Long-term Protective Changes in Adipose Tissue After Gastric Bypass

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OBJECTIVE

Although long-term weight regain may occur after bariatric surgery, many patients are protected against relapse or development of type 2 diabetes. The study objective was to investigate whether this involves beneficial changes in adipose function.

RESEARCH DESIGN AND METHODS

Forty-nine obese women were investigated before and 2 and 5 years after Roux-en-Y gastric bypass (RYGB). At the 5-year follow-up, 30 subjects were pairwise matched for BMI and age to 30 control women. Clinical parameters and fine-needle biopsies from subcutaneous abdominal adipose tissue were obtained; fat cell size and number, lipolysis, adiponectin, and proinflammatory protein secretion were determined.

RESULTS

After 2 years, BMI decreased from 43 to 29 kg/m^2, which was accompanied by improvements in insulin sensitivity (HOMA of insulin resistance [HOMA-IR]), increased circulating and adipose secreted adiponectin, and decreased adipose lipolysis and fat cell size but no change in adipocyte number. Between 2 and 5 years after surgery, BMI had increased to 31 kg/m^2. This was associated with slightly increased HOMA-IR and unaltered circulating or adipose secreted adiponectin but higher secretion of tumor necrosis factor-α and increased lipolysis and number of fat cells but no change in adipocyte size. All these parameters, except lipolysis, were significantly more favorable compared with those in matched control subjects. Furthermore, the relationship between HOMA-IR and circulating adiponectin was less steep than in control subjects.

CONCLUSIONS

RYGB improves long-term insulin sensitivity and adipose phenotypes beyond the control state despite weight regain. Postoperative beneficial alterations in adipose function may be involved in the diabetes-protective effect of bariatric surgery.

Type 2 diabetes in obesity can be efficiently cured and prevented by bariatric surgery (1). Although some relapse of type 2 diabetes does occur postoperatively, the remission and protection from type 2 diabetes is sustained over long periods of time, even after considerable weight regain (1,2). This suggests that factors independent of changes in BMI may be protective. It is conceivable that alterations in white adipose tissue (WAT) function may be of importance. The hypothesis is

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based on the fact that a number of WAT-specific phenotypes are closely linked to insulin sensitivity/type 2 diabetes, including the size/number of fat cells (adipocytes), WAT inflammation, adipocyte lipolysis, and secretion of paracrine/endocrine proteins (3). It has also been suggested that adaptive mechanisms in WAT are activated upon pronounced weight loss to facilitate a return to the preoperative body weight, which results in a remodeled WAT with antidiabetic properties (4,5).

Herein, we examined four aspects of WAT that could be involved in protecting obese individuals from developing type 2 diabetes after bariatric surgery, namely WAT morphology and inflammation, secretion of adipokines, and lipolysis. WAT mass expands by enlarging pre-existing fat cells and/or by increasing the number of adipocytes. This can lead to the development of two different types of adipose morphologies, hypertrophy with fewer but larger fat cells or hyperplasia characterized by many small fat cells (6). Adipose hypertrophy is, independently of BMI, linked to insulin resistance (6) and increased risk of future development of type 2 diabetes (7,8). Furthermore, human fat cells are in a highly dynamic state, which is characterized by a constant renewal and cell death throughout adult life (9). A high turnover of fat cells may be protective since it is related to adipose hyperplasia (10). After weight reduction induced by gastric bypass, there is marked remodeling of adipose morphology (11). Fat cell size decreases markedly while adipocyte number remains unchanged so that a state of relative hyperplasia develops that is linked to improved insulin sensitivity (11). Thus, changes in adipose tissue morphology could be protective against type 2 diabetes development/relapse after gastric bypass.

Adipocytes secrete numerous polypeptides, collectively termed adipokines, that influence energy expenditure and glucose metabolism/insulin sensitivity. Adiponectin is the most abundantly secreted protein by WAT, and unlike most other adipokines, it displays an inverse correlation with obesity, insulin resistance, and type 2 diabetes (12). In addition, low adiponectin levels are associated with an increased risk of future type 2 diabetes development (13).

Obese WAT is characterized by a chronic low-grade inflammatory state with an increased secretion of a number of proinflammatory proteins, some of which may reach the circulation (3,6). Tumor necrosis factor-α (TNF-α) may be particularly important for type 2 diabetes. Although some studies suggest that adipose inflammation may be an adaptive response that enables safe storage of excess nutrients (14,15), adipose TNF-α stimulates lipolysis and inhibits insulin signaling in adipocytes (3). Elevated circulating levels of TNF-α–associated inflammatory markers predict future risk of type 2 diabetes (16), and a meta-analysis suggests that anti–TNF-α therapy reduces the risk of developing type 2 diabetes among patients with rheumatoid arthritis (17).

Fat cells provide energy-rich fatty acids through lipolysis (hydrolysis of triglycerides). The lipolytic activity is increased in WAT of obese subjects, which may induce insulin resistance (18). Cross-sectional and longitudinal examinations suggest an inverse relationship between human WAT lipolysis and insulin sensitivity, and direct inhibition of lipolysis in vivo improves glucose metabolism and insulin function in mice (19). Thus, altered lipolysis after bariatric surgery may influence type 2 diabetes through effects on insulin sensitivity.

To assess whether changes in WAT function can be linked to long-term protective effects against development/relapse of insulin resistance/type 2 diabetes, we investigated abdominal subcutaneous WAT from obese women before and 2 and 5 years after Roux-en-Y gastric bypass surgery (RYGB). The 5-year results were compared with those from age- and BMI-matched control women. Lipolysis, adipose morphology, and secretion of adiponectin or proinflammatory proteins were set in relation to clinical variables, including body shape, fat mass, insulin sensitivity, and lipid profile. This approach enabled us to determine whether bariatric surgery induces BMI-independent changes in WAT phenotype that could be of importance for long-term protection against metabolic complications.

RESEARCH DESIGN AND METHODS

Subjects

The subjects participated in a longitudinal trial of RYGB because of obesity (DEOSH, trial registration number NCT01785134). Initially, 82 women were enrolled and their baseline characteristics were published (20). Three patients with type 2 diabetes, who were not on insulin therapy, were included. No subject had followed any hypocaloric diet prior to the first examination and all had been weight stable (weight change $<$2 kg) for at least 1 year before their first preoperative visit. Postsurgery, subjects reported their actual body weight every 6 months and were reexamined after 2 years when they had reached a new weight-stable level. Sixty-two women returned for this examination and some clinical and adipose morphology data have been reported (11). They were asked to return for a third examination after an additional 3 years and 49 women accepted. Herein, we only report data from the women who participated in all three investigations. There were no significant differences in clinical variables at baseline between completers (Table 1) and dropouts (Supplementary Table 1). At the third examination, body weight had been stable for at least 3 months according to self-report. The patients were asked for their dietary habits. There were no important changes between the 2- and 5-year examinations. To obtain a control group, we included 30 women recruited by local advertising who were pairwise matched for BMI ($\pm$1 kg/m^2) and age ($\pm$2 years) with 30 postsurgery women at the 5-year follow-up. The inclusion of control subjects was made in parallel with the patient examinations at the 5-year follow-up. The matching was done by a research nurse who was completely blinded for all other measures except age and BMI. None of the control women reported any significant weight change ($\pm$5%) for several years prior to the examination. The study was approved by the regional ethics board and explained in detail to each woman. Written informed consent was obtained.

Examinations and Calculations

The women arrived to the laboratory in the morning after an overnight fast. The subject filled in a questionnaire about physical activity, which was graded in three levels: 1 = sedentary lifestyle and no important physical activity at work, score 2 = moderate physical activity at

Table 1—Findings in 49 women before and 2 and 5 years after gastric bypass

Measure	Before = A	2 years = B	5 years = C	*P* value A-B	*P* value B-C	*P* value A-C
Age (years)	43 ± 9	45 ± 9	48 ± 9	—	—	—
BMI (kg/m^2)	43.0 ± 4.8	28.8 ± 4.2	31.3 ± 5.6	<0.0001	<0.0001	<0.0001
WHR	1.00 ± 0.07	0.91 ± 0.06	0.92 ± 0.08	<0.0001	0.0003	<0.0001
Waist circumference (cm)	130 ± 11	96 ± 11	101 ± 14	<0.0001	0.11	<0.0001
Hip circumference (cm)	132 ± 10	104 ± 9	109 ± 12	<0.0001	<0.0001	<0.0001
Body fat (percentage)	53 ± 3	38 ± 8	43 ± 8	<0.0001	<0.0001	<0.0001
Android fat (kg)	63 ± 13	25 ± 13	32 ± 16	<0.0001	<0.0001	<0.0001
Gynoid fat (kg)	10 ± 2	5 ± 2	6 ± 2	<0.0001	<0.0001	<0.0001
EVAT (kg)	2.4 ± 0.8	0.7 ± 0.4	0.9 ± 0.6	<0.0001	<0.0001	<0.0001
ESAT (kg)	3.8 ± 1.1	1.8 ± 0.9	2.3 ± 1.2	<0.0001	<0.0001	<0.0001
S-insulin (mU/L)	15.4 ± 8.5	4.8 ± 1.9	5.7 ± 2.6	<0.0001	0.014	< 0.001
P-glucose (mmol/L)	5.6 ± 1.2	4.9 ± 0.5	5.1 ± 0.6	<0.0001	0.04	0.0006
HOMA-IR (units)	3.9 ± 2.7	1.0 ± 0.4	1.3 ± 0.6	<0.0001	0.007	<0.0001
P-TG (mmol/L)	1.4 ± 0.6	0.9 ± 0.4	1.0 ± 0.5	<0.0001	0.76	<0.0001
P-HDL cholesterol (mmol/L)	1.16 ± 0.29	1.46 ± 0.34	1.60 ± 0.48	<0.0001	0.003	<0.0001
P-total cholesterol (mmol/L)	4.8 ± 1.0	4.0 ± 0.7	4.2 ± 0.8	<0.0001	0.002	0.0001
P-glycerol (μmol/L)	123 ± 49	80 ± 28	125 ± 47	<0.0001	<0.0001	0.79
S-adiponectin (μg/mL)	7.4 ± 3.0	13.3 ± 12.2	12.9 ± 5.6	0.001	0.83	<0.0001
Fat cell volume (pL)	974 ± 193	437 ± 167	470 ± 183	<0.0001	0.19	<0.0001
Fat cell number × 10^7	442 ± 149	449 ± 159	589 ± 412	0.71	0.019	0.018
Glycerol release (μmol/10^7 cells)	3.4 ± 1.7	1.9 ± 0.9	4.4 ± 1.9	<0.0001	<0.0001	0.018
Glycerol release (mmol/total ESAT)	1.5 ± 0.8	0.8 ± 0.5	2.4 ± 1.5	0.0005	<0.0001	0.0003
Physical activity (score)	1.57 ± 0.55	2.13 ± 0.53	2.19 ± 0.40	<0.0001	0.043	0.003

Values are mean ± SD and compared by paired Student *t* test. P, fasting plasma; S, fasting serum; TG, triglycerides.

free time or work, and score 3 = intense and frequent physical activity at free time or work. Height and weight were determined for assessment of BMI. Circumferences of waist and hip were measured to calculate waist-to-hip ratio (WHR). A venous blood sample was obtained and plasma levels of insulin, glucose, triglycerides, cholesterol, and HDL cholesterol were determined, as previously described (20). Insulin and glucose values were used to calculate an insulin sensitivity index according to the HOMA of insulin resistance (HOMA-IR), as previously described (21). Total and regional amounts of body fat mass were measured by DEXA with the aid of GE Lunar iDXA using the EnCore software (version 14.10.022) supplemented with the CoreScan item (GE Healthcare, Chalfont St. Giles, U.K.). CoreScan is an automated method for segmenting total adipose fat within the android region into subcutaneous fat and visceral fat. The estimation of visceral adipose tissue (EVAT) with this software has been approved for clinical use by the U.S. Food and Drug Administration (22). The amount of estimated subcutaneous abdominal adipose tissue mass in the android region (ESAT) was calculated from the following formula: [total adipose fat mass in the android region] − [EVAT] = [ESAT], as previously described (22). Determination of EVAT with this method shows a strong correlation ($r^2 \geq 0.95$) with measures using computed tomography (22). Because only total android fat mass and EVAT are used to determine ESAT and both are valid measures, it also follows that the calculation of ESAT should be valid. Automatic calibration checks of the DEXA were performed daily throughout the study, and three times a week, calibrations using a spine phantom (for bone mineral density, provided by the manufacturer) were performed. The coefficient of variation for the spine phantom testing was 1.5%. No hardware or software changes were made during the course of the trial. The subjects were scanned using standard imaging and positioning protocols, and the same scan mode (set for obese subjects) was used throughout the study. A subcutaneous fat biopsy was obtained from the abdominal area at the same level as the measured ESAT. Mean fat cell weight and volume of fat cells were determined as previously described (20). In brief, fat cells were collagenase isolated, and the diameter of 100 cells was measured. It has been demonstrated that increasing the number of fat cells for diameter measurement to several hundred does not improve the reliability of mean fat cell size (23). The histograms of adipocyte diameter distributions were created using R provided by The R Foundation for Statistical Computing (https://www.r-project.org/foundation/). All data were concatenated into groups based on 100 measured fat cell diameters from each biopsy and plotted using the package ggplot2 for graphics (https://cran.r-project.org/web/packages/ggplot2). The number of fat cells in the ESAT region was determined by dividing ESAT weight with mean subcutaneous fat cell weight. ESAT does not measure total subcutaneous abdominal adipose tissue but only the segment representative for the region where the fat biopsy is taken. Adipose secretion of proteins and glycerol (lipolysis index) were determined exactly as previously described (24–27). In brief, pieces of WAT (100 mg/mL incubation medium) were incubated for 2 h at 37°C in a shaking

water bath with air as gas phase using a Krebs-Ringer phosphate buffer (pH 7.4) supplemented with glucose (1 mg/mL) and BSA (20 mg/mL). At the end of incubation, aliquots of the medium were removed and stored at −80°C for subsequent analyses. Release of adiponectin (24), TNF-α (25), monocyte attractant protein-1 (MCP-1) (26), and interleukin-6 (IL-6) (25) were determined using commercially available ELISA kits from Mercodia (Uppsala, Sweden) for adiponectin and R&D Systems (Minneapolis, MN) for the other ones. Glycerol release was determined by bioluminescence (27). We used the same detection methods to determine fasting plasma glycerol and serum adiponectin. Since glycerol and adiponectin are solely produced by fat cells in WAT, their secretion was related to the number of fat cells incubated, which was determined as weight of lipids extracted from incubated WAT divided by mean fat cell weight. As inflammatory proteins are mainly produced by cells in the stroma-vascular fraction of adipose tissue (28), their secretion was normalized to the weight of the incubated WAT. We also calculated the release of glycerol and proteins per total ESAT mass. Using tissue pieces instead of collagenase-isolated fat cells enables the measure of spontaneous glycerol release (lipolysis activity), which correlates with in vivo insulin sensitivity and is linear for at least 4 h of incubation (19,29).

Statistics

Group values are expressed as mean ± SD in the text and tables and were compared by paired Student *t* test or paired sign test or linear regression. As each control woman was compared with a specific BMI/age-matched postsurgery woman at 5 years, it is appropriate to use paired tests (30). However, in this case, we also used the less sensitive unpaired Student *t* test. Individual values were compared by linear regression and ANCOVA. For statistical power calculations, we used published data on subcutaneous fat cell volume from the entire obese cohort at the 2-year follow-up (11). This showed that if 50 returned at 2 years after surgery, we could detect a 0–2-year difference of 15% with 80% power at $P < 0.05$. Using the same data, we calculated that we could detect a difference of 20% between 2 and 5 years with 80% power ($P < 0.05$) if 30 women returned at 5 years or between 30 postoperative and 30 control women. A *P* value of <0.05 was considered to be statistically significant in all analyses.

RESULTS

Table 1 shows the longitudinal findings in the cohort. At the 2-year follow-up after RYGB, there was a dramatic decrease in mean BMI from morbid obesity (i.e., ≥40 kg/m^2) to a nonobese state. This was accompanied by a significant reduction in WHR, hip and waist circumferences, and all measures of regional fat depots. Furthermore, serum adiponectin, fat cell size, and lipolysis in vivo as well as in vitro (determined by glycerol levels) decreased markedly but there was no change in fat cell number. After 5 years, BMI had increased by ~2.5 kg/m^2 back to an obese state. This was accompanied by increased fat accumulation in all measured regions, a slight but significant deterioration of HOMA-IR, minor changes in the lipid profile, but no change in serum adiponectin. Lipolysis increased dramatically to similar levels observed before surgery, as determined by circulating concentrations of glycerol, and to higher levels than initially using glycerol release per fat cell or per total ESAT. Subcutaneous fat cell size did not change, but there was a 30% increase in fat cell number. Whereas physical activity increased from 0 to 2 years, it did not change further at 5 years.

Table 2 shows the results at the 5-year examination when 30 postsurgery women were compared with 30 matched control women. The measured variables are the same as in Table 1, and values were similar in the postoperative subgroup as in the whole cohort at 5 years. The two groups in Table 2 had similar levels of physical activity, as well as body shape, and their lipolytic activities did not differ by any measure. However, the postsurgery women displayed markedly better insulin sensitivity and lipid profiles than

Table 2—Findings 5 years after gastric bypass in 30 obese women compared with 30 control women pair-matched for age and BMI

Measure	5 years postsurgery	Control	*P* value
Age (years)	49 ± 9	48 ± 9	—
BMI (kg/m^2)	32 ± 7	32 ± 7	—
WHR	0.93 ± 0.07	0.94 ± 0.06	0.76 (0.86)
Waist circumference (cm)	104 ± 17	106 ± 17	0.25 (0.65)
Hip circumference (cm)	111 ± 14	112 ± 13	0.23 (0.68)
Body fat (percentage)	44 ± 9	45 ± 10	0.19 (0.66)
Android fat (kg)	35 ± 18	37 ± 18	0.25 (0.58)
Gynoid fat (kg)	7 ± 3	6 ± 2	0.23 (0.62)
EVAT (kg)	1.0 ± 0.7	1.5 ± 0.9	0.00006 (0.02)
ESAT (kg)	2.6 ± 1.4	2.4 ± 1.0	0.21 (0.62)
S-insulin (mU/L)	5.9 ± 2.7	10.6 ± 7.3	0.001 (0.003)
P-glucose (mmol/L)	5.1 ± 0.6	5.1 ± 0.4	0.48 (0.49)
HOMA-IR (units)	1.4 ± 0.7	2.5 ± 1.7	0.002 (0.003)
P-TG (mmol/L)	0.9 ± 0.5	1.2 ± 0.6	0.02 (0.04)
P-HDL cholesterol (mmol/L)	1.63 ± 0.51	1.34 ± 0.36	0.02 (0.001)
P-total cholesterol (mmol/L)	4.2 ± 0.7	4.8 ± 1.0	0.003 (0.003)
P-glycerol (μmol/L)	116 ± 42	108 ± 69	0.59 (0.57)
P-adiponectin (μg/mL)	12.9 ± 5.5	9.1 ± 3.1	0.0009 (0.001)
Fat cell volume (pL)	492 ± 210	711 ± 243	<0.0001 (0.0008)
Fat cell number × 10^7	653 ± 518	366 ± 122	0.02 (0.06)
Glycerol release (μmol/10^7 cells)	4.5 ± 1.9	4.7 ± 2.72	0.45 (0.68)
Glycerol release (mmol/total ESAT)	2.4 ± 2.0	1.9 ± 0.8	0.08 (0.20)
Physical activity (score)	2.3 ± 0.5	2.1 ± 0.7	0.74 (0.21)

Values are mean ± SD and compared by paired and unpaired (within parentheses) Student *t* test. In some cases, *n* is <30 due to missing values. P, fasting plasma; S, fasting serum; TG, triglycerides.

control subjects as well as higher adiponectin levels. Furthermore, the body fat levels in all regions were similar between the groups except for EVAT, which was increased in the control group. Finally, the fat cells in ESAT were smaller and almost twice as many in postoperative patients as in control women. The outcome was independent of whether unpaired or paired Student *t* test was used.

The individual relationship between HOMA-IR and serum adiponectin values was investigated in Fig. 1 using the data in Table 2. There was a strong inverse relationship between the two measures in both groups of women. However, it was much steeper in the control subjects, implying that in the low range of circulating adiponectin (<12 μg/mL), HOMA-IR values in control individuals were higher than in postoperative patients. In contrast to the postsurgery subjects, none of the control individuals had high adiponectin levels (>16 μg/mL). The relationship between adiponectin and HOMA-IR was not influenced by BMI (values not shown).

Weight loss improves the expression and secretion of proinflammatory adipokines. The release of inflammatory protein and adiponectin from WAT was examined at 2 and 5 years after gastric bypass and in control women (Table 3). There was no change over time in the release of MCP-1, which did not differ from the control group. IL-6 increased when expressed per total ESAT to the same level as in control subjects, and TNF-α secretion increased markedly between 2 and 5 years, albeit to levels that were only about half of those observed in control individuals. During this time period, there was no change in adiponectin secretion, which at 5 years, was nevertheless higher than in the control subjects (fivefold when expressed per cell and 30% when expressed per total ESAT). Similar results were obtained with paired or unpaired Student *t* test.

The predictive value of measurements at 2 years in Table 1 for changes in corresponding variables between 5 and 2 years in the same table was examined by single regression. There were no significant relationships between baseline values for body composition and metabolic changes during body weight regain (values not shown). Likewise, there were no significant relationships between changes between 2 and 5 years in lipolysis and adiponectin/chemokine release or between changes in adipocyte size/number and metabolic measures (values not shown).

Finally, we evaluated the distribution of the adipocyte diameters in the different groups (Fig. 2). To this end, all cell diameters (100 from each individual) were grouped according to the time of investigation for the obese (0, 2, and 5 years) and the control group. Thus, a total of 4,900 diameters were counted at each examination for patients and a total of 3,000 diameters in control subjects. This showed a normal distribution of the adipocyte diameters for all groups with postsurgery women positioned to the left of the other groups. We also examined each of the individual histograms for fat cell diameter. In no case was there any evidence of a bimodal distribution.

A few case and control subjects received oral treatment for type 2 diabetes and hyperlipidemia during the course of the study (Supplementary Table 2). However, the results were not influenced in any important way if these women were excluded from the analyses.

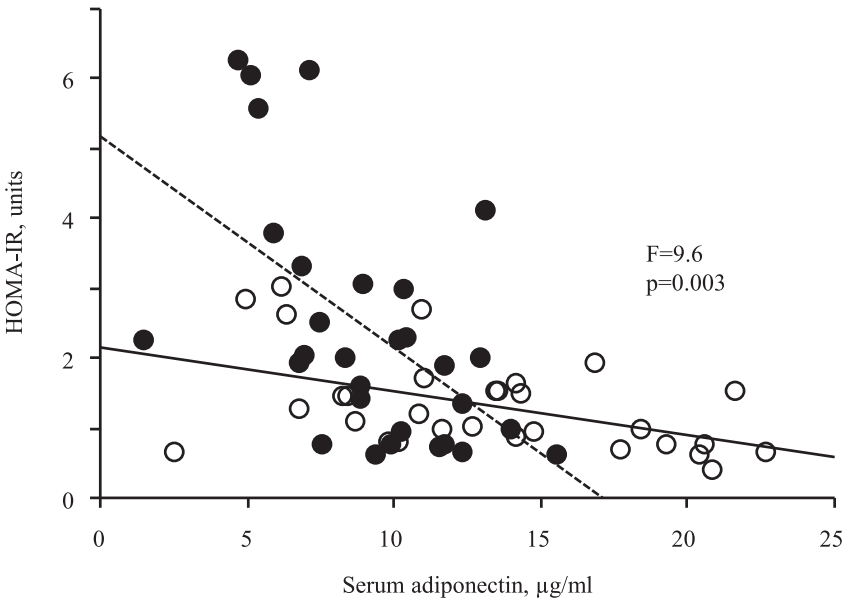

Figure 1—Relationship between serum adiponectin and HOMA-IR in obese women 5 years after gastric bypass (open circles) and control subjects (filled circles). Lines represent the linear relationship between groups. Values were compared by ANCOVA. The slopes of the two lines differed significantly ($F = 6.0$; $P = 0.018$).

CONCLUSIONS

This study was undertaken to investigate whether adipose factors beyond weight reduction could be important for restoration of insulin sensitivity in obese patients undergoing bariatric surgery. We confirm the pronounced effect of RYGB on BMI reduction, converting the subjects from a morbidly obese into a nonobese state after 2 years (1). As expected, this was accompanied by marked metabolic improvements, increase in circulating adiponectin, and reduced lipolytic activity and fat cell size. In agreement with our earlier study (11), we found that obesity prior to surgery was associated with both fat cell hyperplasia and hypertrophy and that the number of fat cells did not decrease after surgery. As we did not include matched control subjects at the 2-year follow-up, we could not establish whether type 2 diabetes–protective events beyond BMI reduction have developed in WAT at this early postoperative stage.

Between the 2- and 5-year follow-up, there was a significant BMI increase, which was of the same magnitude as previously reported in a large Swedish cohort (1). This was accompanied by a small but significant worsening of glucose metabolism (as determined by fasting plasma glucose and HOMA-IR). However, the blood lipid profile did

Table 3—Adipose secretion of inflammatory proteins and adiponectin

	Whole obese cohort (n = 49)			Obese subgroup		
Measure	2 years	5 years	P value	5 years (n = 30)	Control (n = 30)	P value
TNF-α (ng/g tissue)	563 ± 590	867 ± 843	0.10**	755 ± 648	1,892 ± 1,498	0.005 (0.003)
TNF-α (mg/total ESAT)	10 ± 10	23 ± 21	0.003	24 ± 21	59 ± 50	0.004 (0.006)
MCP-1 (ng/tissue)	8.4 ± 4.8	7.7 ± 5.3	0.54	7.8 ± 5.8	7.5 ± 3.2	0.67 (0.88)
MCP-1 (mg/total ESAT)	1.6 ± 1.2	2.2 ± 2.3	0.27	2.4 ± 2.6	2.2 ± 1.2	0.35 (0.75)
IL-6 (ng/g tissue)	10.3 ± 6.6	11.0 ± 4.2	0.27	10.7 ± 4.4	12.1 ± 5.4	0.56 (0.42)
IL-6 (mg/total ESAT)	2.0 ± 1.7	2.9 ± 1.4	0.02	3.0 ± 1.5	3.6 ± 2.0	0.37 (0.46)
Adiponectin (ng/10^7 cells)	997 ± 436	849 ± 374	0.21	829 ± 379	163 ± 61	<0.0001 (<0.0001)
Adiponectin (mg/total ESAT)	433 ± 215	508 ± 348	0.34	511 ± 385	396 ± 213	0.19* (0.21)

Values are mean ± SD and compared by paired and unpaired (within parentheses) Student t test. In some cases, n is <49 or <30 due to missing values. *P = 0.009 by paired sign test. **P = 0.01 by paired sign test.

not deteriorate apart from a minor increase in total cholesterol, which is in line with earlier long-term studies of bariatric surgery (31).

The inclusion of a matched control group with similar age, BMI, body shape, and regional or total fat mass allowed us to evaluate the long-term effects on adipose phenotypes and metabolic control in the absence of these confounding factors. We observed that the postsurgery group had reached a state of "super-normality" at 5 years and was significantly more insulin sensitive and displayed better plasma lipid profiles compared with control women. In a previous 4-year-long study, weight loss improved insulin sensitivity to a higher level compared with BMI-matched control subjects (32). These marked metabolic improvements should encourage people struggling with weight loss.

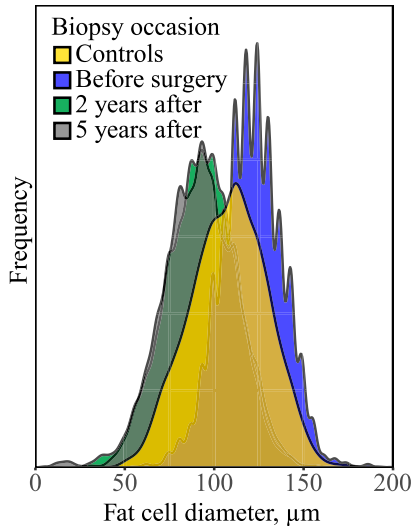

Figure 2—Histograms of the distribution of mean fat cell diameters of each group investigated (i.e., obese before surgery, 2 and 5 years postsurgery, and control subjects). See RESEARCH DESIGN AND METHODS for details of the construction of the histograms. The mean histograms depicted represent 4,900 diameters of the obese patients at each examination and 3,000 diameters in control subjects.

Could long-term improvements in metabolism after gastric bypass involve WAT-specific factors? Our present results support this notion. Although WAT secreted or circulating levels of adiponectin did not change after weight regain (between 2 and 5 years), these values were significantly higher than in control subjects. In addition, the relationship between circulating adiponectin and insulin sensitivity was much steeper in the latter group in the low range of circulating adiponectin concentrations. This implies that control subjects are more insulin resistant at low adiponectin levels and/or that postoperative women are "protected" from the potential adverse effect of low adiponectin. Thus, increased production and perhaps improved function of/sensitivity to adiponectin could constitute a diabetes-protective mechanism after RYGB. The low amount of visceral adipose tissue in patients compared with the control subjects could also play a role besides adiponectin.

It is well established that WAT inflammation is ameliorated after weight reduction induced by bariatric surgery (33,34). In this study we investigated only the weight regain phase after surgery. MCP-1 or IL-6 secretion showed no or minor increase by weight regain, and these levels were similar to the control state. This was in contrast to the perhaps most pernicious inflammatory WAT protein, TNF-α. Although WAT release of TNF-α increased markedly after weight regain, the secretion rate was still only about half of that in control subjects. It is therefore possible that attenuated TNF-α WAT inflammation could be another protective factor after RYGB. Unfortunately, the amount of available WAT was too small for additional histological examinations of, for example, macrophage number/subtypes and fibrosis.

Human WAT morphology has previously been investigated after short-term experimental overfeeding (35,36). Fat cell size increases markedly and significantly, whereas fat cell number only increases in the gluteal-femoral region. This is in marked contrast to the current study in subcutaneous abdominal WAT, where long-term regain of body weight did not alter fat cell size but caused a dramatic increase in fat cell number, leading to a much more prominent hyperplastic phenotype than in control subjects. The mechanisms promoting the generation of new, presumably small, fat cells after 5 years are not clear, although, as suggested by results from rodent studies, it is possible that weight cycling (weight reduction followed by weight gain) triggers adipogenesis (37).

It is interesting to note that there was no evidence of bimodal distribution of fat cells during weight regain. Furthermore, effects of long-term moderate weight gain on adipose morphology might be different from those after short-term weight increases. Adipose hyperplasia is linked to better insulin sensitivity in a large cross-sectional study (10) and to a reduced propensity

of developing insulin resistance upon overfeeding in mice (38). However, a recent study in 29 nonobese men suggested that subjects with small fat cells have a worsened metabolic response to overfeeding (39). Thus, it is for the moment unclear to what extent the long-term development of WAT hyperplasia postsurgery observed herein can explain the higher insulin sensitivity compared with control subjects. Nevertheless, prospective studies of WAT hypertrophy and hyperplasia speak strongly in favor for a diabetes-protective effect of the latter phenotype (7,8).

Fat cell number was presently determined using an indirect measure of regional body fat mass by DEXA, which correlates perfectly with computed tomography (see RESEARCH DESIGN AND METHODS). Admittedly, none of these or other indirect measures have been fully validated in vivo as this would necessitate dissection of fat depots from deceased people. However, all measures of subcutaneous fat and total fat were similar between control subjects and postoperative patients at 5 years. Lipid amounts in adipose tissue are regulated by the size and number of fat cells and accounts for >90% of the adipose volume. Thus, it follows that the decreased fat cell size in the postoperative patients, in spite of a normal amount of subcutaneous fat, is due to increased subcutaneous fat cell number.

Lipolysis was markedly increased after weight regain and was similar to that in control subjects. This demonstrates that lipolysis displays a particular sensitivity to moderate weight gain. However, it also suggests that lipolysis is less likely to be a protective factor against metabolic complications associated with weight regain after RYGB. There is no consensus which denominator to use when expressing adipose lipolysis rate. We used two different and physiological measures (per number of fat cells and per total amount of adipose tissue). Both methods yielded similar results.

We found no significant relationship between changes in body fat distribution, adipose morphology, and metabolic factors during weight regain, and there was no evidence that the values obtained at 2 years could predict the outcome at 5 years. The most likely explanation is that the changes in adipose morphology and in vivo metabolism were relatively small during this phase of investigation (from a few percentages to maximum 30%).

There are some weaknesses with this study. We only measured spontaneous lipolytic activity in intact adipose tissue. Findings with hormone-induced lipolysis (insulin, catecholamines, and natriuretic peptides) could be different but would require fat cell isolation, which may reflect in vivo lipolysis less accurately. We cannot extrapolate the present findings to men or other WAT regions (e.g., the visceral depot). For the examined abdominal subcutaneous region, we only determined the region corresponding to the adipose biopsy. Moreover, we cannot establish whether other bariatric surgical techniques besides RYGB, such as sleeve gastrectomy, or weight loss by means other than bariatric surgery would have yielded similar results. Additionally, a number of other mechanisms in nonadipose tissues (e.g., in the gut, liver, pancreas, and skeletal muscle) may also have important protective antidiabetic effects after bariatric surgery. Finally, bariatric surgery can improve physical activity and causes changes in dietary habits that, in turn, may influence adipose function. However, our examinations, albeit not detailed, suggest that such factors are not of great importance for the findings with postoperative patients at 5 years and in the comparison with control subjects.

In summary, RYGB surgery in obese women leads to long-term improvements in metabolic and WAT phenotype beyond the normal state despite weight regain. These changes involve an attenuated inflammatory response, high adiponectin secretion, as well as development of WAT hyperplasia. The molecular mechanisms that mediate the transition into a "super-normal" WAT after bariatric surgery remain to be established but could be related to changes in the expression of specific WAT genes, as recently observed in metabolically healthy obese individuals after overfeeding (40).

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Author Contributions. J.H. designed the study and participated in recruitment and examination of the subjects. D.P.A. participated in recruitment and examination of the subjects and performed the statistical analyses. D.E.H. and M.R. participated in recruitment and examination of the subjects. J.T. and A.E. performed the statistical analyses. E.N. and A.T. participated in recruitment and examination of the subjects and performed the bariatric surgery. P.A. designed the study, participated in recruitment and examination of the subjects, performed the statistical analyses, and wrote the first version of the manuscript. All authors were involved in the writing and revision of the manuscript and approved the final version. P.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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