1 **Effects of long-term exercise on plasma adipokine levels and inflammation-related**
2 **gene expression in subcutaneous adipose tissue in sedentary men with and without**
3 **overweight and dysglycaemia**

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11 **Tweet:** (Figure 6) Findings after 12 weeks of combined strength and endurance exercise suggest reduced
12 macrophage-related cytokines expression in subcutaneous adipose tissue from overweight men with
13 dysglycaemia but not in control men.
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Aims/hypothesis: Obesity and insulin resistance may be associated with altered expression and secretion of adipokines. Physical activity can improve insulin sensitivity markedly, but the association to adipokines remains largely unknown. We examined effects of physical activity on the subcutaneous white adipose tissue (scWAT) secretome, and its relationship to insulin sensitivity.

Methods: As reported previously, we included 26 sedentary middle-aged men with or without overweight and dysglycaemia to a 12 w supervised intensive physical exercise intervention, including two endurance and two resistance sessions weekly. Insulin sensitivity was measured as glucose infusion rate (GIR) from a hyperinsulinaemic euglycaemic clamp. We measured maximum oxygen uptake, upper and lower body strength, and a range of circulating biomarkers and quantified adipose tissue depots using magnetic resonance imaging and 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 w of exercise, as reported previously. mRNA sequencing of scWAT revealed 90 transcripts responding to exercise in dysglycaemic men, whereas only marginal changes were observed in normoglycaemic men. These results were validated using microarrays and RT-PCR. Sixty-three out of 90 transcripts encoded secreted proteins. Seventeen transcripts were up regulated and 73 transcripts were down regulated. Down regulated transcripts included several macrophage-markers, and were related to inflammatory and immune-related pathways. These immune-related transcripts also displayed enhanced levels in dysglycaemic men at baseline, but were partly normalized after intervention as compared to normoglycaemic men. Performing principal component and correlations analyses revealed inverse correlations between levels of these immune-related transcripts and insulin sensitivity, both at baseline, after intervention and between changes. In addition, levels of these transcripts at baseline could predict exercise-improvement in insulin sensitivity. ScWAT, but not skeletal muscle, adipokine levels were significantly correlated to corresponding plasma adipose concentrations as exemplified by leptin, high-molecular weight adiponectin and secreted frizzled-related protein 4 (SFRP4). The *SFRP4* mRNA was the most exercise-responsive transcript in scWAT from dysglycaemic men, and plasma SFRP4 concentrations were reduced in dysglycaemic, but not in normoglycaemic men after 12 w of exercise.

Conclusion/interpretation: ScWAT may be an important mediator of exercise-induced improvements in insulin sensitivity, especially in overweight dysglycaemic subjects at increased risk of developing type 2 diabetes.

Keywords: adipokines, subcutaneous adipose tissue, long term exercise, prediabetes, humans, insulin sensitivity

53 # Abbreviations

54 Adipose tissue (AT)
55 Subcutaneous white adipose tissue (scWAT)
56 Interleukin 6 (IL-6)
57 Tumour necrosis factor alpha (TNFα)
58 Enzyme-linked immunosorbent assays (ELISA)
59 Dysglycaemia (DG)
60 Glucose infusion rate (GIR)
61 Magnetic resonance imaging/spectroscopy (MRI/MRS)
62 False discovery rate (FDR)
63 Reads per kilo base of transcript per million mapped reads (RPKM)
64 Secreted frizzled-related protein (SFRP4)
65 Electronical supplementary material (ESM)

Research in context

What is already known about this subject?

- Studies on adipokines and insulin resistance indicate beneficial effects of life style interventions but have focused on few adipokines 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 such studies are lacking in humans.

What is the key question?

- How does 12 w of physical exercise influence global expression of secreted factors in scWAT and plasma concentrations of such factors in men with and without overweight and dysglycaemia?

What are the new findings?

- By global mRNA sequencing, we discovered a distinct effect of 12 w of exercise on the scWAT secretome specifically for dysglycaemic men, with minimal alterations observed in normoglycaemic men.
- These secreted factors related to inflammation, and correlated negatively to insulin sensitivity.
- Alterations 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.

87 # Introduction

88 Adipose tissue (AT) is an active endocrine organ that expresses and secretes multiple metabolically active
89 factors such as leptin, adiponectin, interleukin 6 (IL-6) and tumour necrosis factor alpha (TNFα) [1-3]. These
90 secreted factors are involved in metabolic and inflammatory processes and may act in a paracrine or endocrine
91 way, altering metabolism in the liver, pancreas, skeletal muscle, and the central nervous system [3, 4].
92 Dysregulation of these signal molecules is closely related to adipocyte hypertrophy and insulin resistance [5-
93 7], and has been characterized in several studies [3, 8-10].

94 Insulin resistance is a hallmark of type 2 diabetes mellitus and is closely linked to lifestyle variables such
95 as diet and physical activity [11-14]. Physical activity can increase insulin sensitivity substantially, and
96 exercise-induced alterations in subcutaneous white adipose tissue (scWAT) may affect whole body metabolic
97 health [4, 15, 16]. Mediators of these effects may involve extensive adaptations in adipokine expression [4,
98 16]. Numerous studies have focused on the effect of different types of exercise on circulating levels of adipose
99 tissue-derived factors [3, 17-19]. However, the main body of literature on adipokines and exercise are either
100 limited to plasma analyses of one or a few targets and effects of acute exercise [3, 19-22], confounded by
101 weight loss [23] or only focusing on visceral adipose tissue [3, 17, 18]. Few studies addressed long-term
102 exercise-induced alterations in scWAT expression of such factors [22, 24], and these studies were only
103 performed in insulin sensitive women [22, 24]. In addition, whereas the skeletal muscle is extensively studied
104 in regards to physical exercise [25], significantly less focus has been attributed to AT in this aspect.

105 In our present study we investigate the effect of long-term physical exercise on scWAT transcript levels
106 and potential links to insulin sensitivity. We performed global mRNA sequencing on biopsies together with
107 enzyme-linked immunosorbent assays (ELISA) measurements of selected plasma adipokines and quantified
108 insulin sensitivity using hyperinsulinaemic euglycaemic clamp. We hypothesised that 12 w combined
109 endurance and strength training would promote distinct alterations in the scWAT transcriptome among men
110 either with or without dysglycaemia (DG) and overweight, and correlate to insulin sensitivity.

Subjects and methods

Participants and experimental methods standardization

MyoGlu is a controlled clinical exercise intervention trial (Clinical Trials registration: NCT01803568) in 26 sedentary (<1 exercise session/w) men aged 40 to 65 years of Scandinavian origin performed in 2011 to 2012, Oslo, Norway (Fig. 1) [26]. All subjects gave informed consent and the study was approved by the Regional Committee for Medical and Health Research Ethics North (Ref. no.: 2011/882). The study was designed to in depth phenotypically characterize the effects of an intensive exercise intervention across the glucometabolic spectrum. We included 13 dysglycaemic overweight men [fasting glucose $\geqslant$5.6 mmol/l and/or 2 h glucose $\geqslant$7.8 mmol/l and/or insulin resistance (HOMA-IR>2.0)], with body mass index (BMI) 26.8 to 32.5 kg/m^2] and 13 normoglycaemic men (fasting glucose <5.6 mmol/l and 2 h glucose <7.8 mmol/l without family history of diabetes, and BMI 20.9 to 26.7 kg/m^2).

Exclusion criteria included smoking, family history of diabetes (for controls only), known hypertension, liver or kidney disease, chronic inflammatory diseases, or using medication expected to affect glucose metabolism (lipid lowering, anti-hypertensive, acetylsalicylic acid, corticosteroids, etc.).

Before intervention, the participants refrained from physical exercise and alcohol for two days before testing (Fig. 1). After intervention, the last session of the 12 w intervention consisted of a low-intensity endurance session performed three days prior to testing (Fig. 1). VO_2max tests and the maximum strength tests were performed several days (more than three) before clamp and tissue sampling (Fig. 1). Test were performed under similar conditions at separate days both before and after intervention, with some exceptions for magnetic resonance imaging/spectroscopy (MRI/MRS) due to scanner availability.

Diet

Habitual diet was registered using a validated food frequency questionnaire [27, 28]. Calculations were performed using the food database AE-10 and KBS food and nutrients calculation system (KBS Version 7.1, 2013). Alcohol intake was not allowed to exceed two units per day. During testing at baseline and after 12 w of exercise the participants consumed a standardized meal after an over-night fast. A carbohydrate-rich meal including bread, apple juice, cheese, and jam was adjusted depending on individual energy requirement and provided 23% of estimated total daily energy expenditure 90–120 min prior to the test. Tests were typically performed in the morning; the standardized meal was the only intake after overnight fast. Water could be consumed freely.

Exercise intervention

Strength and endurance exercise

The participants performed four hours of intensive exercise weekly for 12 w under professional supervision. Two whole body strength training sessions and two spinning bike interval sessions lasted one hour each (Fig 1.). The 12 w intervention included linear progression in work-load for both strength and endurance exercises. For strength exercises during weeks one throughout three a load that could be lifted a maximum of 12 times [12 repetition maximum (RM)] was used, which progressed to 10 RM in weeks four throughout eight, and to 8 RM weeks nine throughout 12. Abdominal crunches and back extension were performed with 12–20 repetitions the whole intervention period. For endurance exercise during week one, three intervals were performed during the high intensity session and six intervals during the low intensity session (Fig. 1). From weeks two throughout five, four intervals were performed during the high intensity session and seven intervals during the low intensity session. From week six throughout 12, five intervals were performed during the high intensity session and ten intervals during the low intensity session.

Physical fitness and insulin sensitivity

VO_2max tests

VO_2max tests were performed after standardized 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 one min followed by a 15 watt increased workload every 30 s until exhaustion. Test success was based on O_2 consumption increased <0.5 $mL \cdot kg^{-1} \cdot min^{-1}$ over a 30 watt increase in workload, respiratory exchange ratio values >1.10, and blood lactate >7.0 mmol/l.

Euglycaemic hyperinsulinaemic clamp

Euglycaemic hyperinsulinaemic clamp was performed after an overnight fast. A fixed dose of insulin 40 $mU/m^2 \cdot min^{-1}$ was infused, and glucose (200 mg/mL) was injected to maintain euglycaemic (5.0 mmol/l) for 150 min [29]. Insulin sensitivity is reported as glucose infusion rate (GIR) during the last 30 min relative to body weight. Whole blood glucose concentration was measured using a glucose oxidase method (YSI 2300, Yellow Springs, OH) and plasma glucose concentration was calculated as whole blood glucose x 1.119.

Magnetic resonance imaging/spectroscopy (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 workflow.

Tissue sampling

As described previously [26], we obtained scWAT, skeletal muscle biopsies and blood samples before and after the 12 w exercise intervention (Fig. 1). Biopsies were obtained from the periumbilical subcutaneous tissue and from *m. vastus lateralis*. After sterilization, a lidocaine based local anaesthetic was injected in the skin and

174 sub cutis prior to both skeletal muscle and scWAT biopsies [26]. Biopsies were dissected on a cold aluminium
175 plate to remove blood etc. before freezing.

176 Transcriptomics

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

184 TaqMan real-time quantitative RT-PCR

185 Quantitative real-time PCR was performed with reagents and instruments from Applied Biosystems in the 96-
186 well format using a 7900HT Fast instrument and the SDS 2.3 software (Applied Biosystems). Pre-developed
187 primers and probe sets (TaqMan assays, Applied Biosystems) were used to analyse mRNA levels of secreted
188 frizzled-related protein 4 (*SFRP4*, Hs00180066_m1), leptin (*LEP*, Hs00174877_m1), OPG (*TNFRSF11*,
189 Hs00900358_m1), interleukin-6 (*IL6,* Hs00985639_m1) and adiponectin (*ADIPOQ,* Hs00605917_m1).
190 Relative target mRNA levels were calculated as $2^{-\Delta Ct}$, and normalized to beta-2 microglobulin (*B2M*,
191 Hs00984230_m1).

192 Microarrays

193 Purified RNA was labelled with the Affymetrix WT PLUS reagent kit (Affymetrix, Santa Clara, CA, USA) and
194 hybridized to an Affymetrix Human Gene 1.1 ST array plate (Affymetrix, Santa Clara, CA, USA). Hybridization,
195 washing and scanning were carried out on an Affymetrix GeneTitan platform according to the manufacturer's

196 instructions. Arrays were analysed using the R package Oligo [30] following standard procedures for quality
197 checks and calculation of normalized expression values.

198 High throughput mRNA sequencing

199 All muscle and scWAT samples were deep-sequenced using the Illumina HiSeq 2000 system with multiplex
200 at the Norwegian Sequencing Centre, University of Oslo. Illumina HiSeq RTA (real-time analysis) v1.17.21.3
201 was used. Reads passing Illumina's recommended parameters were demultiplexed using CASAVA v1.8.2.
202 For prealignment quality checks, we used the software FastQC v0.10.1. The mean library size was ~44 millions
203 unstranded 51 bp single-ended reads for muscle tissue and ~52 millions for scWAT with no differences
204 between groups or time points. No batch effects were present. cDNA sequenced reads alignment was done
205 using Tophat v2.0.8, Samtools v0.1.18, and Bowtie v2.1.0 with default settings against the UCSC hg19
206 annotated transcriptome and genome dated 14th of May 2013. Post-alignment quality controls were performed
207 using the Integrative Genome Viewer v2.3 and BED tools v2.19.1. Reads were counted using the intersection
208 strict mode in HTSeq v0.6.1.

209 Differential transcript expression using mRNA sequencing

210 The edger v3.4.2, DESeq2 v1.4.5 and Cuffdiff v2.1.1 workflows were performed. Statistical significance was
211 set at false discovery rate (FDR) < 15% for each approach, and then intersected to find coherent results from
212 the three approaches. TaqMan real-time RT-PCR and microarrays were subsequently used to validate the
213 results. Expression levels are presented as reads per kilo base of transcript per million mapped reads (RPKM).

214 Pathway analysis of mRNA sequencing results

215 Pathway analysis was performed using MSigDB KEGG pathways. Differentially expressed transcripts were
216 tested for significant overlaps with these pathways using hypergeometric tests [31]. *P*-values were corrected

217 using the Benjamini-Hochberg procedure [32]. Up-stream transcriptional regulators of the observed transcript
218 changes were identified using Qiagen up-stream regulator analysis.

219 Secretory proteins

220 We used the MetazSecKB knowledgebase to identify transcripts encoding secreted proteins. MetazSecKB
221 identifies secretory proteins based on either curated evidence of secretion (annotated and reviewed in the
222 UniProtKB/Swiss-Prot dataset) or computationally predicted secretory protein sequences, without containing
223 trans membrane domains or endoplasmic reticulum retention signals, by several tools (SignalP4, Phobius,
224 TargetP and WoLF PSORT).

225 ScWAT cell type transcript markers

226 We selected high specificity markers of scWAT cell types (adipocytes, macrophages, leukocytes and
227 progenitor cells) based on the study by Ehrlund et al 2017 [33], and markers of M1-like and M2-like
228 macrophages from the study by Hill et al 2014 [34].

229 Plasma analyses

230 Plasma samples of secreted frizzled-related protein (SFRP4) (Catalog # SEF878, Cloud-Clone Corp, Houston,
231 TX), total and high molecular weight adiponectin (R&D systems), IL-6 (Catalog # HS600B, R&D systems,
232 Minneapolis, MN), and leptin (Catalog # KAC2281, Invitrogen, CA) were measured in duplicates using
233 enzyme-linked immune-sorbent assays (ELISA) according to the manufacture's protocol. Optical density was
234 determined using a micro plate reader (Titertec Multiscan Plus; EFLAB, Helsinki, Finland), which was set to
235 450 or 490 nm depending on the specific protocol. Standard curves for all proteins were generated using a
236 best-fit curve.

External data sets

We compared our results with two other independent studies in obese insulin resistant subjects by using Array Express (EMBL-EBI), and the E-GEOD-70529 [35] and E-GEOD-26637 [36] data sets, respectively. The R packages Oligo and *LIMMA* 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 v.3.3.3 (R Development Core Team, 2009). We performed principal components analysis to enable the study of inter-correlated measures by producing linear combinations (principal components), where the 1st principal component is the linear sum of the measures that has the largest total variance [37].

Results

Subject characteristics are presented in Table 1, showing more adipose tissue, lower physical fitness, and impaired glucose metabolism, and a tendency to higher plasma hsCRP levels ($P<0.06$) in DG vs. control at baseline. Body composition, physical fitness, and glucose metabolism improved similarly for both groups in response to 12 w of exercise (Table 1), as reported previously [26].

ScWAT transcriptomics response to 12 w of exercise

Intersected results from all three approaches to mRNA sequencing analyses revealed seven transcripts responding to 12 w of exercise among control men (Fig. 2 a and c), as compared to 90 transcripts in men with DG (Fig. 2 b and d). Three transcripts were up regulated and four transcripts down regulated in control men (Fig. 2 a and Table 2), while 17 transcripts were up regulated and 73 transcripts were down regulated in men with DG (Fig. 2 b and Table 3). The 73 down regulated transcripts after 12 w of exercise in men with DG were

258 highly related to immune- and macrophage-related processes, based on gene set enrichment analyses (Fig.
259 6 a). Evidence for encoding secretory proteins existed for 5/7 and 62/90 transcripts for control men (Table 2)
260 and men with DG (Table 3), respectively.

261 For the 73 transcripts down regulated in men with DG, absolute levels for most mRNAs were higher
262 in men with DG as compared to control at baseline (Fig. 3). The differences in absolute transcript levels
263 between the two groups were attenuated after 12 w of exercise (Fig. 3).

264 Our mRNA sequencing data were highly coherent across statistical approaches, and across different
265 technologies, such as RT-PCR (Table 4 and Fig. 4), but also cDNA microarrays from a subset of the samples
266 (control men: -76.5%, P=0.13, n=4, and DG men: -55.3%, P<0.001, n=7).

267 ScWAT mRNA levels and insulin sensitivity

268 We applied principal component analyses on the 73 down regulated transcripts in men with DG after 12 w of
269 exercise, and correlated the 1st principal component to insulin sensitivity, measured as GIR (Fig. 5). The 1st
270 principal component correlated to GIR at baseline (Fig. 5 a) and after 12 w intervention (Fig. 5 b). We also
271 observed significant correlations between changes in GIR to the 12 w intervention and changes in levels of
272 these transcripts (Fig. 5 c), and baseline transcript levels predicted GIR change in response to intervention
273 (Fig. 5 d). The transcripts exhibiting the strongest Spearman's correlations to GIR are presented in Fig. 5 e,
274 and all transcript correlations to GIR are presented in the electronic supplementary material (ESM) Table 1.

275 ScWAT pathway and up-stream regulator analyses

276 Transcripts responding to 12 w of exercise in men with DG after (Table 3) overlapped with several immune-
277 related pathways, such as leukocytes, trans-endothelial migration, toll-like receptor signaling, and B cell and
278 T cell receptor signaling (Fig. 6 a). These transcripts (Table 3) also correlated with plasma hsCRP levels, as
279 presented in the ESM Table 2, and might to part of a cytokine signaling network regulated by the transcription
280 factors SOX9, TCIM, and RORC (Fig. 6 b).

281 ## ScWAT cell populations

282 We monitored the top 100 high specificity transcript markers of human scWAT adipocytes, macrophages,
283 leukocytes and progenitor cells, according to Ehrlund et al 2017 [33]. Adipocyte-related transcript levels were
284 lower, and macrophage-related transcript levels were higher in men with DG as compared to control (Fig. 6
285 c). Macrophage- and leukocyte-related transcript levels decreased in men with DG after 12 w of exercise
286 intervention, whereas no changes were observed in control men (Fig. 6 c). We also analysed high specificity
287 markers of human scWAT M1-like and M2-like macrophages, according to Hill et al 2014 [38]. M2-like, but not
288 M1-like, macrophage-related transcripts were expressed higher in men with DG as compared to control men
289 (Fig. 6 d), and decreased in men with DG after 12 w of exercise intervention, whereas no change was observed
290 in controls (Fig. 6 d). M2-like macrophage transcript levels correlated negatively to GIR and plasma
291 adiponectin levels (Fig. 6 e) and scWAT *ADIPOQ* transcript levels (Fig. 6 f) at baseline. However, the
292 correlation between M2-like macrophage transcript levels and GIR disappeared adjusting for adiponectin
293 levels (Fig. 6 e-f). Group differences and responses to 12 w of exercise for all the top 100 transcript markers
294 for each scWAT cell type are presented in the ESM Tables 3-6.

295 ## Effects of weight loss and insulin

296 To explore potential mechanisms behind the DG-specific effects of 12 w of exercise intervention on scWAT,
297 we analysed the transcripts changed in response to exercise in DG men only (Table 3). We investigated if the
298 change in transcripts could be due to weight loss by comparing our exercise-changed transcripts to the ones
299 changed in scWAT after weight loss in obese and insulin resistant participants in the study of Magkos et al
300 2016 [35] (Fig. 7 a). We also evaluated if the change in these transcripts might be due to hyperinsulinemia by
301 studying data from human scWAT before and after 3 h of insulin infusion in obese and insulin resistant
302 compared to lean and insulin sensitive participants from the study by Soronen et al 2012 [36] (Fig. 7 b-c).
303

304 Thirteen transcripts were associated with genes changed in response to weight loss (Fig. 7 a), and
305 these exhibited the same direction of regulation as seen after 12 w of exercise intervention (Table 3). Eight
306 transcripts in the study by Soronen et al 2012 [36] were associated with hyperinsulinaemia (Fig. 7 b) and
307 exhibited opposite directions of regulation as seen after 12 w of exercise intervention (Table 3). Eight
308 transcripts were differently regulated in response to insulin infusion between insulin resistant and insulin
309 sensitive participants (Fig. 7 c).

310 ScWAT mRNA levels and plasma adipokine concentrations

311 mRNA levels of *LEP* and *ADIPOQ* displayed significant correlations to plasma leptin and high molecular weight
312 adiponectin concentrations in both groups of participants at baseline (Fig. 8 a-b). Similar results were also
313 observed for IL-6 and total adiponectin, and for values after 12 w and for changes observed during the
314 intervention (not shown).

315 Plasma adipokines response to exercise

316 Because *SFRP4* mRNA showed the largest change in response to exercise intervention (lowest *P*-values)
317 across all applied methods (Table 3), and has a known signalling peptide (Table 3), we investigated the effect
318 of exercise on plasma SFRP4 levels. Whereas scWAT *SFRP4* mRNA levels did not correlate to plasma
319 SFRP4 concentrations in control men (Fig. 9 a) (left panel), a significant correlation was seen in men with DG
320 (Fig. 9 a) (right panel). Furthermore, no change in plasma SFRP4 concentration was seen in control men after
321 12 w of exercise (Fig. 9 b) (left panel), whereas a reduction was observed in men with DG (Fig. 9 b) (right
322 panel).

323 Skeletal muscle mRNA levels and plasma adipokine levels

324 No correlations were observed between skeletal muscle mRNA levels and plasma concentrations of leptin,
325 total or high molecular weight adiponectin, IL-6 or SFRP4 (not shown).

326 # Discussion

327 Our main findings were: *1)* a substantial difference between change in scWAT transcript levels in response to
328 12 w of exercise among men with DG as compared to controls; *2)* some DG-specific transcripts were up-
329 regulated, but most transcripts were down-regulated after 12 w of exercise, and related to inflammation and
330 macrophages; *3)* absolute levels of these immune-related transcripts were elevated in men with DG at
331 baseline, but were partly normalized after 12 w of exercise, as compared to the control participants; *4)* levels
332 of these immune-related transcripts correlated negatively with insulin sensitivity in several comparisons; *5)*
333 exercise responses in the scWAT secretome may be reflected in altered plasma concentration of adipokines
334 such as SFRP4.

335 Whereas several scientists have described AT responses to different weight loss regimens in a variety
336 of human populations and animal models [15, 24, 35, 39], there is a lack of studies concerning exercise,
337 especially reporting the results after long-term exercise in humans [15, 20, 21]. One study applied microarrays
338 on scWAT before and after 6 months exercise intervention among 14 post-menopausal women with no
339 apparent alterations in transcript levels [24]. Another study among 25 obese pre-menopausal women revealed
340 no changes in mRNA levels of leptin, adiponectin, IL-6 and TNF*α* after 12 w of bicycle ergometer training [22].
341 Although large differences existed between these studies and ours concerning exercise protocols, sex, age,
342 BMI, ethnicity and applied technologies, the most important difference may be that these women were
343 normoglycaemic and insulin sensitive. Hence, the results were in line with the results seen among our
344 normoglycaemic and insulin sensitive control group.

345 One striking observation in our data was the effect of long-term exercise on reducing levels of
346 inflammatory mRNAs in scWAT among the overweight/DG participants. Our pathway analysis, and
347 assessment of high specificity transcript markers of scWAT cell populations [33] indicated less immune cell
348 infiltration, especially leukocytes and macrophages after 12 w of exercise intervention in men with DG. This is
349 in line with previous studies suggesting that exercise may reduce immune cell infiltration in the stromal vascular

350 fraction of adipose tissue as well as positively influence AT macrophage phenotypes [17, 18, 23]. However,
351 these studies focus on visceral AT [17, 18, 23], not scWAT.

352 It should be noted that the different transcriptional alterations in scWAT between the two groups in our
353 study occurred without significant changes in diet, BMI, plasma fasting insulin or C-peptide concentrations
354 (Table 1). There were also similar reductions in both the amount of scWAT and visceral AT [26], and similar
355 increases in GIR and VO_2max between the two groups (Table 1). Thus, we can only speculate as of why
356 exercise-responses in scWAT differed between the two groups, but we suggest that it might relate to
357 differences in fat tissue at baseline (Table 1). Our men with DG had larger scWAT (Table 1) and increased
358 scWAT inflammation-related transcript levels (Fig. 3 and Fig. 6) compared to control men at baseline. Whereas
359 reduced amounts of scWAT after 12 w of exercise in men with DG might counteract inflammation-responses
360 associated with expanded AT [5, 6], no such alterations occurred in normal weight men, perhaps because
361 their scWAT only changed within a physiological range. We analyzed scWAT transcript responses to weight
362 loss (mostly fat mass) and hyperinsulinaemia (insulin infusion for three hours) in obese and insulin resistant
363 humans [35, 36]. These analyses demonstrated that some transcripts responding to 12 w of exercise
364 intervention in men with DG also responded to fat loss or hyperinsulinaemia. Thus, for these transcripts the
365 observed responses in our data might not be due to exercise *per se,* but rather due to loss of fat mass and/or
366 improved insulin sensitivity. However, for the majority of transcripts (Table 3), there may be a direct effect of
367 long-term exercise and alleviation of insulin resistance in men with DG.

368 Although human data are generally lacking regarding scWAT and exercise, reports from experiments
369 in rodents demonstrate profound effects of exercise on scWAT [4]. Transplanting scWAT from mice performing
370 11 days of cage wheel exercise into sedentary mice dramatically improved glucose tolerance [4]. This effect
371 lasted for 9 days and was related to increased skeletal muscle and intra-scapular brown adipose tissue glucose
372 uptake [4], perhaps related to alterations in >250 putative adipokines, possible mediating the effect on glucose
373 tolerance [4]. Although the relevance of these findings to humans remains uncertain, they are in line with our
374 results showing that especially macrophage-related transcript expression seems negatively correlated with

375 insulin sensitivity, and that much of this effect may relate to interplay with adipocytes through e.g. adiponectin
376 [40] (Fig. 6 and the ESM Tables 1-6). Thus, both data from Stanford et al. 2015 [4] and our study suggest that
377 some effects of long-term exercise on insulin sensitivity are mediated by alterations in scWAT.

378 Previous studies on exercise and adipokines describe minor effects, at least compared to different
379 weight loss regimes [20]. These observations are in agreement with our study revealing modest changes in
380 mRNA levels (Fig. 2) and minor changes in plasma adipokines concentrations (Fig. 9). However, subtle effects
381 might still reflect important adaptations, i.e. SFRP4 has been shown secreted from human scWAT explants
382 [41], and scWAT is the major contributor to plasma SFRP4 concentrations, correlating with indices of obesity
383 and insulin resistance [42]. A mechanism linking elevated plasma SFRP4 concentration to type 2 diabetes
384 involves inflammation in pancreatic islets and reduced insulin secretion [43].

385 The main limitation in our study is the small sample size of Caucasian men only, providing low
386 statistical power and reduced generalizability. However, we applied state-of-the art technologies on human
387 subjects with and without overweight and DG undergoing a strictly controlled high intensity exercise
388 intervention, and compared several statistical approaches across multiple technological platforms to minimize
389 bias. Moreover, the published literature on adipokines and exercise is limited and incomplete, especially in
390 regards to scWAT.

391 Conclusion

392 We discovered a pronounced effect of long-term exercise on scWAT in men with overweight and DG, whereas
393 small alterations were observed in men with normoglycaemia. The effect included normalization of
394 macrophage-related transcript levels, and was closely related to improved insulin sensitivity. The alterations
395 in scWAT involved several secreted factors, and may be mirrored in alterations of plasma adipokine
396 concentrations, at least for SFRP4. ScWAT may be an important mediator of exercise-induced improvements
397 in insulin sensitivity for subjects at risk of developing type 2 diabetes.

Appendix

Ethics approval

The study adhered to the Declaration of Helsinki and was approved by the National Regional Committee for Medical and Health Research Ethics North, Tromsø, Norway. Written informed consent was obtained for all participants after full explanation of the purpose and procedures used.

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Author contributions

SL analysed and prepared the data, and wrote the first draft of the manuscript. All authors interpreted the data, and reviewed, revised and approved the manuscript. KIB and CAD initiated, designed and supervised the study.

Duality of interest

The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement

CAD is the guarantor of this work.

416 ## Data availability

417 The data are available on request from the authors.

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536

537 **Table 1** Subject characteristics

	Baseline		**%-change**	
	Control (n = 13)	DG (n = 13)	Control (n = 13)	DG (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 volume (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				
VO_2max (ml/kg/min)	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				
HbA1c (mmol/mol)	33 (4)	37 (4) *	N.A.	N.A.
HbA1c (%)	5.2 (0.2)	5.5 (0.4) *	N.A.	N.A.
F-glucose (mmol/l)	5.4 (0.5)	5.9 (0.3) *	3.1 (4.5) †	1.8 (6.8)
F-C-Peptide (pmol/l)	588.0 (117.8)	932.8 (248.9) *	7.3 (23.8)	12.3 (45.3)
F-Insulin (pmol/l)	38.5 (18.6)	65.3 (27.1) *	15.1 (49.2)	27.6 (66.2)
FFA (mmol/l)	0.3 (0.1)	0.2 (0.1)	−21.7 (31.1) †	16.0 (53.1)
GIR (mg/kg/min)	7.6 (1.6)	4.2 (1.8) *	37.8 (30.1) †	44.4 (58.8) †
Plasma protein				
hsCRP (mg/l)	1.0 (0.8)	2.8 (3.3)	41.0 (96.7)	30.1 (121.2)

538 **Notes:** [a] n = 12 control, [b] n = 10 control, and n = 9 DG. * $p < 0.05$ between groups (DG vs. control), and † $p < 0.05$ baseline vs. 12
539 w within group. Between groups comparison were performed using unpaired t-tests, and within group comparisons were performed
540 using paired t-tests. Logarithmic transformation was performed to approximate normal distribution, if necessary, and back
541 transformed for presentation. Data represent means (SD).
542 **Abbreviations:** DG = dysglycaemic. N.A. = not available, AU = arbitrary units, BMI = body mass index, FFM = fat free mass, AT =
543 adipose tissue, S = subcutaneous, IA = intra-abdominal, GIR = glucose infusion rate. F = fasting. FFA = plasma free fatty acids.
544 hsCRP = high-sensitivity C-reactive protein.

Table 2 ScWAT transcripts responding to 12 w of exercise intervention in control men

Gene symbol	Adipokine?	RPKM	Cuffdiff *lg*2FC	*P*	FDR	edgeR *lg*2FC	*P*	FDR	DESeq2 *lg*2FC	SE	*P*	FDR	UniProt Name
EGFL6	Yes	19,63	-0,95	0,00	0,02	-0,79	0,00	0,00	-0,71	0,15	0,00	0,01	Epidermal growth factor-like protein 6
UCHL1	WL	71,69	-0,48	0,00	0,02	-0,43	0,00	0,00	-0,43	0,08	0,00	0,00	Ubiquitin carboxyl-terminal hydrolase
CETP	WL	12,71	0,74	0,00	0,02	0,87	0,00	0,00	0,80	0,23	0,00	0,13	Cholesteryl ester transfer protein
BDKRB2	WL	3,03	0,54	0,00	0,06	0,51	0,00	0,00	0,50	0,14	0,00	0,13	B2 bradykinin receptor
OSGIN1	WL	12,62	-0,47	0,00	0,07	-0,46	0,00	0,00	-0,46	0,12	0,00	0,07	Oxidative stress-induced growth inhibitor 1
DRD1	No	0,85	-1,15	0,00	0,04	-1,02	0,00	0,00	-0,98	0,23	0,00	0,02	D(1A) dopamine receptor
MEG3	No	30,22	0,40	0,00	0,06	0,39	0,00	0,00	0,37	0,10	0,00	0,08	-

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

Abbreviations: RPKM, reads per kilo base per million mapped reads; *lg*2FC, log2 of the fold-change; SE, standard error (of the fold-change); FDR, false discovery rate; WL, weakly likely.

Table 3 ScWAT transcripts responding to 12 w of exercise intervention in men with DG

				Cuffdiff			edgeR			DESeq2			
Gene symbol	UniProt Name	Secreted?	RPKM	*lg2*FC	*P*	FDR	*lg2*FC	*P*	FDR	*lg2*FC	SE	*P*	FDR
SFRP4	Secreted frizzled-related protein 4	Yes	16,57	-0,54	0,00	0,01	-0,56	0,00	0,00	-0,55	0,12	0,00	0,01
EMILIN2	Elastin microfibril interface-located protein 2	Yes	16,09	-0,53	0,00	0,01	-0,61	0,00	0,00	-0,53	0,12	0,00	0,01
ADAMTS4	A disintegrin and metalloproteinase with thrombospondin motifs 4	Yes	5,74	0,58	0,00	0,01	0,61	0,00	0,00	0,59	0,16	0,00	0,05
CCL22	C-C motif chemokine 22	Yes	2,48	-0,78	0,00	0,01	-0,57	0,00	0,05	-0,57	0,17	0,00	0,09
C1QC	Complement C1q subcomponent subunit C	Yes	132,33	-0,38	0,00	0,01	-0,33	0,00	0,01	-0,33	0,10	0,00	0,12
ANGPT2	Angiopoietin-2	Yes	4,86	-0,64	0,00	0,01	-0,56	0,00	0,00	-0,55	0,11	0,00	0,01
PENK	Proenkephalin-A	Yes	4,60	-0,71	0,00	0,01	-0,65	0,00	0,03	-0,63	0,16	0,00	0,03
CCL3	C-C motif chemokine 3	Yes	4,94	-0,86	0,00	0,02	-0,74	0,00	0,01	-0,72	0,21	0,00	0,07
THBS4	Thrombospondin-4	Yes	43,45	0,33	0,00	0,02	0,38	0,00	0,00	0,37	0,09	0,00	0,02
TGFBI	Transforming growth factor-beta-induced protein ig-h3	Yes	49,74	-0,32	0,00	0,03	-0,30	0,00	0,02	-0,30	0,08	0,00	0,05
NPTX2	Neuronal pentraxin-2	Yes	0,72	0,76	0,00	0,06	0,72	0,00	0,01	0,70	0,18	0,00	0,02
STC2	Stanniocalcin-2	Yes	6,10	-0,33	0,00	0,07	-0,33	0,00	0,00	-0,32	0,09	0,00	0,07
CFH	Complement factor H	Yes	55,64	-0,31	0,00	0,10	-0,28	0,00	0,04	-0,29	0,08	0,00	0,04
TNFRSF11B	Tumour necrosis factor receptor superfamily member 11B	Yes	4,25	-0,44	0,00	0,12	-0,43	0,00	0,05	-0,43	0,12	0,00	0,07
FBLN1	Fibulin-1	HL	73,17	-0,33	0,00	0,01	-0,32	0,00	0,01	-0,32	0,08	0,00	0,02
FST	FST protein	HL	10,73	0,43	0,00	0,01	0,45	0,00	0,00	0,45	0,11	0,00	0,02
BMP3	Bone morphogenetic protein 3 F	HL	0,60	0,82	0,00	0,01	0,79	0,00	0,00	0,75	0,20	0,00	0,03
SLAMF7	SLAM family member 7 F	HL	3,47	-0,72	0,00	0,01	-0,63	0,00	0,00	-0,62	0,16	0,00	0,03
HBA2	Alpha-2 globin F	HL	50,28	-1,35	0,00	0,01	-1,22	0,00	0,00	-1,12	0,29	0,00	0,03
CD4	CD4 protein	HL	26,32	-0,39	0,00	0,01	-0,33	0,00	0,00	-0,33	0,10	0,00	0,07
LIPA	Lysosomal acid lipase F	HL	139,10	-0,65	0,00	0,01	-0,52	0,00	0,00	-0,52	0,16	0,00	0,10
PDE11A	Dual 3',5'-cyclic-AMP and -GMP phosphodiesterase 11A	HL	2,43	0,42	0,00	0,02	0,49	0,00	0,00	0,49	0,11	0,00	0,01
ITGAL	Integrin alpha-L	HL	3,29	-0,45	0,00	0,02	-0,37	0,00	0,00	-0,37	0,11	0,00	0,08
ADAMTS18	A disintegrin and metalloproteinase with thrombospondin motifs 18	HL	1,03	0,55	0,00	0,02	0,58	0,00	0,00	0,56	0,16	0,00	0,08
LILRA2	Leukocyte immunoglobulin-like receptor subfamily A member 2	HL	2,12	-0,73	0,00	0,03	-0,67	0,00	0,03	-0,66	0,18	0,00	0,04
CD86	T-lymphocyte activation antigen CD86 F	HL	3,57	-0,51	0,00	0,03	-0,42	0,00	0,07	-0,43	0,12	0,00	0,05
GALNT6	Polypeptide N-acetylgalactosaminyltransferase 6 F	HL	1,01	-0,82	0,00	0,04	-0,71	0,00	0,02	-0,69	0,19	0,00	0,05
PILRA	Paired immunoglobulin-like type 2 receptor alpha	HL	9,96	-0,49	0,00	0,04	-0,49	0,00	0,01	-0,48	0,14	0,00	0,08
GRN	Granulins F	HL	182,11	-0,32	0,00	0,06	-0,28	0,00	0,01	-0,28	0,08	0,00	0,09
ACE	Angiotensin-converting enzyme	HL	18,74	-0,29	0,00	0,07	-0,27	0,00	0,02	-0,28	0,08	0,00	0,05
PTPRJ	Receptor-type tyrosine-protein phosphatase eta F	HL	5,02	-0,31	0,00	0,08	-0,31	0,00	0,00	-0,31	0,09	0,00	0,07
TSKU	Tsukushin F	HL	166,42	0,31	0,00	0,09	0,29	0,00	0,00	0,28	0,09	0,00	0,13
PAMR1	Inactive serine protease PAMR1 F	HL	5,82	-0,39	0,00	0,09	-0,41	0,00	0,00	-0,41	0,11	0,00	0,03
CD22	B-cell receptor CD22 F	HL	1,19	-0,62	0,00	0,12	-0,56	0,00	0,12	-0,57	0,16	0,00	0,05
BCAT1	Alternative protein BCAT1	L	2,90	-0,50	0,00	0,01	-0,43	0,00	0,00	-0,42	0,12	0,00	0,06
FCER1G	High affinity immunoglobulin epsilon receptor subunit gamma	L	92,32	-0,56	0,00	0,01	-0,50	0,00	0,00	-0,48	0,16	0,00	0,15
TBXAS1	Thromboxane-A synthase	L	6,95	-0,46	0,00	0,02	-0,40	0,00	0,00	-0,40	0,10	0,00	0,03
ITGAM	Integrin alpha-M F	WL	9,36	-0,53	0,00	0,01	-0,46	0,00	0,00	-0,45	0,11	0,00	0,02
LBH	Protein LBH	WL	11,35	-0,42	0,00	0,01	-0,40	0,00	0,00	-0,40	0,10	0,00	0,02
GM2A	Ganglioside GM2 activator F	WL	35,68	-0,45	0,00	0,01	-0,39	0,00	0,00	-0,39	0,10	0,00	0,02

HBB	Mutant beta-globin	WL	343,81	-1,29	0,00	0,01	-1,12	0,00	0,00	-1,06	0,28	0,00	0,03
KYNU	Kynureninase	WL	2,70	-0,81	0,00	0,01	-0,69	0,00	0,02	-0,68	0,18	0,00	0,03
PTPN6	Tyrosine-protein phosphatase non-receptor type 6	WL	16,46	-0,48	0,00	0,01	-0,42	0,00	0,00	-0,41	0,11	0,00	0,04
NDRG4	Protein NDRG4 F	WL	9,51	0,76	0,00	0,01	0,83	0,00	0,00	0,78	0,22	0,00	0,05
ARHGAP30	Rho GTPase-activating protein 30	WL	9,27	-0,45	0,00	0,01	-0,39	0,00	0,00	-0,38	0,11	0,00	0,09
LGALS9	Galectin F	WL	30,71	-0,43	0,00	0,01	-0,39	0,00	0,00	-0,39	0,12	0,00	0,09
LSP1	Lymphocyte-specific protein 1 F	WL	99,05	-0,34	0,00	0,01	-0,31	0,00	0,01	-0,31	0,10	0,00	0,11
STXBP2	Syntaxin-binding protein 2 F	WL	8,19	-0,50	0,00	0,01	-0,39	0,00	0,00	-0,39	0,12	0,00	0,12
TRPV2	Transient receptor potential cation channel subfamily V member 2 F	WL	14,33	-0,38	0,00	0,02	-0,34	0,00	0,00	-0,33	0,10	0,00	0,09
DOK2	Docking protein 2	WL	15,34	-0,40	0,00	0,02	-0,36	0,00	0,00	-0,35	0,11	0,00	0,14
SOX9	SRY-box 9 F	WL	3,39	0,43	0,00	0,03	0,45	0,00	0,00	0,44	0,09	0,00	0,01
DHCR24	Delta	WL	44,49	-0,32	0,00	0,03	-0,32	0,00	0,01	-0,32	0,09	0,00	0,06
PAQR5	Alternative protein PAQR5	WL	0,72	-0,70	0,00	0,04	-0,63	0,00	0,06	-0,61	0,19	0,00	0,10
CHST15	Alternative protein CHST15	WL	5,47	-0,38	0,00	0,04	-0,37	0,00	0,00	-0,37	0,09	0,00	0,02
C8orf4	Alternative protein C8orf4	WL	6,84	0,45	0,00	0,04	0,37	0,00	0,00	0,36	0,12	0,00	0,14
RORC	RAR-related orphan receptor C, isoform CRA_a	WL	0,45	0,78	0,00	0,06	0,76	0,00	0,00	0,71	0,22	0,00	0,11
MYO1E	Unconventional myosin-Ie F	WL	8,42	-0,32	0,00	0,07	-0,31	0,00	0,00	-0,31	0,07	0,00	0,01
RAB11FIP4	Rab11 family-interacting protein 4 F	WL	0,43	-0,66	0,00	0,09	-0,59	0,00	0,09	-0,59	0,14	0,00	0,02
PLXNC1	Plexin-C1	WL	2,47	-0,44	0,00	0,14	-0,38	0,00	0,04	-0,38	0,12	0,00	0,13
RCAN2	Calcipressin-2	WL	9,45	-0,30	0,00	0,15	-0,29	0,00	0,00	-0,29	0,10	0,00	0,14
CYBA	Cytochrome b-245 light chain F	WL	163,49	-0,27	0,00	0,15	-0,25	0,00	0,05	-0,25	0,08	0,00	0,10
PSD4	PH and SEC7 domain-containing protein 4 F	WL	2,38	-0,39	0,00	0,15	-0,33	0,00	0,06	-0,33	0,11	0,00	0,13
ABCB11	Bile salt export pump	No	0,49	0,71	0,00	0,06	0,68	0,00	0,03	0,66	0,17	0,00	0,03
ADRA2A	Alpha-2A adrenergic receptor	No	50,86	-0,29	0,00	0,05	-0,28	0,00	0,04	-0,28	0,09	0,00	0,09
ARHGDIB	Rho GDP-dissociation inhibitor 2	No	133,16	-0,28	0,00	0,09	-0,27	0,00	0,05	-0,28	0,05	0,00	0,00
CD180	CD180 antigen	No	1,39	-0,86	0,00	0,02	-0,71	0,00	0,02	-0,70	0,19	0,00	0,05
CD53	Leukocyte surface antigen CD53	No	23,25	-0,57	0,00	0,01	-0,48	0,00	0,00	-0,46	0,12	0,00	0,04
CORO1A	CoroninCoronin-1A	No	16,74	-0,39	0,00	0,02	-0,36	0,00	0,00	-0,35	0,10	0,00	0,05
CTSZ	Cathepsin Z	No	275,21	-0,28	0,00	0,11	-0,25	0,00	0,05	-0,25	0,08	0,00	0,15
CYBB	Cytochrome b-245 heavy chain	No	11,44	-0,50	0,00	0,01	-0,44	0,00	0,00	-0,43	0,11	0,00	0,03
CYSLTR2	Cysteinyl leukotriene receptor 2	No	0,56	0,84	0,00	0,04	0,87	0,00	0,00	0,83	0,18	0,00	0,01
DARC	-	No	101,21	-0,31	0,00	0,08	-0,27	0,00	0,06	-0,27	0,09	0,00	0,13
DOCK2	Dedicator of cytokinesis protein 2	No	3,87	-0,37	0,00	0,07	-0,32	0,00	0,00	-0,32	0,10	0,00	0,14
FPR3	N-formyl peptide receptor 3	No	8,85	-0,43	0,00	0,02	-0,39	0,00	0,00	-0,37	0,12	0,00	0,14
GSDMB	Gasdermin-B	No	10,92	0,45	0,00	0,01	0,38	0,00	0,00	0,37	0,11	0,00	0,10
HBA1	Hemoglobin subunit alpha	No	11,41	-1,27	0,00	0,01	-1,17	0,00	0,00	-1,08	0,28	0,00	0,03
IFITM1	Interferon-induced transmembrane protein 1	No	208,95	-0,36	0,00	0,01	-0,30	0,00	0,01	-0,30	0,08	0,00	0,02
KCNJ5	G protein-activated inward rectifier potassium channel 4	No	2,18	-0,80	0,00	0,01	-0,62	0,00	0,03	-0,62	0,17	0,00	0,04
LHFPL2	LHFPL tetraspan subfamily member 2 protein	No	9,84	-0,28	0,00	0,15	-0,27	0,00	0,00	-0,27	0,08	0,00	0,09
MAPK13	Mitogen-activated protein kinase 13Mitogen-activated protein kinase	No	2,29	-0,48	0,00	0,01	-0,47	0,00	0,04	-0,47	0,15	0,00	0,13
MARCO	Macrophage receptor MARCO	No	13,15	-0,36	0,00	0,08	-0,34	0,00	0,00	-0,34	0,09	0,00	0,03
NUP210	Nuclear pore membrane glycoprotein 210	No	1,17	-0,53	0,00	0,04	-0,46	0,00	0,06	-0,46	0,11	0,00	0,02
PKD1L2	Polycystic kidney disease protein 1-like 2	No	11,89	0,36	0,00	0,06	0,35	0,00	0,00	0,34	0,10	0,00	0,08
PLEK	Pleckstrin	No	15,44	-0,50	0,00	0,01	-0,38	0,00	0,00	-0,37	0,12	0,00	0,14

RASSF2	Ras association domain-containing protein 2	No	6,06	-0,33	0,00	0,08	-0,32	0,00	0,00	-0,32	0,08	0,00	0,02
SAMHD1	Deoxynucleoside triphosphate triphosphohydrolase SAMHD1	No	32,08	-0,27	0,00	0,12	-0,28	0,00	0,02	-0,28	0,08	0,00	0,04
SASH3	SAM and SH3 domain-containing protein 3	No	5,91	-0,58	0,00	0,01	-0,48	0,00	0,00	-0,46	0,14	0,00	0,09
SCARA3	Scavenger receptor class A member 3	No	13,86	-0,36	0,00	0,02	-0,36	0,00	0,00	-0,36	0,11	0,00	0,12
SLC25A18	Mitochondrial glutamate carrier 2	No	5,65	0,40	0,00	0,09	0,40	0,00	0,00	0,40	0,08	0,00	0,01
SYK	Tyrosine-protein kinaseTyrosine-protein kinase SYK	No	5,18	-0,42	0,00	0,01	-0,38	0,00	0,00	-0,37	0,11	0,00	0,09

Notes: 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 false discovery rate (FDR) was set to <15% for each approach, and the results were intersected between all three approaches. MetazSecKB defined secreted proteins.

Abbreviations: DG, dysglycaemia; RPKM, reads per kilo base per million mapped reads; lg_2FC, log2 of the fold-change; SE, standard error (of the fold-change); FDR, false discovery rate; F, fragment; HL, highly likely; L, likely; WL, weakly likely.

Table 4 mRNA sequencing compared to RT-PCR

	Control		DG	
	lg_2(FC)	*P*	lg_2(FC)	*P*
ADIPOQ				
RT-PCR	-0.152	0.635	-0.022	0.910
Cuffdiff2	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
Cuffdiff2	-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
Cuffdiff2	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
Cuffdiff2	-0.341	0.005	-0.536	0.000
DESeq2	-0.388	0.052	-0.553	0.000
edgeR	-0.418	0.013	-0.563	0.000

Notes: Three statistical approaches to mRNA-sequencing differential expression analyses were compared to each other, as well to results obtained using quantitative RT-PCR.
Abbreviations: FC, fold-change; DG, dysglycaemia; P, *P*-value

Figure legends

Fig. 1. Study design. (a) Twenty-six sedentary men with or without overweight and dysglycaemia were recruited into two groups. (b) The participants donated tissue samples, and underwent several tests at baseline, including hyperinsulinaemic euglycaemic clamp, before being subjected to 12 w of intensive physical exercise intervention followed by re-testing. Three days passed between the last bout of exercise and the hyperinsulinaemic euglycaemic clamp after intervention. (c) The intervention consisted of four exercise sessions each week. ScWAT, subcutaneous white adipose tissue; SkM, skeletal muscle; MRI/MRS, magnetic resonance imaging/spectrometry.

Fig. 2. Exercise-responsive transcripts in subcutaneous white adipose tissue. We compared transcript levels in biopsies obtained from subcutaneous white adipose tissue before and after 12 w 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 men and (b) dysglycaemic men. Volcano plots show fold changes and q-values for (c) normoglycaemic men and (d) dysglycaemic men. On the x-axis, data points below zero indicate down regulation, and data points above zero indicate up regulation after 12 w 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 colour signifies statistical significance. q-values; Benjamini-Hochberg corrected *P*-values.

Fig. 3. Twelve weeks of exercise partly normalized enhanced transcript levels in subcutaneous white adipose tissue from men with dysglycaemia. Median levels of the 73 transcripts being down regulated in men with dysglycaemia after 12 w of exercise intervention. Each row represent the median level across all subjects in that particular comparison, and each column represents one gene. The scale bar below the heat map represents centred and scaled reads per kilo base of transcript per million mapped reads (RPKM).

Fig. 4. Validation of mRNA sequencing results. We analysed the transcript most down regulated in men with dysglycaemia after 12 w of exercise (*SFRP4,* see Table 3) using (a) mRNA sequencing and (b) real-time quantitative RT-PCR. RPKM, reads per kilo base of transcript per million mapped reads; ct, cycle threshold. Paired *t*-tests were used for comparisons. **$P<0.01$.

Fig. 5. Correlations between subcutaneous white adipose tissue transcript levels and the hyperinsulinaemic euglycaemic clamp. Principal component analysis was performed on the 73 transcripts down regulated after 12 w of exercise in men with dysglycaemia (DG; Figure 3). The 1st principal component (PC1) correlated to glucose infusion rate (GIR) at (a) baseline, (b) after 12 w of exercise intervention, (c) to change in GIR in response to 12 w of exercise intervention, and (d) transcript levels at baseline predicted the change in GIR in response to 12 w of exercise. (e) Top 10 strongest 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. Trend lines with standard errors (shaded areas) are presented. DG, dysglycaemia. ***$P<0.001$ and **$P<0.01$.

Fig. 6. Pathways, cell types and mediation analyses. (a) Pathway enrichment analysis of subcutaneous adipose tissue transcripts regulated after 12 w of exercise in dysglycaemic men, and (b) up-stream regulatory network analysis. Analyses of main cell populations in subcutaneous adipose tissue: (c) adipocytes, (d) macrophages, (e) leukocytes, (f) progenitor cells, (g) M1-like and (h) M2-like macrophages. (i) Mediation analyses between M2-like macrophage transcript levels, plasma adiponectin levels or (j) subcutaneous adipose tissue *ADIPOQ* transcript levels, and glucose infusion rate (GIR). Bar plots represent mean±SEM. *$P<0.05$, and ***$P<0.001$ between groups (DG vs. control) comparison at baseline using unpaired *t*-tests. †$P<0.05$ and †† $P<0.01$ within

group [after (right bar) vs. before (left bar) 12 w intervention] comparison using paired *t*-tests. ‡ *P*<0.05 and ‡‡*P*<0.01. RPKM = Reads per kilo base of transcript per million mapped reads.

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. 2016*. (b) Effects of three h of insulin infusion, and (c) differences in response to insulin infusion between insulin resistant and insulin sensitive participants based on data from the study by *Soronen et al. 2012*. The bar plot represents log2(fold-change)±95 % confidence intervals for the response to insulin infusion in insulin resistant vs. insulin sensitive participants. **P*<0.05, ***P*<0.01 and ****P*<0.001. IR, insulin resistant. IS, insulin sensitive.

Fig. 8. Correlations between transcript levels in subcutaneous adipose tissue and protein concentrations in plasma. mRNA sequencing quantification of subcutaneous adipose tissue transcript levels correlated to 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. Trend lines with standard errors (shaded areas) are presented. DG, dysglycaemia.

Fig. 9. Secreted frizzled-related protein 4. (a) Plasma SFRP4 concentrations did not correlate to *SFRP4* levels in subcutaneous white adipose tissue in men with normoglycaemia, but did correlate in (b) men with dysglycaemia. The results were similar using either Spearman's and Pearson's correlations. Trend lines with standard errors (shaded areas) are presented. (c) Plasma SFRP4 concentration did not change after 12 w of exercise in men with normoglycaemia, but (d) decreased in men with dysglycaemia. Paired *t*-tests were performed. DG, dysglycaemia; RPKM, reads per kilo base of transcript per million mapped reads. **P*<0.05.