.9 ± 15.1[†]	−19.4 ± 10.8[†]
Hepatic fat (AU)[b]	2.8 ± 2.2	9.1 ± 5.9*	−23.3 ± 50.7[†]	−27.4 ± 15.8[†]
Thigh muscle area (AU)[a]	20.3 ± 2.9	24.4 ± 3.1*	9.7 ± 4.7[†]	7.1 ± 6.7[†]
Physical fitness				
$\dot{V}O_{2max}$ (ml kg^{-1} min^{-1})	44.1 ± 4.4	37.1 ± 4.9*	13.2 ± 9.7[†]	13.3 ± 7.7[†]
Chest press (kg)	65.6 ± 16.8	68.7 ± 13.7	18.4 ± 8.7[†]	13.6 ± 8.4[†]
Pull down (kg)	68.8 ± 9.3	75.6 ± 15.1	18.3 ± 10.1[†]	13.7 ± 7.3[†]
Leg press (kg)	199.6 ± 36.9	248.7 ± 30.3*	9.8 ± 7.6[†]	12.5 ± 8.4[†]
Glucose metabolism				
HbA$_{1c}$ (mmol/mol)	33 ± 4	37 ± 4*	NA	NA
HbA$_{1c}$ (%)	5.2 ± 0.2	5.5 ± 0.4*	NA	NA
Fasting glucose (mmol/l)	5.4 ± 0.5	5.9 ± 0.3*	3.1 ± 4.5[†]	1.8 ± 6.8
Fasting C-peptide (pmol/l)	588.0 ± 117.8	932.8 ± 248.9*	7.3 ± 23.8	12.3 ± 45.3
Fasting insulin (pmol/l)	38.5 ± 18.6	65.3 ± 27.1*	15.1 ± 49.2	27.6 ± 66.2
Plasma NEFA (mmol/l)	0.3 ± 0.1	0.2 ± 0.1	−21.7 ± 31.1[†]	16.0 ± 53.1
GIR (mg kg^{-1} min^{-1})	7.6 ± 1.6	4.2 ± 1.8*	37.8 ± 30.1[†]	44.4 ± 58.8[†]
Plasma protein				
hsCRP (nmol/l)	9.5 ± 7.6	26.7 ± 21.9	41.0 ± 96.7	30.1 ± 121.2

Data are means ± SD

Between-group comparisons were performed using unpaired t tests and within-group comparisons were performed using paired t tests. Logarithmic transformation was performed to approximate normal distribution, if necessary, and back-transformed for presentation

[a] n=12 control participants

[b] n=10 control participants and n=9 dysglycaemic men

*p<0.05 between groups (dysglycaemic vs control), [†] p<0.05 baseline vs 12 weeks within group

AU, arbitrary units; FFM, fat free mass; IAAT, intra-abdominal adipose tissue; NA, not available; hsCRP, high-sensitivity C-reactive protein
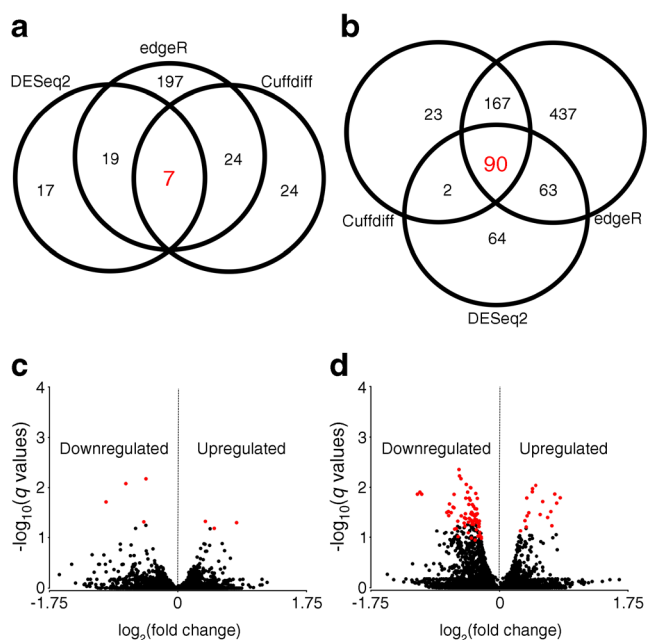
Fig. 2 Exercise-responsive transcripts in scWAT. We compared transcript levels in biopsies obtained from scWAT before and after the 12 week exercise intervention using three commonly applied workflows for mRNA sequencing data: DESeq2, edgeR and Cuffdiff. The results from these three approaches were intersected for both (**a**) normoglycaemic and (**b**) dysglycaemic men. Volcano plots show fold changes and q values for (**c**) normoglycaemic and (**d**) dysglycaemic men. On the x-axis, data points below and above zero indicate downregulation and upregulation, respectively, after the 12 week exercise intervention. On the y-axis, large values represent low q values, represented as the mean from all three workflows. The logarithmic transformations used in volcano plots are necessary for comprehensive and symmetrical representation of the large amount of data. Red coloured points indicate statistical significance, whereas black points signifies statistical insignificance within each group, respectively. q values: Benjamini–Hochberg corrected p values

Exercise intervention

Strength and endurance exercise The participants performed 4 h of intensive exercise each week for 12 weeks under professional supervision. Two whole-body strength training sessions and two spinning bike interval sessions lasted 1 h each (Fig. 1). The 12 week intervention included linear progression in workload for both strength and endurance exercises. For strength exercises, during weeks 1–3 a load that could be lifted a maximum of 12 times (i.e. 12 repetition maximum) was used, which progressed to ten repetition maximum in weeks 4–8 and to eight repetition maximum in weeks 9–12. Abdominal crunches and back extensions were performed with 12–20 repetitions for the whole intervention period. For endurance exercise, during week 1, three intervals were performed during endurance session one (intervals at >90% of maximum heart rate) and six intervals during endurance session two (intervals at 85% of maximum heart rate) (Fig. 1). For weeks 2–5, four intervals were performed during session one and seven intervals during session two. For weeks 6–12, five intervals were performed during session one and ten intervals during session two.

Physical fitness and insulin sensitivity

$\dot{V}O_{2max}$ tests were performed after a standardised warm-up at a workload similar to the final load of an incremental test in which the relationship between work (watt) and oxygen uptake was established. Participants cycled for 1 min followed by a 15 W increased workload every 30 s until exhaustion. Test success was based on oxygen consumption increased

Table 2 scWAT transcripts responding to 12 weeks of exercise intervention in control participants

Gene symbol	UniProt name	Secreted?	RPKM	Cuffdiff			edgeR			DESeq2			
				lg_2FC	p	FDR	lg_2FC	p	FDR	lg_2FC	SE	p	FDR
EGFL6	EGF-like protein 6	Yes	19.63	–0.95	<0.01	0.02	–0.79	<0.01	<0.01	–0.71	0.15	<0.01	0.01
UCHL1	Ubiquitin carboxyl-terminal hydrolase	WL	71.69	–0.48	<0.01	0.02	–0.43	<0.01	<0.01	–0.43	0.08	<0.01	<0.01
CETP	Cholesteryl ester transfer protein	WL	12.71	0.74	<0.01	0.02	0.87	<0.01	<0.01	0.80	0.23	<0.01	0.13
BDKRB2	B2 bradykinin receptor	WL	3.03	0.54	<0.01	0.06	0.51	<0.01	<0.01	0.50	0.14	<0.01	0.13
OSGIN1	Oxidative stress-induced growth inhibitor 1	WL	12.62	–0.47	<0.01	0.07	–0.46	<0.01	<0.01	–0.46	0.12	<0.01	0.07
DRD1	D(1A) dopamine receptor	No	0.85	–1.15	<0.01	0.04	–1.02	<0.01	<0.01	–0.98	0.23	<0.01	0.02
MEG3	–	No	30.22	0.40	<0.01	0.06	0.39	<0.01	<0.01	0.37	0.10	<0.01	0.08

Data were modelled as a (paired) before–after design for control participants using three common statistical approaches to mRNA sequencing differential expression analyses (Cuffdiff, edgeR and DESeq2). The FDR was set to <15% for each approach, and the results were intersected between all three approaches. MetazSecKB defined secreted proteins

SEs are of the fold change

FDR, false discovery rate; lg_2FC, log$_2$ of the fold change; RPKM, reads per kilobase per million mapped reads; WL, weakly likely

Table 3 scWAT transcripts responding to 12 weeks of exercise intervention in men with dysglycaemia

Gene symbol	UniProt Name	Secreted?	RPKM	Cuffdiff			edgeR			DESeq2			
				lg_2FC	p	FDR	lg_2FC	p	FDR	lg_2FC	SE	p	FDR
SFRP4	Secreted frizzled-related protein 4	Yes	16.57	−0.54	<0.01	0.01	−0.56	<0.01	<0.01	−0.55	0.12	<0.01	0.01
EMILIN2	Elastin microfibril interface-located protein 2	Yes	16.09	−0.53	<0.01	0.01	−0.61	<0.01	<0.01	−0.53	0.12	<0.01	0.01
ADAMTS4	A disintegrin and metalloproteinase with thrombospondin motifs 4	Yes	5.74	0.58	<0.01	0.01	0.61	<0.01	<0.01	0.59	0.16	<0.01	0.05
CCL22	C-C motif chemokine 22	Yes	2.48	−0.78	<0.01	0.01	−0.57	<0.01	0.05	−0.57	0.17	<0.01	0.09
C1QC	Complement C1q subcomponent subunit C	Yes	132.33	−0.38	<0.01	0.01	−0.33	<0.01	0.01	−0.33	0.10	<0.01	0.12
ANGPT2	Angiopoietin-2	Yes	4.86	−0.64	<0.01	0.01	−0.56	<0.01	<0.01	−0.55	0.11	<0.01	0.01
PENK	Proenkephalin-A	Yes	4.60	−0.71	<0.01	0.01	−0.65	<0.01	0.03	−0.63	0.16	<0.01	0.03
CCL3	C-C motif chemokine 3	Yes	4.94	−0.86	<0.01	0.02	−0.74	<0.01	0.01	−0.72	0.21	<0.01	0.07
THBS4	Thrombospondin-4	Yes	43.45	0.33	<0.01	0.02	0.38	<0.01	<0.01	0.37	0.09	<0.01	0.02
TGFBI	TGF-β-induced protein ig-h3	Yes	49.74	−0.32	<0.01	0.03	−0.30	<0.01	0.02	−0.30	0.08	<0.01	0.05
NPTX2	Neuronal pentraxin-2	Yes	0.72	0.76	<0.01	0.06	0.72	<0.01	0.01	0.70	0.18	<0.01	0.02
STC2	Stanniocalcin-2	Yes	6.10	−0.33	<0.01	0.07	−0.33	<0.01	<0.01	−0.32	0.09	<0.01	0.07
CFH	Complement factor H	Yes	55.64	−0.31	<0.01	0.10	−0.28	<0.01	0.04	−0.29	0.08	<0.01	0.04
TNFRSF11B	TNF receptor superfamily member 11B	Yes	4.25	−0.44	<0.01	0.12	−0.43	<0.01	0.05	−0.43	0.12	<0.01	0.07
FBLN1	Fibulin-1	HL	73.17	−0.33	<0.01	0.01	−0.32	<0.01	0.01	−0.32	0.08	<0.01	0.02
FST	FST protein	HL	10.73	0.43	<0.01	0.01	0.45	<0.01	<0.01	0.45	0.11	<0.01	0.02
BMP3	Bone morphogenetic protein 3 F	HL	0.60	0.82	<0.01	0.01	0.79	<0.01	<0.01	0.75	0.20	<0.01	0.03
SLAMF7	SLAM family member 7 F	HL	3.47	−0.72	<0.01	0.01	−0.63	<0.01	<0.01	−0.62	0.16	<0.01	0.03
HBA2	α-2 globin F	HL	50.28	−1.35	<0.01	0.01	−1.22	<0.01	<0.01	−1.12	0.29	<0.01	0.03
CD4	CD4 protein	HL	26.32	−0.39	<0.01	0.01	−0.33	<0.01	<0.01	−0.33	0.10	<0.01	0.07
LIPA	Lysosomal acid lipase F	HL	139.10	−0.65	<0.01	0.01	−0.52	<0.01	<0.01	−0.52	0.16	<0.01	0.10
PDE11A	Dual 3′,5′-cyclic-AMP and -GMP phosphodiesterase 11A	HL	2.43	0.42	<0.01	0.02	0.49	<0.01	<0.01	0.49	0.11	<0.01	0.01
ITGAL	Integrin α-L	HL	3.29	−0.45	<0.01	0.02	−0.37	<0.01	<0.01	−0.37	0.11	<0.01	0.08
ADAMTS18	A disintegrin and metalloproteinase with thrombospondin motifs 18	HL	1.03	0.55	<0.01	0.02	0.58	<0.01	<0.01	0.56	0.16	<0.01	0.08
LILRA2	Leucocyte Ig-like receptor subfamily A member 2	HL	2.12	−0.73	<0.01	0.03	−0.67	<0.01	0.03	−0.66	0.18	<0.01	0.04
CD86	T-lymphocyte activation antigen CD86 F	HL	3.57	−0.51	<0.01	0.03	−0.42	<0.01	0.07	−0.43	0.12	<0.01	0.05
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6 F	HL	1.01	−0.82	<0.01	0.04	−0.71	<0.01	0.02	−0.69	0.19	<0.01	0.05
PILRA	Paired Ig-like type 2 receptor α	HL	9.96	−0.49	<0.01	0.04	−0.49	<0.01	0.01	−0.48	0.14	<0.01	0.08
GRN	Granulins F	HL	182.11	−0.32	<0.01	0.06	−0.28	<0.01	0.01	−0.28	0.08	<0.01	0.09
ACE	ACE	HL	18.74	−0.29	<0.01	0.07	−0.27	<0.01	0.02	−0.28	0.08	<0.01	0.05
PTPRJ	Receptor-type tyrosine-protein phosphatase eta F	HL	5.02	−0.31	<0.01	0.08	−0.31	<0.01	<0.01	−0.31	0.09	<0.01	0.07
TSKU	Tsukushin F	HL	166.42	0.31	<0.01	0.09	0.29	<0.01	<0.01	0.28	0.09	<0.01	0.13
PAMR1	Inactive serine protease PAMR1 F	HL	5.82	−0.39	<0.01	0.09	−0.41	<0.01	<0.01	−0.41	0.11	<0.01	0.03
CD22	B cell receptor CD22 F	HL	1.19	−0.62	<0.01	0.12	−0.56	<0.01	0.12	−0.57	0.16	<0.01	0.05
BCAT1	Alternative protein BCAT1	L	2.90	−0.50	<0.01	0.01	−0.43	<0.01	<0.01	−0.42	0.12	<0.01	0.06
FCER1G	High-affinity Ig epsilon receptor subunit γ	L	92.32	−0.56	<0.01	0.01	−0.50	<0.01	<0.01	−0.48	0.16	<0.01	0.15
TBXAS1	Thromboxane-A synthase	L	6.95	−0.46	<0.01	0.02	−0.40	<0.01	<0.01	−0.40	0.10	<0.01	0.03
ITGAM	Integrin α-M F	WL	9.36	−0.53	<0.01	0.01	−0.46	<0.01	<0.01	−0.45	0.11	<0.01	0.02
LBH	Protein LBH	WL	11.35	−0.42	<0.01	0.01	−0.40	<0.01	<0.01	−0.40	0.10	<0.01	0.02
GM2A	Ganglioside GM2 activator F	WL	35.68	−0.45	<0.01	0.01	−0.39	<0.01	<0.01	−0.39	0.10	<0.01	0.02
HBB	Mutant β-globin	WL	343.81	−1.29	<0.01	0.01	−1.12	<0.01	<0.01	−1.06	0.28	<0.01	0.03
KYNU	Kynureninase	WL	2.70	−0.81	<0.01	0.01	−0.69	<0.01	0.02	−0.68	0.18	<0.01	0.03
PTPN6	Tyrosine-protein phosphatase non-receptor type 6	WL	16.46	−0.48	<0.01	0.01	−0.42	<0.01	<0.01	−0.41	0.11	<0.01	0.04
NDRG4	Protein NDRG4 F	WL	9.51	0.76	<0.01	0.01	0.83	<0.01	<0.01	0.78	0.22	<0.01	0.05
ARHGAP30	Rho GTPase-activating protein 30	WL	9.27	−0.45	<0.01	0.01	−0.39	<0.01	<0.01	−0.38	0.11	<0.01	0.09
LGALS9	Galectin F	WL	30.71	−0.43	<0.01	0.01	−0.39	<0.01	<0.01	−0.39	0.12	<0.01	0.09
LSP1	Lymphocyte-specific protein 1 F	WL	99.05	−0.34	<0.01	0.01	−0.31	<0.01	0.01	−0.31	0.10	<0.01	0.11
STXBP2	Syntaxin-binding protein 2 F	WL	8.19	−0.50	<0.01	0.01	−0.39	<0.01	<0.01	−0.39	0.12	<0.01	0.12
TRPV2	Transient receptor potential cation channel subfamily V member 2 F	WL	14.33	−0.38	<0.01	0.02	−0.34	<0.01	<0.01	−0.33	0.10	<0.01	0.09
DOK2	Docking protein 2	WL	15.34	−0.40	<0.01	0.02	−0.36	<0.01	<0.01	−0.35	0.11	<0.01	0.14
SOX9	SRY-box 9 F	WL	3.39	0.43	<0.01	0.03	0.45	<0.01	<0.01	0.44	0.09	<0.01	0.01
DHCR24	Delta(24)-sterol reductase	WL	44.49	−0.32	<0.01	0.03	−0.32	<0.01	0.01	−0.32	0.09	<0.01	0.06
PAQR5	Alternative protein PAQR5	WL	0.72	−0.70	<0.01	0.04	−0.63	<0.01	0.06	−0.61	0.19	<0.01	0.10

Table 3 (continued)

Gene symbol	UniProt Name	Secreted?	RPKM	Cuffdiff			edgeR			DESeq2			
				lg_2FC	p	FDR	lg_2FC	p	FDR	lg_2FC	SE	p	FDR
CHST15	Alternative protein CHST15	WL	5.47	−0.38	<0.01	0.04	−0.37	<0.01	<0.01	−0.37	0.09	<0.01	0.02
C8orf4	Alternative protein C8orf4	WL	6.84	0.45	<0.01	0.04	0.37	<0.01	<0.01	0.36	0.12	<0.01	0.14
RORC	RAR-related orphan receptor C, isoform CRA_a	WL	0.45	0.78	<0.01	0.06	0.76	<0.01	<0.01	0.71	0.22	<0.01	0.11
MYO1E	Unconventional myosin-Ie F	WL	8.42	−0.32	<0.01	0.07	−0.31	<0.01	<0.01	−0.31	0.07	<0.01	0.01
RAB11FIP4	Rab11 family-interacting protein 4 F	WL	0.43	−0.66	<0.01	0.09	−0.59	<0.01	0.09	−0.59	0.14	<0.01	0.02
PLXNC1	Plexin-C1	WL	2.47	−0.44	<0.01	0.14	−0.38	<0.01	0.04	−0.38	0.12	<0.01	0.13
RCAN2	Calcipressin-2	WL	9.45	−0.30	<0.01	0.15	−0.29	<0.01	<0.01	−0.29	0.10	<0.01	0.14
CYBA	Cytochrome b-245 light chain F	WL	163.49	−0.27	<0.01	0.15	−0.25	<0.01	0.05	−0.25	0.08	<0.01	0.10
PSD4	PH and SEC7 domain-containing protein 4 F	WL	2.38	−0.39	<0.01	0.15	−0.33	<0.01	0.06	−0.33	0.11	<0.01	0.13
ABCB11	Bile salt export pump	No	0.49	0.71	<0.01	0.06	0.68	<0.01	0.03	0.66	0.17	<0.01	0.03
ADRA2A	α-2A adrenergic receptor	No	50.86	−0.29	<0.01	0.05	−0.28	<0.01	0.04	−0.28	0.09	<0.01	0.09
ARHGDIB	Rho GDP-dissociation inhibitor 2	No	133.16	−0.28	<0.01	0.09	−0.27	<0.01	0.05	−0.28	0.05	<0.01	<0.01
CD180	CD180 antigen	No	1.39	−0.86	<0.01	0.02	−0.71	<0.01	0.02	−0.70	0.19	<0.01	0.05
CD53	Leucocyte surface antigen CD53	No	23.25	−0.57	<0.01	0.01	−0.48	<0.01	<0.01	−0.46	0.12	<0.01	0.04
CORO1A	Coronin-1A	No	16.74	−0.39	<0.01	0.02	−0.36	<0.01	<0.01	−0.35	0.10	<0.01	0.05
CTSZ	Cathepsin Z	No	275.21	−0.28	<0.01	0.11	−0.25	<0.01	0.05	−0.25	0.08	<0.01	0.15
CYBB	Cytochrome b-245 heavy chain	No	11.44	−0.50	<0.01	0.01	−0.44	<0.01	<0.01	−0.43	0.11	<0.01	0.03
CYSLTR2	Cysteinyl leukotriene receptor 2	No	0.56	0.84	<0.01	0.04	0.87	<0.01	<0.01	0.83	0.18	<0.01	0.01
ACKR1	Atypical chemokine receptor 1	No	101.21	−0.31	<0.01	0.08	−0.27	<0.01	0.06	−0.27	0.09	<0.01	0.13
DOCK2	Dedicator of cytokinesis protein 2	No	3.87	−0.37	<0.01	0.07	−0.32	<0.01	<0.01	−0.32	0.10	<0.01	0.14
FPR3	N-formyl peptide receptor 3	No	8.85	−0.43	<0.01	0.02	−0.39	<0.01	<0.01	−0.37	0.12	<0.01	0.14
GSDMB	Gasdermin-B	No	10.92	0.45	<0.01	0.01	0.38	<0.01	<0.01	0.37	0.11	<0.01	0.10
HBA1	Haemoglobin subunit α	No	11.41	−1.27	<0.01	0.01	−1.17	<0.01	<0.01	−1.08	0.28	<0.01	0.03
IFITM1	IFN-induced transmembrane protein 1	No	208.95	−0.36	<0.01	0.01	−0.30	<0.01	0.01	−0.30	0.08	<0.01	0.02
KCNJ5	G protein-activated inward rectifier potassium channel 4	No	2.18	−0.80	<0.01	0.01	−0.62	<0.01	0.03	−0.62	0.17	<0.01	0.04
LHFPL2	LHFPL tetraspan subfamily member 2 protein	No	9.84	−0.28	<0.01	0.15	−0.27	<0.01	<0.01	−0.27	0.08	<0.01	0.09
MAPK13	Mitogen-activated protein kinase 13Mitogen-activated protein kinase	No	2.29	−0.48	<0.01	0.01	−0.47	<0.01	0.04	−0.47	0.15	<0.01	0.13
MARCO	Macrophage receptor MARCO	No	13.15	−0.36	<0.01	0.08	−0.34	<0.01	<0.01	−0.34	0.09	<0.01	0.03
NUP210	Nuclear pore membrane glycoprotein 210	No	1.17	−0.53	<0.01	0.04	−0.46	<0.01	0.06	−0.46	0.11	<0.01	0.02
PKD1L2	Polycystic kidney disease protein 1-like 2	No	11.89	0.36	<0.01	0.06	0.35	<0.01	<0.01	0.34	0.10	<0.01	0.08
PLEK	Pleckstrin	No	15.44	−0.50	<0.01	0.01	−0.38	<0.01	<0.01	−0.37	0.12	<0.01	0.14
RASSF2	Ras association domain-containing protein 2	No	6.06	−0.33	<0.01	0.08	−0.32	<0.01	<0.01	−0.32	0.08	<0.01	0.02
SAMHD1	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	No	32.08	−0.27	<0.01	0.12	−0.28	<0.01	0.02	−0.28	0.08	<0.01	0.04
SASH3	SAM and SH3 domain-containing protein 3	No	5.91	−0.58	<0.01	0.01	−0.48	<0.01	<0.01	−0.46	0.14	<0.01	0.09
SCARA3	Scavenger receptor class A member 3	No	13.86	−0.36	<0.01	0.02	−0.36	<0.01	<0.01	−0.36	0.11	<0.01	0.12
SLC25A18	Mitochondrial glutamate carrier 2	No	5.65	0.40	<0.01	0.09	0.40	<0.01	<0.01	0.40	0.08	<0.01	0.01
SYK	Tyrosine-protein kinase SYK	No	5.18	−0.42	<0.01	0.01	−0.38	<0.01	<0.01	−0.37	0.11	<0.01	0.09

Data were modelled as a (paired) before–after design for men with dysglycaemia using three common statistical approaches to mRNA sequencing differential expression analyses (Cuffdiff, edgeR and DESeq2). The FDR was set to <15% for each approach, and the results were intersected between all three approaches. MetazSecKB defined secreted proteins

SEs are of the fold change

F, fragment; FDR, false discovery rate; HL, highly likely; L, likely; lg_2FC, $\log_2$ of the fold change; RPKM, reads per kilobase per million mapped reads; WL, weakly likely

by <0.5 ml kg^{-1} min^{-1} over a 30 W increase in workload, respiratory exchange ratio values >1.10 and blood lactate >7.0 mmol/l.

Euglycaemic–hyperinsulinaemic clamp Euglycaemic–hyperinsulinaemic clamps were performed after an overnight fast. A fixed dose of insulin 40 mU m^{-2} min^{-1} was infused, and glucose (200 mg/ml) was injected to maintain euglycaemia (5.0 mmol/l) for 150 min [29]. Insulin sensitivity is reported as the glucose infusion rate (GIR) during the last 30 min relative to body weight. The whole blood glucose concentration was measured using a glucose oxidase method

(YSI 2300, YSI, Yellow Springs, OH, USA) and the plasma glucose concentration was calculated as whole blood glucose ×1.119.

MRI/MRS

MRI methods were used to quantify fat mass [26]. The ankle-to-neck MRI protocol included a 3D Dixon acquisition providing water and lipid quantification. Data were then analysed using the nordicICE software package (NordicNeuroLab, Bergen, Norway) and the jMRUI v.5.2 [30] software package.

Tissue sampling

As described previously [26], we obtained scWAT, skeletal muscle biopsies and blood samples before and after the 12 week exercise intervention (Fig. 1). Biopsies were obtained from the periumbilical subcutaneous tissue and from the vastus lateralis muscle. After sterilisation, a lidocaine-based local anaesthetic was injected into the skin and subcutis prior to both skeletal muscle and scWAT biopsies [26]. Biopsies were dissected on a cold aluminium plate to remove blood and other materials before freezing.

Transcriptomics

Biopsies were frozen in liquid nitrogen, crushed to powder with a pestle in a liquid nitrogen-cooled mortar, transferred into 1 ml QIAzol Lysis Reagent (Qiagen, Hilden, Germany) and homogenised using TissueRuptor (Qiagen) at full speed for 15 s, twice. Total RNA was isolated from the homogenate using the miRNeasy Mini Kit (Qiagen). RNA integrity and concentration were determined using Agilent RNA 6000 Nano Chips on a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster, CA, USA). The cDNA reaction mixture was diluted in water and a cDNA equivalent of 25 ng RNA was used for each sample.

TaqMan real-time quantitative RT-PCR Quantitative real-time PCR was performed with reagents and instruments from Applied Biosystems in the 96-well format using a 7900HT Fast instrument and SDS 2.3 software (Applied Biosystems). Predeveloped primers and probe sets (TaqMan assays, Applied Biosystems) were used to analyse mRNA levels of *SFRP4* (Hs00180066_m1), *LEP* (Hs00174877_m1), *TNFRSF11B* (Hs00900358_m1), *IL6* (Hs00985639_m1) and *ADIPOQ*
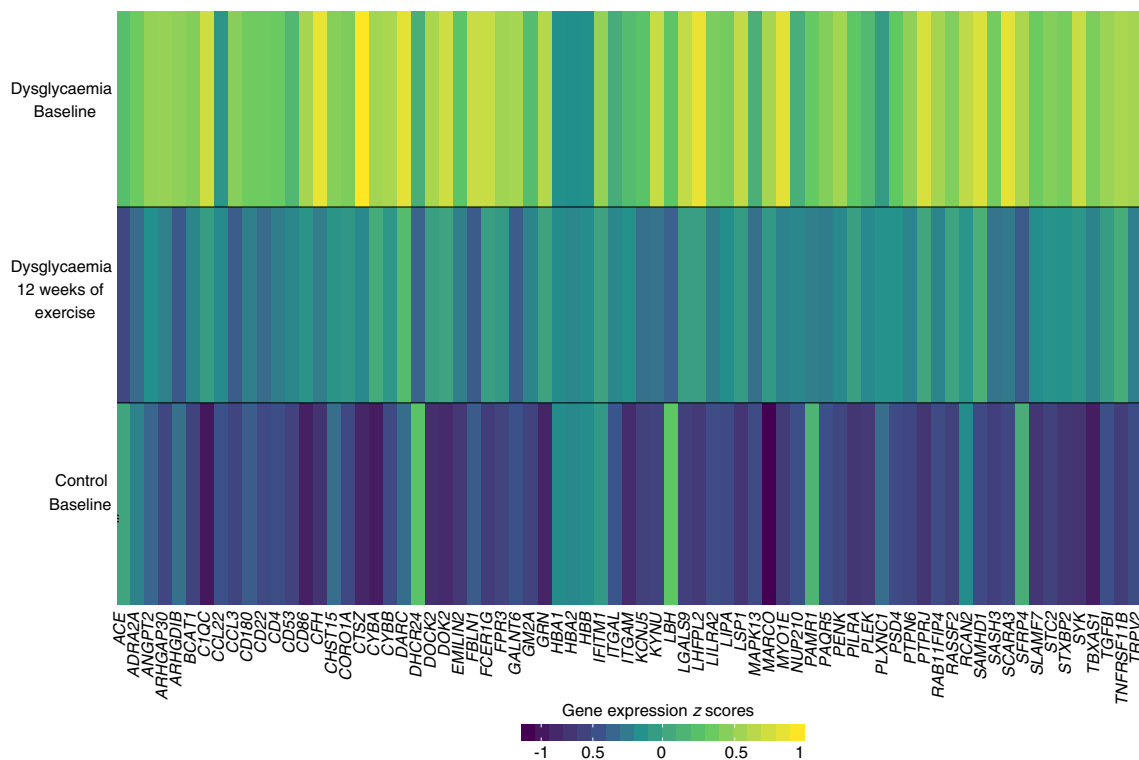
Fig. 3 Twelve weeks of exercise normalised enhanced transcript levels in scWAT from men with dysglycaemia. The figure shows median levels of the 73 transcripts that were downregulated in men with dysglycaemia after 12 weeks of the exercise intervention. Each row represents the median level across all participants in the specified group, and each column represents one gene. The scale bar below the heat map represents centred and scaled reads per kilobase of transcript per million mapped reads

Table 4 mRNA sequencing compared with RT-PCR

Gene	Control men		Men with dysglycaemia	
	lg_2FC	p value	lg_2FC	p value
ADIPOQ				
RT-PCR	−0.152	0.635	−0.022	0.910
Cuffdiff	0.000	1.000	0.000	1.000
DESeq2	0.066	0.379	0.086	0.239
edgeR	0.067	0.464	0.086	0.291
LEP				
RT-PCR	−0.222	0.015	−0.278	0.122
Cuffdiff	−0.153	0.333	−0.294	0.015
DESeq2	−0.146	0.057	−0.296	0.002
edgeR	−0.145	0.086	−0.299	0.002
IL6				
RT-PCR	−0.144	0.510	0.018	0.965
Cuffdiff	0.251	0.496	0.799	0.006
DESeq2	0.057	0.838	0.401	0.121
edgeR	0.041	0.886	0.395	0.139
SFRP4				
RT-PCR	−0.374	0.052	−0.495	0.004
Cuffdiff	−0.341	0.005	−0.536	<0.001
DESeq2	−0.388	0.052	−0.553	<0.001
edgeR	−0.418	0.013	−0.563	<0.001

Three statistical approaches to mRNA sequencing differential expression analyses were compared with each other, as well with results obtained using quantitative RT-PCR.

lg_2FC, log$_2$ of the fold change

(Hs00605917_m1). Relative target mRNA levels were calculated as $2^{-\Delta C_t}$ and normalised to *B2M* (Hs00984230_m1).

Microarrays Purified RNA was labelled using the Affymetrix WT PLUS reagent kit (Affymetrix, Santa Clara, CA, USA) and hybridised to an Affymetrix Human Gene 1.1 ST array plate. Hybridisation, washing and scanning were carried out on the Affymetrix GeneTitan platform according to the manufacturer's instructions. Arrays were analysed using the R package Oligo v.1.46 [31] following standard procedures for quality checks and calculation of normalised expression values.

High-throughput mRNA sequencing All muscle and scWAT samples were deep-sequenced using the Illumina HiSeq 2000 system (San Diego, CA, USA) with multiplex at the Norwegian Sequencing Centre, University of Oslo. Illumina HiSeq RTA (real-time analysis) v1.17.21.3 was used. Reads passing Illumina's recommended parameters were demultiplexed using CASAVA v1.8.2 (Illumina). For pre-alignment quality checks, we used the software FastQC v0.10.1 (Illumina). The mean library size was approximately 44 million unstranded 51 bp single-ended reads for muscle tissue and approximately 52 million for scWAT, with no differences between groups or time points. No batch effects were present. cDNA sequenced reads alignment was performed using Tophat v2.0.8 [32], Samtools v0.1.18 [33] and Bowtie v2.1.0 [34] with default settings against the UCSC hg19 (University of California Santa Cruz, Santa Cruz, CA, USA) annotated transcriptome and genome dated 14 May 2013. Post-alignment quality controls were performed using the Integrative Genome Viewer v2.3 (Illumina) and BED tools v2.19.1 [35]. Reads were counted using the intersection strict mode in HTSeq v0.6.1 [36].

Differential transcript expression using mRNA sequencing The edgeR v3.4.2 [37], DESeq2 v1.4.5 [38], and Cuffdiff v2.1.1 [32] workflows were performed. Statistical significance was set at a false discovery rate of <15% for each approach, and then intersected to find coherent results from the three approaches. TaqMan real-time RT-PCR and microarrays were subsequently used to validate the results. Expression levels are presented as reads per kilobase of transcript per million mapped reads.
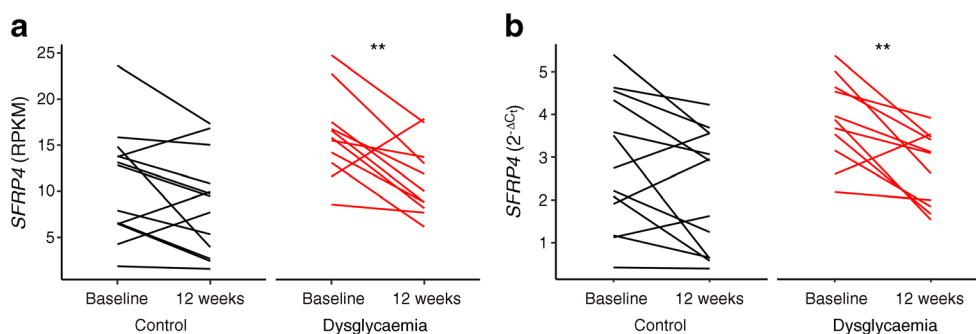
Fig. 4 Validation of mRNA sequencing results. We analysed the transcript most downregulated in men with dysglycaemia after 12 weeks of exercise (*SFRP4*; see Table 3) using (**a**) mRNA sequencing (−28.9% [*p*>0.05] and −67.8 % [**p*<0.01] in control individuals and men with dysglycaemia, respectively) and (**b**) real-time quantitative RT-PCR (−66.1% [*p*>0.05] and −112.4 % [***p*<0.01] in control individuals and men with dysglycaemia, respectively. Paired *t* tests were used for comparisons. RPKM, reads per kilobase of transcript per million mapped reads
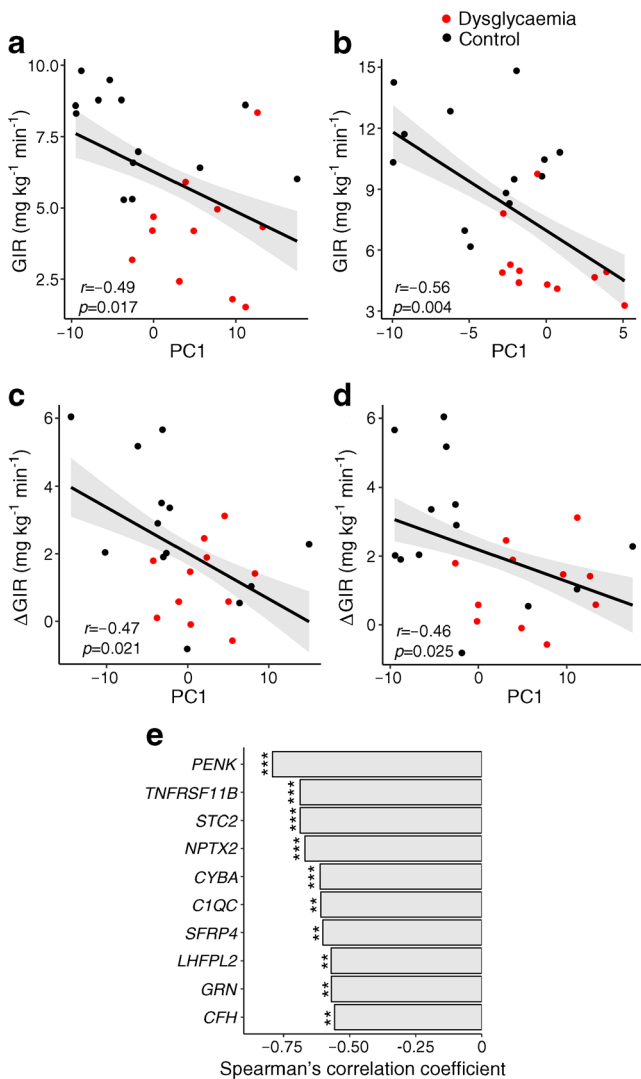
Fig. 5 Correlations between scWAT transcript levels and euglycaemic–hyperinsulinaemic clamp results. Principal component analysis was performed on the 73 transcripts downregulated after 12 weeks of exercise in men with dysglycaemia (see Fig. 3). The first principal component (PC1) correlated with GIR at (**a**) baseline and (**b**) after 12 weeks of exercise intervention, and (**c**) with change in GIR in response to 12 weeks of exercise intervention (principal component analysis was performed on change scores). (**d**) Transcript levels at baseline predicted the change in GIR in response to 12 weeks of exercise. Trend lines with standard error of estimates (SEEs; grey areas around the black regression lines) are presented. (**e**) The top ten most significant Spearman's correlations between transcript levels and GIR (see also ESM Table 1). The results were similar using either Spearman's or Pearson's correlations. **p<0.01, ***p<0.001 for the correlation between the gene and GIR

Pathway analysis of mRNA sequencing results Pathway analysis was performed using MSigDB (The Molecular Signatures Database; http://software.broadinstitute.org/gsea/msigdb) KEGG pathways. Differentially expressed transcripts were tested for significant overlaps with these pathways using hypergeometric tests [39]. The Benjamini–Hochberg procedure was used to correct *p* values [40].

Upstream transcriptional regulators of the observed transcript changes were identified using Qiagen Upstream Regulator Analysis (Hilden, Germany).

Secretory proteins

We used the MetazSecKB (the metazoa [human & animal] protein subcelluar location, secretome and subcellular proteome database; http://bioinformatics.ysu.edu/secretomes/animal/index.php) knowledgebase to identify transcripts encoding secreted proteins. MetazSecKB identifies secretory proteins based on either curated evidence of secretion (annotated and reviewed in the UniProtKB/Swiss-Prot dataset) or computationally predicted secretory protein sequences, without containing transmembrane domains or endoplasmic reticulum retention signals, using several tools (SignalP4 [41], Phobius [42], TargetP [43] and WoLF PSORT [44]).

scWAT cell type transcript markers

We selected high-specificity markers of scWAT cell types (adipocytes, macrophages, leucocytes and progenitor cells) based on the study by Ehrlund et al [45], and markers of M1-like and M2-like macrophages from the study by Hill et al [46].

Plasma analyses

Plasma samples of secreted frizzled-related protein 4 (SFRP4) (catalogue no. SEF878Hu, Cloud-Clone, Houston, TX, USA), total and high-molecular-weight adiponectin (catalogue no. DRP300 and DHWAD0, R&D Systems, Minneapolis, MN, USA), IL-6 (catalogue no. HS600B, R&D Systems) and leptin (catalogue no. KAC2281, Invitrogen, Carlsbad, CA, USA) were measured in duplicate using ELISA according to the manufacturer's protocol. Absorbance was determined using a microplate reader (Titertec Multiscan Plus; EFLAB, Helsinki, Finland), which was set to 450 or 490 nm depending on the specific protocol. Standard curves for all proteins were generated using a best-fit curve.

External datasets

We compared our results with those from two other independent studies in obese, insulin-resistant participants by using the Array Express (EMBL-EBI), and the E-GEOD-70529 [47] and E-GEOD-26637 [48] datasets, respectively. The R packages Oligo [31] and *LIMMA* [49] were used for analysis.

Statistics

Data were modelled using parametric or non-parametric methods, as appropriate and specified along with each

analysis. p values were considered significant at $\alpha = 0.05$. All data were analysed using R v3.3.3 [50]. We performed principal component analysis to enable the study of intercorrelated measures by producing linear combinations (principal components), where the first principal component is the linear sum of the measures that has the largest total variance [51].

Results

GIR increased by 45% and 38% in men with dysglycaemia and normoglycaemia, respectively (Table 1). Participant characteristics are presented in Table 1. At baseline, men with dysglycaemia had significantly more adipose tissue, lower $\dot{V}O_{2max}$ and impaired glucose metabolism, and a non-significant tendency to higher plasma hsCRP levels ($p < 0.06$) vs control individuals. Body composition, physical fitness and glucose metabolism improved similarly for both groups in response to 12 weeks of exercise (Table 1), as reported previously [26].

scWAT transcriptomics response to 12 weeks of exercise

Intersected results from all three approaches to mRNA sequencing analyses revealed seven transcripts responding to 12 weeks of exercise among control participants (Fig. 2a,c), as compared with 90 transcripts in men with dysglycaemia (Fig. 2b,d). Three transcripts were upregulated and four transcripts were downregulated in control participants (Fig. 2a and Table 2), while 17 transcripts were upregulated and 73 transcripts were downregulated in men with dysglycaemia (Fig. 2b and Table 3). Evidence for encoding secretory proteins existed for five of seven transcripts for control participants (Table 2) and for 62 of 90 transcripts for men with dysglycaemia (Table 3).

For the 73 transcripts that were downregulated in men with dysglycaemia, absolute levels of most mRNAs were higher in men with dysglycaemia as compared with control participants at baseline (Fig. 3). The differences in absolute transcript levels between the two groups were attenuated after 12 weeks of exercise (Fig. 3).

Our mRNA sequencing data were highly coherent across statistical approaches and across different technologies, including as RT-PCR (Table 4 and Fig. 4), but also cDNA microarrays from a subset of the samples (after 12 weeks of exercise, *SFRP4* expression in control participants was reduced by 76.5% [$p = 0.13$; $n = 4$], whilst in men with dysglycaemia it was reduced by 55.3% [$p < 0.001$ $n = 7$]).

scWAT mRNA levels and insulin sensitivity

We applied principal component analyses to the 73 downregulated transcripts in men with dysglycaemia after 12 weeks of exercise, and correlated the first principal component to insulin sensitivity, measured as GIR (Fig. 5). The first principal component correlated with GIR at baseline (Fig. 5a) and after the 12 week intervention (Fig. 5b). We also observed significant correlations between changes in GIR in response to the 12 week intervention and changes in the levels of these transcripts (Fig. 5c), and baseline transcript levels predicted GIR change in response to the intervention (Fig. 5d). The transcripts exhibiting the most significant Spearman's correlations with GIR are presented in Fig. 5e, and all transcript correlations with GIR are presented in the electronic supplementary material (ESM) Table 1.

scWAT pathway and upstream regulator analyses

Transcripts responding to 12 weeks of exercise in men with dysglycaemia (Table 3) overlapped with several immune-related pathways, such as leucocytes, transendothelial migration, toll-like receptor signalling, and B and T cell receptor signalling (Fig. 6a). These transcripts (Table 3) also correlated with plasma hsCRP levels (ESM Table 2), and might form a part of a cytokine signalling network regulated by the transcription factors SRY-box 9 (SOX9), transcriptional and immune response regulator (TCIM) and RAR-related orphan receptor C, isoform CRA_a (RORC) (Fig. 6b).

scWAT cell populations

The 73 downregulated transcripts after 12 weeks of exercise in men with dysglycaemia were highly related to immune- and macrophage-related processes, based on gene-set enrichment analyses (Fig. 6a). We monitored the top 100 high-specificity transcript markers of human scWAT adipocytes, macrophages, leucocytes and progenitor cells, as according to Ehrlund et al [45]. Adipocyte-related transcript levels were lower and macrophage-related transcript levels were higher in men with dysglycaemia as compared with the control group (Fig. 6c,d). Macrophage- and leucocyte-related transcript levels decreased in men with dysglycaemia after 12 weeks of exercise intervention, whereas no changes were observed in the control participants (Fig. 6d,e). We also analysed high-specificity markers of human scWAT M1-like and M2-like macrophages, as according to Hill et al [46]. M2-like, but not M1-like, macrophage-related transcripts were more highly expressed in men with dysglycaemia as compared with control participants (Fig. 6g,h), and M2-like macrophage-related transcripts decreased in men with dysglycaemia after 12 weeks of the exercise intervention, whereas no change was observed in the control participants (Fig. 6h). M2-like macrophage transcript levels correlated negatively to GIR and plasma adiponectin levels (Fig. 6i) and scWAT *ADIPOQ* transcript levels (Fig. 6j) at baseline. However, the correlation between M2-like macrophage transcript levels and GIR disappeared
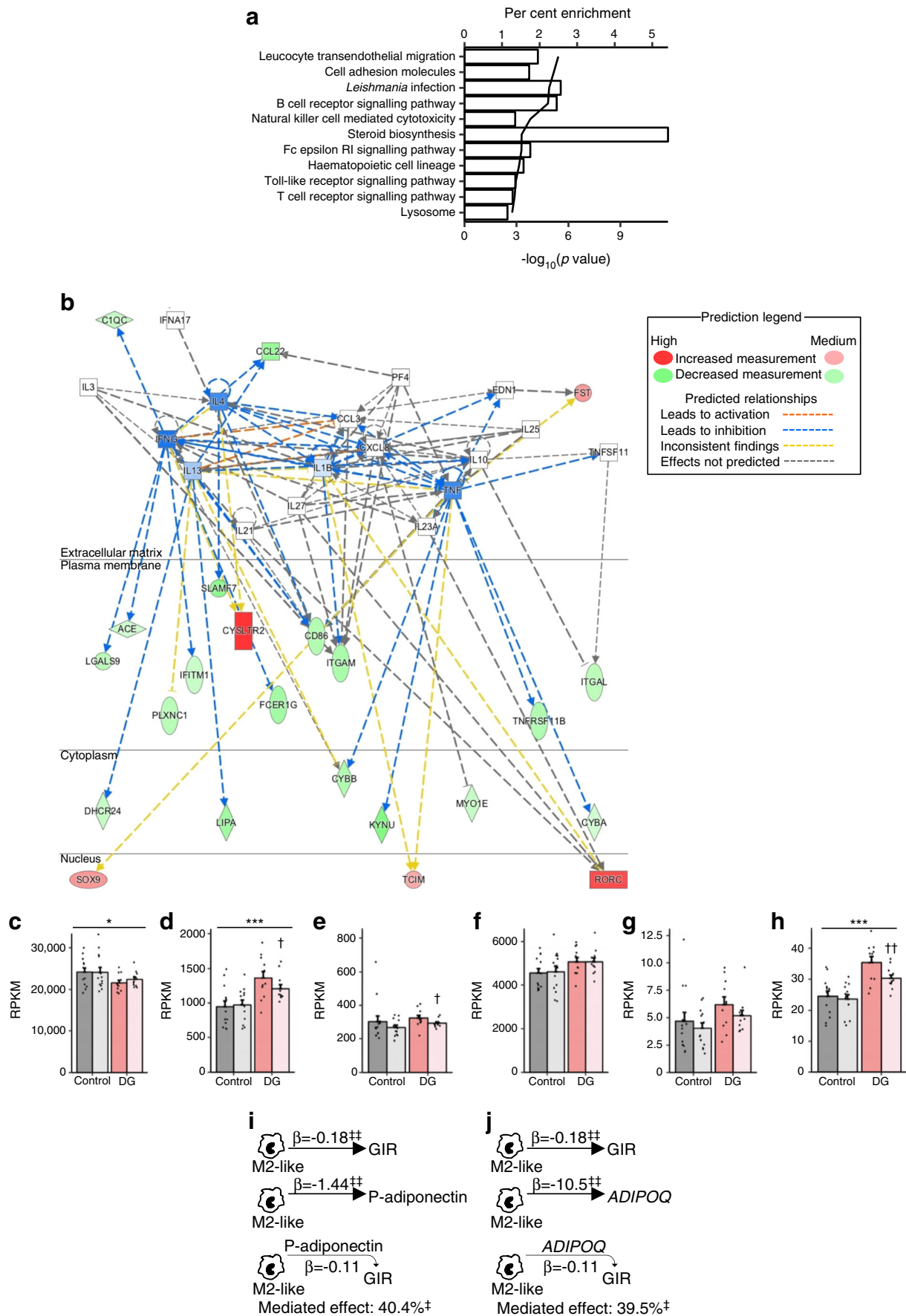
◀ **Fig. 6** Pathways, cell types and mediation analyses. (**a**) Pathway-enrichment analysis of scWAT transcripts regulated after 12 weeks of exercise in dysglycaemic men, and (**b**) upstream regulatory network analysis. (**c**-**h**) Analyses of main cell populations in scWAT: (**c**) adipocytes, (**d**) macrophages, (**e**) leucocytes, (**f**) progenitor cells, (**g**) M1-like macrophages and (**h**) M2-like macrophages. Bar plots represent means ± SEM. *$p<0.05$, ***$p<0.001$ for between-group comparison (dysglycaemia vs control participants) at baseline using unpaired t tests. †$p<0.05$, ††$p<0.01$ for within-group comparison (after [light grey/light red] vs before [dark grey/dark red] the 12 week intervention) using paired t tests. (**i**, **j**) Mediation analyses between M2-like macrophage transcript levels and (**i**) plasma (P) adiponectin levels or (**j**) scWAT *ADIPOQ* transcript levels, and GIR. ‡ $p<0.05$, ‡‡$p<0.01$. DG, participants with dysglycaemia; RPKM, reads per kilobase of transcript per million mapped reads

after adjusting for adiponectin levels (Fig. 6i,j). Group differences and responses to 12 weeks of exercise for all of the top 100 transcript markers for each scWAT cell type are presented in ESM Tables 3–6.

Effects of weight loss and insulin

To explore potential mechanisms behind the dysglycaemia-specific effects of 12 weeks of exercise intervention on scWAT, we analysed the transcripts that changed in response to exercise in men with dysglycaemia only (Table 3). We investigated if the change in transcripts could be due to weight loss by comparing our exercise-changed transcripts to those changed in scWAT after weight loss in obese and insulin-resistant participants in the study of Magkos et al [47] (Fig. 7a). We also evaluated if the change in these transcripts might be due to hyperinsulinaemia by studying data from human scWAT before and after 3 h of insulin infusion in obese and insulin-resistant participants compared with lean and insulin-sensitive participants in the study by Soronen et al [48] (Fig. 7b,c).

Thirteen transcripts were associated with genes changed in response to weight loss (Fig. 7a), and these exhibited the same direction of regulation as seen after 12 weeks of exercise intervention (Table 3). Eight transcripts in the study by Soronen et al [48] were associated with hyperinsulinaemia (Fig. 7b) and exhibited the opposite directions of regulation as seen after 12 weeks of exercise intervention (Table 3). Eight transcripts were differently regulated in response to insulin infusion between insulin-resistant and insulin-sensitive participants (Fig. 7c).

scWAT mRNA levels and plasma adipokine concentrations

LEP and *ADIPOQ* mRNA levels displayed significant correlations with plasma leptin and high-molecular-weight adiponectin concentrations in both groups of participants at baseline (Fig. 8). Similar results were also observed for
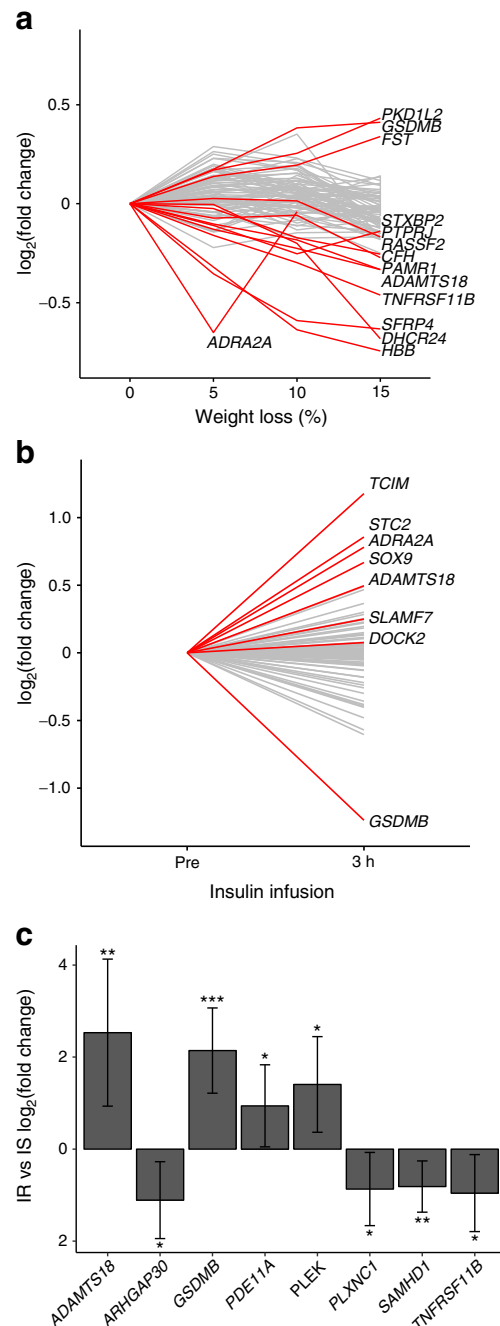
Fig. 7 Effects of weight loss and hyperinsulinaemia on subcutaneous adipose tissue transcript levels. (**a**) Effects of moderate and subsequent progressive weight loss from the study by Magkos et al [47]. Grey lines, $p>0.05$; red lines, $p<0.05$ (5, 10 or 15% weight loss vs baseline). (**b**) Effects of 3 h of insulin infusion (grey lines, $p>0.05$; red lines, $p<0.05$ 3 h insulin infusion vs baseline), and (**c**) differences in response to insulin infusion between insulin-resistant (IR) and insulin-sensitive (IS) participants based on data from the study by Soronen et al [48]. The bar plot represents log_2(fold change) ± 95% CIs for the response to insulin infusion in insulin-resistant vs insulin-sensitive participants. *$p<0.05$, **$p<0.01$, ***$p<0.001$

IL-6 and total adiponectin, and for values after 12 weeks and for changes observed during the intervention (data not shown).

Plasma adipokines response to exercise

Because *SFRP4* mRNA showed the largest change in response to the exercise intervention (i.e. lowest *p* values) across all applied methods (Table 3), and has a known signalling peptide (Table 3), we investigated the effect of exercise on plasma SFRP4 levels. Whereas scWAT *SFRP4* mRNA levels did not correlate to plasma SFRP4 concentrations in control participants (Fig. 9a), a significant correlation was seen in men with dysglycaemia (Fig. 9b). Furthermore, no change in plasma SFRP4 concentration was seen in the control group after 12 weeks of exercise (Fig. 9c), whereas a reduction was observed in men with dysglycaemia.

Skeletal muscle mRNA levels and plasma adipokine levels

No correlations were observed between skeletal muscle mRNA levels and plasma concentrations of leptin, total or high-molecular-weight adiponectin, IL-6 or SFRP4 (data not shown).
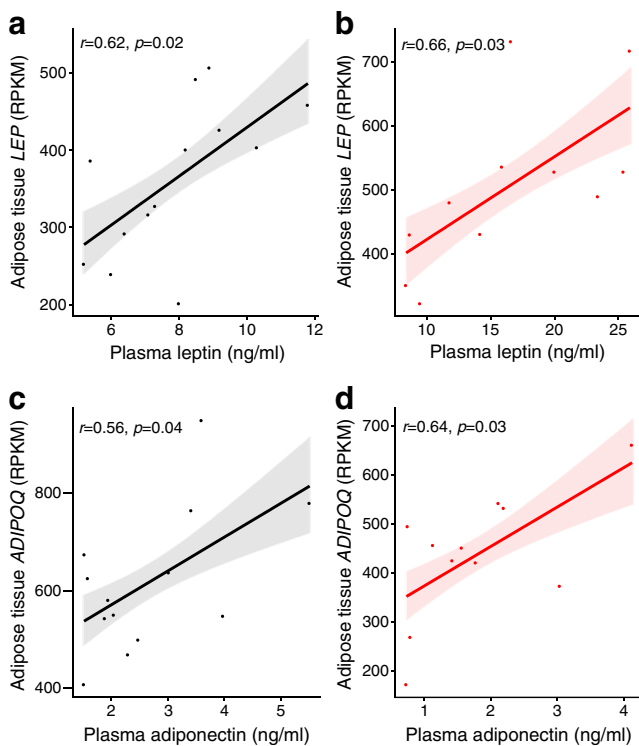
Fig. 8 Correlations between transcript levels in scWAT and protein concentrations in plasma. scWAT transcript levels (quantified by mRNA sequencing) correlated with corresponding plasma protein concentrations for (**a**,**b**) leptin and (**c**,**d**) high-molecular-weight adiponectin in both groups at baseline. The results were similar using either Spearman's or Pearson's correlations. Black lines and dots, control participants; red lines and dots, men with dysglycaemia. Trend lines with standard error of estimates (SEEs; shaded areas) are presented. RPKM, reads per kilobase per million mapped reads

Discussion

Our main findings were: (1) a substantial difference between change in scWAT transcript levels in response to 12 weeks of exercise among men with dysglycaemia as compared with the control group; (2) some dysglycaemia-specific transcripts were upregulated, but most transcripts were downregulated after 12 weeks of exercise, and were related to inflammation and macrophages; (3) absolute levels of these immune-related transcripts were elevated in men with dysglycaemia at baseline, but were normalised after 12 weeks of exercise, as compared with control participants; (4) levels of these immune-related transcripts correlated negatively with insulin sensitivity in several comparisons; and (5) exercise responses in the scWAT secretome may be reflected in altered plasma concentration of adipokines such as SFRP4.

Whereas several scientists have described adipose tissue responses to different weight-loss regimens in a variety of human populations and animal models [15, 24, 35, 52], there
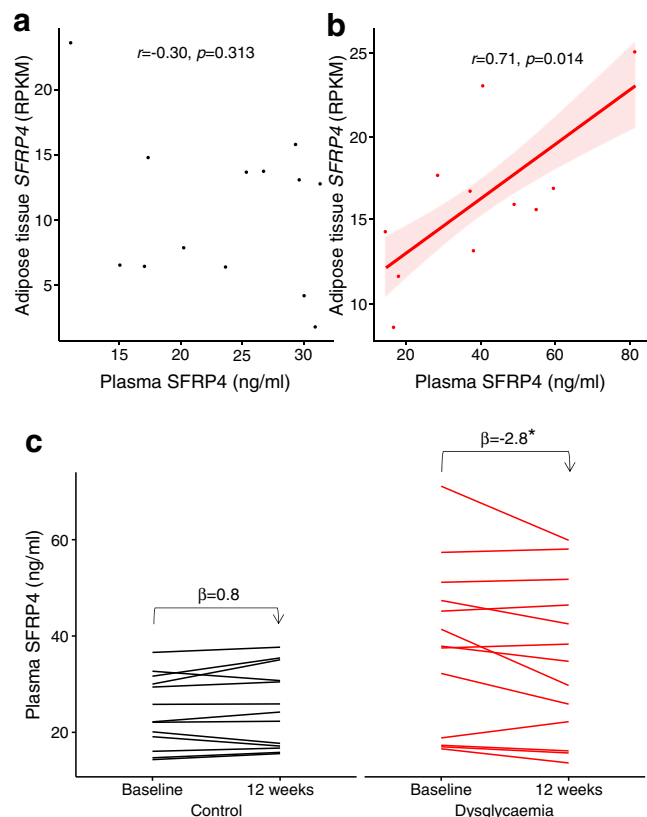
Fig. 9 Analysis of SFRP4. (**a**) Plasma SFRP4 concentrations did not correlate with *SFRP4* levels in scWAT in men with normoglycaemia (control), but did correlate in (**b**) men with dysglycaemia. The results were similar using either Spearman's and Pearson's correlations. Trend lines with standard error of estimates (SEEs; shaded areas) are presented. (**c**) Plasma SFRP4 concentrations did not change after 12 weeks of exercise in men with normoglycaemia, but decreased in men with dysglycaemia. Black lines and dots, control participants; red lines and dots, men with dysglycaemia. RPKM, reads per kilobase of transcript per million mapped reads. *$p<0.05$, paired *t* tests were performed

is a lack of studies on the response to exercise, and especially in reporting results after a long-term period of exercise in humans [15, 20, 21]. One study applied microarrays on scWAT before and after 6 months of an exercise intervention in 14 postmenopausal women and found no apparent alterations in transcript levels [24]. Another study among 25 obese, premenopausal women revealed no changes in the mRNA levels of LEP, ADIPOQ, IL6 and TNF after 12 weeks of bicycle ergometer training [22]. Although large differences exist between these studies and ours with respect to exercise protocols, the sex, age, BMI and ethnicity of the participants and the applied technologies, the most important difference may be that the women in these earlier studies were normoglycaemic and insulin sensitive. Hence, the results were in line with those seen among our normoglycaemic and insulin-sensitive control group.

One striking observation in our data is the effect of long-term exercise on reducing levels of inflammatory mRNAs in scWAT among the overweight, dysglycaemic participants. Our pathway analysis and assessment of high-specificity transcript markers of scWAT cell populations [45] indicated less immune cell infiltration, especially of leucocytes and macrophages, after 12 weeks of the exercise intervention in men with dysglycaemia. This is in line with previous studies suggesting that exercise may reduce immune cell infiltration in the stromal vascular fraction of adipose tissue and positively influence adipose tissue macrophage phenotypes [17, 18, 23]. However, these studies focused on visceral adipose tissue [17, 18, 23], not scWAT.

It should be noted that the different transcriptional alterations in scWAT between the two groups in our study occurred without significant changes in diet, BMI, plasma fasting insulin or C-peptide (Table 1). There were also similar reductions in the amount of scWAT and visceral adipose tissue [26] and similar increases in GIR and $\dot{V}O_{2max}$ between the two groups (Table 1). Thus, we can only speculate as to why exercise responses in scWAT differed between the two groups, but we suggest that it might relate to differences in fat tissue at baseline (Table 1). Our men with dysglycaemia had more scWAT (Table 1) and increased scWAT inflammation-related transcript levels (Figs. 3 and 6) compared with the control group at baseline. Whereas reduced amounts of scWAT after 12 weeks of exercise in men with dysglycaemia might counteract inflammation-responses associated with expanded adipose tissue [5, 6], no such alterations occurred in normal-weight men, perhaps because their scWAT only changed within a physiological range. We analysed scWAT transcript responses to weight loss (mostly fat mass) and hyperinsulinaemia (insulin infusion for 3 h) in obese and insulin-resistant humans [47, 48]. These analyses demonstrated that some transcripts responding to 12 weeks of exercise intervention in men with dysglycaemia also responded to fat loss or hyperinsulinaemia. Thus, for these transcripts, the observed responses in our data might not be due

to exercise per se, but rather due to loss of fat mass and/or improved insulin sensitivity. However, for the majority of transcripts (Table 3), there may be a direct effect of long-term exercise and alleviation of insulin resistance in men with dysglycaemia.

Although human data are generally lacking regarding scWAT and exercise, reports from experiments in rodents have demonstrated profound effects of exercise on scWAT [4]. Transplanting scWAT from mice performing 11 days of cage wheel exercise into sedentary mice dramatically improved glucose tolerance [4]. This effect lasted for 9 days and was related to increased skeletal muscle and intrascapular brown adipose tissue glucose uptake, perhaps related to alterations in more than 250 putative adipokines, possible mediating the effect on glucose tolerance [4]. Although the relevance of these findings to humans remains uncertain, they are in line with our results showing that especially macrophage-related transcript expression seems to be negatively correlated with insulin sensitivity, and that much of this effect may relate to interplay with adipocytes through, for example, adiponectin (Fig. 6 and ESM Tables 1–6) [53]. Thus, both data from Stanford et al [4] and our study suggest that some of the effects of long-term exercise on insulin sensitivity are mediated by alterations in scWAT.

Previous studies on exercise and adipokines have described minor effects, at least compared with different weight-loss regimes [20]. These observations are in agreement with our study, which revealed modest changes in mRNA levels (Fig. 2) and minor changes in plasma adipokine concentrations (Fig. 9). However, subtle effects might still reflect important adaptations; for example, SFRP4 has been shown to be secreted from human scWAT explants [54], and scWAT is the major contributor to plasma SFRP4 concentrations, correlating with indices of obesity and insulin resistance [55]. A mechanism linking elevated plasma SFRP4 concentration to type 2 diabetes involves inflammation in pancreatic islets and reduced insulin secretion [56].

The main limitation of our study is the small sample size of white men only, providing low statistical power and reduced generalisability. However, we applied state-of-the art technologies on human participants who were overweight and of normal weight, and with and without dysglycaemia, who underwent a strictly controlled high-intensity exercise intervention, and compared several statistical approaches across multiple technological platforms to minimise bias. Moreover, the published literature on adipokines and exercise is limited and incomplete, especially in regard to scWAT.

Conclusion

We discovered a pronounced effect of long-term exercise on scWAT in overweight men with dysglycaemia, whereas small alterations were observed in normal-weight men with

normoglycaemia. The effect included normalisation of macrophage-related transcript levels, and was closely related to improved insulin sensitivity. The alterations in scWAT involved several secreted factors, and may be mirrored in alterations of plasma adipokine concentrations, at least for SFRP4. These findings indicate that scWAT may be an important mediator of exercise-induced improvements in insulin sensitivity for individuals at risk of developing type 2 diabetes.

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