Tissue-specific insulin sensitivity in anorexia nervosa, a cross-sectional positron emission tomography study

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Abstract

Background and aim: Anorexia nervosa (AN) has been associated with both impaired and improved whole-body insulin sensitivity, but the associations with metabolism in different tissues has not been investigated before. Here, we aimed to study the association between AN and insulin sensitivity in different tissues.

Methods: A sample of 12 female volunteers with recently diagnosed AN and 13 healthy, lean female controls were studied cross-sectionally. We performed a whole-body positron emission tomography (PET) study with the glucose analogue radiotracer 2-deoxy-2[^{18}F]fluoro-D-glucose ([^{18}F]FDG) during hyperinsulinemic, euglycemic clamp to quantify tissue insulin-stimulated glucose uptake, and a whole-body DIXON magnetic resonance imaging (MRI) scans to measure tissue proton density fat fraction (PDFF%).

Results: Tissue-specific insulin sensitivity was higher in the duodenum of individuals with AN (3.0 [SD 0.4] *vs.* 2.6 [SD 0.4] μmol/100 g/min, but similar in other tissues. In five individuals with AN, glucose uptake rate was significantly higher compared to decreased skeletal muscle glucose utilization. In the control group growth hormone (GH) levels correlated with skeletal muscle, liver and brain insulin sensitivity, but this association was absent in individuals with AN.

Conclusions: While individuals with AN did not present altered whole-body insulin sensitivity, we found varying associations with tissue-specific glucose metabolism. These differences could not be directly predicted by the duration of AN or the severity of weight loss, so they may also depend on the individual genetic background and the behavioural pattern.

1. Introduction

Anorexia nervosa (AN) is a severe psychiatric disorder characterized by self-imposed starvation, excessive weight loss, and a distorted body image. The estimated lifetime prevalence of AN is 1.4 % in women and 0.2 % in men[1], and it has a high rate of mortality partly due to medical complications associated with the condition[2]. Recent studies have revealed that individuals with AN experience metabolic changes concerning insulin sensitivity and glucose metabolism in both acute and recovery phases of AN[3–6].

The relationship between insulin sensitivity and anorexia nervosa is multifaceted. In controlled trials using hyperinsulinemic, euglycemic clamp to assess whole-body insulin sensitivity[7], there has been no significant increase in insulin-stimulated glucose disposal in the acute phase of AN[8–11]. In a calorimetry study, glucose oxidation was reported to be upregulated and storing reduced[11], and this was interpreted to represent diminished skeletal muscle glycogen content in AN. Another study investigating skeletal muscle biopsies from individuals with AN showed, however, that glycogen is more likely accumulated in skeletal muscle due to its inefficient degradation[12]. During the recovery period insulin sensitivity has been reported to decrease, which is possibly driven by increased visceral adiposity[9,13]. Studies using HOMA-IR as a proxy for insulin sensitivity have reached contrasting conclusions, indicating improved insulin responsiveness in AN[14–16]. However, this may be attributed to the typically low fasting glucose and insulin levels observed in AN, which might not accurately reflect heightened responses to insulin.

The relationship between AN and insulin sensitivity likely involves a complex interplay between genetic, epigenetic, biological, psychological, and social factors. For instance, certain genetic factors that reduce the risk of developing obesity or type 2 diabetes are also linked to a higher genetic risk for AN[14,17]. The hormonal changes in AN, which reflect the body's adaptive responses to severe undernutrition, also result in altered metabolism. In addition to low insulin levels and relative hypercortisolism[18], individuals with AN typically present elevated GH levels[19], but they also develop resistance to GH action, resulting in decreased IGF-1 levels, which can contribute to insulin resistance. Other hormonal changes often reported in AN are reduced leptin levels, elevated ghrelin and peptide YY levels and hypothalamic-pituitary-gonadal axis dysfunction[3,4], but their effects on insulin sensitivity are less well characterized in the context of AN.

These previous findings suggest a redefinition of AN as a metabo-psychiatric disorder, and therefore elucidating both its metabolic and psychiatric components is a key to improving outcomes and developing novel treatment strategies. Additionally, insulin resistance not only complicates the recovery process but also poses risks for long-term health outcomes, including the development of metabolic syndrome[20,21]. However, it is currently not known to which extent AN associates with metabolic changes in different tissues. Therefore, the current study was aimed to explore the associations between AN and tissue-specific insulin sensitivity in several different organs by using positron emission tomography (PET) with the glucose analogue radiotracer 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) under hyperinsulinemic, euglycemic clamp conditions.

2. Materials and methods

2.1 General study outline

The study was a cross-sectional, observational study to evaluate the metabolic alterations in AN by comparing results from participants with AN and healthy controls. The measures were made on three separate visits: a screening visit, a [^{18}F]FDG-PET/CT scan visit, and a whole-body MRI scan visit, all performed within 28 days of each other. The study was conducted at the Turku PET Centre, Turku University Hospital, Turku, Finland from August 2020 to March 2023. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland and the study was conducted according to the principles of the Declaration of Helsinki. All study participants gave written informed consent prior to any study procedures.

2.2 Study participants

Participants with AN were recruited by psychiatrists from the Eating Disorder Unit at the Turku University Hospital, whereas the control participants were recruited via university and hospital internet page message boards.

All participants with AN were to meet the following criteria: 1) modified DSM-IV diagnosis of AN with or without amenorrhea; 2) AN onset before the age of 25 years and not more than two years prior to screening; 3) age 18–30 years; 4) BMI less than 17.5 kg/m^2 ; 5) no lifetime history of binge eating. Due to difficulties in the recruitment process, longer disease duration was, however, allowed. In the control group the participants were to be age-matched to the AN participants, and to have no reported history of eating disorders or obesity (BMI over 30 kg/m^2), first degree relatives with an eating disorder, or psychiatric disorders. All participants were female, had no use of tobacco or narcotics, had no significant previous exposure to radiation, and were required to use reliable contraception during the study.

2.3 Screening visit

On the screening visit, concomitant medications and medical history were recorded, and the participants underwent a physical examination. Fasting blood samples were collected, followed by a 75 g oral glucose tolerance test (OGTT). Body fat mass and fat free mass were quantified with air displacements plethysmograph (BOD POD, version 5.4.0, COSMED Inc., CA, USA) after a minimum of 4 hours of fasting.

2.4 PET study visit protocol

The study visit was performed after an overnight fast. First, two venous catheters were inserted in opposite forearms, one for the insulin and glucose infusions and for injecting [^{18}F]FDG, and the other for collecting venous blood samples, arterialised by placing a hot water bottle distally on the arm. After the collection of fasting plasma blood samples, hyperinsulinemic, euglycemic clamp was started[7]. Insulin (Actrapid, Novo Nordisk A/S, Bagsvaerd, Denmark) was administered with a reduced dose of 37 mU/m^2 body surface area/min, and a variable rate of 20% glucose was infused based on plasma glucose measurements performed every 5–10 min to maintain euglycemia (plasma glucose 5.0 mmol/L).

The participants were then transferred to a combined PET/CT scanner (Discovery 690 or Discovery MI; General Electric Medical Systems, Milwaukee, WI), and 75 minutes (SD 11) into the clamp, 150 MBq (SD 7) of [^{18}F]FDG was administered together with the start of dynamic scans of the thoracic region (32 min) and the brain (15 min), followed by static frames of the neck (10 min), abdomen (10 min) and the thighs (10 min). Next, the participants were transferred from the scanner, insulin and glucose infusions

were ceased, and the study participants had a meal and were monitored until the plasma glucose levels were adequate before being discharged.

2.5 PET data analysis

Before analysis, all PET data was corrected for dead time, decay, and photon attenuation. Input function was obtained by combining activity data from PET images from inside the left ventricle, and plasma samples collected at 8–11 time points during the scan and analysed with an automated gamma counter (Wizard 1480 3, Wallac, Turku, Finland). Tissue radioactivity was determined using Carimas software (Version 2.9, Turku PET Centre)[23], by manually applying 3D volumes of interest to the right lobe of the liver, quadriceps femoris muscles, left ventricular myocardium, waistline and mid-thigh subcutaneous adipose tissue, supraclavicular brown adipose tissue, humeral shaft bone marrow and in the descending part of duodenal wall. Tissue tracer uptake rate was then calculated with graphical analysis (K_i or fractional uptake rate [FUR]) by comparing tissue radioactivity accumulation rate to the integral of plasma radioactivity[24,25].

Tissue glucose uptake (GU, μmol/100 g/min) was then calculated by multiplying K_i or FUR with the mean plasma glucose level during the scan, and dividing it by tissue density and a constant accounting for the differences in the metabolic rates of [^{18}F]FDG and glucose (1.2 for skeletal muscle[26], 1.0 for liver[27], 1.14 for adipose tissue[28], 0.65 for the brain [29]).

Glucose uptake in the brain was obtained using SPM12-based toolbox Magia, with the data spatially normalised to MNI space using an in-house [^{18}F]FDG template and finally smoothed with a Gaussian kernel with 8-mm full-width at half-maximum.

EGP was assessed by subtracting the rate of glucose infused during the [^{18}F]FDG-PET/CT study from the whole-body glucose disappearance rate (R_d) derived from [^{18}F]FDG consumption[30].

2.6 Whole-body MRI

A whole-body MRI scan was performed at 3T using the MRI part of a clinical PET-MR system (Philips Ingenuity TF, Philips Healthcare, OH, USA) using a 2-point Dixon sequence (LAVA Flex 3D, flip angle 12°, echo time 1.7 ms, repetition time 4.4 ms, slice thickness 1.5 mm). Volumes of visceral and subcutaneous adipose tissues were defined manually from Th12-L1 to L5-S1 level using sliceOmatic (version 5.0, Tomovision, Canada). Tissue fat fractions (FF%) were analyzed from maps calculated using in-house software developed in MATLAB2015b (Mathworks Inc, Natick, MA) and SPM8 (Wellcome Trust, UCL) using the formula fat fraction = fat component / (fat component + water component). Using Carimas software (version 2.9, Turku PET Centre), 3D volumes were drawn similarly to PET data analyses, with the addition of visceral adipose tissue volumes of interest being drawn in lower retroperitoneal space.

2.7 Laboratory analyses

On the screening visit during OGTT, glucose and insulin were measured every 30 minutes for 2 hours. Cholesterols, thyroid stimulating hormone (TSH), free T4 and T3 were also measured at screening, whereas other laboratory samples were analyzed from samples collected during the PET visit. During clamp, plasma glucose was measured in duplicates using the glucose oxidase method. Plasma insulin

was determined every 30 minutes, and serum free fatty acids (FFA) and growth hormone (GH) every 60 minutes during the clamp. Plasma radioactivity was determined every 5–15 minutes using an automatic gamma counter, and the amount of [^{18}F]FDG lost into urine was measured from a urine sample given at the end of scan using an isotope dose calibrator.

2.8 Statistical analyses

The sample size was set to detect a significant difference between individuals with AN and controls in μ opioid receptor availability, reported separately, and no separate power calculations for metabolic endpoints were determined. Normality of the data was evaluated with Shapiro-Wilk test. Square root or logarithmic transformations were performed for non-normally distributed data. Differences between groups were compared using independent samples *t* test, except for the screening visit HDL cholesterol and TSH analysed with Mann-Whitney *U* test. Associations were studied with Pearson correlation. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 29.0, IBM Corp, Armonk, NY, USA).

3 Results

3.1 Participant characteristics

The characteristics of the two study groups are presented in Table 1. Individuals with AN were younger than healthy controls, and in addition to having lower fat and lean tissue mass also had higher insulin sensitivity in the OGTT-based measures. The duration of eating disorders in the AN group ranged from 1 to 11 years (mean 4.3 [SD 3.1] years), and 9/11 had amenorrhea.

3.2 Whole-body and tissue glucose metabolism in AN

During the steady state of clamp, mean glucose (5.0 [SD 0.3] mmol/L *vs.* 5.0 [SD 0.2] mmol/L, $P = .50$) and insulin levels (442.7 [SD 90.0] pmol/L *vs.* 417.4 [SD 58.1] pmol/L, $P = .42$) were similar in individuals with AN and healthy controls, respectively. After 60 minutes of clamp, serum FFA levels were slightly lower in individuals with AN (0.04 [SD 0.02] mmol/L *vs*. 0.06 [SD 0.03] mmol/L, $P = .04$), but the levels were more similar between the groups at fasting (Table 1), and 120 minutes into the clamp. Also, the change from fasting to 60 minutes of hyperinsulinemia was equal between the groups (-0.50 [SD 0.30] mmol/L in individuals with AN *vs.* -0.59 [SD 0.16] mmol/L in controls, $P = .38$).

Levels of fasting insulin, cortisol, TSH, free T4, free T3, IGF-1 or leptin did not correlate with peripheral measures of insulin sensitivity in the whole study group or separately in participants with AN (*P* values >.20).

Whole-body insulin-stimulated glucose disposal measured as *M* value was higher in participants with AN when using total body weight, but not fat free mass, as a reference. Rate of endogenous glucose production (EGP) during hyperinsulinemic euglycemia was not different between groups (Figure 1A, Supplemental Table 1). Tissue-specific glucose uptake was statistically significantly higher only in the duodenal wall (3.0 [SD 0.4] μmol/100 g/min in the participants with AN *vs*. 2.6 [SD 0.4] μmol/100 g/min in controls, $P = .013$). (Figure 1B, Supplemental Table 1).

There was a negative correlation with the duration of AN and glucose uptake in the duodenal wall (r = -0.64, P = .04), but not with other tissues (P values above .41).

There were no significant differences between individuals with AN and controls in brain glucose uptake rates during hyperinsulinemia in the whole brain (23.0 [SD 4.3] µmol/100 g/min *vs.* 24.6 [SD 2.5] µmol/100 g/min, respectively, P = .30), white matter (19.6 [SD 3.8] µmol/100 g/min *vs.* 20.8 [SD 2.1] µmol/100 g/min, respectively, P = .36), or gray matter (26.9 [SD 5.2] µmol/100 g/min *vs.* 28.6 [SD 3.2] µmol/100 g/min, respectively, P = .36), or in specific anatomical brain regions.

3.3 Varying tissue insulin-stimulated glucose uptake in skeletal muscle and adipose tissue

Although glucose uptake to subcutaneous adipose tissue (AT) was numerically slightly higher in participants with AN, uptake rate in the total subcutaneous AT depot was higher in the control participants (44.2 [SD 15.3] µmol/min *vs*. 29.2 [SD 16.6] in participants with AN, P=.05). Based on *post hoc* analyses performed after visually evaluating the PET data, five participants with AN, glucose uptake rate was higher in subcutaneous AT (5.0 [SD 1.0] µmol/100 g/min) than in skeletal muscle (1.8 [SD 0.6] µmol/100 g/min) (Figure 2). This was mostly driven by increased subcutaneous AT glucose uptake, while the difference in skeletal muscle GU was only lower compared to other participants with AN (4.0 [SD 1.9] µmol/100 g/min, P = .04) and not with healthy controls (3.0 [SD 1.7] µmol/100 g/min, P = .17). Glucose uptake to the total waistline subcutaneous adipose tissue depot was also numerically higher in the five participants (46.2 [SD 8.4] µmol/min *vs.* 34.7 [SD 23.1] µmol/min in other participants with AN and 35.2 [SD 17.0] µmol/min in controls), but the differences were not statistically significant (P = .29 and P = .24, respectively). In the whole study group, mean insulin levels during the clamp correlated negatively with subcutaneous adipose tissue GU (r = -0.76, P = .001).

3.4 The relationship with GH and tissue glucose metabolism

There was no difference in fasting GH levels between groups, but 60 minutes into hyperinsulinemic, euglycemic clamp GH remained at a higher level in individuals with AN (Table 1). One healthy control had exceptionally high GH levels both in fasting conditions (9.5 µg/L) and during hyperinsulinemia (4.9 µg/L), but was included in the analyses.

In the normal-weight control group, higher GH during hyperinsulinemic euglycemia was associated with impaired insulin sensitivity. GH correlated negatively with M value (r = -0.87, P <.001) and skeletal muscle glucose uptake (r = -.76, P = .006), and positively with EGP (r = 0.64, P = .02) and brain glucose uptake (r = 0.79, P = .004). These association were absent in individuals with AN (P values ≥ .37) (Figure 3). In the whole group, fasting GH levels correlated negatively with free fatty acid levels (r = -0.48, P = .02), whereas during hyperinsulinemic clamp the correlation was absent (r = 0.01, P = .96) also when looking at the groups separately (P values >.60).

3.5 Different profiles in adipose tissue fat fractions

Participants with AN had significantly lower volumes of abdominal and femoral subcutaneous adipose tissue, visceral adipose tissue and femoral skeletal muscle (Table 2). Tissue proton density fat fractions were similar in the liver, skeletal muscle and visceral adipose tissue. While adipose tissue fat fraction

was lower in the subcutaneous abdominal and femoral depots in individuals with AN, it was higher in the femoral bone marrow (Table 2).

4 Discussion

This is the first study to investigate the association between AN and glucose metabolism in several different tissues within the same individuals. The results on the whole-body level are in line with several previous reports, but this study also improves our understanding on the altered glucose homeostasis and organ crosstalk in AN, with special emphasis on adipose tissue, skeletal muscle and the brain.

Previous studies using hyperinsulinemic, euglycemic clamp have shown that AN is not associated with improved insulin sensitivity[8–11]. As skeletal muscle is the main site of glucose uptake during hyperinsulinemia, uptake measured with [^{18}F]FDG-PET was expected to show no difference between individuals with AN and normal-weight controls.

In five individuals with AN, insulin-stimulated glucose uptake into skeletal muscle was impaired compared to subcutaneous adipose tissue, which is in contrast to the rest of the study population and a larger dataset of 326 individuals studied earlier in Turku PET Centre[31]. This could not be directly explained by differences in levels of circulating substrates, but there was an association between higher adipose tissue GU and lower plasma insulin levels during the clamp, which can be interpreted as higher insulin clearance. This is in line with previous data, as prolonged fasting has been reported to induce insulin resistance in skeletal muscle[32,33], and to potentiate insulin binding to adipocytes *in vitro*[34]. Also, in a rat model repeated cycles of starvation and refeeding result in significant upregulation of white adipose tissue lipogenesis, which would require higher glucose supply to the tissue. Therefore, it is possible that the individuals with higher adipose tissue glucose uptake had had a longer period of low-calorie intake than others, have a history of repeating starvation-refeeding periods, or they might *e.g.* express starvation myopathy or a different profile on myocyte fibre types[12]. Also, both skeletal muscle insulin resistance and increased subcutaneous AT glucose uptake can be caused or incremented by hypercortisolism[34,35] and elevated GH levels[36], which are common findings in AN. While we did not observe differences in fasting cortisol levels between individuals with AN and controls, this can be attributed to the use of oral contraceptives in the control group.

For other AT depots, the increase in bone marrow fat content has been reported by several groups previously and suggested to be associated with impaired bone health in AN[37]. Also, in line with previous studies, we observed a more significant reduction in subcutaneous AT volumes but a lesser difference in the volume of visceral AT and no difference in visceral AT fat fraction[38]. Young females with AN have previously been shown to express less cold-activated brown AT than healthy controls[39], and this is not in disagreement with our studies as cold-activation remains the only method to reliably detect brown AT using PET imaging.

Changes in intestinal metabolism in anorexia have not been reported earlier, but gastrointestinal complications, such as delayed gastric emptying, delayed small bowel transit time, or constipation, are common in individuals with AN[2]. In animal models, extended fasting has been associated with increased glucose transporter expression on the luminal surface of enterocytes[40,41], but the uptake of D-glucose from circulation has gained even less attention. As a result, we can only speculate that chronic undernourishment in AN results in upregulated nutrient uptake from the circulation, and note that due to

the small sample size the current finding must be considered exploratory. Lower duodenal glucose uptake in the individuals with longer disease duration might be explained by a reduction in mucosal surface area as seen in chronic malnutrition, but this cannot be confirmed from the data collected in the current study.

Brain glucose metabolism in AN has previously been studied only in fasting conditions. Delvenne et al. reported globally lowered cerebral glucose uptake in the acute phase, and this change was reversed after weight restoration[42]. Other studies using predetermined rate constants or measuring relative changes in the brain have reported regional hyper- or hypometabolism, but due to methodological differences, the results from these studies are difficult to compare[43,44]. As we saw no significant differences between groups, the increased availability of free fatty acids or ketone bodies may compete with glucose uptake in the fasted state, and as lipolysis and ketone production are suppressed during hyperinsulinemia, these differences in glucose uptake also diminish. However, we also noted hypometabolism in some of the individuals with AN, and second, as the participants in the control group were slightly older and aging has been shown to reduce brain glucose uptake under the hyperinsulinemic, euglycemic condition[45], it is possible that the current small observational study lacked the power to detect a difference.

Similarly to previous studies using oral or intravenous glucose tolerance tests to study hormonal responses in AN[46,47], in our study the growth hormone level remained elevated in individuals with AN after 60 minutes of hyperinsulinemic euglycemia. The lost association between GH levels and skeletal muscle insulin-stimulated glucose uptake, endogenous glucose production during hyperinsulinemia, and as a result, whole-body insulin sensitivity in individuals with AN can be interpreted to associate with the previously established GH resistance and the disrupted pulsatile secretion of GH in individuals with AN[3,48]. Considering the association between brain glucose uptake and GH, we have previously shown in a larger dataset that obesity and insulin resistance are associated with increased brain glucose uptake during hyperinsulinemia[45]. The higher GH levels and increased brain glucose uptake observed during hyperinsulinemia might both result from peripheral or hepatic insulin resistance. However, it is also possible that an increased cerebral demand for glucose stimulates GH secretion, which in turn induces peripheral insulin resistance to ensure sufficient glucose availability for the brain [36] – and that this regulatory system is disrupted in AN.

While this study offers exciting insights into metabolic alterations in AN, some of the results are limited by the small sample size. This also prevents us from being able to differentiate between educational level, or varying behavioural patterns in AN, with restricted feeding or excessive physical exercise possibly explaining some of the variations within the AN group. Due to the cross-sectional approach, we cannot determine a causal relationship between the hormonal changes and tissue glucose metabolism. Also, for the interpretation of the GH results, collecting samples for ghrelin measurement could have been of interest.

To conclude, AN is not associated with improved insulin sensitivity, but the results provide foundation for further investigation into the interplay between glucose metabolism, hormonal regulation, and organ crosstalk. It is likely that different genetic, epigenetic, behavioural and hormonal factors can cause a varying metabolic phenotype, with some individuals with AN showing heightened and others impaired insulin sensitivity in skeletal muscle and subcutaneous adipose tissue. Of all the hormonal and metabolic factors studied, the level of growth hormone during steady hyperinsulinemic euglycemia – and its association with glucose metabolism in skeletal muscle, the liver, and the brain – was the most distinctive

difference between individuals with AN and healthy controls.These insights may ultimately inform more targeted approaches to managing the metabolic consequences of AN.

Author contributions

Conceptualization: E.R., S.L., H.K.K., M.K., L.N., P.N.
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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

The data is available per reasonable request from the corresponding author.

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Tables

Table 1. Participant characteristics

	Individuals with AN ($N = 12$)	Controls ($N = 13$)	*P* value
Age	20.6 ± 1.7	23.9 ± 3.2	.005
BMI	17.0 ± 0.7	22.8 ± 1.9	<.001
Weight	48.3 ± 3.8	62.6 ± 5.0	<.001
Fat percentage (%)	19.6 ± 7.5	33.6 ± 5.4	<.001
Fat mass (kg)	9.6 ± 4.3	21.1 ± 4.6	<.001
Lean mass (kg)	38.6 ± 2.6	41.5 ± 3.5	.03
WHR	0.75 ± 0.05	0.74 ± 0.05	.73
Fasting plasma glucose (mmol/L)	4.7 ± 0.3	5.0 ± 0.4	.03
Fasting plasma insulin (pmoL/L)	26.4 ± 10.4	54.2 ± 26.0	.002
Fasting free fatty acids (mmol/L)	0.54 ± 0.31	0.65 ± 0.18	.11
Fasting β-hydroxybutyrate (mmol/L)	0.18 ± 0.13	0.16 ± 0.08	.61
HOMA-IR	0.8 ± 0.3	1.7 ± 1.0	.003
Matsuda-ISI	9.6 ± 3.6	5.5 ± 1.9	.001
Total cholesterol (mmol/L)	3.8 ± 0.7	4.3 ± 0.7	.13
LDL cholesterol (mmol/L)	2.1 ± 0.7	2.5 ± 0.4	.17
HDL cholesterol (mmol/L)	1.8 ± 0.7	1.7 ± 0.3	.89
Triglycerides (mmol/L)	0.6 ± 0.3	0.9 ± 0.5	.07
TSH (mIU/L)	1.9 ± 0.6	2.0 ± 1.8	.32
Free T4 (pmol/L)	13.4 ± 1.9	14.6 ± 1.5	.08
Free T3 (pmol/L)	3.7 ± 0.6	5.0 ± 0.9	<.001
IGF-1 (nmol/L)	28.0 ± 8.2	28.4 ± 8.8	.91
Fasting GH (µg/L)	2.7 ± 1.6	2.4 ± 2.9	.11
GH during HEC (µg/L)	2.3 ± 1.9	1.0 ± 1.4	.02
Cortisol (nmol/L)	261 ± 76	356 ± 250	.21
Leptin (µg/L)	2.3 ± 2.0	13.7 ± 7.0	<.001

WHR Waist-hip-ratio, OGTT oral glucose tolerance test, TSH thyroid stimulating hormone, IGF-1 insulin-like growth factor 1, GH growth hormone, HEC hyperinsulinemic, euglycemic clamp. The data are means and SDs, and the *P* values are from *t* test comparisons between groups.

Table 2. Tissue volumes and fat fractions.

	Individuals with AN	Controls	*P* value
Tissue volumes (ml)			
Abdominal subcutaneous AT	776 ± 289	2321 ± 787	<.001
Femoral subcutaneous AT	2023 ± 555	4560 ± 1243	<.001

Visceral AT	110 ± 60	404 ± 297	<.001
Thigh skeletal muscle	3426 ± 580	4185 ± 660	.003
Proton-density fat fractions (%)			
Abdominal subcutaneous AT	80.5 ± 8.9	90.4 ± 3.8	.01
Femoral subcutaneous AT	83.8 ± 8.9	90.3 ± 3.4	.03
Visceral AT	84.7 ± 6.5	87.7 ± 4.1	.18
Femoral bone marrow AT	94.0 ± 2.5	91.0 ± 3.1	.02
Brown AT	79.8 ± 6.8	76.9 ± 8.9	.39
Thigh skeletal muscle	4.8 ± 2.5	4.3 ± 2.2	.65
Liver	3.8 ± 1.4	3.7 ± 1.2	.87

AT adipose tissue. The data are means and SDs, and the *P* values are from *t* test comparisons between groups.

Figures

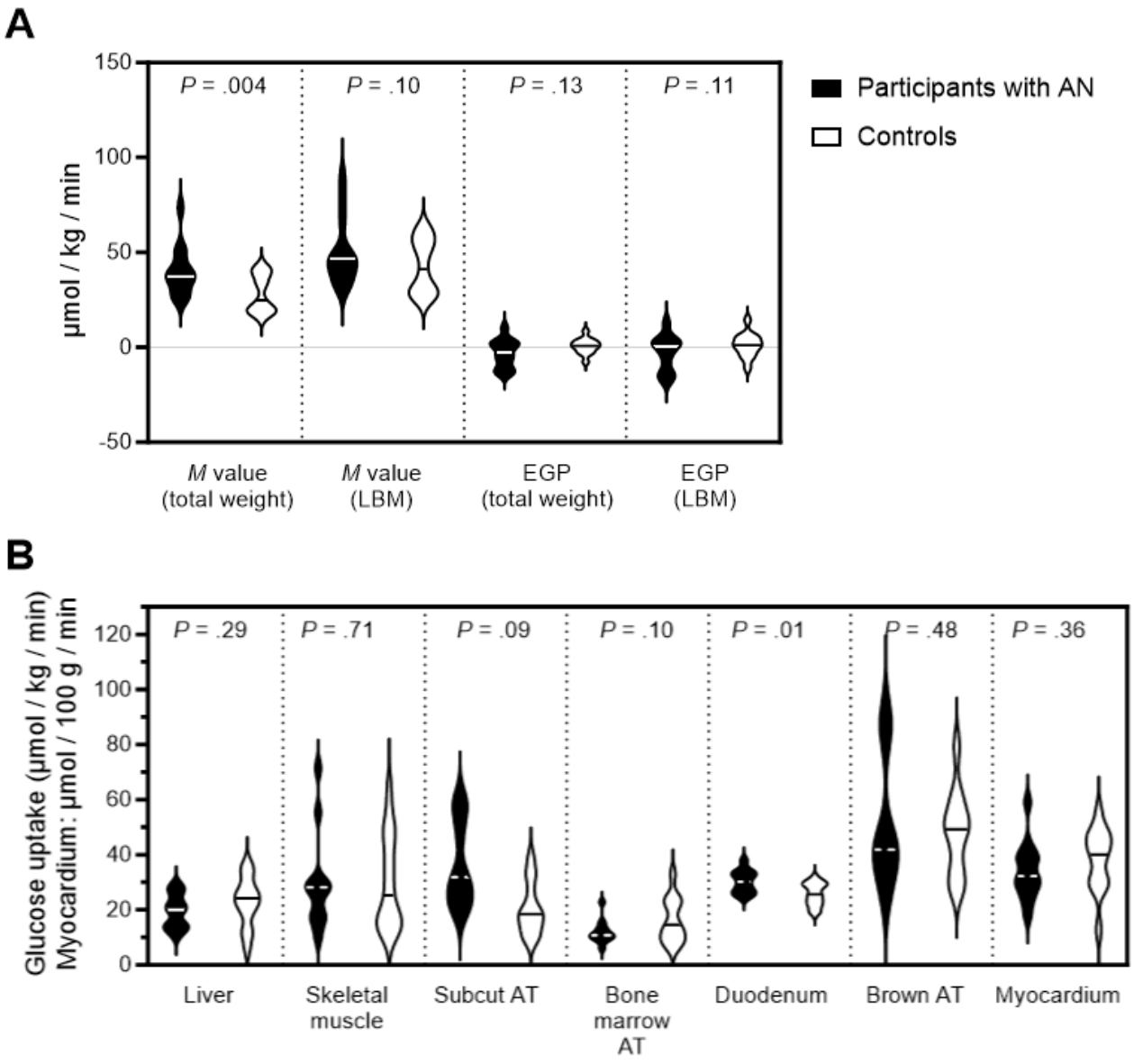

Figure 1. **A.** *M* value was higher in the participants in AN when using total body weight, but not lean body mass (LBM) as reference. Endogenous glucose production (EGP) during hyperinsulinemia did not differ between groups. **B.** Insulin-stimulated glucose uptake was statistically higher only in the duodenal wall in participants with AN. AT, adipose tissue.

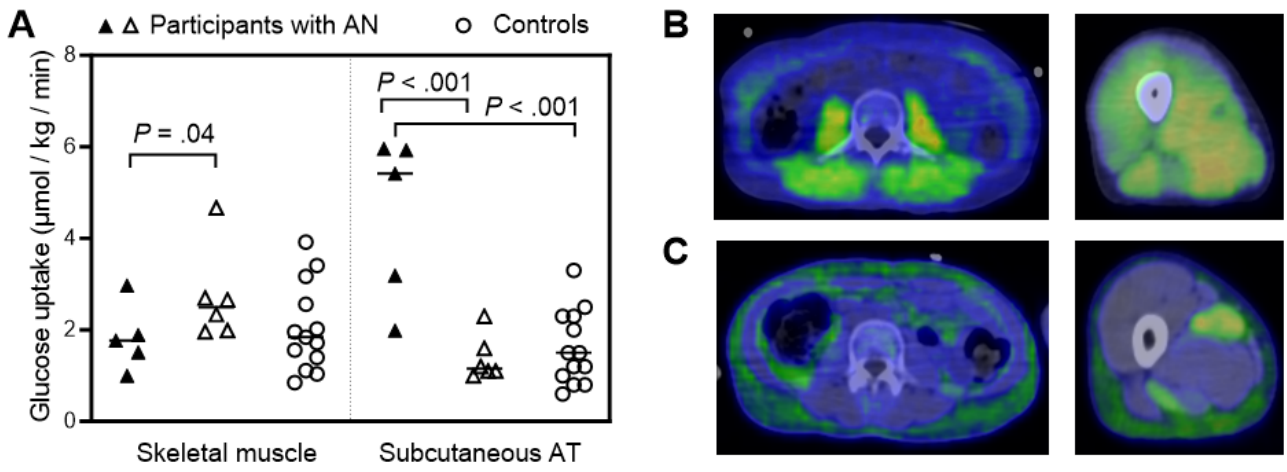

Figure 2. A. In five participants with AN, the rate of glucose uptake was increased in subcutaneous adipose tissue in comparison to skeletal muscle (black triangles), mostly driven by increased adipose tissue glucose uptake, as skeletal muscle glucose uptake was only lower compared to other participants with AN (white triangles) but not healthy controls (P =.17, circles). B. and C. [^{18}F]FDG-PET/CT images from a waistline transverse section (left) and from right mid-thigh (right), with B showing a typical pattern of insulin-stimulated glucose uptake and C increased glucose uptake to adipose tissue compared to skeletal muscle.

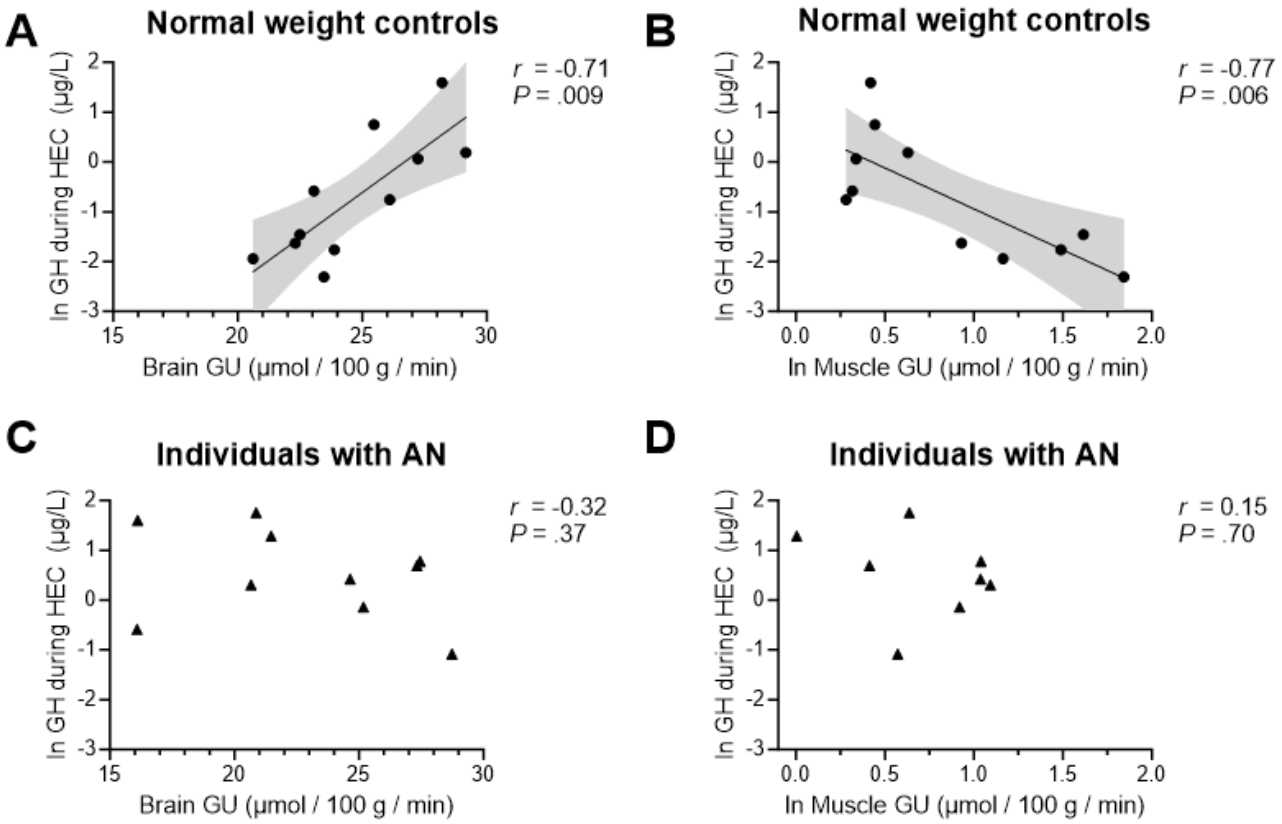

Figure 3. During hyperinsulinemic euglycaemia, higher growth hormone (GH) levels correlated typical characteristics of insulin resistance, such as elevated brain glucose metabolism and lower skeletal muscle glucose uptake, in the normal weight controls (A and B), but not in individuals with AN (C and D). GU, glucose uptake. GH and skeletal muscle GU values are ln transformed to reach normal distribution.

SUPPLEMENTAL MATERIAL

Supplemental table 1. Whole-body glucose uptake rates, endogenous glucose production rate and insulin-stimulated glucose uptake rates in peripheral tissues

	Participants with AN (*N* = 12)	Controls (*N* = 13)	*P* value
M value (μmol/kg/min)	40.2 ± 13.4	28.4 ± 10.0	.004
M value per lean mass (μmol/kg_{FFM}/min)	50.5 ± 18.1	42.7 ± 14.4	.10
EGP (μmol/kg/min)	-3.3 ± 7.6	0.6 ± 4.1	.12
EGP per lean mass (μmol/kg_{FFM}/min)	-4.4 ± 9.9	1.1 ± 6.4	.11
Tissue glucose uptake rates (μmol/100 g/min)			
Myocardium	34.1 ± 10.7	38.2 ± 11.5	.36
Liver	2.0 ± 0.6	2.3 ± 0.9	.29
Skeletal muscle	3.0 ± 1.8	3.0 ± 1.7	.71
Duodenum	3.0 ± 0.4	2.6 ± 0.4	.013
Waistline subcutaneous AT	2.7 ± 2.3	1.4 ± 1.0	.09
Bone marrow AT	1.2 ± 0.4	1.8 ± 0.8	.10
Brown AT	5.1 ± 2.4	4.8 ± 1.6	.48

EGP endogenous glucose production, R_d rate of disappearance, AT adipose tissue. The data are means and SDs, and the *P* values are from *t* test comparisons between groups.