Original Article

Fatty Acids, Obesity and Insulin Resistance

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Abstract

Objective: Although elevated free fatty acid (FFA) levels in obesity have been considered to be of importance for insulin resistance, a recent meta-analysis suggested normal FFA levels in obese subjects. We investigated fasting circulating FFA and glycerol levels in a large cohort of non-obese and obese subjects. ***Methods:*** Subjects recruited for a study on obesity genetics were investigated in the morning after an overnight fast (n = 3,888). Serum FFA (n = 3,306), plasma glycerol (n = 3,776), and insulin sensitivity index (HOMA-IR,n = 3,469) were determined. Obesity was defined as BMI ≥ 30 kg/m^2 and insulin resistance as HOMA-IR ≥ 2.21. ***Results:*** In obese subjects, circulating FFA and glycerol levels were higher than in non-obese individuals (by 26% and 47%, respectively; both p < 0.0001). Similar results were obtained if only men, women or medication-free subjects were investigated. Insulin resistance and type 2 diabetes were associated with a further minor increase in FFA/glycerol among obese subjects. When comparing insulin-sensitive non-obese with insulin-sensitive or -resistant obese individuals, FFA and glycerol were 21–29% and 43–49% higher in obese individuals, respectively. ***Conclusion:*** Circulating FFA and glycerol levels are markedly elevated in obesity but only marginally influenced by insulin resistance and type 2 diabetes. Whether these differences persist during diurnal variations in circulating FFA/glycerol, remains to be established.

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Introduction

It has been generally acknowledged that the concentration of circulating free fatty acids (FFAs), often also referred to as non-esterified fatty acids, is increased in obesity and that this constitutes an important causal factor for the association between obesity and type 2 diabetes.

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As previously reviewed, FFAs may induce peripheral insulin resistance and inhibit production/release of insulin from pancreatic beta cells, two corner stones in the etiology of type 2 diabetes [1].

Recently, some eminent experts in lipid metabolism challenged the view of elevated circulating FFA levels among obese [2]. They performed a thorough literature search and found 43 original articles where FFA levels had been compared in obese/overweight versus control subjects. The final data set included 1,410 obese/overweight and 953 non-obese subjects. Overall, there was only a modest average increase (~0.07 mmol/l) in circulating FFAs which the authors suggested was unrelated to fat mass estimated by an algorithm including BMI, gender, and height [3]. They subsequently investigated retrospectively FFA levels in the probably largest known FFA cohort, namely the Paris Prospective Study, consisting of 5,790 subjects [4]. When the authors divided this study material into quintiles based on BMI, there was no relationship between fasting plasma FFA levels and BMI. Finally, circulating FFA levels were compared in their own data set (the Oxford Biobank) derived from a population-based cohort of 1,591 healthy subjects aged between 30 and 50 years [5]. Using BMI or fat mass as independent regressors, they observed no correlation with fasting plasma FFA in men and a very modest relationship in women (Spearman's rank correlation coefficients 0.091 and 0.081, for BMI and fat mass, respectively). In addition, there was no significant association between FFA levels and insulin sensitivity [2].

Altogether, the meta-analysis by Karpe and colleagues [2] suggested no important influence of excess body fat on circulating FFA levels. This prompted the authors to conclude that the role of FFA for obesity-induced insulin resistance should be re-evaluated. In order to obtain an independent validation of this notion, we analyzed fasting circulating FFA levels in an ongoing study of obesity genetics. The predominant source of circulating FFA is fat cell lipolysis, which is a process where the intracellular lipids (mainly triglycerides) are broken down to FFA and glycerol. Since glycerol is also released into the circulation, the present study included analyses of fasting plasma glycerol as well. Finally, we determined the relationship between circulating FFA/glycerol and insulin resistance in obese and non-obese subjects.

Subjects and Methods

Subjects

The subjects were recruited for an ongoing study of obesity genetics where part of the data pertaining to the genetic results has been published elsewhere [6]. The study was constructed to compare obese with non-obese individuals (case-control). Subjects were included irrespective of BMI and were subsequently subdivided into obese/non-obese groups. The study has been approved by the regional committee on ethics in Stockholm (Regionala Etikprövningsnämnden, *www.epn.se/sv/stockholm*). It was explained in detail to each subject, and informed written consent was obtained. So far, about 4,000 subjects have been enrolled by local advertisement or from our out-patient clinic as well as surgery clinics in the Stockholm area (Danderyd and Ersta Hospitals). The latter were recruited among patients scheduled for abdominal surgery for non-malignant diagnoses (obesity and gallbladder disease). We have no data on menopausal state but the majority of the women (up to the 75th percentile) were below 50 years of age suggesting that only a minority had attained menopause. Data on fasting serum FFA and/or plasma glycerol were available from 3,888 subjects (2,800 women/1,088 men), and these subjects were included in the present examination. This group was composed of healthy subjects free of continuous medication (n = 2,981) while the remaining 907 were diagnosed with hypertension, dyslipidemia, polycystic ovarian syndrome (no treatment), or thyroid disorder (goiter or hypothyreodism currently euthyreoid with levo-thyroxin substitution). These 907 individuals were classified as 'unhealthy' and out of these, a total of 279, 245 obese (138 women, 107 men) and 34 non-obese (16 women, 18 men), were diagnosed with type 2 diabetes (treated with diet alone or in combination with metformin). All included subjects were categorized based on their BMI as being non-obese or obese (BMI < or ≥ 30 kg/m^2). Clinical data are given in table 1.

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Table 1. Clinical characteristics[a]

Measure (data available from n individuals)	Whole cohort	Men	Women
Age, years (n = 3,885)	41 ± 13	43 ± 15 (n = 1,088)	41 ± 12 (n = 2,797)
BMI, kg/m^2 (n = 3,888)	33 ± 9.5	33 ± 10 (n = 1,088)	33 ± 9 (n = 2,800)
Total body fat, %	40.6 ± 16	32.6 ± 15 (n = 1,088)	43.7 ± 15 (n = 2,800)
Waist circumference, cm	104 ± 24	111 ± 25 (n = 1,019)	101 ± 22 (n = 2,582)
Serum FFA, µmol/l (n = 3,306)	0.66 ± 0.24	0.62 ± 0.23 (n = 871)	0.68 ± 0.24 (n = 2,435)
Plasma glycerol, µmol/l (n = 3,776)	95 ± 49	75 ± 34 (n = 1,064)	103 ± 52 (n = 2,712)
Plasma glucose mmol/l (n = 3,532)	5.6 ± 1.9	5.9 ± 2.0 (n = 1,014)	5.5 ± 1.8 (n = 2,518)
Plasma insulin, mU/l (n = 3,797)	14.7 ± 17.1	18.7 ± 23.3 (n = 1,067)	13.2 ± 13.6 (n = 2,730)
HOMA-IR, index (n = 3,469)	4.0 ± 6.2	5.4 ± 8.6 (n = 997)	3.4 ± 4.8 (n = 2,472)
Healthy/unhealthy* (n = 3,888)	2,981/907	727/361 (n = 1,088)	2,254/546 (n = 2,800)
Type 2 diabetes	279	125	154
Obese/non-obese (n = 3,888)	2,327/1,561	637/451 (n = 1,088)	1,690/1,110 (n = 2,800)

[a]Values are mean ± SD or n = number of subjects.

*Healthy was defined as no relevant chronic disorder except obesity as detailed in 'Subjects and Methods'.

Examination

The subjects were instructed to fast from 10 p.m. the day before the examination. They arrived to the laboratory the following day at 07.30–08.00 a.m. Height and weight were determined. After a 15-min rest in the supine position , a venous blood sample was obtained, and plasma and serum were stored at –70 °C for a maximum of 3 weeks after which serum FFA was measured using the NEFA C kit (Wako Chemicals, Neuss, Germany) while plasma glycerol was determined using an established bioluminescence method [7]. Plasma glucose was determined by the hospital's routine clinical chemistry laboratory, and insulin was measured using an ELISA kit (Mercodia, Uppsala, Sweden). Glucose and insulin values were used to calculate the insulin sensitivity index Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR), as described earlier [8]. Based on the data from a large cohort [9] studied as in the present paper, insulin resistance was defined as HOMA-IR ≥ 2.21. The same two technicians performed all FFA, glycerol and insulin measures throughout the study. The percentage total body fat was estimated using a previously described formula based on age, gender, and BMI [10].

Statistical Analysis

Values are given as mean ± standard deviation (SD). They were compared by simple linear or multiple regression, analysis of co-variance (ANCOVA) or Student's unpaired t-test (assuming unequal variances). Calculations were performed using JMP (v10.0.2; SAS Institute Inc., Cary, NC, USA)

Results

The values for serum FFAs were normally distributed (fig. 1A) and in agreement with previous data and slightly but significantly higher in women compared with men (table 1; p < 0.0001). In the whole material, FFA levels were 0.574 ± 0.23 and 0.714 ± 0.23 mmol/l in the non-obese and obese subjects, respectively (p < 0.0001), representing a 26% difference. Similar results were obtained in women, men, or healthy subjects (fig. 1 B–D). In the entire cohort, there were linear relationships between BMI, total fat mass or waist circumference and serum FFA (r = 0.30, 0.33 and 0.31, respectively; all p < 0.0001), implying that these parameters explained approximately 10% of the inter-individual variation in serum FFA (i.e. adjusted r^2).

Plasma glycerol values were normally distributed (fig. 2A) and higher in women compared with men (table 1; p < 0.0001). The average values were 74 ± 34 µmol/l and 110 ± 52 µmol/l

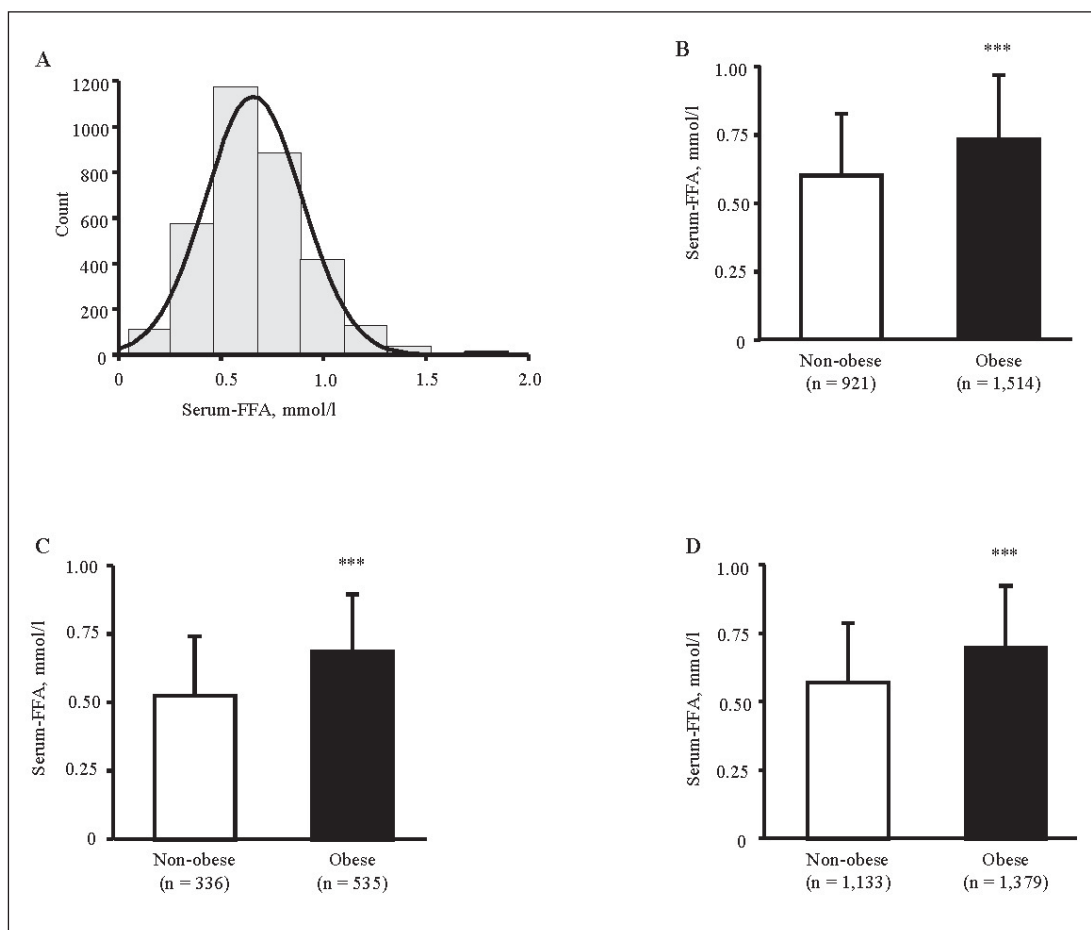Arner and Rydén: Fatty Acids, Obesity and Insulin Resistance

Fig. 1. Fasting serum FFA levels. **A** is distribution of values. **B** is findings in women. **C** is findings in men. **D** is findings in healthy men and women put together. Values were compared by unpaired t-test.

in non-obese and obese subjects, respectively (p < 0.0001), representing a 47% difference. Similar results were observed in women, men, and healthy subjects (fig. 2 B–D). In analogy with the results on serum FFA, plasma glycerol correlated significantly with BMI, total fat mass and waist circumference in the whole material (r = 0.40, 0.47 and 0.32, respectively; all p < 0.0001). Thus, the regressors explained 10–22% of the inter-individual variation in circulating glycerol levels.

The majority of the cohort was of female gender which reflects the relative difficulties in recruiting men to this type of studies. Unfortunately, we did not have any information on menopausal state. However, when subdividing women into subjects <45 (n = 1,629) or >55 (n = 331) years of age, the differences in FFA and glycerol levels remained in the same order of magnitude in non-obese and obese subjects (all p < 0.0001, graph not shown).

Values for HOMA-IR were available from 3,469 subjects. They were used to categorize the cohort into four groups based on the obese/non-obese and insulin-sensitive/insulin-resistant phenotypes (fig. 3). There was a highly significant overall difference in FFA and glycerol levels between the four groups (fig. 3). Subgroup analyses demonstrated that, while FFA concentrations did not differ between non-obese subjects discordant for insulin sensitivity, insulin-resistant obese indivduals displayed slightly (6%) but significantly higher levels than insulin-sensitive obese subjects (fig. 3 A). Compared with non-obese insulin-
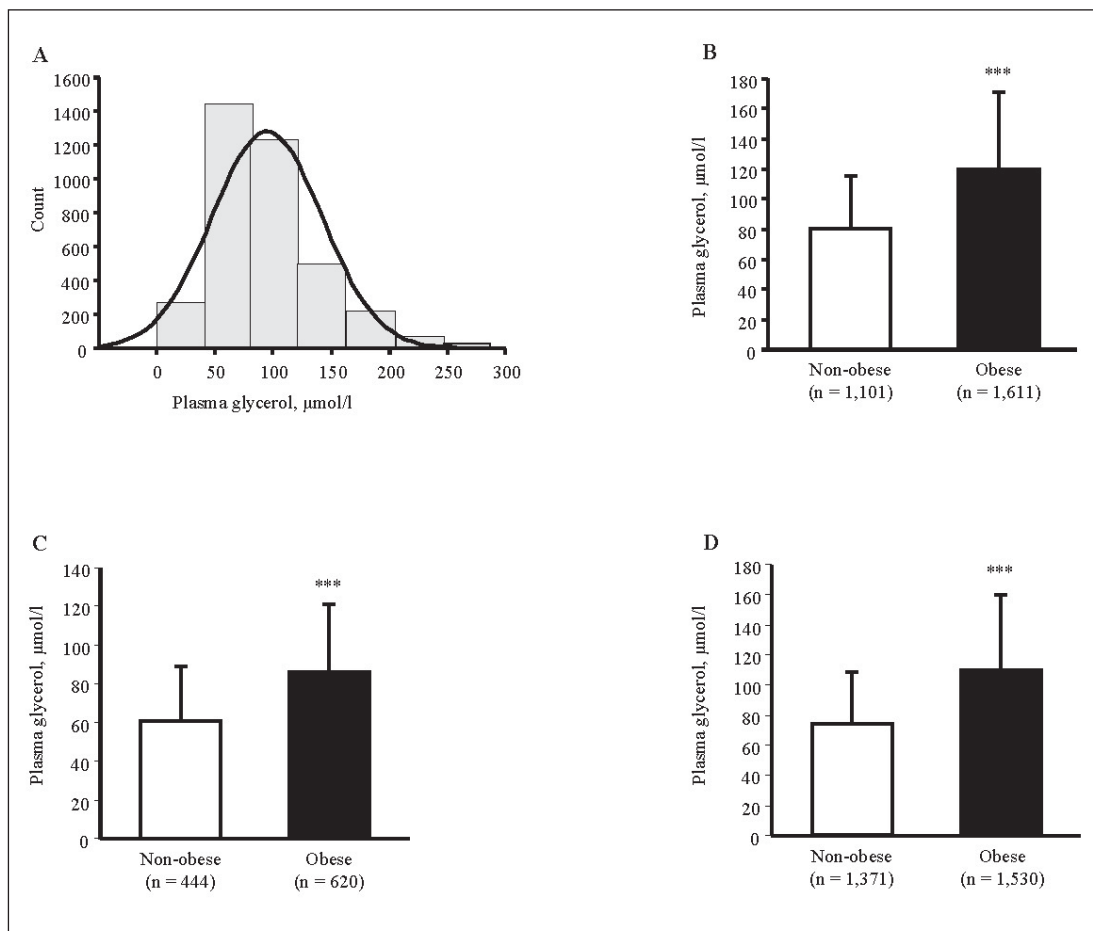
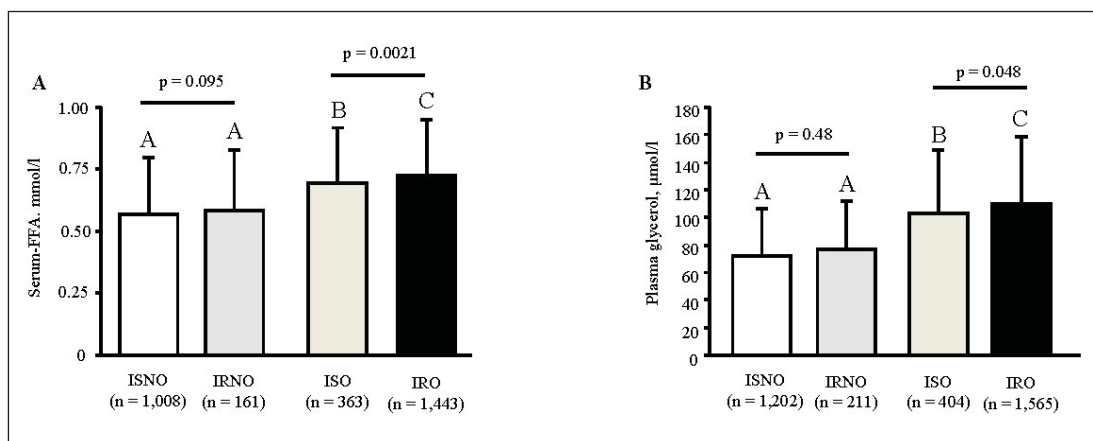**Fig. 2.** Fasting plasma glycerol levels. See text to figure 1 for further details.

Fig. 3. Fasting serum FFA (**A**) and plasma glycerol (**B**) in non-obese or obese subjects with or without insulin resistance. INSO = Insulin sensitive non-obese; IRNO = insulin resistant non-obese; ISO =insulin sensitive obese; IRO = insulin resistant obese. Values were compared by ANCOVA (p < 0.0001) and Fishers PLSD post-hoc test (values in graphs). Letters (A–C) denote statistically significant differences between groups.

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sensitive subjects, obese insulin-sensitive and insulin-resistant individuals had 21 and 29% higher serum FFA levels (both p < 0.0001), respectively. Similar results were observed for glycerol (fig. 3 B), i.e., for individuals discordant in insulin sensitivity there was a significant difference in plasma levels in the obese but not in the non-obese subjects. Insulin-sensitive and -resistant obese individuals displayed 43 and 49% higher glycerol values, respectively, than insulin-sensitive non-obese subjects (both p < 0.0001). There was a linear correlation between serum FFA or plasma glycerol and $\log_{10}$ HOMA-IR (r = 0.25 or r = 0.28; both p < 0.0001). These associations remained significant in multiple regression analyses after correction for total fat mass and waist circumference (beta coefficient = 0.046, p = 0.0015 for FFA and beta coefficient = 0.072, p < 0.0001 for glycerol). The 279 subjects with known type 2 diabetes displayed significantly higher circulating $\log_{10}$ HOMA-IR (p < 0.0001), FFA (0.75 ± 0.27 vs. 0.65 ± 0.23 µmol/l; p < 0.0001). and glycerol (105 ± 53 vs. 94 ± 49 µmol/l; p = 0.0014) levels than the rest of the cohort.

Discussion

The recent notion that obesity does not impact on fasting plasma FFA levels [2] is somewhat challenged by the present investigation performed in a large cohort of >3,000 subjects. Our data demonstrate that FFA concentrations were almost 30% higher in obese compared with non-obese subjects. Similar results were obtained in the whole cohort (including some subjects with metabolic disorders) or in the about three quarters of the study group that were healthy and free of any continuous medication. Furthermore, there was a linear relationship between BMI, fat mass or waist circumference and FFA/glycerol levels.

Admittedly, our results are based on FFA levels determined during a narrow (i.e., 30-min time window) sampling period in the morning after an overnight fast. Because there are pronounced diurnal variations of circulating FFAs [11, 12], we cannot establish whether similar differences between obese and non-obese individuals are present at other times during the day, e.g., postprandially.

Women have more body fat than men, and FFA kinetics are subjected to gender variation [13]. We could confirm that FFA levels in women were slightly higher than in men but the influence of obesity was similar in both sexes. In the present cohort, we did not have information on menopausal state. However, the observation that FFA and glycerol levels were significantly higher in obese compared with non-obese women in the age groups <45 or >55 years of age (all at p < 0.0001), suggests that menopause does not have a major impact on the observed differences in circulating FFA/glycerol between weight-discordant individuals.

Fasting plasma glycerol, the second end product of lipolysis, was not investigated in the study by Karpe and colleagues [2]. Previous investigations on small study groups have reported conflicting results; i.e., either no differences or increased levels in obesity were detected [14–24]. In the present study, glycerol levels were almost 50% higher in obese indivuals, which constitutes a considerable difference. Although women displayed higher values than men, there was a similar impact of obesity in both genders. Thus, the fasting circulating levels of both end products of lipolysis, FFA and glycerol, are significantly higher in obesity. Taking our results together, we suggest that an increased lipid release from adipose tissue into the circulation is an important factor causing elevated FFA and glycerol in obesity. If this is primarily due to a mass effect of adipose tissue and/or altered lipolytic activity remains to be established.

Karpe and colleagues [2] reported that insulin sensitivity did not associate with FFA levels. This is in part confirmed presently where non-obese subjects discordant for insulin sensitivity displayed similar FFA and glycerol values. However, in obese participants, FFA and

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glycerol concentrations were slightly but significantly higher in subjects with insulin resistance. In individuals with or without type 2 diabetes the differences were even more pronounced (12–15%). Moreover, in the whole cohort, there was a linear association between serum FFA or plasma glycerol and $\log_{10}$ HOMA-IR which remained significant after correction for total fat mass and waist circumference. Admittedly, the beta coefficients for FFA/glycerol in multiple regression were small, suggesting that, although significant, the circulating levels of these metabolites influence insulin resistance only to a minor extent. A study combining different in vitro and in vivo models in human and murine systems as well as a prospective clinical trial demonstrated that attenuation white adipose tissue (WAT) lipolysis results in improved glucose metabolism and insulin sensitivity without increasing WAT mass [25]. The main mechanism appeared to be increased glucose uptake and stimulated de novo lipogenesis. Also, both metformin and thiazolidinediones have been shown to inhibit lipolysis in human adipocytes which could constitute an additional mechanism contributing to their beneficial effects on glucose tolerance [26]. These data indicate that therapies targeting WAT lipolysis reduce FFA levels and insulin resistance via direct/indirect mechanisms that improve WAT function and that the link between obesity, lipolysis and insulin resistance may not depend solely on circulating FFA levels per se but rather on altered FFA fluxes. Needless to say, this notion needs to be proven in clinical studies. Interestingly, a recent study described a small number of human subjects with null mutations in the gene encoding hormone sensitive lipase (HSL), the rate-limiting enzyme in in human adipocyte lipolysis [27]. Individuals homozygous for the mutation displayed insulin resistance / type 2 diabetes, dyslipidemia and ectopic fat deposition while heterozygous subjects displayed an intermediate phenotype compared with non-carriers. This observation suggests that inhibition of adipocyte lipolysis via HSL may not be a feasible therapeutic strategy.

Who is right or wrong in the story about FFA levels in obesity? Perhaps the truth lies somewhere in between. The Oxford study was retrospective [2], and neither that nor the present investigation were designed with the primary goal to investigate the impact of obesity on fasting FFA levels. As discussed by Karpe and co-workers [2], overweight/obesity is not likely to be a disease entirely based on continuous variation in BMI, total body fat, or body fat distribution. Several other factors such as adipose morphology (size and number of fat cells), inflammation, endocrine/secretory activity as well as non-adipose aspects may influence the relationship between obesity and circulating FFAs. Such factors have not been taken into account in the present and previous investigations. Differences in how FFAs were sampled and analyzed could also contribute. Furthermore, unlike the previous study [2], we investigated a case-control cohort enriched for subjects with obesity.

In conclusion, this large study suggests that circulating FFA levels, determined after an overnight fast, correlate with body weight and are indeed increased to a significant extent (almost 30%) in obese subjects. Although insulin resistance and type 2 diabetes did not impact on FFA levels to any large degree, recent mechanistic insights into the effects of lipolysis inhibition on FFA fluxes should encourage investigators to continue research on the putative importance of FFA as a link between obesity, insulin resistance and type 2 diabetes.

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Disclosure Statement

The authors have no conflict of interest to report.

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