Long-term changes in adipose tissue gene expression following bariatric surgery

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Abstract. Kerr AG, Andersson DP, Rydén M, Arner P, Dahlman I (Lipid Laboratory, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden). Long-term changes in adipose tissue gene expression following bariatric surgery (Original Article). J Intern Med 2020; 288: 219–233.

Objective. Patients undergoing bariatric surgery present long-term metabolic improvements and reduced type 2 diabetes risk, despite long-term weight regain. We hypothesized that part of these protective effects could be linked to altered gene expression in white adipose tissue (WAT).

Methods. Transcriptomic profiling by gene microarray was performed in abdominal subcutaneous WAT from women before (n = 50) and two (n = 49) and five (n = 38) years after Roux-en-Y gastric bypass (RYGB) surgery as well as in 28 age-matched nonoperated women.

Results. In the obese women, the average body weight decrease was 38 kg 2 years postsurgery followed by an 8 kg weight regain between 2 and 5 years. Most of the long-term changes in WAT gene expression occurred during the first 2 years. However, a subset of genes encoding proteins involved in inflammation displayed a continued decrease between baseline, 2 and 5 years, respectively; that is an expression pattern independent of body weight regain. Expression of 71 of these genes correlated with measurements of adipocyte morphology or serum adipokine levels.

Conclusion. The continuous improvement in WAT inflammatory gene expression, despite body weight relapse, may contribute to the sustained effects on adipose morphology after bariatric surgery.

Keywords: Roux-en-Y gastric bypass surgery, subcutaneous white adipose tissue, inflammation, inflammatory gene expression.

Introduction

Obesity is a major risk factor for type 2 diabetes (T2D). Marked body weight reduction through bariatric surgery can at best reverse T2D and confer long-term protection against disease relapse despite subsequent weight regain [1-4]. The T2D-protective effect is driven by several mechanisms which, in part, involve changes in the secretion of gut hormones collectively termed incretins amongst which glucagon-like peptide-1 (GLP-1) is the most well-studied [5]. Nevertheless, factors in white adipose tissue (WAT) may also contribute. In obesity, WAT displays pernicious alterations in metabolic/endocrine function, enlarged fat cells and a chronic low-grade inflammation [6]. All these factors may contribute to increasing the risk of T2D [6]. We have previously shown that in obese subjects undergoing Roux-en-Y gastric bypass (RYGB) surgery, WAT function is improved 2 years postsurgery and further improved at a 5-year follow-up despite significant weight regain [7,8]. However, the mechanisms underlying these long-term improvements in WAT phenotype remain unclear.

Global gene expression profiling can provide clues to the factors promoting improved WAT function after gastric bypass. Several studies have examined the global WAT transcriptome following weight reduction induced by diet or bariatric surgery [9-13]. A caveat with most of these studies is that they have only included short-term follow-ups, with few publications reporting findings up to 2 years postsurgery [14]. Thus, patients in these studies may still be in a catabolic nonweight stable state [3].

In order to identify mechanisms underlying the long-term beneficial effect of bariatric surgery on abdominal subcutaneous WAT, we performed gene microarray analyses in a previously described cohort of obese women [7]. Adipose tissue biopsies were obtained before RYGB, and then 2 and 5 years thereafter. To evaluate the long-term effect of RYGB surgery on WAT, we also compared the
WAT gene expression at 5 years postsurgery with that of age-matched nonoperated women.

Methods

Clinical cohort

The baseline and postsurgery examinations in the cohort have been described previously [7,15]. In the present study, we included all women from which we had a WAT sample for two or more time points, pre- and 2 and/or 5 years post-RYGB. From 37 women, we had WAT samples at each time point. At baseline, three women were diagnosed with type 2 diabetes of which two received metformin and one diet only. Diabetes was absent at 2 and 5 years follow-up. At baseline, 16 women were diagnosed with hypertension of which 15 were treated with antihypertensive drugs (ACE- or angiotensin two receptor blockers, beta blockers, thiazide diuretics and/or calcium channel antagonist); nine and eight women remained on antihypertensive treatment at 2 and 5 years follow-up, respectively. Four women were diagnosed with asthma and treated with inhalable cortisone at baseline. Only one woman was on cortisone treatment at follow-up. Two women were treated with statins at baseline and one at follow-up. Patients were instructed to adhere to a protein-rich diet for the first six postoperative weeks. Thereafter, instructions were given to eat three main meals daily, each a small serving with high-protein and whole-grain product content, and up to a total of four snacks in between these meals. No specific recommendations were given regarding the relative energy content of fat, carbohydrates and proteins. All patients reported no important changes in the proportional intake of these components.

For comparison of data at 5 years postsurgery, we included a nonoperated group of 28 women matched for age and recruited by local advertisement. Their body weight had been stable for at least 2 years prior to examination according to self-report, and none had undergone attempts to reduce body weight. No woman in the nonoperated group was diagnosed with diabetes or hypertension, whereas one woman was diagnosed with asthma and treated with inhalable cortisone. None was treated with a statin. The study was approved by the local committee on ethics. It was explained in detail to each woman, and informed written consent was obtained.

Clinical procedures

All subjects were examined at 8 AM following overnight fasting. Patients completed a questionnaire on physical activity and menstrual status. Physical activity was graded as score 1 to 3 where 1 = sedentary lifestyle and no important physical activity at work, 2 = moderate physical activity at free time or work, and 3 = intense and frequent physical activity at free time or work. Menopause was graded as pre- or postmenopausal. Anthropometric and clinical chemistry variables were determined as described [7,16]. Height and weight were used to calculate BMI. Dual-energy X-ray absorptiometry (DXA) using a GE lunar iDXA (GE Healthcare, Madison, WI, USA) with the software EnCore (version 14.10.022) was used to measure total fat mass, as previously described [8]. The EnCore software supplemented with the CoreScan item was used to segment total adipose fat within the android region into estimated subcutaneous (ESAT) and visceral adipose tissue (EVAT). Homeostasis model assessment of insulin resistance (HOMA-IR), which is frequently used as an indirect measure of insulin sensitivity, was calculated as fasting plasma glucose in mmol L^{-1} × fasting serum insulin in mU L^{-1} /22.5 [17,18].

An abdominal subcutaneous WAT biopsy was obtained from the same region each time, lateral to the umbilicus, by needle aspiration. WAT samples were rapidly rinsed in sodium chloride (9 mg mL^{-1}). Thereafter, one portion of 300 mg unfractionated WAT was immediately frozen in liquid nitrogen and kept at −70°C. Another portion was used to isolate adipocytes by collagenase treatment and to determine mean fat cell size as previously described [19]. The number of fat cells was calculated by dividing ESAT by mean subcutaneous fat cell weight. ESAT weight represents the subcutaneous abdominal adipose tissue where the biopsy was taken, not the whole abdominal subcutaneous depot. Adipose secretion of proteins and glycerol as index of lipolysis index were determined exactly as previously described [20-22].

Microarray experiment

Total RNA was extracted from frozen WAT samples using the RNeasy kit (217004, Qiagen, Hilden, Germany) and subjected to global transcriptome analysis using Clarion™ D arrays (Affymetrix, Santa Clara, CA, USA) according to the manufacturer’s instructions. Briefly, amplified and
biotinylated sense-strand DNA targets were generated from total RNA using the WT Plus Kit (Thermo Fisher, Waltham, MA, USA). A total of 5.5 µg fragmented and biotinylated sense-strand DNA target was hybridized to the arrays in GeneChip Hybridization Oven 645 at 45°C for 16–18 h. Arrays were washed and stained in GeneChip Fluidics Station 450 prior to scanning in Affymetrix GeneChip Scanner 7.

**Microarray analysis**

The Affymetrix Expression Console (v. 1.4.1) was used to preprocess and analyse CEL files based on the SST-RMA method. The Affymetrix probe IDs were mapped to the FANTOM-CAT gene annotation, a robust atlas of protein-coding genes as well as long noncoding RNA [23]. A total of 54 980 probe sets mapped to 40 590 unique gene IDs; this has been explained previously [24]. In this study, we were interested in protein-coding genes and therefore filtered for 19 418 genes annotated as locus type 'mRNA coding' and excluded all non-coding RNAs from analysis.

Qlucore Omics Explorer (http://www.qlucore.com) was used to compare log2-transformed gene expression levels between two groups. For analyses over time, paired comparison was made in Qlucore by eliminating the effect of sample and, as indicated, concomitant medications, physical activity score and menstrual status. Comparison between five-year and control samples was made using a two-group unpaired method. A mixed-design ANOVA was used to test for interaction effect between the weight stable and weight gain cohort over time; the effect of sample, time point and group were eliminated. Threshold for differentially expressed genes was set at a false discovery rate (FDR) ≤ 0.05. We have previously reported that differentially expressed genes from these arrays can be confirmed using real-time PCR [16].

**Gene ontology analysis**

Gene ontology (GO) analyses were performed on genes differentially expressed between two groups. ToppGene Suite software (http://toppgene.cchmc.org) [25], date of use 29/11/2018, was used to identify significantly enriched GOs amongst differentially expressed genes. The analysis was limited to GO ‘biological process’ terms as these appeared the more relevant in relation to adipocyte biology. Only GOs containing more than 20 or fewer than 800 genes were used for analysis. A hypergeometric distribution with Bonferroni correction was used for determining statistical significance with an FDR of ≤ 0.05. Reduce and visualize gene ontology (REVIGO) software was used to summarize gene ontology terms and network plot the resulting broader GO terms [26].

**Statistical analysis**

Clinical parameters were compared using a paired t-test between examinations in the same subject and with unpaired t-test between controls and five-year follow-up. Values in Table 1 are mean ± standard deviation.

**Results**

**Clinical findings**

The clinical characteristics of the women undergoing RYGB have been described before and are given in Table 1 [7]. At the two-year follow-up, there was a pronounced reduction in body weight, total fat mass, as well as hip and waist circumference. The average weight at one year postsurgery was 81.4 kg ± 13.7 (results not shown) compared to 80.1 kg ± 14.3 at 2 years postsurgery, indicating the cohort was weight stable at examination at 2 years postsurgery. Fasting levels of insulin, glucose, HOMA-IR and leptin decreased significantly, and the circulating lipid profile and adiponectin levels improved as compared to baseline. Between 2 and 5 years postsurgery there was on average a significant weight regain and expansion of total body fat. Leptin and HOMA-IR increased with body weight. Total cholesterol also increased between 2–5 years; it was the HDL fraction which increased with no change in LDL cholesterol levels. From baseline to 2 years postsurgery there was a decrease in fat cell volume whilst fat cell number remained unchanged. Following weight regain, fat cell number increased whilst fat cell volume did not change. The plasma glycerol levels and glycerol release from the WAT followed the changes in body weight. Physical activity increased following surgery and remained constant between 2 and 5 years.

**WAT gene expression 2 years postsurgery**

Comparison of WAT gene expression between baseline and 2 years postsurgery revealed 2420 upregulated and 3576 downregulated genes (Fig. 1, Table S1). Many key adipocyte genes
### Table 1. Clinical Characteristics in women undergoing bariatric surgery and nonoperated subjects

|                      | Baseline (n = 50) | 2 y's postsurgery (n = 49) | 5 y's postsurgery (n = 38) | Nonoperated (n = 28) | 0 vs 2 y's P | 0 vs 5 y's P | 2 vs 5 y's P | 5 y's vs nonoperated P |
|----------------------|-------------------|-----------------------------|-----------------------------|----------------------|--------------|--------------|--------------|------------------------|
| **Age (years)**      | 43 ± 9            | 45 ± 9                      | 47 ± 10                     | 44 ± 9               | <0.0001      | <0.0001      | <0.0001      | 0.20                   |
| **Weight (kg)**      | 118 ± 15          | 80 ± 14                     | 88 ± 18                     | 76 ± 12              | <0.0001      | <0.0001      | <0.0001      | 0.0041                 |
| **Body mass index (kg m⁻²)** | 43 ± 5           | 29 ± 4                      | 32 ± 6                      | 27 ± 5               | <0.0001      | <0.0001      | <0.0001      | 0.0011                 |
| **Waist circumference (cm)** | 130 ± 11          | 98 ± 12                     | 102 ± 14                    | 92 ± 11              | <0.0001      | <0.0001      | <0.0001      | 0.0017                 |
| **Hip circumference (cm)** | 130 ± 10          | 106 ± 10                    | 111 ± 13                    | 103 ± 9              | <0.0001      | <0.0001      | <0.0001      | 0.0050                 |
| **Total fat mass (kg)** | 61 ± 10           | 31 ± 11                     | 38 ± 14                     | 29 ± 10              | <0.0001      | <0.0001      | <0.0001      | 0.014                  |
| **Android fat mass (kg)** | 6.2 ± 1.3         | 2.6 ± 1.3                   | 3.3 ± 1.7                   | 2.4 ± 1.1            | <0.0001      | <0.0001      | <0.0001      | 0.0060                 |
| **Gynoid fat mass (kg)** | 9.3 ± 2.1         | 5.2 ± 1.7                   | 6.4 ± 2.7                   | 5.4 ± 1.7            | <0.0001      | <0.0001      | <0.0001      | 0.0060                 |
| **EVAT (kg)**        | 2.3 ± 0.9         | 0.8 ± 0.4                   | 0.9 ± 0.6                   | 0.6 ± 0.4            | <0.0001      | <0.0001      | 0.0054       | 0.33                   |
| **ESAT (kg)**        | 3.8 ± 1.0         | 1.9 ± 0.9                   | 2.5 ± 1.1                   | 1.8 ± 0.8            | <0.0001      | <0.0001      | <0.0001      | 0.0068                 |
| **Systolic blood pressure (mmHg)** | 136 ± 17          | 124 ± 15                    | 129 ± 18                    | 119 ± 14             | <0.0001      | 0.0072       | 0.19         | 0.10                   |
| **Diastolic blood pressure (mmHg)** | 84 ± 8            | 76 ± 10                     | 79 ± 10                     | 74 ± 7              | <0.0001      | 0.0036       | 0.026        | 0.015                  |
| **P-Glucose (mmol L⁻¹)** | 5.6 ± 1.4         | 4.9 ± 0.6                   | 5.0 ± 0.5                   | 5.2 ± 0.5            | <0.0001      | 0.0051       | 0.13         | 0.090                  |
| **S-Insulin (mU L⁻¹)** | 15.5 ± 8.4        | 4.9 ± 1.9                   | 5.8 ± 2.7                   | 5.5 ± 2.8            | <0.0001      | <0.0001      | 0.086        | 0.60                   |
| **P-Glycerol (mmol L⁻¹)** | 122 ± 47          | 79 ± 29                     | 126 ± 48                    | 62 ± 32              | <0.0001      | 0.88         | <0.0001      | <0.0001                 |
| **P-Cholesterol (mmol L⁻¹)** | 4.8 ± 1.0         | 4.0 ± 0.8                   | 4.2 ± 0.7                   | 5.0 ± 1.0            | <0.0001      | <0.0001      | 0.041        | 0.0003                 |
| **P-Triglycerides (mmol L⁻¹)** | 1.5 ± 0.7         | 0.9 ± 0.4                   | 0.9 ± 0.3                   | 0.9 ± 0.6            | <0.0001      | <0.0001      | 0.38         | 0.47                   |
| **P-HDL cholesterol (mmol L⁻¹)** | 1.2 ± 0.3         | 1.5 ± 0.3                   | 1.6 ± 0.5                   | 1.5 ± 0.3            | <0.0001      | <0.0001      | 0.013        | 0.17                   |
| **P-LDL cholesterol (mmol L⁻¹)** | 3.0 ± 0.8         | 2.1 ± 0.7                   | 2.1 ± 0.7                   | 3.1 ± 0.8            | <0.0001      | <0.0001      | 0.080        | <0.0001                 |
| **HOMA-IR (units)**  | 4.0 ± 2.6         | 1.0 ± 0.4                   | 1.3 ± 0.6                   | 1.3 ± 0.9            | <0.0001      | <0.0001      | 0.025        | 0.04                   |
| **Fat cell volume (µL)** | 970 ± 185         | 466 ± 174                   | 458 ± 168                   | 584 ± 199            | <0.0001      | <0.0001      | 0.52         | 0.0093                 |
| **Fat cell number × 10⁷** | 449 ± 150         | 439 ± 153                   | 554 ± 176                   | 330 ± 119            | 0.8          | 0.0004       | 0.0005       | 0.0013                 |
| **Glycerol release (mmol / 10⁷ cells)** | 3.5 ± 1.7         | 2.0 ± 1.1                   | 4.4 ± 1.9                   | NA                   | 0.0006       | 0.029        | <0.0001      | NA                    |
| **Glycerol release (mmol) from ESAT** | 1.5 ± 0.8         | 0.9 ± 0.5                   | 2.9 ± 1.6                   | NA                   | 0.0033       | 0.0005       | <0.0001      | NA                    |
| **P-Adiponectin (µg mL⁻¹)** | 7.3 ± 3.0         | 13.4 ± 12.9                 | 12.9 ± 5.7                  | NA                   | 0.0038       | <0.0001      | 0.0013       | NA                    |
| **S-Leptin (ng mL⁻¹)** | 63 ± 29           | 29 ± 21                     | 40 ± 28                     | NA                   | <0.0001      | 0.0006       | 0.0002       | NA                    |
| **Physical activity (score)** | 1.52 ± 0.54       | 2.14 ± 0.50                 | 1.98 ± 0.5                  | NA                   | <0.0001      | 0.0002       | 0.058        | NA                    |

**EVAT**, estimated visceral adipose tissue; **ESAT**, estimated subcutaneous abdominal adipose tissue; **NA**, not available.

Clinical Characteristics in women before, two and five years post bariatric surgery, and in nonoperated women. Baseline refers to examinations made just before bariatric surgery with 2 and 5 years postoperatively denoting the time post bariatric surgery. Values presented are mean ± SD. Statistical comparisons were made using paired Student t-test for same subjects at different time points and unpaired Student t-test for comparisons between 5 y’s postsurgery and controls.
| Gene symbol | Gene name                                      | Average expression 0 year | Average expression 2 Year | Average expression 5 Year | Average expression controls |
|-------------|-----------------------------------------------|---------------------------|---------------------------|----------------------------|----------------------------|
| AIF1        | Allograft inflammatory factor 1               | 10.19                     | 9.04                      | 8.58                       | 9.29                       |
| ALOX5AP     | Arachidonate 5-lipoxygenase activating protein| 9.15                      | 8.32                      | 7.88                       | 8.46                       |
| ARRB2       | Arrestin beta 2                               | 9.31                      | 8.47                      | 8.14                       | 8.64                       |
| BIN2        | Bridging integrator 2                         | 6.49                      | 5.68                      | 5.10                       | 5.63                       |
| C1QA        | Complement C1q A chain                        | 8.75                      | 7.97                      | 7.63                       | 8.05                       |
| CD4         | CD4 molecule                                  | 7.21                      | 6.20                      | 5.80                       | 6.51                       |
| CD48        | CD48 molecule                                 | 5.39                      | 4.89                      | 4.50                       | 4.89                       |
| CD53        | CD53 molecule                                 | 9.16                      | 8.02                      | 7.38                       | 8.02                       |
| CD68        | CD68 molecule                                 | 10.09                     | 8.97                      | 8.66                       | 9.12                       |
| CLEC7A      | C-type lectin domain containing 7A            | 6.83                      | 5.39                      | 4.70                       | 5.73                       |
| CNPY3       | Canopy FGF signalling regulator 3             | 7.38                      | 7.08                      | 6.92                       | 7.11                       |
| CORO1A      | Coronin 1A                                    | 7.65                      | 7.08                      | 6.57                       | 7.11                       |
| CR1         | Complement C3b/C4b receptor 1 (Knops blood group) | 5.30                      | 4.39                      | 4.04                       | 4.51                       |
| CTSC        | Cathepsin C                                   | 9.75                      | 9.35                      | 9.02                       | 9.44                       |
| CTSS        | Cathepsin S                                   | 9.36                      | 8.08                      | 7.57                       | 8.26                       |
| CXCR4       | C-X-C motif chemokine receptor 4              | 8.11                      | 7.49                      | 6.74                       | 7.39                       |
| CYBB        | Cytochrome b-245 beta chain                   | 9.22                      | 7.97                      | 7.45                       | 8.18                       |
| CYLD        | CYLD lysin 63 deubiquitinase                  | 6.95                      | 6.86                      | 6.66                       | 6.88                       |
| EZR         | Ezrin                                         | 8.34                      | 7.97                      | 7.69                       | 8.03                       |
| FCER1A      | Fc fragment of IgE receptor Ia                | 5.37                      | 4.87                      | 4.24                       | 4.87                       |
| FCGR2A      | Fc fragment of IgG receptor Ila               | 9.98                      | 8.70                      | 8.18                       | 9.02                       |
| FCGR3A      | Fc fragment of IgG receptor IIIa              | 6.81                      | 5.68                      | 4.95                       | 5.75                       |
| FCGR3B      | Fc fragment of IgG receptor IIIb              | 6.76                      | 5.77                      | 4.96                       | 5.85                       |
| FGL2        | Fibrinogen-like 2                             | 8.84                      | 8.70                      | 8.40                       | 8.73                       |
| FOLR2       | Folate receptor beta                           | 8.05                      | 7.06                      | 6.52                       | 7.30                       |
| FPR1        | Formyl peptide receptor 1                     | 5.99                      | 5.25                      | 4.42                       | 5.27                       |
| FUCA1       | Alpha-L-fucosidase 1                          | 7.44                      | 6.98                      | 6.71                       | 6.99                       |
| GLIPR1      | GLI pathogenesis-related 1                    | 8.98                      | 7.71                      | 7.28                       | 7.77                       |
| GMFG        | Glia maturation factor gamma                  | 9.75                      | 9.48                      | 9.17                       | 9.48                       |
| GPR183      | G protein-coupled receptor 183                | 8.05                      | 6.51                      | 5.93                       | 6.76                       |
| HGF         | HEPATOCYTE growth factor                     | 6.51                      | 6.02                      | 5.63                       | 5.97                       |
| IQGAP2      | IQ motif containing GTPase-activating protein 2| 6.98                      | 5.98                      | 5.63                       | 6.08                       |
| ITGAM       | Integrin subunit alpha M                      | 6.92                      | 6.02                      | 5.70                       | 6.19                       |
| LCP1        | Lymphocyte cytosolic protein 1                | 9.19                      | 7.75                      | 7.21                       | 7.84                       |
| LGMN        | Legumain                                      | 10.01                     | 9.36                      | 9.08                       | 9.46                       |
| LY86        | Lymphocyte antigen 86                         | 6.64                      | 5.80                      | 5.44                       | 5.94                       |
| LY96        | Lymphocyte antigen 96                         | 4.10                      | 3.61                      | 3.31                       | 3.58                       |
displayed higher expression postsurgery including genes encoding factors involved in adipogenesis (*PPARG*, *EBF1*), insulin signalling (*IRS1*, *IRS2*) and endocrine function (*ADIPOQ*). Gene Ontology (GO) analysis was employed on the differentially expressed genes to identify enriched biological processes. A table of the 60 genes assigned to an inflammatory GO ‘biological processes’, continually downregulated after bariatric surgery and compared to age-matched control subjects. Expressions relate to average log2-transformed array signal at each time point.

| Gene symbol | Gene name                                      | Average expression 0 year | Average expression 2 Year | Average expression 5 Year | Average expression controls |
|-------------|------------------------------------------------|---------------------------|---------------------------|---------------------------|-----------------------------|
| LYZ         | Lysozyme                                       | 10.74                     | 9.22                      | 8.56                      | 9.58                        |
| MNDAS       | Myeloid cell nuclear differentiation antigen    | 5.26                      | 4.19                      | 3.51                      | 4.25                        |
| MYO1F        | myosin IF                                      | 7.94                      | 7.17                      | 6.66                      | 7.31                        |
| NCKAP1L      | NCK-associated protein 1-like                  | 7.49                      | 6.15                      | 5.76                      | 6.37                        |
| NPC2         | NPC intracellular cholesterol transporter 2     | 9.44                      | 9.01                      | 8.72                      | 9.02                        |
| PIK3AP1      | Phosphoinositide-3-kinase adaptor protein 1     | 7.06                      | 6.63                      | 6.39                      | 6.67                        |
| PLEK         | Pleckstrin                                      | 6.71                      | 5.46                      | 5.02                      | 5.58                        |
| PRKCB        | Protein kinase C beta                          | 5.95                      | 5.12                      | 4.57                      | 5.19                        |
| PTPRC        | Protein tyrosine phosphatase receptor type C    | 8.78                      | 7.83                      | 7.36                      | 7.99                        |
| QPCT         | glutaminyl-peptide cyclotransferase            | 5.24                      | 4.74                      | 4.46                      | 4.81                        |
| RNASET2      | ribonuclease T2                                 | 7.30                      | 6.67                      | 6.36                      | 6.70                        |
| RPS6KA1      | Ribosomal protein S6 kinase A1                 | 5.52                      | 4.71                      | 4.37                      | 4.76                        |
| S100A8       | S100 calcium-binding protein A8                | 6.99                      | 6.41                      | 5.30                      | 6.47                        |
| SAMHD1       | SAM and HD domain containing deoxyxynucleoside triphosphate triphosphohydrolase 1 | 9.42                      | 8.84                      | 8.58                      | 8.84                        |
| SAMSN1       | SAM domain, SH3 domain and nuclear localization signals 1 | 5.49                      | 4.48                      | 3.88                      | 4.35                        |
| SELLS        | Selectin L                                      | 5.74                      | 5.39                      | 4.57                      | 5.38                        |
| SYK          | Spleen-associated tyrosine kinase              | 5.34                      | 4.62                      | 4.22                      | 4.61                        |
| TLR8         | Toll-like receptor 8                            | 5.11                      | 3.91                      | 3.50                      | 4.12                        |
| TNFRSF1B     | TNF receptor superfamily member 1B             | 8.71                      | 8.52                      | 8.27                      | 8.50                        |
| TYROBP       | Transmembrane immune signalling adaptor TYROBP | 10.72                     | 9.53                      | 8.98                      | 9.78                        |
| VAMP8        | Vesicle-associated membrane protein 8          | 6.65                      | 5.70                      | 5.39                      | 5.81                        |
| VSIG4        | V-set and immunoglobulin domain containing 4   | 8.51                      | 7.07                      | 6.51                      | 7.34                        |
| ZC3HAV1      | Zinc finger CCCH-type containing, antiviral 1   | 7.79                      | 7.64                      | 7.43                      | 7.63                        |
processes. The full lists of significantly up- or downregulated pathways are provided in Tables S2 and S3, respectively. The five most significantly up-and downregulated GO terms are given in Fig. 1. Two years after surgery, increased expression of genes related to protein translation and metabolism was observed. Amongst other relevant upregulated pathways ‘fat cell differentiation’ displayed 43 genes with increased expression at the two-year time point ($P = 4.08 \times 10^{-4}$) (Table S2). Conversely, RYGB surgery was accompanied by a significant decrease in immune response and inflammatory gene expression (Fig. 1).

**WAT gene expression 5 years postsurgery**

Comparison between baseline and 5 years postsurgery revealed 5583 differentially expressed genes, out of which 1653 were up- and 3930 were downregulated (Fig. 1, Table S4). Many of the upregulated genes mapped to GOs related to lipid metabolism (Table S5, and Fig. 1 where the latter shows the top five most significant GO terms). Twenty-eight genes within ‘fat cell differentiation’ were also increased at the five-year time point ($P = 7.58 \times 10^{-7}$) (Table S5). Genes of central importance for adipocyte function such as PPARG, EBF1, IRS1, IRS2, and ADIPOQ remained upregulated. Amongst the downregulated genes, the enrichment in immune response processes observed at the two-year follow-up persisted, including processes related to leucocyte function, as well as interferon-$\gamma$ and TNF-$\alpha$ cytokine production (Table S6 and Fig. 1 where the latter shows the top five most significant GO terms).

Bariatric surgery was accompanied by changes in physical activity, medication and the menstrual status of some patients. To assess the impact of these variables, the baseline to five-year comparison was re-analysed using the physical activity score, use of concomitant medication (scored yes if the patient was taking any cardiometabolic drugs) or menstrual status as covariates. These analyses resulted in 4407 (physical activity), 5350 (medication), and 5492 (menstrual status) differentially expressed genes, respectively. Over 95% of the significant genes found with each covariate overlapped with the 5583 genes identified in the
nonadjusted comparison. We therefore did not take these potential confounders into account in subsequent analyses.

To assess the long-term effects of bariatric surgery on the WAT transcriptome and weight-dependent or -independent regulation, the expression of the 5583 significant genes in the baseline to five-year comparison were examined in more detail over time. Between zero and 2 years, in conjunction with weight loss, 1264 of these genes were up- and 2993 downregulated (Table S4). During the weight regain period, from 2–5 years, 202 were upregulated and 373 downregulated (Table S4). Based on these findings the 5583 genes were subgrouped (Fig. 2a) into (i) early responders (significantly regulated zero to two years only, green lines), (ii) late responders (significantly regulated 2–5 years only, black lines), (iii) body weight-dependent (significantly regulated in one direction zero to two and then the opposite two to five, red lines), and (iv) continually changing independent of weight (significantly regulated zero to two and further in the same direction two to five, blue lines) (Lists of genes in each group are given in Table S4). Amongst the early responding genes, 1174 were upregulated and enriched for GO terms involved in lipid metabolism whilst 2735 were downregulated and enriched for inflammatory pathways (Tables S7–S8). The 85 weight-tracking genes were enriched for ‘lipid biosynthetic process’ and ‘steroid biosynthetic processes’ (Table S9). Amongst the continuously changing set, the 174 downregulated genes were enriched for inflammatory processes including ‘cytokine production’, ‘cell chemotaxis’, and ‘neutrophil activation’ (Fig. 2b and Table S10). Summarizing the GO terms for the 174 genes revealed core inflammatory pathways. The continuously increasing genes were enriched for ‘ion transport’ and ‘fatty acid metabolic pathways’ (Table S11). The remaining subgroups in Fig. 2a were not overrepresented for any pathways.

**Comparison of weight stable versus weight gaining patients between 2 to 5 years postbariatric surgery**

The RYGB cohort displayed a heterogenous change in body weight. Some remained weight stable whilst others significantly regained body weight between the 2 and 5-year follow-up. To assess whether this could impact our results, we...
subdivided the group according to the proportional weight gain. There is no consensus on how to define weight stability. A separate analysis of the population-based Stockholm Pregnancy And Women’s Nutrition study [27] showed that the 25th percentile in long-term body weight increase corresponded to 7%. We therefore defined body weight stability during the two to five-year follow-up period as a change < 7% (n = 19) and weight gain as ≥ 7% increase (n = 18). Six of the weight regain patients had an increase greater than 5 kg m⁻² in BMI, a threshold used to classify significant weight regain following surgery [28]. At baseline and 2 years postsurgery, anthropometric measures showed no difference between the weight gain and weight stable groups (Table S12). Neither did the lipid profile, glucose, adiponectin or insulin levels, HOMA-IR, fat cell number or volume differ between groups. However, 5 years postsurgery anthropometric and body fat measures were elevated in weight gainers. Fat cell volume was also significantly increased in the weight gain group whereas fat cell number remained similar. Plasma glucose, lipids and systemic insulin resistance showed no difference between groups.

The 5583 significant genes in the baseline to five-year postsurgery comparison were examined for differential expression between the weight gain and stable groups. Only five genes; CD151, GPD1L, NQO1, SYNPO and UCHL1 were significantly different between the groups (Table S13). Thus, the vast majority of the long-term differentially regulated genes after RYGB surgery were not affected by weight regain.

**WAT gene expression 5 years after bariatric surgery versus nonoperated subjects**

To validate that effects on gene expression 5 years after surgery was not primarily the consequence of ageing, we compared the transcriptomic data with that in 28 nonoperated women matched for age. The nonoperated group had a lower BMI and fat depot measurements, and higher total and LDL cholesterol than the five-year postsurgery group. No difference in fasting glucose or insulin levels was observed (Table 1). Nonoperated women had fewer but larger fat cells.

Transcriptome analysis was focused on the 5583 differentially expressed genes mentioned previously. Out of these, 965 were differentially expressed between the operated versus the nonoperated women. 66 genes displayed a higher expression and 899 genes a lower expression in the postsurgery group (Table S14). GO biological process terms associated with ‘fatty acyl-CoA biosynthesis’ and lipid metabolism were significantly enriched amongst the genes with higher expression in the five-year postsurgery group (Table S15). The top 50 GO terms enriched amongst the genes expressed at lower levels in the postsurgery cohort are summarized and displayed in Fig. 3 (Full list of GO terms Table S16). Pathways associated with inflammation including ‘cell chemotaxis’, ‘leucocyte proliferation’ and ‘cytokine metabolic process’ were all lower in the five-year postsurgery group. Next, we wanted to assess how the group of genes continually changing independent of weight in the surgery cohort behaved in the nonoperated women, and whether expression of these genes constitutes a marker of previous bariatric surgery. We therefore overlapped the 965 differentially expressed genes between the five-year postsurgery group and the controls, with the genes continually changing over time independent of weight change in the RYGB group. This revealed eleven genes displaying continued upregulation after bariatric surgery which were also increased in the five-year surgery group compared to controls; GYS2, PLEKHH2, MLXIPL, AP1G1, PLEKHH1, ME1, SLC6A8, OLFM2, TMEM164, PCYT2 and FASN. Furthermore, 113 genes downregulated at each time point after bariatric surgery also displayed lower expression in the five-year postsurgery group compared to the nonoperated women. GO analysis of these genes demonstrated that many are involved in immune response and inflammatory pathways (Table S17). The 60 genes mapping to inflammation-related GO terms are listed in Table 2, which thus represent a set of inflammatory genes downregulated post-bariatric surgery independent of weight regain and found to be downregulated compared to nonoperated subjects.

**Correlation between genes continually regulated after bariatric surgery and adipocyte phenotypes**

Bariatric surgery has weight loss independent effects on metabolic health [29]. We examined the 220 genes continually up or downregulated, independent of weight regain, against measures of WAT function. The change in gene expression between 2–5 years was correlated with the change in adipocyte cell volume, adipocyte cell number, adiponectin and leptin levels (Fig. 4).
**Fig. 3** GO ‘biological processes’ summary terms for genes downregulated in five years postbariatric surgery patients compared with age-matched nonoperated controls. The log₁₀ P-value is given for each GO term.

**Fig. 4** Correlation of the 220 genes continually upregulated or downregulated postbariatric surgery with clinical parameters of WAT. Genes correlating with serum leptin levels (yellow circle), serum adiponectin levels (blue circle) adipocyte cell number (red circle) and adipocyte cell volume (green circle) are displayed in respective circles. All significant genes continually downregulated following surgery positively correlated with adipocyte volume and leptin and negatively with adiponectin. ARRB2, TLR8, CD163L1, BASP1 and CHST15 negatively correlated with adipocyte cell number and M6PR, PLEKHH2 and MCOLN3 positively.

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Adipocyte cell volume

Expression of 32 genes positively and three genes negatively correlated with average fat cell volume (Table S18). PRKAR2B, a gene known to influence lipid metabolism and continually upregulated following surgery, was negatively correlated with fat cell volume. 16 of the genes positively correlated with cell volume were inflammatory genes continually downregulated after bariatric surgery. This points to a continued resolution of inflammatory gene expression associating with a smaller cell volume, indicative of a healthier adipose tissue.

Adiponectin

34 genes correlated with serum adiponectin levels (Table S19). 32 were continually downregulated postsurgery and negatively correlated with serum adiponectin, of which nine also correlated with cell volume.

Leptin

Increased serum leptin correlated positively with 26 genes and negatively with ECHS1 (Table S20). Six inflammatory genes (HCSL1, CORO1A, CXCR4, SELL, LCP1 and CD48) correlating positively with leptin also inversely correlated with adiponectin.

Adipocyte cell number

Adipocyte cell number, a marker of hyperplastic and metabolically healthier adipose tissue, correlated inversely with five genes, ARRB2, CD163L1, BASP1, CHST15 and TLR8, and positively with three genes, M6PR, PLEKHH2 and MCOLN3 (Table S21). Three of the genes correlating with adipocyte cell number also correlated with a change in total fat mass (Table S22). The other four genes (CD163L1, CHST1,5 MCOLN3, M6PR, TLR8) not correlating with a gross change in fat mass may be indicative of a hyperplastic adipose expansion.

The gene ARRB2 was significantly correlated in all five clinical parameters, with higher expression linked to a worsening phenotype. The majority of genes (81 of the 93) found to correlate with an adipocyte phenotype, were continually downregulated after surgery and increased expression associated with a pathological state. This is in line with inflammation previously shown to regulate adipose tissue expansion and adiponectin secretion [30,31]. Overall there is an association of decreased inflammatory and increased lipid metabolism gene expression with a healthier adipocyte profile, despite the weight regain.

Discussion

In the present study, the dramatic changes in the clinical phenotype of the patients postsurgery were reflected in a large number of differentially expressed genes at the two- and five-year time points compared with baseline. Most of the long-term changes in WAT gene expression occurred during the 2 years of early dramatic body weight loss. This suggests that changes in body weight per se are the major driving force behind long-term alterations in gene expression after bariatric surgery, at least for RYGB operations.

Previous studies of gene expression following the initial rapid weight loss after dietary or surgical intervention in obese people reported similar data as the two-year findings herein [9-12]; i.e. altered expression of genes involved in metabolic and immune processes. Genes involved in protein trafficking and translation were also reported previously to be upregulated following bariatric surgery [10]. Many of the proteins involved in protein trafficking and translation are regulated by insulin and it is possible that changes in insulin sensitivity underlie the changes in expression [32,33]. There is, however, no previous report on weight regain, which is evident 5 years postbariatric surgery. In short-term [1–2 months] experimental overfeeding studies resulting in 2–3 kg weight gain, increased expression of genes regulating extracellular matrix, lipid metabolism and angiogenesis but not inflammation was observed in WAT [34,35]. Capel et al., report in a dietary restriction study a decrease in inflammatory gene expression during a low-calorie diet and weight stabilization period [9]. It would be of interest to compare if inflammation continues to decrease during weight regain after dietary intervention or if this decrease is specific to RYGB surgery.

Obesity is associated with low-grade inflammation [36]. As expected, expression of inflammatory genes decreased at 2 years during weight loss. However, despite the subsequent weight regain, there was a further lowering in expression of a subset of these genes over this three year period. Our data suggest that bariatric surgery may have long-term effects on expression of inflammatory markers in WAT that are independent of weight.
changes. In support of this, none of the immune genes mentioned were found to be differentially expressed between weight stable and weight gainer subgroups. Many of the continually downregulated immune response genes were also down when compared to an age-matched nonoperated group. This was despite the control group having a significantly lower BMI and total fat mass. The nonoperated women had a larger fat cell volume than the five-year postsurgery group, which may be related to the higher expression of immune response genes. However, there was a significant difference in fat cell volume between weight stable and weight gainer in the five-year postsurgery biopsies and no differences in the expression of inflammatory genes. Thus, differences in fat cell volume may still not explain the gene expression changes.

There was also a continuous change in the expression of lipid metabolic genes. An increase was first observed at the two-year weight loss time point and further developed during the period of body weight relapse. Eleven genes were continually upregulated after surgery at each time point and increased compared to controls. Fatty Acid Synthase (FASN) was one of these genes, a gene involved in lipogenesis which predicts insulin sensitivity [37]. The glycerol release from WAT decreased 2 years postsurgery before rebounding above presurgery level at 5 years. Interestingly, the three lipase genes, MGLL, ATGL and LIPE were unchanged during the zero to two-year time point but upregulated long term at the five-year time point. Hormone-sensitive lipase (LIPE) has been shown to regulate the rate of lipolysis in the adipocyte [38] and the increased gene expression is consistent with the clinical findings.

WAT is heterogeneous in regards to cellular makeup, with the majority of cells not being adipocytes [39]. Due to the limited amount of available WAT, we were unable to assess the cellular composition of the WAT and if this changed over time. A decreased macrophage content of WAT has previously been shown three months post- bariatric surgery [10]. In the cited study, CANCELLO et al. found AIF1, CD4 and CSF1R to be upregulated at three months following RYGB surgery; genes we find downregulated at 2 and 5 years. CD4 is a marker for T cells and monocytes; changes in expression may indicate a change in composition of the WAT during weight loss postsurgery where an initial inflammatory response during the catabolic state at three months resolves after long-term weight stabilization at 2 years. Many of the GO biological processes enriched for genes significantly downregulated at all time points postsurgery contained T-cell terms (Table S19). In addition, pathways involving IFN-γ, a known pro-inflammatory factor secreted by T cells and natural killer cells in adipose tissue [40], were also significantly downregulated over time. In type 2 diabetic mice, bariatric surgery ameliorated IFN-γ mediated adipose tissue inflammation and improved insulin sensitivity [41]. Furthermore, IFN-γ⁻/⁻ mice, despite exhibiting similar WAT mRNA levels of TNFα and MCP-1 as controls, had decreased expression of important T-cell-related genes, and lower plasma triglycerides and glucose [42]. Taken together previous and present results suggest an important role of macrophages and perhaps T cells within WAT as mediators of the long-term downregulation of inflammatory gene expression after bariatric surgery. A recent study demonstrated a decrease in pro-inflammatory gene expression in the liver one year postbariatric surgery [43], it would be interesting to see if the continued dampening of pro-inflammatory gene expression in WAT extended to other organs such as the liver.

We identified ARRB2, a β-arrestin previously shown to modulate macrophage chemotaxis [44], as a gene which was continuously downregulated after bariatric surgery and correlated with all measured detrimental WAT phenotypes. Interestingly, ARRB2 interacts with the GLP-1 receptor [45], however, further investigatory work will be needed to establish if this interaction influences WAT biology.

One limitation of the current study is that all study subjects were women. Previously, no differences were seen in the transcriptional response between males and females in response to bariatric surgery [11]. Another limitation is that we did not validate the changes in gene expression with more quantitative methods. However, we have already shown that changes in gene expression of observed magnitude using the present platform can be confirmed by qPCR [46]. Limited sample availability prevented confirmation at the protein level. Recent high-quality datasets have shown that protein level variation is primarily dependent on mRNA concentration [47], but any specific protein of interest would still need to be confirmed. Although the women were weight stable at second examination, we cannot exclude that some had subclinical manifestations of mineral and/or vitamin...
deficiency due to malabsorption. Hypothetically this might influence WAT gene expression though we are not aware of any studies supporting this notion. Finally, we did not study visceral WAT; however, omentectomy has no impact on the outcome of bariatric surgery which supports the notion that it is the reduction in subcutaneous WAT that primarily determines the beneficial metabolic effects of bariatric surgery [48].

Conclusion

There is a distinct signature for long-term WAT gene expression changes following RYGB-induced weight loss. Many gene expression changes occur in the context of body weight variation, but there is a continuous decrease and increase, respectively, in expression of genes regulating inflammation and lipid metabolism independent of body weight changes. The anti-inflammatory improvement goes beyond the control state. This may hypothetically constitute a protective mechanism against type 2 diabetes after bariatric surgery as WAT inflammation is considered an important pathogenic factor behind diabetes [6,36,49].

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Conflict of interest

None declared.

Author contributions

A.G.K analysed the gene expression data, generated the figures and tables and wrote the first version of the manuscript. D.P.A, M.R., I.D. and P.A. participated in recruitment and examination of the patients. D.P.A generated clinical tables. P.A designed the study, participated in recruitment and examination of patients and wrote the first version of the manuscript. I.D designed the study and wrote the first version of the manuscript. All authors were involved in the writing and revision of the manuscript and contributed to the final submitted version. I.D is the guarantor of this work.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:

**Table S1.** Genes differentially expressed between baseline and 2 years post bariatric surgery

**Table S2.** GO terms enriched among genes increased between baseline and 2 years post bariatric surgery

**Table S3.** GO terms enriched among genes decreased between baseline and 2 years post bariatric surgery

**Table S4.** Genes differentially expressed between baseline and 5 years post bariatric surgery with expression pattern over time

**Table S5.** GO terms enriched by genes increased between 0 and 5 years post bariatric surgery

Table S6. GO terms enriched by genes decreased between 0 and 5 years post bariatric surgery

**Table S7.** GO terms enriched by upregulated early responder genes

**Table S8.** GO terms enriched by downregulated early responder genes

**Table S9.** GO terms enriched by genes tracking weight

**Table S10.** GO terms enriched by genes continually downregulated

**Table S11.** GO terms enriched by genes continually upregulated

**Table S12.** Clinical characteristics of the weight stable and weight gain subgroups

**Table S13.** Genes significantly different between the weight stable and weight gain subgroups over two to five years post-surgery

**Table S14.** Genes differentially expressed between subjects 5 years post bariatric surgery and non-operated controls

**Table S15.** GO terms enriched by genes upregulated in 5 year post-surgery patients compared to age-matched controls

**Table S16.** GO terms enriched by genes downregulated in 5 year post-surgery patients compared to age-matched controls

**Table S17.** GO terms enriched by genes downregulated at each time point post surgery and at 5 year post-surgery compared to control subjects

**Table S18.** Genes significantly correlating with changes in adipocyte cell volume

**Table S19.** Genes significantly correlating with changes in adiponectin

**Table S20.** Genes significantly correlating with changes in leptin

**Table S21.** Genes significantly correlating with changes in adipocyte cell number

**Table S22.** Genes significantly correlating with changes in fat mass.