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Clinical Research Article

NEFA Dynamics in Adults With Severe Obesity and Insulin Resistance: No Coupling to the rs9939609 *FTO* Risk Allele

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Abstract

Context: The *FTO* gene is highly expressed in adipose tissues; however, whether nonesterified fatty acids (NEFA) dynamics are impacted by *FTO* has not been rigorously tested for in a uniformly obese study population comprising both sexes.

Objective: To test for associations of the rs9939609 *FTO* risk allele with NEFA suppression.

Methods: We investigated 97 subjects with severe obesity but without diabetes, having genotype TT (n = 32), AT (n = 31), or AA (n = 34) in a cross-sectional observation study. NEFA suppression was assessed from a low-dose hyperinsulinemic euglycemic clamp with glucose-tracer as well as from the response to a standardized meal. Insulin sensitivity was assessed by hepatic and total insulin sensitivity measurements in the clamp and by the Matsuda index during the meal. Variables of possible importance for NEFA dynamics were primarily assessed by linear regression.

Results: No genotype associations with fasting or suppressed NEFA were found, whether in the clamp or meal situation ($P > .7$ for all comparisons). Independent of genotype, higher fasting concentrations of NEFA and larger NEFA suppression were found in female compared with male subjects. Fasting NEFA or degree of suppression were not associated with total fat mass or body mass index. The respiratory quotient was negatively associated with NEFA suppression.

Conclusion: In a gender-mixed adult population of obese individuals, an *FTO* obesity-risk allele did not affect fasting NEFA nor suppression thereof. These negative results on NEFA dynamics appear strengthened by the documentation of gender influence and associations with parameters reflective of insulin resistance.

Key Words: NEFA suppression, *FTO* rs9939609, insulin sensitivity, RQ, euglycemic clamp, meal test

Abbreviations: BMI, body mass index; BMR, basal metabolic rate; DXA, dual-energy x-ray absorptiometry; FFM, fat-free mass; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCR, metabolic clearance; NEFA, nonesterified fatty acid; Ra, rate of appearance; Rd, rate of disappearance; RQ, respiratory quotient.

Certain alleles of the *FTO* locus are linked to risk of obesity [1-3]. The impact of these risk alleles on different aspects of metabolism has been much studied; however, a complete understanding of the molecular events that underlie the *FTO*-linked risk for obesity is still lacking. The fact that the *FTO* gene is highly expressed in adipose tissue [4] makes it plausible that aspects of lipid metabolism such as nonesterified fatty acid (NEFA) dynamics could be regulated by FTO.

Obesity per se affects intermediary metabolism [5, 6] with a well-recognized coupling to insulin resistance, which in turn is a major risk factor for type 2 diabetes. The molecular mechanisms behind obesity-induced insulin resistance have not been fully elucidated. An important factor is that adipose tissue buffering of lipid fluxes is impaired in obesity through defects in the ability of adipose tissue to respond rapidly to the dynamic situation that occurs after meals [7]. It would therefore seem advantageous for any study on NEFA dynamics to focus on a study population that is homogeneous in terms of being either not obese or uniformly obese.

We have previously examined a possible association of a *FTO* risk allele with different metabolic parameters in an obese study population [8] and reported a genotype effect on the respiratory quotient (RQ) [9] by the *FTO* rs9939609 risk allele. Here we have proceeded to assess NEFA dynamics by fasting levels of NEFA and insulin suppression thereof in our study population. In order to gain as much as possible a complete understanding of any putative influence, we assessed NEFA dynamics in 2 different ways, that is, by euglycemic clamp and postprandially after a standard meal. Our results on suppression were put in context with concomitant measures of insulin sensitivity in terms of glucose metabolism, energy metabolism, selected lipid parameters, and body composition. Further, we took into consideration any sex-dependent modifications of the results.

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Methods

Participants and Study Design

The study population has been described [8]. Briefly, in this cross-sectional observation study we included adults aged 20 years or older with a body mass index (BMI) ≥35 kg/m^2 without a diagnosis of diabetes. Individuals that were recruited had newly been referred to the hospital's outpatient obesity clinic. We aimed for 100 participants in the study with an equal number of participants that carried 2, 1, or no copies of the *FTO* risk allele rs9939609. Enrollment and genotype allocations are shown in Fig. 1. All volunteers gave their signed informed consent to participate. The study was conducted according to the guidelines of the Declaration of Helsinki. The regional ethics committee approved the study (registration number 2013/642).

Nonesterified Fatty Acids

Clamp study

Details of the clamp protocol are reported elsewhere [8]. Briefly, NEFA suppression by insulin was tested in a hyperinsulinemic euglycemic clamp situation. Blood sampling took place after overnight fasting (0 minutes) and at minutes 100, 110, 120, 150, 220, 230 and 240 minutes following the start of a continuous administration of insulin (0.3- mU·kg^{-1}·min^{-1}) from minute 120.

Standardized meal

The meal consisted of 600 kcal (containing 72 g CHO); details of the meal test protocol are reported elsewhere [8, 10]. Briefly, the meal contained whole grain bread, butter, cheese, jam, orange juice, and either milk or a sweetened yogurt drink. Blood samples were collected into EDTA vials in the overnight fasted state and then every 30 minutes for 2.5 hours. Samples were immediately centrifuged (2110 RCF, 10 minutes, 18 °C) and plasma stored at −80 °C pending analyses.

NEFA measurements and calculations

NEFA were measured by an enzymatic colorimetric method (WAKO kit [NEFA-HR (2)], FUJIFILM Wako Chemicals Europe GmbH, Neuss, Germany). Blank, standard, and control samples were assayed in triplicate. We assayed fasting samples in duplicate in the clamp and meal tests, and in the 150-minute samples of the meal test. Samples at other time points were assayed in single wells. Fasting NEFA was measured at 2 occasions 1 week apart, on each of the meal and clamp test days, in that order. The average of these measurements was used for calculations. The average of the time points 100, 110, and 120 minutes was selected as the basal NEFA concentration (mmol/L) in the clamp. The average of time points 220, 230, and 240 minutes was selected as the NEFA concentration that was suppressed by insulin. We calculated insulin-induced NEFA suppression during the clamp as the difference between basal and insulin-suppressed NEFA concentrations. For meal-induced NEFA suppression we chose the decrease from minute 0 to minute 120 as the main variable. In addition, NEFA suppression was analyzed as percentage decrease during the clamp and the meal test.

Mean and (SD) concentration of the standard and quality control were within the range recommended by the manufacturer. The intra- and inter-assay coefficient of variation for the standard in the clamp test NEFA analyses were both 3.2%, and in the meal test analyses were both 7.6%. Intra- and inter-assay coefficient of variation for the quality control in the clamp NEFA analyses were 4.8% and 4.9% respectively, and in the meal analyses 6.0% and 6.2%, respectively.

Parameters of Insulin Sensitivity

Insulin sensitivity was assessed in the clamp by (i) μmol·kg$_{FFM}^{-1\cdot min-1}$glucose Ra (rate of appearance); (ii) μmol·kg$_{FFM}^{-1\cdot min-1}$glucose Rd (rate of disappearance); and (iii) mL·kg$_{FFM\cdot min-1}$ glucose MCR (metabolic clearance). In addition, we calculated hepatic and total insulin sensitivity as % insulin-induced changes of Ra, Rd, and MCR. Details about measurements and adjustments made to fat-free mass (FFM) are given elsewhere [8]. Insulin sensitivity was assessed in the meal test in 96 of 97 participants by the Matsuda index which is derived from glucose and insulin values [8].

Other Measurements

Height, weight, and body composition were measured manually and also by Hologic dual-energy x-ray absorptiometry (DXA) Systems following procedures reported elsewhere [8]. Basal metabolic rate (BMR) (kcal) and respiratory quotient (RQ) were measured in the fasting and resting mode by indirect calorimetry [9].

Triglycerides, total, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol, C-peptide, high-sensitivity C-reactive protein, and glycated hemoglobin (HbA1c) were measured in the overnight fasting state in connection with a previous visit at the outpatient clinic. The median (25th-75th percentiles) time from sampling of the mentioned parameters to performance of the study tests was 160 (89-196) days. For one-fifth of participants (n = 22, being representative of the total study sample, results not shown) measurements were available in medical records from 0 to 5 years earlier (median 2 years). Concentrations of LDL-cholesterol used in the current study were median (25th, 75th percentile) 3.20 (2.73, 3.73) compared with 3.19 (2.59, 3.82) mmol/L measured earlier, $P = .590$ for difference. The respective data for HDL-cholesterol were 1.24 (1.00, 1.45) compared with 1.21 (1.01, 1.35) mmol/L, $P = .795$ for difference.

Statistics

We performed all statistics in IBM SPSS Statistics for Windows, Version 29.0. Armonk, NY: IBM Corp, released 2022. Summary statistics are presented with medians and 25th and 75th percentile values due to the presence of outliers and skewed data. We used the Kruskal-Wallis method for testing the null hypothesis that the distribution of the variables was the same across the 3 different genotypes, and Mann-Whitney for testing that the distribution of the variables were the same across the 2 categories of sex. A *P* value (two-sided) below .05 was considered significant.

Linear regression analysis was performed using the method ENTER, listwise. Dependent (outcome) variables were levels of NEFA fasting and NEFA suppression. For both NEFA fasting and NEFA suppression, the independent (predictor) variables were variables of anthropometry, body composition, fasting RQ and BMR, HbA1c, insulin C-peptide,

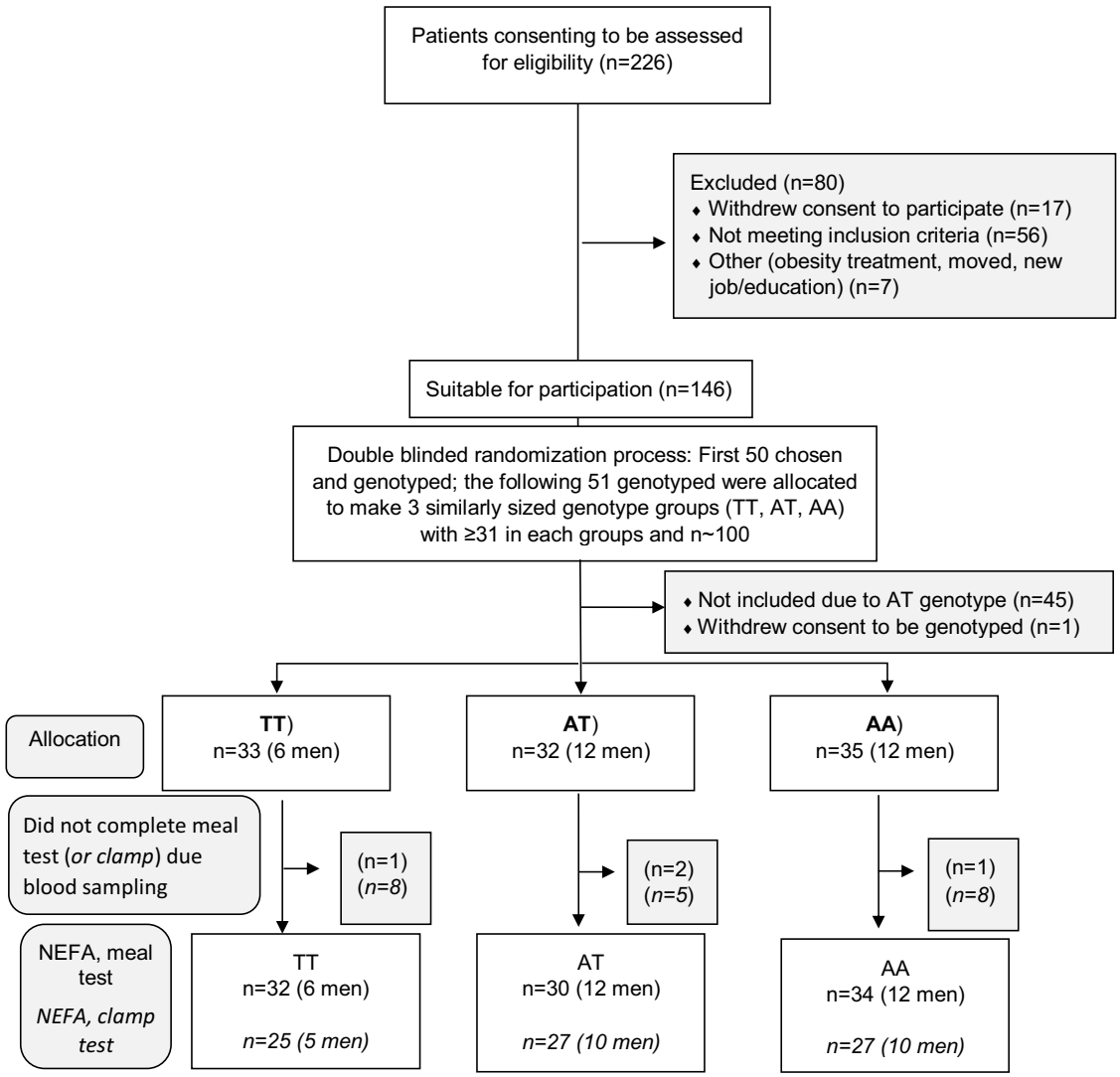

Figure 1. Flow diagram, participant selection, and group allocation. Figure 1 is adapted from Fig. 1 by AKH de Soysa et al (The fat mass and obesity-associated (FTO) gene allele rs9939609 and glucose tolerance, hepatic and total insulin sensitivity, in adults with obesity), in PLoS ONE, 2021 Fig. 1 [8], used under the terms of CC BY 4.0. The adapted Figure 1 is licensed under CC BY 4.0 (IL Mostad and V Grill).

high-sensitivity C-reactive protein, triglycerides, LDL- and HDL-cholesterol. For NEFA suppression during the clamp, in addition the independent variables fasting NEFA, insulin and glucose, and the hepatic and total insulin sensitivity variables of the clamp, were included. For NEFA suppression during the meal, in addition the independent variables fasting NEFA, insulin and glucose, and the Matsuda index were included. The variables eventually included in the respective regression analysis were those being in linear relationship (but without collinearity, results not shown) with the outcome variables. The eventually included independent variables are presented with unstandardized coefficient B with 95% CI, standardized coefficient BETA, *P* value for the significance, analysis of variance (ANOVA), and unadjusted R square. For NEFA fasting and NEFA suppression meal we included n = 92 and for NEFA suppression clamp n = 75 in the analyses. (The listwise method ENTER required data on both the dependent and all independent variables; this requirement was not met in all 97 participants). Finally, we adjusted the regression analyses with the possible confounders of age and sex in separate analyses.

Results

Participants

Of 97 participants, we obtained complete data for 79 from the clamp and for 96 in the meal test. We found no genotype differences except for higher RQ in genotype AT than TT, Table 1. For sex differences, see Supplementary Table S1 [13]. A total of 79% of female participants were premenopausal as defined by the mean menopausal age of 53 years for Norway [14]. There was no difference in the distribution of these women between the genotype groups (data not shown). No significant differences were found when comparing the 79 participants who performed the clamp (54 women and 25 men) with the 18 participants who did not perform the clamp (13 women and 5 men). Medications at the time of the study are given in Supplementary Table S2 [13].

NEFA Results During the Clamp

Genotype

Fasting NEFA concentrations did not differ between genotypes, nor did the insulin-induced NEFA suppression (Table 2,

Table 1. Characteristics of study population

Females/males, n=	All *n* = 97 67/30		TT *n* = 32 26/6		AT *n* = 31 19/12		AA *n* = 34 22/12	
	median	25th, 75th perc.	Median	25th, 75th perc.	median	25th, 75th perc.	median	25th, 75th perc.
Age, y	43	32, 50	40	31, 47	44	37, 51	44	31, 53
Height, cm	170	164, 177	170	165, 176	173	164, 180	170	164, 178
Weight, kg	120.9	109.7, 142.3	119.8	106.0, 141.3	120.9	109.5, 142.3	127.1	115.4, 142.4
BMI, kg/m^2	42.8	39.5, 46.5	43.1	38.2, 46.6	40.9	37.8, 45.4	43.1	40.5, 47.4
Hip[a], cm	130	125, 139	135	127, 147	129	122, 136	130	125, 138
Waist[b], cm	139	130, 147	140	128, 151	133	126, 146	140	133, 147
FM[c,d], kg	45.7	38.8, 53.4	48.3	40.0, 55.8	42.0	37.3, 52.5	46.3	39.9, 52.3
Android fat[c], kg	5.0	4.0, 6.0	5.1	4.0, 6.0	4.3	3.7, 6.1	5.1	4.3, 6.0
Gynoid fat[c], kg	7.8	6.1, 9.4	8.7	7.5, 9.7	7.2	6.0, 8.9	7.0	5.8, 9.1
Android/gynoid ratio[c]	.59	.51, .78	.56	.50, .70	.62	.50, .81	.60	.52, .90
FFM[c,d], kg	64.3	59.3, 78.2	63.4	59.0, 72.0	67.8	59.4, 80.5	63.7	58.2, 77.5
BMR[e], kcal/day	1625	1453, 1806	1584	1422, 1734	1567	1438, 1907	1643	1496, 1806
RQ[e]	0.85	0.82, 0.88	0.84[i]	0.81, 0.86	0.87[i]	0.83, 0.91	0.84[i]	0.81, 0.91
HbA1c, %	5.4	5.2, 5.6	5.3	5.1, 5.5	5.5	5.3, 5.7	5.5	5.2, 5.6
Insulin C-peptide, nmol/L	1.1	0.9, 1.4	1.0	0.8, 1.2	1.2	1.0, 1.4	1.2	0.9, 1.6
Cholesterol[f], mmol/L	4.8	4.3, 5.3	4.8	4.3, 5.4	4.8	4.6, 5.1	4.8	4.1, 5.3
Triglycerides[f], mmol/L	1.3	1.0, 1.7	1.2	1.0, 1.7	1.5	1.1, 2.0	1.2	0.9, 1.8
HDL-cholesterol[f], mmol/L	1.2	1.0, 1.4	1.2	1.0, 1.4	1.2	0.9, 1.5	1.2	1.1, 1.4
LDL-cholesterol[f], mmol/L	3.0	2.5, 3.5	3.3	2.8, 3.7	2.8	2.5, 3.3	2.9	2.5, 3.3
High sensitivity CRP[f], mg/L	5.5	3.0, 10.0	5.9	2.6, 10.7	4.1	2.9, 7.5	6.9	4.0, 11.4

Abbreviations: BMI, body mass index; BMR, basal metabolic rate; CRP, C-reactive protein; FM, fat mass; FFM, fat-free mass; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RQ, respiratory quotient.
[a]Measured manually, $n = 95$.
[b]From DXA measurement, $n = 87$.
[c]From DXA measurement, $n = 96$. The android area is roughly the area around the waist between the mid-point of the lumbar spine and the top of the pelvis; the gynoid area lies roughly between the head of the femur and mid-thigh, for details see the definitions by the Hologic APEX software used in the scan analysis [11, 12]. The fat mass values in those regions are given in kg, and we have given the ratio as android fat mass divided with gynoid fat mass.
[d]Excluding arms.
[e]Measured by indirect calorimetry, $n = 96$. Data underlying these results have been published [9].
[f]Measured fasting as standard at the outpatient obesity clinic, $n = 96$.
Statistics: [i]Kruskal-Wallis test, P value .024 for the distribution of RQ across categories of genotype. Mann-Whitney test for TT vs AT, $P = .005$; AT vs AA, $P = .170$; TT vs AA, $P = .223$.

Table 2. NEFA suppression and hepatic and total insulin sensitivity in the clamp by genotype

Variable Females/males	All n = 79 54/25		TT n = 25 20/5		AT n = 27 17/10		AA n = 27 17/10		Genotype diff.[a]
	median	25th, 75th perc.	median	25th, 75th perc.	median	25th, 75th perc.	median	25th, 75th perc.	*P* value
NEFA 0 minutes (fasting), mmol/L	.70	.59, .87	.67	.61, .89	.78	.59, .89	.70	.52, .87	.813
NEFA 100-120 minutes (basal), mmol/L	.66	.58, .79	.64	.59, .75	.66	.55, .79	.67	.58, .84	.927
NEFA 220-240 minutes, (clamped), mmol/L	.15	.09, .21	.13	.08, .20	.15	.07, .22	.17	.11, .21	.554
NEFA suppression, Δ mmol/L	.51	.40, .63	.49	.40, .63	.53	.40, .63	.51	.41, .63	.953
Insulin 0 minutes, pmol/L	152	110, 207	138	110, 165	149	90, 216	167	118, 227	.278
Glucose 0 minutes, mmol/L	4.8	4.4, 5.1	4.6	4.2, 4.9	4.8	4.6, 5.1	4.9	4.5, 5.2	.271
Glucose Ra basal ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	15.1	14.4, 16.1	15.0	14.0, 15.9	15.5	14.5, 16.5	14.9	14.4, 16.1	.326
Glucose Ra clamped ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	4.6	3.9, 6.3	4.8	4.0, 6.8	4.8	3.9, 6.7	4.5	3.7, 6.0	.681
Ra suppression, ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	10.6	8.5, 11.8	9.1	7.8, 11.5	10.7	8.9, 12.7	10.9	8.5, 12.0	.101
Glucose Rd basal ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	15.3	14.5, 16.3	15.2	14.1, 16.0	15.6	14.6, 16.6	15.1	14.6, 16.3	.318
Glucose Rd clamped ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	17.8	16.0, 20.0	18.6	16.4, 19.9	19.0	16.2, 20.5	17.1	15.2, 19.1	.104
Rd increase, ($\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$)[b]	2.5	.6, 4.2	3.6	1.6, 4.5	3.1	.4, 4.6	1.4	.3, 3.6	.146
Glucose MCR basal ($ml \cdot kg_{FFM}^{\cdot min-1}$)[b]	3.0	2.8, 3.3	3.0	2.8, 3.3	3.1	2.7, 3.4	3.0	2.8, 3.2	.830
Glucose MCR clamped ($ml \cdot kg_{FFM}^{\cdot min-1}$)[b]	3.6	3.1, 4.1	3.8[c]	3.5, 4.2	3.7[c]	3.1, 4.4	3.3[c]	2.9, 3.7	.048
MCR increase($ml \cdot kg_{FFM}^{\cdot min-1}$)[b]	.6	.2, .9	.8[d]	.4, 1.1	.6[d]	.1, 1.1	.3[d]	.1, .7	.042

Abbreviations: MCR, metabolic clearance; NEFA, nonesterified fatty acid; Ra, rate of appearance; Rd, rate of disappearance.
[a]Kruskal-Wallis test.
[b]Adjusted for fat-free mass (FFM), as measured by DXA, and includes lean mass excluding right and left arms. Data underlying these results have been published [8].
[c]Mann-Whitney test for TT vs AT, $P = .615$; AT vs AA, $P = .110$; TT vs AA, $P = .012$.
[d]Mann-Whitney test for TT vs AT, $P = .268$; AT vs AA, $P = .197$; TT vs AA, $P = .010$.

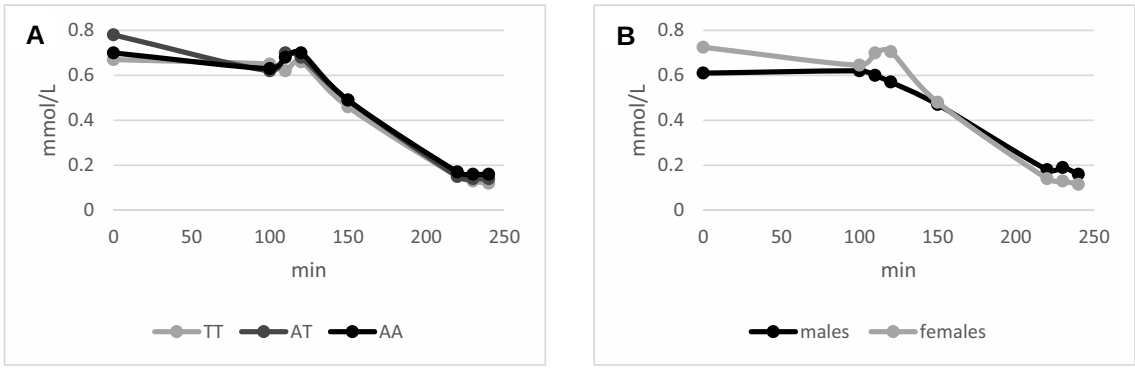

Figure 2. NEFA fasting and insulin induced suppression during the clamp, median value. Panel A depicts data by genotype, Panel B by sex. Horizontal axis shows NEFA fasting (0 minutes), basal (100-120 minutes), and insulin-induced suppression (220-240 minutes).

Fig. 2A). Similar results were obtained when NEFA suppression was calculated as percentage changes from the basal concentration (Supplementary Table S3) [13].

Gender effects

The gender-mixed study population prompted a test for gender-related differences—apart from genotypes. Women displayed higher fasting and basal NEFA concentrations but a more pronounced suppression of NEFA (Fig. 2B, Supplementary Table S4 [13]). Likewise hepatic (Ra suppression) and total insulin sensitivity (Rd increase, MCR increase) were more pronounced in women (Supplementary Table S4) [13].

NEFA Results From the Meal Test

Genotype

The meal test data qualitatively mimicked those of the clamp with no genotype differences during the fasting or postprandial state (Table 3, Fig. 3A). NEFA suppression calculated as percentage changes from fasting showed similar results (Supplementary Table S3) [13].

Gender effects

Women had higher fasting concentrations of NEFA but a larger NEFA suppression at all time points postprandially (Fig. 3B, Supplementary Table S5 [13]). Also, women displayed better insulin sensitivity when measured by the Matsuda index (Supplementary Table S5) [13].

Linear Regression Analysis

As to *NEFA fasting concentrations* we found an association amounting to 31% by RQ and LDL-cholesterol (Table 4, panel 1). The BETA coefficients were both negative (Table 4). Scatterplot by RQ is given as supplementary data (Supplementary Fig. S1A) [13]. Adjusting for age and sex gave similar results (Supplementary Table S6, panel 1) [13].

To investigate whether *NEFA suppression* was coupled to/was part of the hepatic and total insulin sensitivity expressed in the *clamp*, we analyzed the independent variables of relevance (Table 4, panel 2). We found a 30% association with RQ. The negative BETA coefficient indicated that a higher fasting RQ (signifying lower fat oxidation) was associated with less NEFA suppression. Scatterplot are given as supplementary data (Supplementary Fig. S1B) [13]. Adjusting for age and sex gave similar results (Supplementary Table S6, panel 2) [13].

As for *NEFA suppression during the meal test* we found—as for the clamp—associations with RQ (Supplementary Fig. S1C) [13] and LDL-cholesterol but none coupled to the Matsuda index (Table 4, panel 3). Adjusting for age and sex gave similar results (Supplementary Table S6, panel 3) [13].

Correlations

NEFA fasting concentrations did not correlate with BMI (Spearman's correlation coefficient −.014, $P = .892$) or fat mass (Spearman's correlation coefficient .110, $P = .291$).

Neither BMI nor fat mass correlated with variables of NEFA suppression (Supplementary Table S7) [13].

The NEFA suppression in the clamp, expressed as %, correlated positively with hepatic and total insulin sensitivity, and the % NEFA suppression in the meal correlated positively with the Matsuda index (Supplementary Table S7) [13].

The calculated Matsuda index during the meal correlated positively with NEFA suppression during the clamp, and with hepatic and total insulin sensitivity but negatively with BMI (Supplementary Table S7) [13].

Discussion

The present study forms part of an investigation into possible impacts of the *FTO* rs9939609 obesity-risk allele on intermediary metabolism in severely obese subjects without a diagnosis of diabetes. That the *FTO* gene is strongly expressed in adipose tissue makes it plausible that NEFA dynamics could be influenced by an *FTO* obesity-risk allele. Further, our finding of a genotype effect on RQ, published [9] and reproduced here, would be compatible with an obesity-risk allele affecting such dynamics. However, our present results negate any obvious effect whether on basal or insulin-suppressed levels of NEFA.

Our findings concur with recent observations from a small study encompassing 24 young males, all selected by their high physical fitness [15]. Using the same obesity allele as here, the previous study failed to find any genotype effect on fasting lipids as well as on a NEFA response to a standardized meal. We confirm and expand these genotype-negative results. A novel and important expansion is that our results come from a relatively large population of obese subjects. Obesity is coupled to a sedentary lifestyle; this would a priori make it easier to detect

Table 3. NEFA concentrations (mmol/L) and NEFA response to the meal

Females/males	All n = 96 66/30		TT n = 32 26/6		AT n = 30 18/12		AA n = 34 22/12		Genotype diff.[a]
	median	25th, 75th percentile	median	25th, 75th percentile	median	25th, 75th percentile	median	25th, 75th percentile	*P* value
NEFA 0 minutes, mmol/L	**.58**	.49, .73	.57	.48, .72	.58	.46, .81	.61	.49, .72	.944
NEFA 30 minutes, mmol/L	**.46**	.36, .61	.45	.36, .57	.47	.31, .61	.47	.39, .62	.555
NEFA 60 minutes, mmol/L	**.21**	.15, .29	.18	.13, .25	.22	.15, .36	.21	.15, .26	. 590
NEFA 90 minutes, mmol/L	**.13**	.09, .21	.12	.09, .21	.14	.08, .22	.14	.11, .20	.791
NEFA 120 minutes, mmol/L	**.14**	.09, .19	.13	.10, .19	.14	.08, .20	.13	.09, .18	.983
NEFA 150 minutes, mmol/L	**.14**	.11, .20	.15	.12, .21	.14	.12, .20	.14	.11, .19	.881
NEFA suppression from 0 to 30 minutes, mmol/L	**.10**	.02, .18	.12	.02, .18	.13	.05, .23	.06	–.02, .16	.289
NEFA suppression from 0 to 60 minutes, mmol/L	**.35**	.23, .50	.37	.26, .49	.29	.20, .46	.35	.19, .50	.385
NEFA suppression from 0 to 90 minutes, mmol/L	**.42**	.31, .55	.44	.35, .53	.36	.27, .58	.42	.31, .58	.428
NEFA suppression from 0 to 120 minutes, mmol/L	**.43**	.32, .57	.45	.34, .55	.39	.31, .57	.42	.30, .60	.702
NEFA suppression from 0 to 150 minutes, mmol/L	**.42**	.32, .54	.43	.30, .54	.38	.32, .55	.42	.33, .55	.841
Insulin 0 minutes, pmol/L	**129**	96, 181	120	87, 151	127	93, 173	141	110, 191	.335
Glucose 0 minutes, mmol/L	**5.5**	5.2, 5.8	5.3[b]	5.1, 5.6	5.6[b]	5.2, 6.2	5.6[b]	5.3, 5.9	.026
Matsuda index	**2.4**	1.8, 3.2	2.8	1.9, 3.8	2.2	1.9, 3.0	2.1	1.6, 2.9	.130

By genotype for all participants. Abbreviation: NEFA, nonesterified fatty acid.
[a]Kruskal-Wallis test.
[b]Mann-Whitney test for TT vs AT, $P = .017$; AT vs AA, $P = .657$; TT vs AA, $P = .024$.

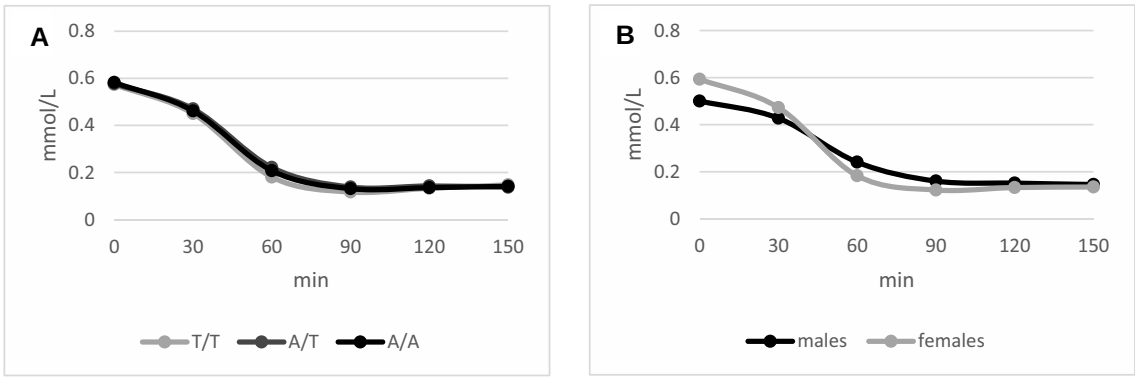

Figure 3. NEFA fasting and meal induced suppression, median values. Panel A depicts data by genotype, Panel B by sex. Horizontal axis shows NEFA fasting (0 minutes) and meal induced NEFA suppression postprandially (30-150 minutes).

Table 4. Linear regression of variables associated with NEFA fasting and NEFA suppression

Dependent variables, median (25th, 75th perc.)	Independent variables	Unstandardized coefficients		Standardized coefficient BETA	Sig.	ANOVA Sig.	R square
		B	95% CI for B				
1. NEFA, fasting[b], mmol/L .66 (.52, .79)	Gynoid mass[a], kg	.005	−.017 to.027	.059	.644	<.001	.312
	Android/gynoid ratio[a]	−.091	−.349 to.168	−.098	.488		
	BMR, kcal/24 hours	−5.778E-5	.000 to.000	−.090	.481		
	RQ	−1.053	−1.637 to −.468	−.337	<.001		
	HDL-cholesterol, mmol/L	.050	−.081 to.181	.085	.449		
	LDL-cholesterol, mmol/L	−.067	−.112 to −.021	−.270	.004		
	High-sensitivity CRP, mg/L	.001	−.005 to.006	.030	.749		
2. NEFA, suppression clamp[c], mmol/L	Insulin fasting 0 minutes, pmol/L	.000	−.001 to.000	−.213	.117	.004	.295
	Rd increase, $\mu mol \cdot kg_{FFM}^{-1 \cdot min-1}$	−.002	−.013 to.009	−.043	.741		
	Gynoid mass[a], kg	.011	−.010 to.032	.158	.288		
	Android/gynoid ratio[a]	.067	−.224 to.358	.081	.646		
	BMR, kcal/24 hours	.000	.000 to.000	−.201	.168		
.51 (.40, .63)	RQ	−.672	−1.313 to −.031	−.234	.040		
	HbA1c	−.080	−.186 to.025	−.179	.134		
	iC-peptide	.026	−.106 to.158	.057	.698		
	HDL-cholesterol, mmol/L	.098	−.052 to.248	.192	.195		
NEFA, suppression meal test[d] mmol/L	Glucose fasting 0 minutes, mmol/L	.010	−.040 to .061	.039	.681	<.001	.374
	Matsuda index	−.001	−.027 to .026	−.007	.950		
	Gyneoid mass[a], kg	.000	−.020 to .021	.004	.971		
	Android/gyneoid ratio[a]	−.121	−.380 to .137	−.129	.353		
	BMR, kcal/24 hours	−7.878E −5	.000 to .000	−.120	.341		
.43 (.32, .57)	RQ	−1.375	−1.952 to −.799	−.430	<.001		
	HDL-cholesterol, mmol/L	.069	−.074 to .211	.113	.342		
	LDL-cholesterol, mmol/L	−.059	−.104 to −.014	−.229	.010		

Abbreviations: ANOVA, analysis of variance; BMR, basal metabolic rate; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid; Rd, rate of disappearance; RQ, respiratory quotient.
[a]Fat mass (kg) in android and gynoid areas as defined by the Hologic APEX software used in the DXA scan analysis [11, 12]. We have given the ratio as android fat mass divided with gynoid fat mass.
[b]Average value of the clamp and meal test day, n = 92.
[c]Suppression from the average NEFA concentrations at minutes 100-110-120 (basal) to minutes 220-230- 240 (insulin-induced), n = 75.
[d]Suppression of NEFA from min 0 (fasting) to min 120 after the meal, n = 92.

effects of an *FTO* risk allele than in physically active individuals, since physical activity attenuates effects of *FTO* [16, 17]. Further, our study is the first to include both men and women and our results were analyzed with due consideration to gender-related differences on NEFA dynamics. Also, failure to detect genotype effects not only in a meal but also in a euglycemic clamp situation would appear to strengthen the validity of our findings.

For a proper evaluation of genotype effects, we deemed it important to characterize factors that influence NEFA

dynamics. Pursuing such characterization, we find considerable individual variation between participants but remarkably similar intra-individual NEFA responses in the 2 test situations (clamp and meal). This indicates a generalized influence of the degree of insulin sensitivity/resistance on NEFA dynamics in each individual. It is also notable that the suppression of NEFA by insulin is strongly correlated with the percentage suppression—or lack of suppression—of hepatic glucose output that was previously reported in the same individuals [8]. These observations reinforce the concept that NEFA and glucose dynamics are closely linked in the case of insulin resistance. On the other hand, we did not find any correlation of parameters of insulin resistance with BMI or total fat mass, at least not during the clamp. The latter finding is in agreement with at least some previous reports [18, 19]. We note that fasting NEFA did not correlate with BMI or fat mass. Such a finding was also previously reported [20].

We performed regression analysis to elucidate metabolic parameters which could possibly contribute to the NEFA dynamics. We find that RQ was linked to NEFA dynamics, in so far that higher fasting RQ was associated with a lesser degree of NEFA suppression. Hence, increased oxidation of carbohydrates (at the expense of fat oxidation) could contribute to a less pronounced NEFA suppression in response to insulin. Except for studies employing intravenous nutrition, we have not found reports that clearly demonstrate such associations in nondiabetic individuals whether obese or not obese.

Our analysis of meal data suggests a role for LDL-cholesterol in relation to fasting NEFA, and NEFA suppression during the meal. It is not clear whether the association with LDL is reflective of causality or signifies a coupling to other not specified molecular events.

Our study confirms a major influence by sex on NEFA dynamics. Our findings seem to increase the overall importance of an influence by sex since we report—to our knowledge for the first time—an important influence by gender in a population of uniformly obese men and women, thus negating a potential attenuating effect of obesity on gender-related differences in NEFA dynamics. Our data also rule out a confounding influence by sex on the findings on genotype effects that we present here.

To what extent are the gender-related differences in NEFA dynamics due to hormones? Evidence indicates that hormones, such as estrogens, do affect the function of adipocyte tissues [21, 22]. In this context it is of interest that we did not observe total fat mass to be different between sexes (Table 1). As to nonhormonal causes, a recent study suggests that genetic influences are also at play [23].

Limitations of our study include the recruitment of the study population. All individuals who could potentially participate in our study were recognized from referrals to a hospital-based obesity clinic. Investigations based on a population-based survey of obese individuals would be a relevant complement to the present study. Also, we acknowledge that the results pertain to a Caucasian and Norwegian population, and similar studies in other populations are needed to confirm the general validity of our findings.

Conclusion

In an adult population of obese individuals, a *FTO* risk allele fails to affect NEFA dynamics as tested both in a clamp and a meal experimental situation. These negative results appear strengthened by the confirmatory—but also extended—documentation of associations between NEFA dynamics and levels of insulin sensitivity as well as of gender.

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Disclosures

The authors have nothing to disclose.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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