# Changes in the Serum Composition of Free-fatty Acids During an Intravenous Glucose Tolerance Test

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**Recent studies suggest that measuring the free-fatty acids (FFA) during an intravenous glucose tolerance test (IVGTT) may provide information about the metabolic associations between serum FFA and carbohydrate and insulin metabolism. We evaluated the FFA profile during an IVGTT and determined whether this test changes the composition and concentration of FFA. An IVGTT was given to 38 severely obese persons before and 7 months after undergoing bariatric surgery and also to 12 healthy, nonobese persons. The concentration and composition of the FFA were studied at different times during the test. The concentration of FFA fell significantly faster during the IVGTT in the controls and in the severely obese persons with normal-fasting glucose (NFG) than in the severely obese persons with impaired-fasting glucose (IFG) or type 2 diabetes mellitus (T2DM) ($P < 0.05$). Significant differences were found in the time to minimum serum concentrations of FFA (control = NFG < IFG < T2DM) ($P < 0.001$). These variables improved after bariatric surgery in the three groups. The percentage of monounsaturated and n-6 polyunsaturated FFA in the control subjects and in the obese persons, both before and after surgery, decreased significantly during the IVGTT. In conclusion, during an IVGTT, severely obese persons with IFG or T2DM experienced a lower fall in the FFA than the severely obese persons with NFG and the controls, becoming normal after bariatric surgery.**

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## INTRODUCTION

Obesity is one of the greatest public health problems in industrialized countries (1). Obese persons have both increased insulin resistance and an increase in free-fatty acids (FFAs) (2). These FFA from adipose tissue are primarily an important energy substrate for a number of organs. In addition, FFA are involved in the regulation of a number of metabolic processes in the body. A change in lipid metabolism can have an important effect on glucose metabolism in different organs (3).

The associations between serum FFA and carbohydrate and insulin metabolism have been known for a long time (4). Different studies have shown that an increase in the serum concentration of FFA results in a change in the action of insulin and this increase can be considered an independent predictive factor for progression to type 2 diabetes mellitus (T2DM) (5,6).

Serum FFA forms a nonhomogeneous group of fatty acids with different lengths of carbon chains and different degrees of unsaturation. The fatty acid composition of the serum FFA can be influenced by the composition of the diet, by the action of insulin and by the metabolic status. Additionally, the effect of fatty acids on peripheral insulin sensitivity (SI) and insulin secretion by β-cells is dependent on the type of fatty acid (7).

The intravenous glucose tolerance test (IVGTT) is a dynamic test that provides a description of the association between insulin action and glucose metabolism. Recent studies have suggested that measuring the FFA concentrations during an IVGTT may provide additional information about the metabolic associations between serum FFA and carbohydrate and insulin metabolism (8,9). However, the response of the FFA concentration during an IVGTT in persons with severe obesity, before and after an important weight loss, and the changes that occur in the fatty acid composition of the serum FFA at different times during an IVGTT have not been studied. The change in FFA composition as a consequence of the different

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metabolism of the fatty acids may have important repercussions (10).

This study therefore had two aims: (i) to assess the response of the FFA concentration during an IVGTT in severely obese persons before and after weight loss and its association with SI and secretion, and (ii) to determine whether the fatty acid composition of the FFA is altered during the course of an IVGTT.

## METHODS AND PROCEDURES

### Subjects

The study included 38 severely obese persons (BMI 54.4 ± 1.72 kg/m$^2$) (14 men and 24 women) and 12 healthy, nonobese persons (BMI 23.1 ± 0.6 kg/m$^2$) (6 men and 6 women). The severely obese persons were classified into three groups, according to their fasting glucose levels prior to bariatric surgery: severely obese with normal-fasting glucose (SO-NFG) (glucose <5.6 mmol/l) ($n$ = 9), severely obese with impaired-fasting glucose (SO-IFG) (glucose ≥5.6 and <7.0 mmol/l) ($n$ = 17), and severely obese with T2DM (SO-T2DM) (glucose ≥7.0 mmol/l) ($n$ = 12). None of the severely obese persons with T2DM were receiving insulin therapy or thiazolidinediones. The patients underwent bariatric surgery with mixed techniques, combining gastric reduction with an intestinal bypass: biliopancreatic diversion ($n$ = 22) or gastric bypass ($n$ = 16). All the participants gave their informed consent and the study was reviewed and approved by the Ethics and Research Committee.

### IVGTT

An IVGTT (11) was performed in the healthy persons and in the severely obese persons prior to surgery and 7 months after bariatric surgery. The study protocol commenced at 08:30 after a 10–12-h fast (12). Baseline blood samples were obtained 15, 10, and 5 min before glucose administration. At point 0 glucose was administered (50% dextrose; 11.4 g/m$^2$ body surface area) in <1 min. Blood samples were taken after 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160, and 180 min for the measurement of concentrations of glucose, insulin, and free fatty acids. The serum was separated, aliquoted within 30 min of extraction and immediately frozen at −80 °C.

### Laboratory measurements

Serum glucose, triglycerides, and glycerol (Randox Laboratories, UK) were measured in duplicate by standard enzymatic methods. FFAs were measured in duplicate by standard enzymatic methods (WAKO Chemicals, Richmond, VA). The intra- and interassay coefficient of variation in our laboratory was 2.1 and 3.2%, respectively. The sensitivity of the technique was 0.025 mmol/l. Glycerol was measured before the administration of glucose, at that point of the IVGTT when the FFA concentration was minimum and at 180 min. The insulin was analyzed in duplicate by an immunoradiometric assay (BioSource Europe S.A., Belgium).

The FFA composition was analyzed at three points during the IVGTT: before the administration of glucose, at the FFA nadir of the IVGTT curve and 180 min after starting the IVGTT. For fatty acid analysis, lipids were extracted with chloroform–methanol 2:1 (vol/vol) (13). The lipid classes were separated by thin-layer chromatography on silica gel plates (Merck, Darmstadt, Germany) with hexane-ethylic ether-acetic acid (80:20:2, vol/vol/vol) as the developing solvent. Fatty acid methyl esters of FFA were prepared according to Lepage and Roy (14) and analyzed in a Hewlett-Packard 4890A gas chromatograph equipped with a flame ionization detector and capillary column Supelco OMEGAWAX 320 (30 m × 0.32 mm × 0.25 µm film thickness). The oven temperature was maintained at 140 °C for the first minute and increased at a rate of 6 °C/min until 240 °C. This temperature was maintained for 4 min. The identity of each fatty acid peak was ascertained by comparison of the retention time of the peak with the retention times of synthetic standards with known fatty acids composition.

### Calculations

The SI and the $AIR_G$ (acute insulin response) were calculated after introduction of the results for glucose and insulin obtained during the IVGTT into the MINMOD program (11). The disposition index (DI) was expressed as $DI = SI \times AIR_G$ (15). The elimination of glucose during the IVGTT, or the glucose tolerance index, was expressed as $K_G$: the slope of the natural logarithm of the concentration of glucose between 20 and 60 min of the IVGTT (15,16). The rate of decline of FFA concentration at the start of the IVGTT was calculated as the slope of the natural logarithm of FFA concentration between 10 and 40 min ($K_{FFA}$). It is reported as the fractional disposal rate of FFA (9,17).

### Statistical analysis

The statistical analysis was done with SPSS (Version 11.5 for Windows; SPSS, Chicago, IL). Comparison between the results of the different groups (control, SO-NFG, SO-IFG, and SO-T2DM) was made with one-way analysis of variance and the *post hoc* analysis was done with Duncan's multiple range tests. The differences in the various study variables within the same group before and after bariatric surgery were compared with the Student's $t$-test for paired samples. The Pearson correlation coefficient was calculated to estimate the linear correlations between variables. Multiple linear regressions were used to determine the association between variables. Inclusion of variables in the multivariate models was made according to the recommendations of Kleimbaun *et al.* (18). Values were considered to be statistically significant when the $P \leq 0.05$. The results are given as the mean ± s.e.m.

## RESULTS

### Anthropometric and biochemical characteristics

**Table 1** summarizes the anthropometric and biochemical characteristics of the severely obese patients and the control subjects. The decrease in weight was similar in the three groups of severely obese patients (SO-NFG: 30.6 ± 1.8%; SO-IFG: 30.1 ± 1.8%; SO-T2DM: 30.4 ± 3.2%). **Figures 1** and **2** show the curves for glucose, insulin, and FFAs obtained during the IVGTT before (**Figure 1**) and after (**Figure 2**) the patients underwent bariatric surgery.

### FFAs before bariatric surgery

The baseline FFA concentration followed the trend control < SO-NFG < SO-IFG < SO-T2DM ($P < 0.05$) (**Table 1**). **Figure 1c** shows the curve for the FFA concentration obtained during the IVGTT before the patients underwent bariatric surgery. The levels of FFA during the first minutes of the IVGTT remained almost constant, falling later. However, the duration of this period was significantly greater in the SO-T2DM (control: 12.2 ± 1.3 min; SO-NFG: 12.7 ± 1.4 min; SO-IFG: 13.4 ± 0.4 min; SO-T2DM: 20.3 ± 1.1 min) ($P = 0.008$).

The FFA concentration fell later in all the study groups. The $K_{FFA}$ was significantly greater in the controls, the SO-NFG, and the SO-IFG than in the SO-T2DM (control: 1.03 ± 0.23; SO-NFG: 1.16 ± 0.14; SO-IFG: 0.97 ± 0.08; SO-T2DM: 0.47 ± 0.10) ($P = 0.002$), the FFA reduction in this last group being slower but more prolonged (**Table 1** and **Figure 1c**). The time to reach the minimum concentrations of FFA during the IVGTT was significantly greater in the SO-T2DM (control: 64 ± 9 min; SO-NFG: 60 ± 6 min; SO-IFG: 88 ± 6 min; SO-T2DM: 115 ± 13 min) ($P < 0.001$).

The $K_{FFA}$ correlated significantly with the different study variables: glucose ($r = -0.66$, $P < 0.001$), insulin ($r = -0.54$,

**Table 1 Variables of the control subjects and the three groups of severely obese patients, before and after bariatric surgery**

| | | Severely obese—preoperative | | | Severely obese—postoperative | | |
|---|---|---|---|---|---|---|---|
| | Controls | NFG | IFG | T2DM | NFG | IFG | T2DM |
| *N* | 12 | 9 | 17 | 12 | — | — | — |
| Age (years) | 36.7 ± 1.9$^{a,b}$ | 35.9 ± 3.4$^{b}$ | 45.2 ± 2.5$^{a}$ | 44.6 ± 2.4$^{a}$ | — | — | — |
| Weight (Kg) | 66.4 ± 3.1$^{c}$ | 133.3 ± 8.4$^{b}$ | 160.1 ± 5.6$^{a}$ | 143.9 ± 6.7$^{a,b}$ | 95.7 ± 5.9$^{*,\dagger}$ | 109.6 ± 4.2$^{*,\S}$ | 97.1 ± 3.6$^{\ddagger}$ |
| BMI (Kg/m$^2$) | 23.1 ± 0.7$^{c}$ | 48.6 ± 1.7$^{b}$ | 58.1 ± 1.4$^{a}$ | 53.9 ± 1.7$^{a}$ | 34.2 ± 1.4$^{*,\dagger}$ | 39.6 ± 1.4$^{*,\S}$ | 36.6±1.5$^{*,\ddagger}$ |
| Waist (cm) | 83.4 ± 2.6 $^{c}$ | 130.6 ± 4.5$^{b}$ | 148.6 ± 4.6$^{a}$ | 141.1 ± 4.1$^{a,b}$ | 105.9 ± 4.1$^{*}$ | 116.0 ± 3.0$^{*}$ | 111.9 ± 3.4$^{*}$ |
| Glucose (mmol/l) | 4.64 ± 0.10$^{c}$ | 4.92 ± 0.12$^{c,b}$ | 5.92 ± 0.09$^{b}$ | 10.59 ± 0.78$^{a}$ | 4.77 ± 0.15 | 4.79 ± 0.15$^{\S}$ | 4.93 ± 0.20$^{\ddagger}$ |
| Triglycerides (mmol/l) | 0.93 ± 0.25$^{b}$ | 1.26 ± 0.19$^{b}$ | 1.37 ± 0.26$^{b}$ | 2.69 ± 0.33$^{a}$ | 0.92 ± 0.12 | 1.20 ± 0.15 | 1.44 ± 0.80$^{*,\dagger}$ |
| FFA (mmol/l) | 0.315 ± 0.030$^{c}$ | 0.605 ± 0.049$^{b}$ | 0.670 ± 0.049$^{a,b}$ | 0.817 ± 0.061$^{a}$ | 0.435 ± 0.064$^{\dagger}$ | 0.562 ± 0.035$^{*,\dagger}$ | 0.587 ± 0.064$^{*,\dagger}$ |
| Insulin (pmol/l) | 55.1 ± 6.1$^{c}$ | 157.3 ± 35.0$^{b}$ | 206.5 ± 25.9$^{a,b}$ | 245.7 ± 35.9$^{a}$ | 78.4 ± 12.6 | 74.7 ± 8.7$^{\S}$ | 83.2 ± 13.4$^{\ddagger}$ |
| SI ((10$^{-4}$/min)/(μU/ml)) | 7.46 ± 1.66$^{a}$ | 3.06 ± 1.28$^{b}$ | 1.42 ± 0.32$^{b}$ | 0.28 ± 0.15$^{b}$ | 7.94 ± 1.32$^{\dagger}$ | 5.09 ± 0.75$^{\ddagger}$ | 5.61 ± 1.22$^{\dagger}$ |
| $AIR_G$ (min pmol/ml) | 4.59 ± 1.02$^{a}$ | 4.61 ± 1.67$^{a}$ | 2.75 ± 0.62$^{a,b}$ | 0.34 ± 0.13$^{b}$ | 3.66 ± 0.77 | 3.75 ± 0.68$^{\dagger}$ | 2.73 ± 0.55$^{\dagger}$ |
| DI (10$^{-3}$) | 3.70 ± 0.44$^{a}$ | 1.21 ± 0.43$^{b}$ | 0.41 ± 0.10$^{c}$ | 0.03 ± 0.01$^{c}$ | 2.82 ± 1.26 | 2.23 ± 0.60$^{\S}$ | 1.93 ± 0.53$^{*,\dagger}$ |
| $K_G$ (%/min) | 1.66 ± 0.08$^{a}$ | 1.27 ± 0.16$^{b}$ | 0.81 ± 0.05$^{c}$ | 0.54 ± 0.08$^{d}$ | 1.57 ± 0.28$^{\dagger}$ | 1.21 ± 0.11$^{*,\ddagger}$ | 1.20 ± 0.13$^{*,\dagger}$ |
| $K_{FFA}$ (%/min) | 1.03 ± 0.23$^{a}$ | 1.16 ± 0.14$^{a}$ | 0.97 ± 0.08$^{a}$ | 0.47 ± 0.10$^{b}$ | 1.55 ± 0.53$^{\dagger}$ | 1.26 ± 0.13$^{\ddagger}$ | 1.18 ± 0.17$^{\ddagger}$ |
| Glycerol (mmol/l) | | | | | | | |
| Baseline | 0.100 ± 0.019$^{b,1}$ | 0.177 ± 0.037$^{a,b,1}$ | 0.185 ± 0.020$^{a,b}$ | 0.252 ± 0.048$^{a,1}$ | 0.140 ± 0.033$^{\dagger}$ | 0.123 ± 0.018$^{\dagger}$ | 0.102 ± 0.020$^{\dagger,1}$ |
| Nadir | 0.052 ± 0.010$^{b,2}$ | 0.068 ± 0.007$^{b,2}$ | 0.144 ± 0.022$^{a}$ | 0.123 ± 0.015$^{a,2}$ | 0.086 ± 0.033 | 0.089 ± 0.011$^{\dagger}$ | 0.063 ± 0.019$^{\dagger,2}$ |
| Final | 0.101 ± 0.025$^{1}$ | 0.152 ± 0.031$^{1}$ | 0.180 ± 0.021 | 0.151 ± 0.032$^{1,2}$ | 0.131 ± 0.009 | 0.132 ± 0.017 | 0.092 ± 0.013$^{1,2}$ |

Data are expressed as mean ± s.e.m. Different letters indicate significant differences between the means of the different groups of preoperative severely obese patients and the controls ($P < 0.05$). Different numbers indicate significant differences within each group of subjects between the means of the baseline, nadir, and final glycerol levels during the IVGTT ($P < 0.05$).
*Significant differences between the means of the different groups of postoperative severely obese patients and the controls ($P < 0.05$).
$^{\dagger}P<0.05$; $^{\ddagger}P < 0.01$; $^{\S}P < 0.001$: significant differences within the same group of obese persons, before and after bariatric surgery.

$P = 0.002$), $K_G$ ($r = 0.76$, $P < 0.001$), SI ($r = 0.59$, $P < 0.001$), and DI ($r = 0.37$, $P = 0.043$). Of all these, $K_G$ was the variable that was most significantly associated with $K_{FFA}$ before surgery ($P = 0.012$), even after adjusting the linear regression model for age ($P = 0.333$), BMI ($P = 0.448$), glucose ($P = 0.519$), insulin ($P = 0.436$), SI ($P = 0.739$), DI ($P = 0.689$), and FFA concentration ($P = 0.845$).

### FFAs after bariatric surgery

After bariatric surgery, the evolution of the FFA concentration curve during the IVGTT was similar to that of the controls (Figure 2c), independently of the type of bariatric surgery undergone.

No significant differences were detected either at the point at which the FFA concentration began to fall (SO-NFG: 12 ± 3 min; SO-IFG: 9 ± 1 min; SO-T2DM: 11 ± 1 min) or the point of minimum level (SO-NFG: 50 ± 8 min; SO-IFG: 50 ± 3 min; SO-T2DM: 67 ± 8 min). The $K_{FFA}$ was similar in all the study groups (Table 1).

After surgery, the $K_{FFA}$ correlated significantly with the SI ($r = 0.70$, $P < 0.001$), DI ($r = 0.53$, $P = 0.006$), and $K_G$ ($r = 0.52$, $P = 0.008$). The variables that best explained the $K_{FFA}$ in a multiple linear regression model were SI ($P < 0.001$) and FFA concentration ($P = 0.035$). This association remained after adjusting the model for postsurgery variables: age ($P = 0.419$), BMI ($P = 0.254$), glucose ($P = 0.507$), insulin ($P = 387$), $K_G$ ($P = 0.206$), and DI ($P = 0.533$).

### Composition of FFAs

The FFA composition varied significantly during the IVGTT in both the controls and in the severely obese patients before and after surgery. The percentage of monounsaturated fatty acids fell significantly during the IVGTT in the control group (baseline: 38.4 ± 1.9%; nadir: 26.2 ± 2.8%; final: 27.7 ± 1.5%, $P < 0.05$) and in the severely obese patients both before surgery (baseline: 36.7 ± 1.3%; nadir: 24.6 ± 2.2%; final: 29.2 ± 2.2%, $P < 0.05$) and after surgery (baseline: 43.7 ± 1.6%; nadir: 35.2 ± 1.6%; final: 28.8 ± 1.7%, $P < 0.05$). The percentage of n-6 polyunsaturated fatty acids (n-6 PUFA) also fell significantly in the control group (baseline: 16.9 ± 0.9%; nadir: 14.4 ± 2.2%; final: 14.3 ± 1.2%, $P < 0.05$) and in the severely obese patients before surgery (baseline: 21.5 ± 1.4%; nadir: 13.3 ± 1.3%; final: 14.9 ± 0.9%, $P < 0.05$), but not after surgery (baseline: 18.4 ± 1.0%; nadir: 16.6 ± 1.3%; final: 15.9 ± 0.8%, $P > 0.05$). The percentage of n-3 polyunsaturated fatty acids (n-3 PUFA), however, rose significantly during the IVGTT in the control group (baseline: 2.8 ± 1.5%; nadir: 10.6 ± 1.6%; final: 8.1 ± 1.6%, $P < 0.05$) and in the severely obese patients both before surgery (baseline:

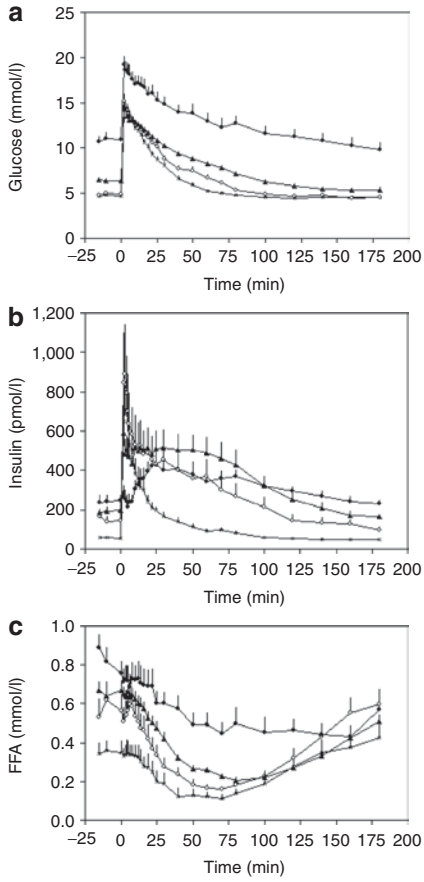


**Figure 1** (**a**) Glucose , (**b**) insulin, and (**c**) free-fatty acid (FFA) concentrations during an intravenous glucose tolerance test before bariatric surgery. Control subjects (×), severely obese patients with normal-fasting glucose (open circles), impaired-fasting glucose (closed triangles), or type 2 diabetes mellitus (closed circles). Data are presented as means + s.e.m.

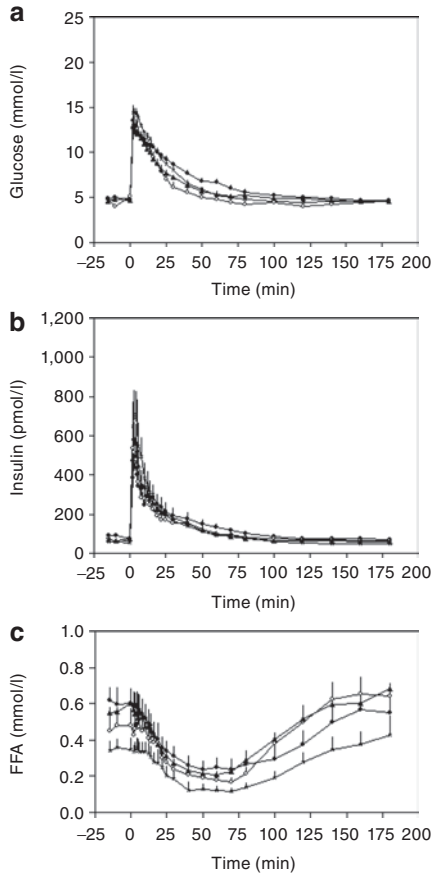


**Figure 2** (**a**) Glucose, (**b**) insulin, and (**c**) free fatty acid concentrations during an intravenous glucose tolerance test after bariatric surgery. Control subjects (×), severely obese patients with normal-fasting glucose (open circles), impaired-fasting glucose (closed triangles), or T2DM (closed circles). Data are presented as means + s.e.m.

1.4 ± 0.3%; nadir: 8.1 ± 0.6%; final: 5.8 ± 1.7%, $P < 0.05$) and after surgery (baseline: 0.7 ± 0.2%; nadir: 8.6 ± 0.6%; final: 3.4 ± 0.6%, $P < 0.05$). The percentage of saturated fatty acids rose significantly in the severely obese patients both before surgery (baseline: 40.3 ± 1.4%; nadir: 53.9 ± 3.7%; final: 50.0 ± 3.0%, $P < 0.05$) and after surgery (baseline: 37.7 ± 1.6%; nadir: 39.6 ± 3.7%; final: 51.8 ± 2.2%, $P < 0.05$), but not in the control group (baseline: 41.9 ± 2.1%; nadir: 48.8 ± 5.0%; final: 49.8 ± 2.7%, $P > 0.05$). The changes seen in the percentages of the different groups of fatty acids were independent of whether the subject belonged to any of the three groups of severely obese patients (data not shown). No differences were seen according to the type of bariatric surgery undergone.

## DISCUSSION

The main conclusions of this study are that (i) severely obese patients with IFG or T2DM experience a lower reduction in FFA concentration during an IVGTT, probably as a result of less inhibition of lipolysis and a reduced reuptake of the FFA by the tissues and (ii) the FFA are cleared selectively during the IVGTT, with a significant decrease in the serum percentage of monounsaturated fatty acids and n-6 PUFA.

The IVGTT is a dynamic test in which glucose and insulin concentrations change during the test. The test also affords a view of the interaction between insulin and FFA (8,9,19). Nevertheless, this test is not performed under physiologic conditions as the glucose is administered intravenously, bypassing the gastrointestinal tract and preventing the enterohormone response. A first phase exists during an IVGTT in which the level of FFA is refractory to the changes produced by the insulin (8,9). This phase represents the time required by the insulin to pass to the interstitium (20) and raise the concentrations necessary to initiate the inhibition of lipolysis (9). However, the time required for the insulin to start inhibiting the hormone-sensitive lipase is unknown. The results of our study show that in severely obese persons the time taken by the insulin to start exerting its antilipolytic effect is longer in the patients with diabetes mellitus (control = SO-NFG = SO-IFG < SO-T2DM).

As expected, during the IVGTT there was an initial fall in the levels of FFA that was closely associated with the increase in glucose and insulin, as suggested by the significant correlations seen between the $K_{FFA}$ slope and glucose, insulin, SI, DI, and $K_G$. Of these, the $K_G$ and the SI seem to provide a better explanation for the variations found in the $K_{FFA}$, indicating that

the reduction in the concentration of FFA induced by insulin ($K_{FFA}$) was directly associated with insulin-stimulated glucose uptake ($K_G$) and SI. $K_G$ has recently been shown to be the variable that best discriminates the degree of tolerance to carbohydrates in severely obese persons, both before and after bariatric surgery (12). Inhibition of insulin-stimulated glucose uptake by FFA has also been suggested by others (21). Although our results show an association between reduction in FFA during an IVGTT ($K_{FFA}$) and SI, no association was found with insulin secretion ($AIR_G$). Nevertheless, another study did find an association between $K_{FFA}$ and insulin secretion, though not with insulin resistance (17).

We show that the behavior of serum FFA at baseline or during an IVGTT differs between severely obese patients and controls. All the severely obese patients had significantly higher fasting serum FFA concentrations than the controls. Additionally, differences were noted between the severely obese patients depending on their carbohydrate metabolism status. The severely obese patients with NFG before surgery had similar patterns of inhibition curve of lipolysis by insulin to the controls: similar $K_{FFA}$, similar duration of the first minutes of the IVGTT with constant FFA levels and similar time to minimum concentrations of FFA during the IVGTT. The patients with IFG had a greater time to minimum concentrations of FFA during the IVGTT. IFG represents a prediabetic state, intermediate between normal glucose tolerance and type 2 diabetes and with distinct metabolic abnormalities. Even though the NFG and the IFG patients had similar fasting levels of FFA, the DI and the $K_G$ were already significantly lower in the IFG than the NFG patients. Similar results have been found in women with gestational diabetes, especially those with significant β-cell dysfunction (17). The alteration in glucose homeostasis was especially obvious in severely obese persons with T2DM, as their $K_G$, SI, and DI were all clearly reduced in comparison with the severely obese persons with normal baseline glucose levels. In those persons with T2DM, the first phase lasted longer, the slope of the reduction in the FFA concentration ($K_{FFA}$) was less pronounced and the FFA levels took longer to recover.

Taken together, these results confirm that the reduction in the FFA concentration during an IVGTT was lower as glucose tolerance worsened, and suggest that the ability of insulin to inhibit lipolysis and reduce serum FFA concentrations is altered as glucose tolerance worsens, as was found in another study (22). Adipocyte insulin resistance in obese persons has recently been shown to be increased in IFG compared with NFG persons (23). The later rise in FFA levels during the IVGTT, even to values above baseline levels, has also been found in other studies (9,24).

Improvement in FFA concentration during an IVGTT with weight loss has also been shown with other types of treatment that improve SI (25). Our results confirm that bariatric surgery tends to normalize the DI, improving β-cell secretion and insulin resistance in patients with severe obesity. The mechanism by which weight loss surgery improves FFA and glucose metabolism and insulin resistance remains controversial. It has been suggested that improvements following bariatric surgery result in the short-term from decreased stimulation of the entero-insular axis by decreased caloric intake and in the long-term by decreased fat mass and resulting changes in the release of adipocytokines (26–28). Despite their great weight loss, the patients in this study were still obese (BMI > 30 $kg/m^2$) 7 months after surgery, and the FFA concentration, DI, and $K_G$ were still altered in those severely obese persons with T2DM. Our results suggest that these persons with T2DM probably require a longer period to fully recover SI and β-cell function. Nevertheless, all these obese patients are still in negative energy balance, and in response to carbohydrate restriction, a marked increase in nonoxidative glucose disposal may help maintain systemic glucose availability (29).

The fatty acid composition did not remain constant during the IVGTT. At the point where the concentration of FFA was minimum there was a reduction in the percentage of monounsaturated fatty acids and n-6 PUFA, whereas the percentage of saturated fatty acids and n-3 PUFA rose. As far as we are aware, this is the first report that during an intravenous glucose overload not only is there a fall in the amount of FFA but also a change in their composition. Although the *ex vivo* findings of Raclot *et al.* (30) indicate the possibility of selective release, Mittendorfer *et al.* (31) found selective changes in FFA release during insulin infusion that were relatively subtle, and were overshadowed by selective changes in clearance. They showed that total FFA levels cannot be representative of the individual dynamics of each individual fatty acid, at least during stimulated or inhibited lipolysis of adipose tissue (31). However, these changes may represent a phenomenon that has few implications in FFA flux or selective uptake. Furthermore, because n-3 PUFA concentrations are much lower than other circulating FFA, relative changes in concentrations of these fatty acids from fasting during IVGTT may have no biological relevance. These changes, which are related to class-specific aspects of cellular fatty acid transport and uptake, are generic and would be expected with any intervention that lowers FFA in any patient population. It should be emphasized that under steady-state conditions, even though there are changes in the relationship between the serum concentrations of different classes of FFA, the relationship between the fluxes of the fatty acids and between the rates of uptake of the fatty acids would not necessarily be expected to change. The observed changes are predictable and a further characterization of the phenomenon would require not only measurement of individual kinetics with tracers, but also careful measurement of concentrations using an internal standard in the analysis.

In summary, the results of this study in severely obese persons show that during an IVGTT there is a lower and more delayed fall of FFA concentrations, consistent with inhibition of lipolysis, as the baseline glucose levels increase. However, our study did not address the tissue(s) or the mechanisms responsible for the changes seen in FFA levels. The dynamic character of the concentration of FFA may have repercussions for the biological and clinical interpretation of the serum FFA levels. Caution should be exercised when choosing a particular fatty acid to

represent all the others in metabolic studies. Others studies with FFA tracers should be carried out to show the kinetics of the different fatty acids.

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## DISCLOSURE

The authors declared no conflict of interest.



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