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Change in abdominal, but not femoral subcutaneous fat CT-radiodensity is associated with improved metabolic profile after bariatric surgery

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Abstract *Background and aims:* Computed tomography (CT)-derived adipose tissue radiodensity represents a potential noninvasive surrogate marker for lipid deposition and obesity-related metabolic disease risk. We studied the effects of bariatric surgery on CT-derived adipose radiodensities in abdominal and femoral areas and their relationships to circulating metabolites in morbidly obese patients.
Methods and results: We examined 23 morbidly obese women who underwent CT imaging before and 6 months after bariatric surgery. Fifteen healthy non-obese women served as controls. Radiodensities of the abdominal subcutaneous (SAT) and visceral adipose tissue (VAT), and the femoral SAT, adipose tissue masses were measured in all participants. Circulating metabolites were measured by NMR. At baseline, radiodensities of abdominal fat depots were lower in the obese patients as compared to the controls. Surprisingly, radiodensity of femoral SAT was higher in the obese as compared to the controls. In the abdominal SAT depot, radiodensity strongly correlated with SAT mass (r = -0.72, $p < 0.001$). After surgery, the radiodensities of abdominal fat increased significantly (both $p < 0.01$), while femoral SAT radiodensity remained unchanged. Circulating ApoB/ApoA-I, leucine, valine, and GlycA decreased, while glycine levels significantly increased as compared to pre-surgical values (all $p < 0.05$). The increase in abdominal fat radiodensity correlated negatively with the decreased levels of ApoB/ApoA-I ratio, leucine and GlycA (all $p < 0.05$). The increase in abdominal SAT density was significantly correlated with the decrease in the fat depot mass (r = -0.66, p = 0.002).
Conclusion: Higher lipid content in abdominal fat depots, and lower content in femoral subcutaneous fat, constitute prominent pathophysiological features in morbid obesity. Further studies are needed to clarify the role of non-abdominal subcutaneous fat in the pathogenesis of obesity.
Clinical trial registration number: NCT01373892.

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Introduction

Excessive lipid deposition into metabolically relevant tissues compromises peripheral insulin sensitivity and contributes to the risk of metabolic diseases seen in obesity [1]. Morbid obesity is associated with elevated systemic biomarkers of metabolic dysfunction [2] as well as with elevated levels of circulating fatty acids, lipoproteins and branch-chained amino acids (BCAA) [3]. These have been recognized to strongly associate with insulin resistance, diabetes and cardiovascular disease [4–6]. Conversely, elevated levels of glycine are associated with an improved glycemic control and reduced inflammation [7,8].

Computed tomography (CT) scans can distinguish different tissue types based on the radiodensity, which is expressed as Hounsfield units (HU) [9]. The radiodensity of white adipose tissue typically ranges from −300 to −10 HU [10]. A smaller negative number (higher absolute number) is indicative of a lower radiodensity (e.g., −100 is a smaller negative number and indicate a lower radiodensity than −90HU). Lower abdominal subcutaneous (SAT) and visceral (VAT) adipose tissue CT radiodensities are thought to reflect increased lipid deposition, decreased tissue vascularity, a lower tissue blood flow rate [11], a lower mitochondrial density [12], low-grade systemic inflammation and an unfavorable metabolic state [13].

Adipose tissue serves as the storage site for energy-rich triglycerides, and has the capacity to expand to accommodate excess energy [14]. The expansion of femoral SAT mass may play a protective metabolic role (i.e. buffering cardiovascular disease and diabetes risk [15]) especially when the tissue delivery of dietary and endogenous fatty acids is elevated. However, fat accumulation in the abdominal SAT and VAT is associated with metabolic abnormalities such as insulin resistance, hyperglycemia, hypertension, and dyslipidemia [16]. Overall, this suggests that the lipid storage profile may play alternative metabolic roles between different adipose compartments.

Bariatric surgery has shown to be of value to achieve marked and sustained weight loss as well as diabetes resolution in morbidly obese individuals [17]. Previous studies by us and others have shown that weight loss following bariatric surgery improves insulin sensitivity in key metabolic tissues [18–20], increases abdominal SAT and VAT radiodensity [11], and is associated with clinical improvements in circulating fatty acid levels, and serum metabolic profiles [21]. However, an extensive non-invasive evaluation of fat lipid content in both the abdominal and femoral adipose tissue depots using CT imaging and its relation to indices of systemic metabolism has not been performed in morbidly obese individuals undergoing bariatric surgery.

We hypothesized that low adipose tissue CT-radiodensity is associated with a poorer metabolic profile prior to bariatric surgery. We also hypothesized that adipose tissue radiodensities increase after surgery and correlate with improved systemic metabolism. Hence, the present longitudinal study aimed to assess the association between abdominal and femoral adipose tissue radiodensities, and circulating metabolite profile in morbidly obese individuals before and after bariatric surgery.

Methods

Subjects and study design

The study included 23 morbidly obese adult women (all >18 years) recruited from a randomized prospective three-center study comparing laparoscopic Roux-en-Y gastric bypass (RYGB) *vs.* sleeve gastrectomy for the treatment of morbid obesity [22]. In addition, 15 age-matched, non-obese non-diabetic women served as controls. Prior to the surgical intervention, 10 obese patients had diabetes, and 13 were nondiabetic. Among nondiabetic patients, 4 had impaired glucose tolerance and 1 had impaired fasting glucose [23].

Nine of the 10 diabetic subjects were treated with either metformin or DPP-IV inhibitors or a combination of these medications, and one was controlled by a dietary regiment. All glucose-lowering treatments were withheld for a minimum of 24 h and a maximum of 72 h before the imaging studies. Clinical screening and physical examination, anthropometric measurements and blood-based biochemical analyses including 2-h oral glucose tolerance test (OGTT) were performed in the study participants as previously described [24]. During the OGTT, samples of plasma glucose, plasma insulin and C-peptide were collected at a 30-min interval for 2 h (i.e. 0, 30, 60, 90, 120 min). The morbidly obese subjects followed a 4-week very low-calorie diet (VLCD, 800 kcal/day), which was discontinued a day before bariatric surgery procedures [24]. The post-procedural evaluation phase was conducted at six months, and the anthropometric, metabolic studies were repeated similarly as in the baseline phase. The 6-month time-point was chosen as the weight loss is most prominent during the first six months after surgery [24] This study has been approved by the local ethics committee of the Hospital District of Southwest Finland and was performed in compliance with the Declaration of Helsinki. All the study subjects provided written informed consent.

CT image acquisition and processing

Patients underwent CT scans after an overnight fast and at room temperature. The CT imaging was performed before the start of the VLDL. The imaging was performed using a Discovery VCT (VCT) PET/CT system (General Electric Medical Systems, Milwaukee, WI, USA). The CT system consists of a multislice CT scanner with a large 70-cm patient port and CT coverage up to 64 slices, 40 mm axial coverage and 0.625 mm slice thickness [25]. High resolution CT imaging was performed at a tube voltage of 120 kVp and a variable current of approximately 50 mA as previously described [25].

Data analysis and calculations

The adipose tissue radiodensity analysis was performed using Carimas version 2.9 (http://turkupetcentre.fi/carimas/download/). Forty-seven slices of the CT-scans were used for the analysis of both the abdominal and femoral regions. The attenuation threshold value of −300 to −10HU was used to define the adipose tissue regions [10]. The mean pixel attenuation within the defined areas of the combined 47 slices was calculated to represent adipose tissue radiodensity (HU) [26].

Abdominal adipose CT-radiodensity measurement. The abdominal CT imaging covered regions from the 12th thoracic to 1st sacral (T12 - S1) vertebrae [27]. Subcutaneous adipose tissue in this region was outlined whilst avoiding skin and abdominal skeletal muscles. The abdominal VAT comprised an average of both the independent intraperitoneal and extraperitoneal fat depots separated by anatomical references such as the kidneys, ascending and descending colon [28]. A single operator performed the abdominal adipose CT radiodensity analysis and the values obtained were similar to values previously reported by Torriani et al., [11].

Femoral subcutaneous adipose CT radiodensity measurement. A total of 47 slices covering the length of 15 cm in the mid-section of the thigh was used for the femoral SAT HU analysis. The subcutaneous fat of both legs was outlined whilst avoiding skin and skeletal muscles as previously performed on PET/MRI scans [18]. The average CT-radiodensity values of the combined slices for both legs were then calculated. The femoral SAT CT radiodensity analysis was performed by two independent operators. The intraclass correlation coefficient value for the two measurements was 0.82.

Distribution of body fat

Abdominal and femoral fat volumes were calculated from a whole body MRI scans (Gyroscan Intera CV Nova Dual; Philips, Amsterdam, the Netherlands) using the SliceOmatic Tomovision software (version 4.3) as previously reported [29].

Biochemical and immunological analyses

Plasma glucose concentrations were measured in duplicate using the glucose oxidase method (Analox GM7 or GM9 Analox Instruments Ltd., London, UK). Glycosylated hemoglobin was determined by HPLC (Variant II; Bio-Rad, Herculas, CA). Serum insulin was determined by time-resolved immunofluorometric assay (AutoDELFIA, PerkinElmer Life and Analytical Sciences). Serum high-sensitivity C-reactive protein was analyzed with the sandwich immunoassay method using an Innotrac Aio1 immunoanalyzer (Innotrac Diagnostics, Turku, Finland). The C-peptide in plasma was measured with an electrochemiluminescence immunoassay (ECLIA) on cobas e602 automatic analyzer (Roche Diagnostics, Mannheim, Germany).

Metabolomics

The procedure for the serum metabolomics profiling analysis has been previously described [30]. Briefly, fasting serum samples were stored at −70 °C. Low molecular weight metabolites including lipoprotein and lipid extracts including serum cholesterol (Serum-C), triglycerides (Serum-TG), amino acids such as branch-chained amino acids (BCAAs: isoleucine, leucine and valine), and aromatic amino acids (AAA) (phenylalanine and tyrosine), glycine and glycoprotein acetyls (GlycA) were measured using a high-throughput nuclear magnetic resonance metabolomic platform [30].

Statistical analysis

Continuous variables are expressed as mean ± SD. The variables that were not normally distributed were logarithmically transformed prior to the analyses. Correlations between variables were evaluated through the Pearson's biserial correlation coefficient. The percentage change concerning continuous variables was calculated for the pre- and the post-surgery values. The percentage change for the continuous variable was calculated as post-minus pre-surgery value divided by the pre-surgery value and the result was expressed as a percentage. A positive value indicated percentage increase, whereas a negative result represented a decrease in the value of the variable after the surgical procedure. Paired-samples t-tests were used to compare the means of the measured variables between the obese patients pre- and post-bariatric surgery, while independent-samples t-tests were performed to compare continuous variables between the obese and control groups. One-way analysis of variance (ANOVA) was used for the comparison of radiodensities and fat volumes in abdominal SAT, VAT and femoral SAT depots. Lastly, we adjusted for the BMI to test whether the association between SAT radiodensity and the depot mass was independent of this variable. All analyses were performed using SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, New York, IBM Corp). A p-value < 0.05 was considered statistically significant.

Results

Adipose tissue volumes, CT-radiodensities and metabolite profiles in obese patients and non-obese controls

Major aspects of the subject characteristics, including measures of adiposity such as body weight, BMI, waist circumference, depot fat volumes, glycemic and lipid parameters in obese patients and non-obese controls have been previously published [18,22] and are summarized in Table 1. In obese patients, abdominal SAT volume was significantly higher as compared with abdominal VAT or femoral SAT volumes (Table 1, $p < 0.001$ by ANOVA). In contrast, CT-radiodensity was higher in femoral SAT than in abdominal SAT or VAT

(Table 1, $p < 0.001$ by ANOVA). Compared to the lean controls, the obese had significantly lower abdominal SAT and VAT, but higher femoral SAT radiodensity values (Table 1, Fig. 1). Levels of serum triglycerides, and ApoB/ApoA1 ratio were significantly higher in the obese patients as compared to the non-obese healthy controls (Table 2). Similarly, essential amino acids and their derivatives such as the BCAAs, phenylalanine, and GlycA were significantly higher in obese patients when compared with non-obese controls (Table 2).

Abdominal SAT CT-radiodensity was negatively correlated also with markers of obesity such as BMI ($r = -0.55$, $p = 0.01$) and waist circumference ($r = -0.80$, $p < 0.001$) (Fig. 2). In contrast, femoral SAT radiodensity was positively correlated with waist circumference ($r = 0.44$, $p = 0.033$) (Fig. 2). Abdominal SAT CT-radiodensity correlated with abdominal SAT mass both in the unadjusted ($r = -0.72$, $p < 0.001$) and after BMI-adjusted ($r = -0.58$, $p = 0.005$) analysis.

Increase in abdominal adipose tissue densities after bariatric surgery associates with improved systemic metabolism

Adiposity measures, adverse metabolic and inflammatory indices decreased after bariatric surgery (Table 1). However, serum lipid parameters remained unchanged when compared to pre-procedural values (Table 1). The CT-radiodensities of abdominal SAT and VAT depots increased after surgery and the values were statistically similar to those observed in the non-obese controls (Table 1, Fig. 1). The decrease in the absolute mass of abdominal VAT was greater than SAT (Table 1, $p < 0.001$). However, there was a difference in change in the densities of abdominal SAT vs. VAT (Table 1). The density of femoral SAT remained unchanged as compared to their pre-surgery values (Table 1, Fig. 1). The profile of circulating metabolites was also improved, as evidenced by the decrements in levels of ApoB/ApoA1, BCAAs, AAAs and GlycA, and the increase levels of glycine compared to the pre-surgery concentrations (Table 2).

To further explore the possible effect of surgery, we calculated the change (post - pre-surgery) of the CT-derived radiodensities, fat volumes, and serum metabolite profiling values. There was a 39% reduction in abdominal SAT mass, which significantly correlated with a 13% decrease in lipid content in the SAT depot ($r = -0.63$, $p = 0.002$). The increase in CT-radiodensities of abdominal SAT and VAT correlated negatively to the change in circulating levels of leucine ($r = -0.57$, $p = 0.005$, and $r = -0.43$, $p = 0.039$, respectively) (Fig. 3). Furthermore, the change in abdominal SAT CT-radiodensity correlated negatively with the change in GlycA ($r = -0.46$, $p = 0.028$), and abdominal VAT CT-radiodensity correlated negatively with ApoB/ApoA-I ratio levels ($r = -0.48$, $p = 0.020$) (Fig. 3).

Discussion

This study shows that abdominal subcutaneous and visceral adipose tissue CT-radiodensities are significantly lower, and femoral adipose CT-radiodensity higher in

Table 1 Anthropometric and clinical parameters of study subjects.

	Controls (n = 15)	Obese surgery patients (n = 23)		Change (%) from pre-surgery	#*P*-value within the obese group
		Pre-surgery	Post-surgery		
Age (years)	44.8 ± 12.4	42.8 ± 9.6	43.4 ± 9.4	–	–
Weight (kg)	61.8 ± 7.1	112.4 ± 15.4***	86.8 ± 13.5***	−22.6 ± 6.2	<0.001
BMI (kg/m^2)	22.6 ± 2.8	41.1 ± 4.2***	31.8 ± 13.5***	−22.6 ± 6.3	<0.001
Waist circumference (cm)	74.7 ± 8.2	115.0 ± 10.6***	94.9 ± 12.2***	−15.9 ± 7.7	<0.001
Abdominal SAT (kg)	3.7 ± 1.5	16.5 ± 4.5***	10.3 ± 4.2***	−38.6 ± 16.7	<0.001
Abdominal VAT (kg)	0.8 ± 0.4	3.5 ± 1.3***	1.9 ± 1.03**	−47.7 ± 20.1	<0.001
Femoral SAT (kg)	5.9 ± 1.8	13.5 ± 3.8***	9.3 ± 3.6***	−31.0 ± 13.2	<0.001
Abdominal SAT (HU)	−97.7 ± 17.1	−112.3 ± 7.1***	−98.1 ± 11.6	−12.7 ± 8.4	<0.001
Abdominal VAT (HU)	−94.9 ± 12.2	−111.9 ± 6.8***	−101.2 ± 11.0	−9.3 ± 10.7	0.001
Femoral SAT (HU)	−107.1 ± 8.2	−97.9 ± 10.6**	−100.6 ± 7.2*	4.9 ± 15.0	0.177
Fasting glucose (mmol/L)	5.3 ± 0.6	6.1 ± 1.0**	5.4 ± 0.7	−11.3 ± 9.8	<0.001
Fasting insulin (mU/L)	5.3 ± 3.5	13.1 ± 8.4***	8.5 ± 5.9	−20.7 ± 57.8	<0.001
Fasting C-peptide (mmol/L)	0.6 ± 0.2	1.1 ± 0.3***	0.8 ± 3.5**	−26.1 ± 15.6	<0.001
2-h glucose (mmol/L)	5.6 ± 1.2	8.4 ± 2.9***	5.2 ± 2.6	−36.7 ± 23.8	<0.001
HbA1c [(%), (mmol/mol)]	5.6 ± 0.3 (37.5 ± 3.4)	6.0 ± 0.7 (41.6 ± 7.3)	5.4 ± 0.4 (35.9 ± 4.6)	−8.3 ± 5.9	<0.001
FFA level (mmol/L)	0.55 ± 0.17	0.80 ± 0.22	0.76 ± 0.17	1.7 ± 34.0	0.535
Triglycerides (mmol/L)	0.7 ± 0.3	1.2 ± 0.4***	1.1 ± 0.5**	−6.7 ± 36.4	0.321
Total cholesterol (mmol/L)	4.42 ± 0.83	4.30 ± 0.88	4.30 ± 0.82	3.5 ± 29.3	0.990
HDL-cholesterol (mmol/L)	1.8 ± 0.4	1.2 ± 0.3***	1.4 ± 0.3***	18.9 ± 26.4	0.008
LDL-cholesterol (mmol/L)	2.4 ± 0.7	2.5 ± 0.8	2.4 ± 0.7	1.5 ± 48.5	0.457
CRP (mg/L)	0.8 ± 1.0	4.0 ± 3.5*	2.0 ± 1.9	−53.1 ± 42	<0.001

Continuous variables presented as mean ± SD; SAT, subcutaneous fat; VAT, visceral adipose tissue; 2-h glucose, glucose levels 2 h after a standardized (75 g) oral glucose tolerance test; HbA1c, glycated hemoglobin; FFA, free fatty acids; HDL, high and LDL, low-density lipoprotein cholesterol; CRP, C-reactive protein; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. controls; #$P < 0.05$, pre-vs post-surgery comparison.

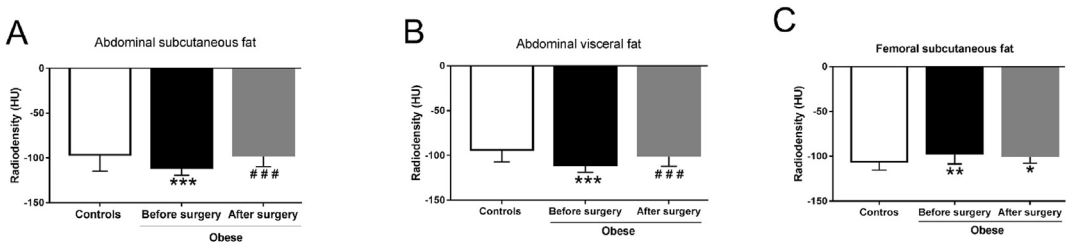

Figure 1 Adipose tissue radiodensity in abdominal subcutaneous [A] and visceral fat [B], and femoral subcutaneous fat [C] in non-obese healthy controls [plain bars], obese before [dark bars] and after surgery [gray bars]; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ obese vs controls; $^{\#\#\#}P < 0.001$ obese before vs after surgery comparison.

Table 2 Serum metabolomics profiling in the study subjects.

	Controls (n = 15)	Obese surgical patients (n = 23)		Change (%) from pre-surgery	#*P*-value within obese group
		Pre-surgery	Post-surgery		
ApoB/ApoA-1	0.46 ± 0.09	0.57 ± 0.13*	0.51 ± 0.13	−8.6 ± 17.7	0.020
Glycine (mmol/L)	0.26 ± 0.1	0.26 ± 0.05	0.29 ± 0.06	14.9 ± 22.1	0.006
Isoleucine (mmol/L)	0.04 ± 0.01	0.05 ± 0.01**	0.04 ± 0.01	−14.5 ± 21.1	0.001
Leucine (mmol/L)	0.06 ± 0.01	0.07 ± 0.01*	0.06 ± 0.01	−16.7 ± 14.9	<0.001
Valine (mmol/L)	0.15 ± 0.03	0.17 ± 0.03**	0.14 ± 0.03	−18.2 ± 15.1	<0.001
Phenylalanine (mmol/L)	0.06 ± 0.01	0.07 ± 0.01**	0.06 ± 0.01	−10.2 ± 13.3	0.001
Tyrosine (mmol/L)	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	−4.7 ± 25.9	0.132
GlycA (mmol/L)	1.13 ± 0.19	1.37 ± 0.14***	1.23 ± 0.15	−9.8 ± 10.5	<0.001

Continuous variables presented as mean ± SD; ApoB/ApoA1, ratio of apolipoprotein B to apolipoprotein A-I; GlycA, glycoprotein acetyls, mainly a1-acid glycoprotein; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. controls; #P < 0.05, pre-vs post-surgery comparison.

morbidly obese patients as compared to non-obese subjects. Secondly, bariatric surgery decreases abdominal adipose lipid content in line with the improved metabolic control and circulating metabolites in morbidly obese individuals. However, bariatric surgery had no effect on the CT-radiodensity in the femoral subcutaneous area.

At baseline prior to surgery, we demonstrated an association between adipose radiodensity and fat volume in our obese population. Specifically, we found that abdominal SAT was strongly correlated with depot mass, a finding that has been previously noted in literature [11,31]. We suggest that these observations may be attributed to the increased size of existing adipocytes [32]. An earlier study demonstrated that women with low abdominal adipose CT-derived radiodensity were characterized by increased adipose area as well as adipocyte hypertrophy [33]. Despite their higher femoral SAT mass, the radiodensity was higher in the obese individuals compared to controls. In the femoral SAT depot, the increasing lipid accumulation confers cardiometabolic protection due to the lower rate of lipolysis, and a greater sensitivity to insulin of adipocytes in this depot [34]. Also, femoral SAT has increased lipoprotein lipase activity which facilitates lipid deposition in adipocytes as well as stimulate the production of new adipocytes [35]. It has also been suggested that the long-term entrapment of circulating non-esterified fatty acids in the newly formed adipocytes prevents non-adipose tissue from excessive exposure to fatty acids [34]. Research shows that the major long chain fatty acid constituent of an expanded femoral SAT is the monounsaturated palmitoleic acid ($C_{16}H_{30}O_2$), which is known to promote beneficial blood lipid profile, insulin sensitivity, and glycemic control [36].

The post-surgery increase in abdominal SAT and VAT radiodensities and the parallel improvements in metabolic biomarkers are similar to findings from a previously reported study [11]. In this report, we further explore the association between the post-surgery change in adipose radiodensity with metabolomics-derived metabolic biomarkers. We found that the increase in abdominal SAT and VAT radiodensities was significantly correlated with decreases in systemic levels of leucine, ApoB/ApoA ratio, and GlycA. It has been suggested that elevated levels leucine [37], ApoB/ApoA-I ratio [38], and GlycA [39] are strongly correlated with insulin resistance and metabolic syndrome. Using ultrasound measurements, Pontiroli et al. [40] demonstrated that the loss of visceral fat area correlated with improvement in metabolic variables after bariatric surgery. In addition, a previous systematic review and meta-analysis has described a greater percentage loss of abdominal fat regardless of the weight loss intervention [41]. These findings provide further evidence that abdominal adipose tissue radiodensity may provide beneficial information about metabolic disease risk. It is noteworthy to mention that the combined effect of weight loss from dietary restriction and from bariatric surgery may have contributed to the observable change in the abdominal adipose tissue densities radiodensities. Viljanen et al. [42] showed that 6 weeks after

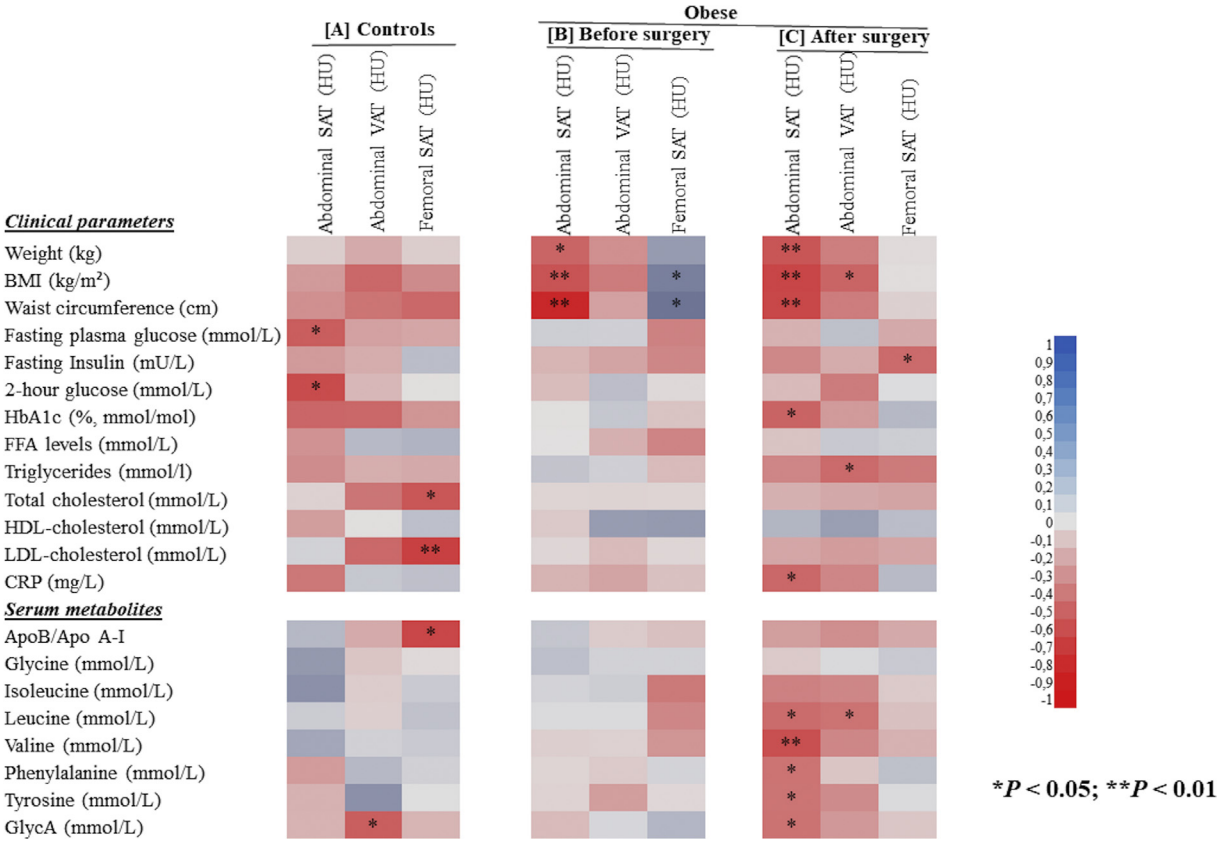

Figure 2 Pearson's correlation coefficient between tissue radiodensity and circulating metabolites in the non-obese healthy control [A], obese before [B], after [C]; SAT, subcutaneous adipose tissue, VAT, visceral adipose tissue; OGTT, glucose levels 2 h after a standardized oral glucose tolerance test; HbA1c, glycated hemoglobin; FFA, free fatty acids; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; CRP, C-reactive protein; ApoB/ApoA-I, ratio of apolipoprotein B to apolipoprotein A-I; GlycA, glycoprotein acetyls, mainly α1-acid glycoprotein;

VLCD, obese subjects recorded significant decrease in abdominal SAT and VAT fat volume along with the change in adipose tissue-specific metabolic profile.

Another important finding of this study was that the radiodensity of femoral SAT was unchanged and remained similar compared to the pre-surgery values despite significant decrease in femoral SAT depot mass. A previous study has shown that there are depot-specific differences in fat mass expansion in response to overfeeding - hypertrophy in abdominal SAT, and mainly hyperplasia in femoral SAT [43]. Moreover, it has been shown that lean individuals have lower prevalence of hyperplasia as compared to obese subjects [44], and that following weight loss a decrease in the adipocyte numbers was not found in obese individuals [45]. On the contrary, following weight loss there is marked decrease in the size of hypertrophic adipocytes [46]. Taken together, our findings may further highlight the differences in adipocyte behavior and regional fat distribution between the abdominal and femoral SAT fat depots following weight loss [47]. For the current study, there were no adipose biopsies to ascertain the possible associations between the post-bariatric surgery change in adipose CT radiodensity and the resident adipocyte morphology and expandability in our obese patients. Physiologically, femoral SAT is known to be less metabolically active in terms of blood flow dynamics and fatty acid metabolism as compared with the abdominal SAT depot [15]. Therefore, the turnover is more robust in the abdominal than in femoral fat depots [47]. Our data also revealed that the capacity to preserve lipid content in the femoral SAT may be a necessary requirement to achieve and maintain healthy metabolic homeostasis in post-surgery obese patients. Of significant note, our obese population were still losing weight 6 months after the surgical intervention as characterized by the lack of change in serum fatty acids levels between the two study visits.

A strength of the current method was that the CT-derived radiodensity measurement has been validated against *ex-vivo* adipose tissue samples for the assessment of tissue lipid content [48]. Our study has some limitations. Adipose lipid content and tissue properties were measured indirectly using computed tomography. We studied a relatively small group of obese women patients with different metabolic phenotypes (i.e. healthy, prediabetes, diabetes). Second, the current data does not include biopsies as well as dietary information and therefore could not be accounted for in the analysis of the associations between fat radiodensities and serum metabolites. Third, these results regarding femoral SAT radiodensity cannot be generalized to men because of the different estrogen/testosterone ratios, which are known regulators of adiposity between fat depots. Fourth, even though the presence of diabetes exerts an influence on certain metabolic parameters, our exploration of the current

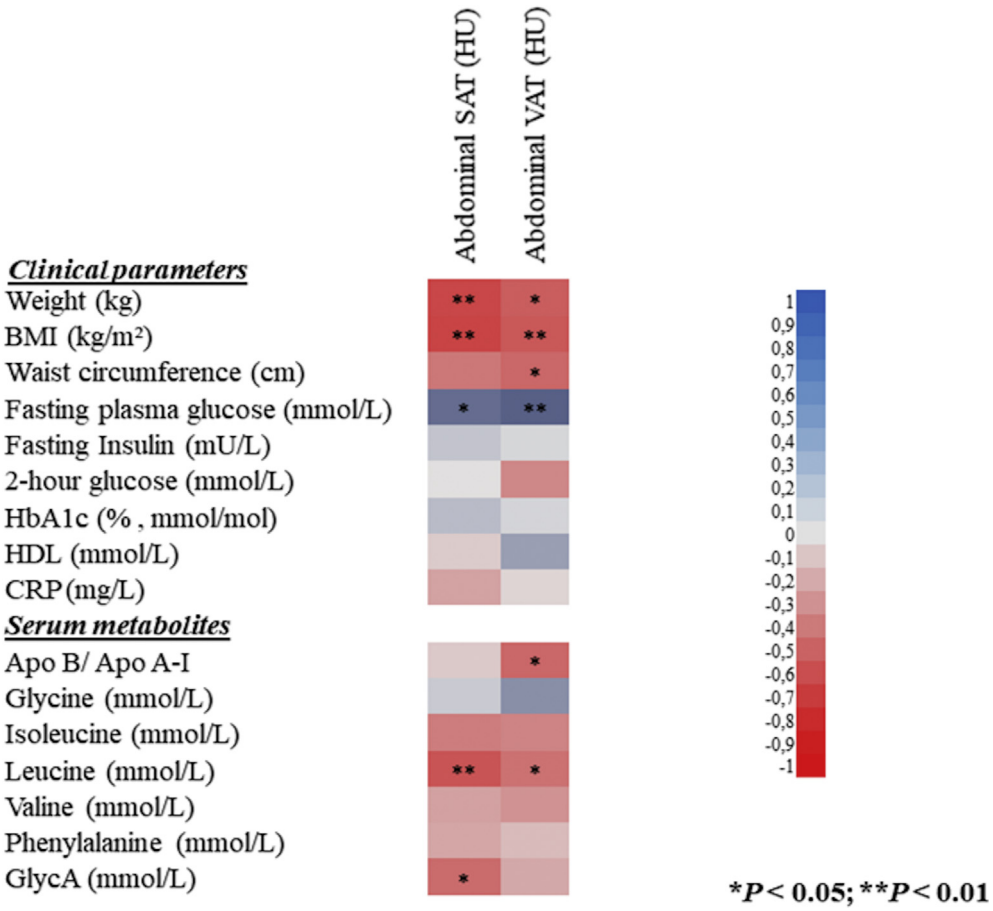

Figure 3 Pearson's correlation coefficient of the change [(post-pre) surgery] in CT-derived tissue radiodensities and serum metabolites in obese patients; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; OGTT, glucose levels 2 h after a standardized oral glucose tolerance test; HbA1c, glycated hemoglobin; HDL, high density cholesterol; CRP, C-reactive protein; ApoB/ApoA-I ratio, ratio of apolipoprotein B to apolipoprotein A-I; GlycA, Glycoprotein acetyls, mainly α1-acid glycoprotein;

data did not show significant differences with respect to baseline diabetes status and hence the obese patients were combined for the analyses. Fifth, the morbidly obese patients underwent two bariatric surgical procedures (sleeve and gastric bypass), which inputs some heterogeneity in the effect of the surgery as a concept. Sixth, the pre-surgery CT scans were conducted before VLCD and therefore we are unable to quantify the contribution of VLCD to the observed changes; however, weight loss due to VLCD was only 8%, compared to the 23% weight loss due to surgery, and the main goal of this study was to examine the relationships between changes in adipose tissue compared to circulating metabolites. Lastly, CT-radiodensity measurements also take into account tissue intracellular water and blood retention as well as the dead cells and other remnants of cellular components [49] and should, therefore, not be conceptualized as an unequivocal proxy to lipid content alone.

In conclusion, we showed that a higher femoral fat radiodensity may be linked to the metabolic disorders in morbid obesity. We further demonstrated that bariatric surgery-induced weight loss does not affect the radiodensity of femoral subcutaneous adipose tissue. However, the change in abdominal fat radiodensities may be linked to the improved systemic metabolism in the obese patients following bariatric surgery. Further studies involving larger sample size and a combination of tissue radiodensity data and tissue biopsies will be required to establish the direct mechanism linking the changes in adipose radiodensities and the improved metabolic health in obese patients after bariatric surgery.

Author contributions

P.D, E.R. performed the image analysis; P. D drafted the manuscript. E.R, H.H, L.E.J-O, K.K.K, P.I, J.T, P.S, J.P, J.C.H and P.N. were involved in the discussion of the results and critical reading of the manuscript. P.S, J.C.H and P.N. were involved in patient recruitment and study design. P. N. is the guarantor of this work and, as such, had full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Declaration of Competing Interest

No potential conflicts of interest related to the article were reported.

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