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# Role of Adipose Tissue Insulin Resistance in the Natural History of Type 2 Diabetes: Results From the San Antonio Metabolism Study

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In the transition from normal glucose tolerance (NGT) to type 2 diabetes mellitus (T2DM), the role of  $\beta$ -cell dysfunction and peripheral insulin resistance (IR) is well established. However, the impact of dysfunctional adipose tissue has not been fully elucidated. The aim of this study was to evaluate the role of resistance to the antilipolytic effect of insulin (adipose tissue IR [Adipo-IR]) in a large group of subjects with NGT, impaired glucose tolerance (IGT), and T2DM. Three hundred two subjects with varying glucose tolerance received an oral glucose tolerance test (OGTT) and euglycemic insulin clamp. We evaluated Adipo-IR (fasting and mean OGTT plasma free fatty acid [FFA]  $\times$  insulin concentrations), peripheral IR ( $1/[\text{Matsuda index}]$  and  $(\text{M/I})^{-1}$  value), and  $\beta$ -cell function (calculated as the ratio of the increment in plasma insulin to glucose [ $\text{OGTT/IR } (\Delta\text{I}/\Delta\text{G} \div \text{IR})$ ]). Fasting Adipo-IR was increased twofold in obese subjects with NGT and IGT versus lean subjects with NGT ( $8.0 \pm 1.1$  and  $9.2 \pm 0.7$  vs.  $4.1 \pm 0.3$ , respectively) and threefold in subjects with T2DM ( $11.9 \pm 0.6$ ;  $P < 0.001$ ). Progressive decline in  $\Delta\text{I}/\Delta\text{G} \div \text{IR}$  was associated with a progressive impairment in FFA suppression during OGTT, whereas the rise in mean plasma glucose concentration only became manifest when subjects became overtly diabetic. The progressive decline in  $\beta$ -cell function that begins in individuals with NGT is associated with a progressive increase in FFA and fasting Adipo-IR.

Adipose tissue is an endocrine organ that influences both glucose and lipid metabolism (1,2) by releasing adipokines, proinflammatory factors, and free fatty acids (FFAs), which impair glucose metabolism and muscle ATP synthesis (3),

promote the synthesis of toxic lipid metabolites, and alter insulin signaling (4,5). Insulin acts on adipose tissue 1) by stimulating glucose uptake and triglyceride synthesis and 2) by suppressing triglyceride hydrolysis and release of FFA and glycerol into the circulation (6,7). Adipose tissue insulin resistance (Adipo-IR), that is, the impaired suppression of lipolysis in the presence of high insulin levels, has been associated with glucose intolerance, and elevated plasma FFA levels have been shown to impair muscle insulin signaling, promote hepatic gluconeogenesis, and impair glucose-stimulated insulin response (7–13). In obese subjects without diabetes and with type 2 diabetes mellitus (T2DM), subcutaneous adipose tissue is resistant to the antilipolytic effect of insulin. Insulin also is an adipogenic hormone that increases the uptake of circulating fatty acids and enhances triglyceride synthesis, thus stimulating the accumulation of subcutaneous fat as well as ectopic fat in liver, muscle, pancreas, heart, and other tissues (14–16). Although the role and natural history of  $\beta$ -cell dysfunction and muscle insulin resistance are well established in the development of T2DM, the impact of Adipo-IR in the transition from normal glucose tolerance (NGT) to T2DM has not been fully elucidated. The *in vivo* assessment of Adipo-IR is still controversial because many different approaches have been used to characterize Adipo-IR. By using tracers, it is possible to quantitate palmitate turnover (17,18) and the rate of glycerol release (19,20) to provide an index of lipolysis. Our group was one of the first to show that in man, the suppression of lipolysis and FFA release is related to plasma insulin concentration in a curvilinear fashion that becomes linear if logarithmically transformed (18). All studies agree that the relationship between the circulating plasma insulin concentration and both

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the lipolytic rate and the plasma FFA concentration becomes linear when plotted on a log-log scale (17,21–25). Thus, the product of the plasma FFA and insulin concentrations provides an index of Adipo-IR, and this index has been used to evaluate adipose tissue sensitivity to insulin in a variety of metabolic conditions (26–30). However, no study research to our knowledge has systematically evaluated adipose tissue sensitivity to insulin during the transition from NGT to impaired glucose tolerance (IGT) to T2DM in a large subject population.

The goal of the current study was to evaluate the impact of resistance to the antilipolytic effect of insulin during the natural history of T2DM (i.e., in a large group of subjects with NGT, IGT, and T2DM). To accomplish this objective, we have analyzed the data from subjects who participated in the San Antonio Metabolism (SAM) study in whom we measured plasma FFA levels during an oral glucose tolerance test (OGTT) and, on a separate day, peripheral insulin sensitivity by using the euglycemic-hyperinsulinemic clamp.

## RESEARCH DESIGN AND METHODS

### Subjects

The SAM study cohort comprised 302 subjects (35 lean and 30 obese with NGT, 44 with IGT, and 193 with T2DM) (31). All subjects were in good general health as judged by medical history, physical examination, and blood tests. Body weight was stable ( $\pm 3$  lb) over the preceding 3 months, and no subject participated in an excessively heavy exercise program. Subjects with NGT and IGT were not taking medications known to affect glucose tolerance. Subjects with T2DM taking sulfonylureas or metformin had their oral hypoglycemic agent discontinued 3 days before the study. No subject with diabetes had received treatment with a thiazolidinedione, glucagon-like peptide 1 receptor agonist, dipeptidyl peptidase 4 inhibitor, sodium-glucose cotransporter 2 inhibitor, or insulin. None of the subjects participated in any regular physical activity program. Obesity was defined as BMI  $>30$  kg/m<sup>2</sup> or body fat  $>35\%$  (measured by using tritiated water as previously described [31]). Patients with T2DM were divided into tertiles according to 2-h plasma glucose values (2h-PG): group I (2h-PG  $<300$  mg/dL), group II (2h-PG  $<360$  mg/dL), and group III (2h-PG  $\geq 360$  mg/dL). The study protocol was approved by the institutional review board of the University of Texas Health Science Center at San Antonio (San Antonio, TX), and informed written consent was obtained from each subject before participation.

### Study Protocol

At 8:00 A.M. after a 10-h overnight fast, all subjects received a 2-h 75-g OGTT with measurement of plasma glucose, insulin, and FFA concentrations at  $-30$ ,  $-15$ , and  $0$  min and then every 30 min after glucose ingestion. On a separate day after an overnight fast, subjects returned at 7:00 A.M. for a euglycemic insulin clamp (31). Catheters were placed in an antecubital vein for the infusion of all test substances and retrogradely into a vein on the dorsum of the hand for

blood withdrawal. The hand was placed in a heated box at 60°C. Two hours (3 h in T2DM) before the start of the insulin clamp, 3-<sup>3</sup>H-glucose (DuPont NEN, Boston, MA) was infused as a primed (40  $\mu$ Ci in subjects without diabetes and  $[\text{fasting plasma glucose (FPG)}/5.6] \times 40$   $\mu$ Ci in subjects with T2DM), continuous infusion (0.4  $\mu$ Ci/min) throughout the study as previously described (31).

### Analytical Methods

Plasma glucose concentration was determined by the glucose oxidase method (Beckman Glucose Analyzer; Beckman Coulter, Fullerton, CA). Plasma insulin and C-peptide concentrations were measured by radioimmunoassay with specific kits (Diagnostic Products Corporation, Los Angeles, CA), and plasma FFA levels were measured spectrophotometrically (Wako Chemicals, Neuss, Germany). Plasma 3-<sup>3</sup>H-glucose levels were measured in Somogyi precipitates as previously described (31). Plasma adiponectin concentration was measured by radioimmunoassay (Linco Research, St. Charles, MO).

### Data Analysis

Insulin sensitivity was assessed by Matsuda index (32) from the plasma glucose and insulin concentrations measured during the OGTT. Insulin sensitivity also was measured as the M/I value from the euglycemic-hyperinsulinemic clamp (31) in 235 subjects (17 of 34 lean subjects with NGT; 21 of 30 obese subjects with NGT; 39 of 44 subjects with IGT; and 53 of 65 subjects with T2DM in group I, 54 of 64 in group II, and 51 of 64 in group III). Insulin resistance was assessed as the inverse of insulin sensitivity. During the postabsorptive state, the rate of endogenous glucose production (EGP) equals  $R_g$  by all tissues in the body and is calculated as the tritiated glucose infusion (DPM/min) divided by the plasma tritiated glucose-specific activity (DPM/mg). After the start of insulin infusion, non-steady-state conditions prevailed, and  $R_a$  was calculated by using Steele's equation. Residual EGP was calculated as  $R_a$  minus the exogenous glucose infusion rate. To calculate total body  $R_d$ , the rate of residual EGP during the last 30 min of the insulin clamp was added to the exogenous glucose infusion rate required to maintain euglycemia during the last 30 min of the insulin clamp. Adipo-IR was calculated as the product of fasting plasma FFA and fasting plasma insulin concentrations and as the product of the mean plasma FFA and insulin concentrations during the OGTT.

$\beta$ -Cell function (insulin secretion/insulin resistance index  $[\Delta I/\Delta G \div IR]$ ) was calculated as the ratio of the incremental area under the curve (AUC) in plasma insulin to the incremental AUC in plasma glucose ( $\Delta AUC-I/\Delta AUC-G$ ) during OGTT divided by insulin resistance, measured as the inverse of the Matsuda index as previously reported (31,33).

### Statistical Analysis

Data are mean  $\pm$  SEM and presented as mean  $\pm$  SE. Non-normally distributed values were log-transformed before analysis.

Group values were compared by ANOVA and Bonferroni-Dunn post hoc analysis. Univariate associations were tested by Spearman rank correlation. Multivariable analysis tested

the association between  $\ln(\text{Adipo-IR})$  (dependent variable) and age, BMI, sex, presence of diabetes, insulin sensitivity ( $\ln[\text{Matsuda index}]$ ), insulin secretion ( $\Delta\text{AUC-I}/\Delta\text{AUC-G}$ ), and  $\beta$ -cell function ( $\Delta\text{AUC-I}/\Delta\text{AUC-G} \div \text{IR}$ ) as independent variables.

## RESULTS

The clinical characteristics of the study subjects are shown in Table 1. Subjects with diabetes were slightly older than those without diabetes but had a similar BMI and percent body fat to obese subjects with NGT and IGT. In subjects without diabetes (NGT and IGT), the FPG concentrations were within the normal range and increased progressively in subjects with T2DM in groups I–III (Table 1). Similarly, the mean plasma glucose concentrations measured during the OGTT increased progressively in T2DM groups I–III (Figs. 1B and 2A).

The fasting plasma FFA concentrations were significantly higher in obese NGT and IGT than in lean NGT. The fasting plasma FFA concentration was the lowest in lean NGT, increased markedly and linearly from obese NGT to IGT, and plateaued without further increase in T2DM (Table 1). During the OGTT, the mean plasma FFA concentration was significantly increased in obese NGT versus lean NGT and rose progressively from NGT to IGT (Fig. 1E) to T2DM in groups I–III (Fig. 1F) with no evidence of

plateau (Fig. 2B). This pattern closely followed the change in Matsuda index of insulin sensitivity and insulin secretion, reflecting the progressive declines in insulin sensitivity (Fig. 2C) and  $\beta$ -cell function (Table 1).

The fasting plasma insulin concentration progressively increased from NGT to IGT (Fig. 1C) to T2DM (Fig. 1D), whereas the mean plasma insulin concentration during OGTT showed the typical inverted U-shaped curve (34), increasing from NGT to IGT and then decreasing progressively in T2DM in groups I–III (Fig. 2D).

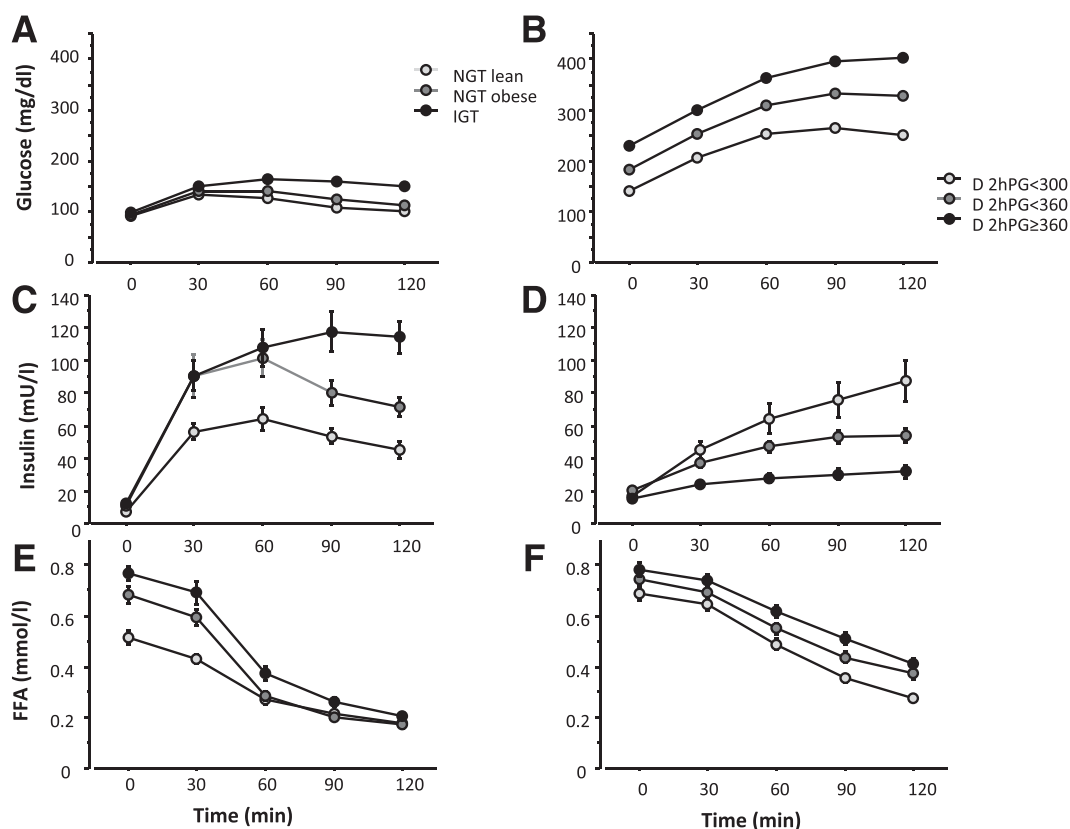
The progressive impairment in FFA suppression during OGTT was strongly correlated with the progressive decline in  $\beta$ -cell function ( $\Delta\text{AUC-I}/\Delta\text{AUC-G} \div \text{IR}$ ) ( $r = -0.52$ ;  $P < 0.0001$ ) (Fig. 3) and with the progressive decline in insulin sensitivity measured as the M-value during the insulin clamp ( $r = -0.42$ ;  $P < 0.0001$ ). In marked contrast, the rises in FPG and mean plasma glucose during the OGTT only became pronounced when subjects became overtly diabetic (Figs. 1 and 2).

Thus, as lean subjects with NGT became obese (but still maintained NGT) or IGT or T2DM, both the fasting plasma and OGTT FFA (as well as glucose) concentrations increased (Table 1 and Fig. 2A and B), which was explained by the progressive decline in insulin sensitivity (Fig. 2C), insulin response during the OGTT (Fig. 2D), and reduced  $\beta$ -cell function (Table 1 and Fig. 3).

**Table 1—Clinical characteristics of the study subjects**

	NGT		IGT	T2DM		
	Lean	Obese		Group I	Group II	Group III
<i>n</i>	34	30	44	65	64	64
Sex						
Female	14	24	26	36	28	36
Male	20	6	18	29	36	28
Age (years)	40 ± 2	38 ± 2	41 ± 2	52 ± 1*§	53 ± 1*§	51 ± 1*§
Weight (kg)	73 ± 2	79 ± 3*	84 ± 3*	86 ± 2*	90 ± 2*§	82 ± 2*
BMI (kg/m <sup>2</sup> )	25.0 ± 0.4	30.5 ± 0.8*	31.2 ± 0.9*	31.5 ± 0.7*	32.3 ± 0.7*	30.8 ± 0.6*
Percent fat	30 ± 1	39 ± 1*	38 ± 1*	38 ± 1*	38 ± 1*	38 ± 1*
Triglycerides (mg/dL)	144 ± 29	97 ± 9	153 ± 17§	145 ± 10§	172 ± 10§	157 ± 16§
Total cholesterol (mg/dL)	178 ± 10	167 ± 7	190 ± 7§	180 ± 4	176 ± 4	179 ± 4
LDL (mg/dL)	108 ± 8	105 ± 6	118 ± 6	112 ± 4	106 ± 4	112 ± 4
HDL (mg/dL)	42 ± 3	42 ± 2	41 ± 2	39 ± 1	37 ± 1	40 ± 2
HbA <sub>1c</sub> (%)	5.1 ± 0.1	5.3 ± 0.2	5.5 ± 0.1	7.3 ± 0.2*§	8.3 ± 0.2*§	9.2 ± 0.2*§
Fasting glucose (mg/dL)	92 ± 1	95 ± 1	98 ± 1*§	141 ± 4*§	183 ± 4*§	229 ± 5*§
2-h glucose (mg/dL)	100 ± 3	112 ± 2*	149 ± 3*§	250 ± 4*§	329 ± 2*§	402 ± 4*§
Fasting insulin (mU/L)	7.2 ± 0.5	11 ± 1.2*	12.5 ± 0.9*	16.7 ± 1.4*§	20.6 ± 1.6*§	15.1 ± 1.1*§
Fasting FFA (mmol/L)	0.51 ± 0.03	0.69 ± 0.03*	0.77 ± 0.03*	0.69 ± 0.03*	0.74 ± 0.02*	0.78 ± 0.03*§
2-h FFA (mmol/L)	0.18 ± 0.01	0.17 ± 0.01	0.21 ± 0.02	0.28 ± 0.01*§	0.37 ± 0.02*§	0.41 ± 0.02*§
Clamp FFA (mmol/L)	0.22 ± 0.03	0.20 ± 0.2	0.24 ± 0.03	0.23 ± 0.02	0.25 ± 0.01	0.22 ± 0.01
$\Delta\text{AUC-I}/\Delta\text{AUC-G}$	251 ± 47	282 ± 37	162 ± 14*§	48 ± 7*§	22 ± 2*§	10 ± 1*§
$\Delta\text{I}/\Delta\text{G} \div \text{IR}$	1,500 ± 281	1,137 ± 238*	415 ± 25*§	87 ± 7*§	33 ± 2*§	17 ± 1*§

Data are mean ± SE. Subjects with T2DM were grouped according to tertiles of 2h-PG concentrations (i.e., group I: 2h-PG <300 mg/dL; group II: 2h-PG <360 mg/dL; group III: 2h-PG ≥360 mg/dL). \* $P < 0.05$  vs. lean subjects with NGT; § $P < 0.05$  vs. obese subjects with NGT.



**Figure 1**—Changes in plasma glucose (A), insulin (C), and FFA (E) concentrations during the OGTT in subjects without diabetes. Changes in plasma glucose (B), insulin (D), and FFA (F) concentrations during the OGTT in subjects with T2DM. D, diabetic.

### Adipo-IR Index

Fasting Adipo-IR increased twofold in obese NGT and IGT versus lean NGT ( $8.0 \pm 1.1$  and  $9.2 \pm 0.7$  vs.  $4.1 \pm 0.3$ ; both  $P < 0.001$ ) and threefold in T2DM ( $P < 0.001$ ) (Fig. 2E). Fasting Adipo-IR was significantly greater in obese T2DM versus lean T2DM ( $13.8 \pm 0.8$  vs.  $8.0 \pm 0.7$ ;  $P < 0.001$ ) and inversely correlated with  $\beta$ -cell function, indicating that high fasting plasma FFA concentrations and impaired FFA suppression were mainly a result of insulin secretion deficiency (Fig. 3). This finding was confirmed by analysis of the euglycemic-hyperinsulinemic clamp data (Table 1) showing that the plasma FFA concentrations at the end of the hyperinsulinemic clamp were similar in all study groups.

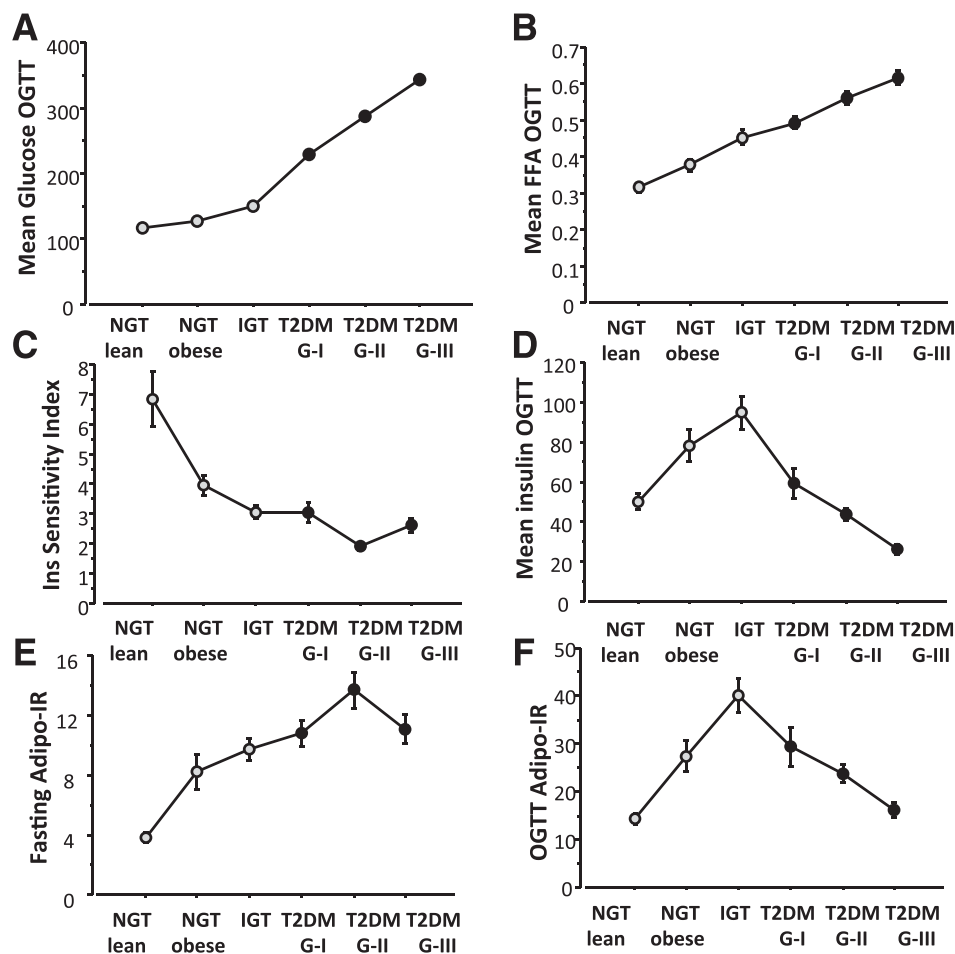
Although fasting Adipo-IR rose continuously in the transition from NGT to IGT to T2DM (Fig. 2E), OGTT Adipo-IR increased from lean NGT to obese NGT to IGT and then decreased progressively in T2DM groups I–III (Fig. 2F), following the U-shaped insulin response curve during the OGTT (Fig. 2D). Thus, markedly deficient insulin secretion during the OGTT results in a paradoxical decline in OGTT Adipo-IR in T2DM (Fig. 2D and F), making the OGTT Adipo-IR unreliable in subjects with diabetes.

In a multivariable regression analysis (with age, BMI, sex, presence of diabetes, insulin sensitivity [ $\ln(\text{Matsuda index})$ ], insulin secretion [ $\Delta\text{AUC-I}/\Delta\text{AUC-G}$ ], and  $\beta$ -cell function [ $\Delta\text{AUC-I}/\Delta\text{AUC-G} \div \text{IR}$ ] as independent variables),

$\ln(\text{Adipo-IR})$  (dependent variable) was found to be independently correlated (total  $r = 0.81$ ;  $P < 0.0001$ ) with BMI ( $P < 0.0001$ ), insulin sensitivity ( $P < 0.0001$ ), and male sex ( $P < 0.0001$ ). By performing the same analysis separately in males and females, we found that in both females (total  $r = 0.82$ ;  $P < 0.0001$ ) and males (total  $r = 0.80$ ;  $P < 0.0001$ ), the association holds with BMI ( $P < 0.0001$ ) and insulin sensitivity ( $P < 0.0001$ ). In a subgroup of 54 subjects for whom plasma was available, adiponectin correlated negatively with  $\ln(\text{Adipo-IR})$  independently of age, sex, and BMI ( $r = -0.49$ ;  $P = 0.009$ ).

### DISCUSSION

We previously have shown that during the transition from NGT to IGT to T2DM,  $\beta$ -cell function progressively declines and peripheral insulin resistance progressively increases (31,35). Adipo-IR also is increased in patients with T2DM, but the natural history of its development as individuals progress from NGT to IGT to T2DM has been poorly studied. As recently reviewed (21), a number of indices of adipocyte insulin resistance have been proposed that are based on tracer turnover (i.e., labeled palmitate or glycerol) or FFA suppression during insulin infusion (euglycemic-hyperinsulinemic clamp) or OGTT. In the current study, we used the product of fasting plasma FFA and fasting plasma insulin concentrations as the index



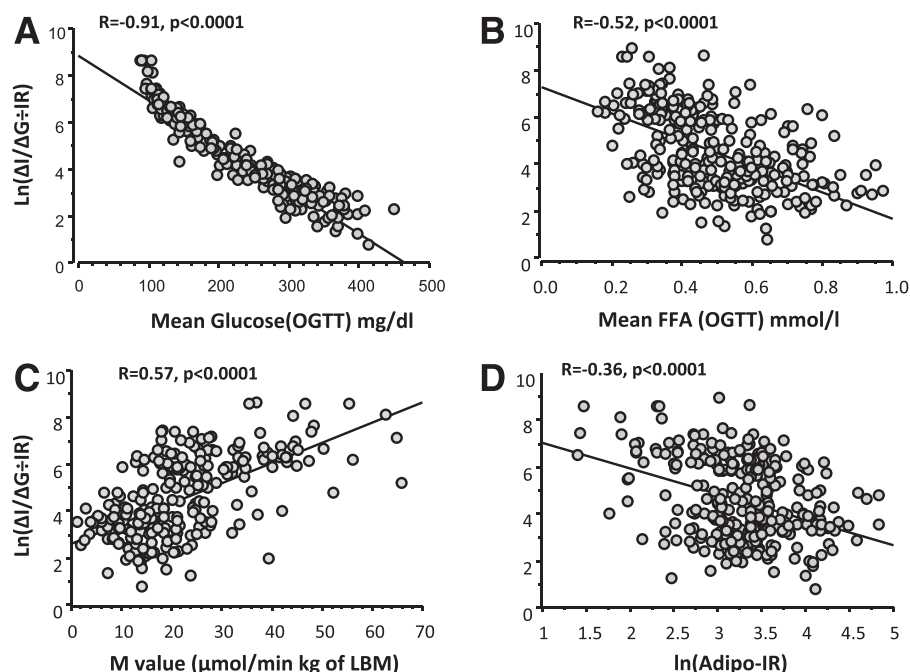
**Figure 2**—Mean plasma glucose (A), FFA (B), and insulin (D) concentrations during OGTT; Matsuda index of insulin sensitivity (C); and fasting (E) and OGTT (F) Adipo-IR index. The figure demonstrates that as lean subjects with NGT become obese (but still maintain NGT) or IGT or T2DM, not only glucose but also FFA concentrations increased during fasting and OGTT, which is explained by the progressive decline in the insulin secretion/insulin resistance index. G, group; Ins, insulin.

of Adipo-IR. Because the circulating plasma FFA concentration closely reflects the rate of peripheral lipolysis, Adipo-IR represents an index for adipose tissue resistance to the antilipolytic effect of insulin. The hyperbolic relationship between plasma insulin and FFA concentrations initially was demonstrated by Groop et al. (18) who examined the relationship between insulin and the inhibition of plasma FFA concentration and rate of lipolysis (measured by  $^{14}\text{C}$ -palmitate) during a five-step euglycemic-hyperinsulinemic clamp. Similar results were reported by Bugianesi et al. (26) who measured the plasma FFA concentration and rate of lipolysis (by  $^2\text{H}$ -glycerol turnover) during a two-step hyperinsulinemic-euglycemic clamp. However, the number of subjects in these previous studies was small, and the changes in Adipo-IR in NGT to IGT to T2DM was not evaluated.

In this cross-sectional study, we evaluated changes in plasma FFA concentration during the fasting state and OGTT in subjects across a wide range of glucose tolerance and insulin resistance. Subjects with insulin resistance (ranging from obese NGT to IGT and T2DM) were compared with lean subjects with NGT. The fasting plasma FFA

concentration increased markedly and linearly as subjects progressed from lean NGT to obese NGT and IGT and plateaued without further increase in T2DM, reflecting the progressive decline in insulin sensitivity. The increase in plasma FFA concentration during the OGTT tracks with worsening whole-body insulin resistance and worsening Adipo-IR during the OGTT over the range of NGT to IGT. With progression of IGT to T2DM, the plasma FFA concentration during the OGTT continues to rise, whereas whole-body insulin resistance plateaus and OGTT Adipo-IR declines. Thus, as we previously have shown, patients with IGT are maximally/near maximally insulin resistant with respect to glucose metabolism, and the rise in fasting plasma FFA concentrations closely follows the increase in insulin resistance. Further progression from NGT to IGT is associated with parallel increases in fasting plasma FFA and worsening whole-body insulin resistance and Adipo-IR. As IGT progresses to T2DM, no further increase in fasting plasma FFA occurs, even though Adipo-IR continues to worsen. A likely explanation is the observation that the fasting plasma insulin concentration increases sufficiently to offset the worsening of





**Figure 3**—Progressive decline in insulin secretion/insulin resistance index was associated with an increase in mean plasma glucose concentration during OGTT (A) and mean plasma FFA concentration (B), a decline in insulin sensitivity (C), and an increase in fasting Adipo-IR (D).  $\Delta I/\Delta G \div IR$ ,  $\Delta AUC-I/\Delta AUC-G \div IR$ ; LBM, lean body mass.

adipocyte insulin resistance. FFA levels during the OGTT continued to rise in all groups without reaching a plateau (as was observed with the fasting plasma FFA concentration) primarily because of  $\beta$ -cell dysfunction and reduced insulin secretion in subjects with T2DM, thus following the Starling curve of the pancreas (34). BMI and insulin sensitivity (Matsuda index) were strongly related to Adipo-IR in both males and females, although Adipo-IR was greater in females than in males. This finding agrees with a previous study by Nielsen et al. (36) who found increased plasma FFA concentrations in females compared with males. The current results, however, are cross-sectional, but they are consistent with those of two prospective studies (e.g., Metabolic Syndrome in Men [METSIM] and Actos Now for the Prevention of Diabetes [ACT NOW]) that showed a significant association between the fasting plasma FFA concentration or Adipo-IR and incidence of T2DM (37,38).

Therefore, the results of the current analysis show that the fasting Adipo-IR index remains a reliable index of insulin resistance in the fat cell over the entire range of glucose tolerance from NGT to IGT to T2DM. In contrast, a different picture is observed with Adipo-IR during the OGTT. This dissociation between impaired FFA suppression during the OGTT (i.e., higher plasma FFA levels) and Adipo-IR is explained by the marked decline in insulin secretion (as a result of  $\beta$ -cell dysfunction) during the OGTT as subjects progressed from IGT to T2DM (groups I–III). Thus, Adipo-IR during the OGTT does not provide a reliable index of adipocyte insulin resistance in patients with T2DM. Caution is needed when using the Adipo-IR index in patients

with severe fasting hyperglycemia in whom marked insulin deficiency may be present. In contrast to the OGTT, studies with the stepped hyperinsulinemic clamp consistently have demonstrated impaired suppression of plasma FFA and glycerol concentrations and  $^{14}\text{C}$ -palmitate turnover (18,22,24) (i.e., adipocyte insulin resistance) in subjects with T2DM.

The results shown in Figs. 2 and 3 emphasize the importance of distinguishing between insulin secretion and  $\beta$ -cell sensitivity to glucose (i.e., the  $\beta$ -cell sensitivity-secretion relationship) and the critical role of the latter in determining not only overall glucose tolerance but also plasma FFA concentration. Thus, with progression from lean NGT to obese NGT to IGT, the insulin secretion/insulin resistance (disposition) index declined and was closely related to the deterioration in OGTT, consistent with previous studies from our group and others (31,34,39,40). This point is evident if one plots the log of the insulin secretion/insulin resistance index against the mean plasma glucose and mean plasma FFA concentrations during the OGTT for the entire subject population (NGT, IGT, T2DM). These variables are strongly and inversely related. A decrease in  $\beta$ -cell secretion of insulin also is associated with an increase in fasting Adipo-IR.

In this analysis, we compared lean subjects with NGT with obese subjects with NGT, IGT, and T2DM who were not only more insulin resistant but also more obese on average. Subjects with T2DM also were slightly older than those with NGT and IGT. The difference in weight and age could influence FFA levels. The greater amount of fat in subjects with insulin resistance is likely to contribute to the higher

plasma FFA levels. Older age also influences body composition because older patients usually have more fat than younger patients with the same BMI. However, the effect of aging on insulin action is small, as shown by Ferrannini et al. (41).

In summary, the results demonstrate that the fasting adipocyte insulin resistance index (fasting FFA  $\times$  fasting insulin) rises progressively over the span of glucose tolerance, ranging from NGT to IGT to T2DM, and provides a valid index of fat cell sensitivity to insulin. In contrast, the adipocyte insulin resistance index during OGTT rises from NGT to IGT and declines with progression of IGT to T2DM because of the progressive deficiency of insulin secretion in the group with diabetes. Thus, in the subjects with diabetes, the fasting but not the OGTT adipocyte insulin resistance index provided a reliable measure of fat cell sensitivity to insulin. In conclusion, the progressive decline in  $\beta$ -cell function that begins in individuals with NGT is associated with a progressive increase in FFA and fasting Adipo-IR.

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**Author Contributions.** A.G. and R.A.D. contributed to the study design and data analysis and wrote the manuscript. M.G. contributed to the data analysis, discussion, and writing of the manuscript. All authors reviewed the manuscript before submission. A.G. and R.A.D. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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