Role of Adipose Tissue Insulin Resistance in the Natural History of T2DM: Results from the San Antonio Metabolism Study

Amalia Gastaldelli, Melania Gaggini and Ralph A DeFronzo

1. University of Texas Health Science Center at San Antonio
2. Cardiometabolic Risk Laboratory, Institute of Clinical Physiology, National Research Council, Pisa, Italy;

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Address all correspondence to

Amalia Gastaldelli, PhD

Research Director of Cardiometabolic Risk Laboratory- Institute of Clinical Physiology, CNR

via Moruzzi 1, Pisa, Italy, 56124

Phone +39 0503152680/79

Fax +39 0503152166

E-mail: amalia@ifc.cnr.it

Adjunct Associate Professor

University of Texas Health Science Center

Medicine/Diabetes Division

San Antonio, TX, USA.

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ABSTRACT

In the transition from normal glucose tolerance (NGT) to type 2 diabetes mellitus (T2DM), the role of β-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 the present study is to evaluate the role of resistance to the antilipolytic effect of insulin (Adipo-IR) in a large group of NGT, IGT and T2DM subjects.

302 subjects with varying glucose tolerance received OGTT and euglycemic insulin clamp. We evaluated: Adipo-IR (fasting and mean OGTT plasma FFA x insulin), peripheral IR (1/(Matsuda index) and $(M/I)^{-1}$), β-cell function (calculated as the ratio of the increment in plasma insulin to glucose (OGTT) divided by IR [ΔI/ΔG÷IR]).

Fasting Adipo-IR was increased 2-folds in obese-NGT and IGT vs lean-NGT (8.0±1.1 and 9.2±0.7 vs 4.1±0.3) and 3-fold in T2DM (11.9±0.6 $p<0.001$). Progressive decline in ΔI/ΔG÷IR was associated with a progressive impairment in FFA suppression during OGTT, while the rise in mean plasma glucose concentration only became manifest when subjects became overtly diabetic.

In conclusion, the progressive decline in β-cell function that begins in "normal" glucose tolerant individuals is associated with a progressive increase in FFA and fasting Adipo-IR.

Introduction

Adipose tissue is an endocrine organ that influences both glucose and lipid metabolism (1; 2) by releasing adipokines, proinflammatory factors and free fatty acids (FFA) 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 (i) by stimulating glucose uptake and triglyceride synthesis and (ii) by suppressing triglyceride hydrolysis and release of FFA and glycerol into the circulation (6; 7). Adipose tissue insulin resistance (Adipo-IR), i.e., the impaired suppression of lipolysis in the presence of high insulin levels, has been associated with glucose intolerance, and elevated plasma FFA have been shown to impair muscle insulin signaling, promote hepatic gluconeogenesis, and impair glucose-stimulated insulin response (7-13). In obese nondiabetic and type 2 diabetic subjects subcutaneous adipose tissue is resistant to the anti-lipolytic effect of insulin. Insulin also is an adipogenic hormone which increases the uptake of circulating fatty acids and enhances triglyceride (TG) synthesis, thus stimulating the accumulation of subcutaneous fat, as well as ectopic fat in liver, muscle, pancreas, heart and other tissues (14-16). While the role and natural history of β-cell dysfunction and muscle insulin resistance (IR) are well established in the development of type 2 diabetes mellitus (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 since many different approaches have been used to characterize adipose tissue insulin resistance. 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 the plasma insulin concentration in a curvilinear fashion, that, if logarithmically transformed, becomes linear (18). All studies agree that the relationship between the circulating plasma insulin concentration and both the lipolytic rate and plasma FFA concentration becomes linear when plotted on a log-log scale (17; 21-25). Thus, the product of the plasma FFA x insulin concentrations provides an index of adipose tissue insulin resistance, and this index has been used in variety of metabolic conditions to evaluate adipose tissue sensitivity to insulin (26-30). However, no study systematically has 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 this 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 we have analyzed the data from subjects who participated in the San Antonio Metabolism study (SAM) in whom we measured plasma FFA levels during an OGTT and on a separate day peripheral insulin sensitivity using the euglycemic hyperinsulinemic clamp.

Materials and methods

Subjects

The study cohort consisted of 302 subjects (35 lean and 30 obese NGT; 44 IGT; 193 T2DM) who participated in the San Antonio Metabolism study (31). All subjects were in good general health as judged by medical history, physical exam, and screening blood tests. Body weight was stable (±3 lbs) over the preceding 3 months and no subject participated in an excessively heavy exercise program. NGT and IGT subjects were not taking any medications known to affect glucose tolerance. T2DM subjects taking sulfonylureas or metformin had their oral hypoglycemic agent discontinued 3 days before the study. No diabetic subject had received treatment with a thiazolidinedione, GLP-1 recpetor agonist, DPP4 inhibitor, SGLT2 inhibitor or insulin. None of the subjects participated in any regular physical activity program. Obesity was defined as BMI >30 or percent body fat >35% (measured using tritiated water as previously described) (31). T2DM patients were divided in tertiles according to 2h-PG values, i.e. Group I (GI, 2h-PG<300), Group II (G2, 2h-PG<360), Group III (G3, 2h-PG≥360). The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and informed written consent was obtained from each subject prior to their participation.

Study protocol

At 8AM following a 10-hour overnight fast, all subjects received a 2-hour 75 gram OGTT with measurement of plasma glucose, insulin, and FFA concentrations at -30, -15, 0 minutes and every 30 minutes after glucose ingestion. On a separate day, following an overnight fast, subjects returned to the CRC at 7AM 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 into a heated box at 60^{o}C. Two hours (3 hours in T2DM) prior to the start of the insulin clamp, 3-^{3}H-glucose (DuPont-NEN, Boston, Mass.,

USA) was infused as a primed (40 uCi in non-diabetic subjects and [FPG/5.6]* 40 uCi in T2DM) - continuous infusion (0.4 uCi/min) throughout the study as previously described (31).

Analytical Methods

Plasma glucose concentration was determined by the glucose oxidase method (Beckman Glucose Analyzer, Fullerton, Calif., USA). Plasma insulin and C-peptide concentrations were measured by radioimmunoassay using specific kits (DPC, Los Angeles, Calif., USA) and plasma FFA were measured spectrophotometrically (Wako, Neuss, Germany). Plasma 3-[3H]glucose levels were measured in Somogyi precipitates as previously described (31). Plasma adiponectin concentration was measured by radioimmunoassay (Linco Research, St Charles, MO, USA).

Data analysis

Insulin sensitivity was assessed using Matsuda index (32) from the plasma glucose and insulin concentrations during the OGTT. Insulin sensitivity also was measured as M/I value from the euglycemic hyperinsulinemic clamp (31) in 235 subjects (17/34 NGT lean, 21/30 NGT obese, 39/44 IGT, 53/65 G1, 54/64 G2 and 51/64 G3 T2DM). Insulin resistance (IR) was assessed as the inverse of insulin sensitivity. During the postabsorptive state, the rate of endogenous glucose production equals the rate of glucose uptake 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). Following the start of insulin infusion, non-steady state conditions prevail and rate of glucose appearance (Ra) was calculated using Steele's equation. Residual EGP was calculated as the Ra of glucose minus the exogenous glucose infusion rate. To calculate total body glucose metabolic rate (Rd) the rate of residual EGP during the last 30 minutes of the insulin clamp was added to the exogenous glucose infusion rate required to maintain euglycemic during the last 30 minutes of the insulin clamp. Adipose tissue IR (Adipo-IR) was calculated as the product of fasting plasma FFA x fasting plasma insulin concentrations and as the product of the mean plasma FFA and insulin concentrations during the OGTT.

β-cell function (insulin secretion/insulin resistance index or ΔI/ΔG÷IR) was calculated as the ratio of the incremental AUC in plasma insulin to the incremental AUC in plasma glucose (ΔAUC-I/ΔAUC-G) during OGTT divided by IR, measured as the inverse of Matsuda index, as previously reported (31; 33).

Statistical analysis

Data are given as the mean ± sem. All data are presented as the means±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 with the use of Spearman rank correlation. Multivariable analysis tested the association between ln(Adipo-IR) (dependent variable) and age, BMI, gender, presence of diabetes, insulin sensitivity (ln[Matsuda index]), insulin secretion (Δ AUC-I/Δ AUC-G) and beta cell function ((Δ AUC-I/Δ AUC-G ÷ IR) as independent variables.

Results

The clinical characteristics of the study subjects are shown in **Table 1**. Diabetic patients were slightly older than non-diabetic subjects, but had a similar BMI and % fat to obese NGT and IGT subjects. In non-diabetic subjects (NGT and IGT) the fasting plasma glucose concentrations were within the normal range and increased progressively in T2DM subjects in Groups I, II, and III (**Table 1**). Similarly, the mean plasma glucose concentrations measured during the OGTT increased progressively in T2DM subjects in Groups I, II, and III **(Figure 1B and Figure 2A).**

The fasting plasma FFA concentrations were significantly higher in obese NGT and IGT subjects compared to lean NGT. The fasting plasma FFA concentration was the lowest in lean NGT and increased markedly and linearly from obese NGT to IGT and plateaued without further increase in T2DM subjects (**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 (**Figure 1A**) to T2DM in groups I, II, and III (**Figure 1B**), with no evidence of plateau (**Figure 2B**). This pattern closely followed the change in Matsuda index of insulin sensitivity and insulin secretion, reflecting the progressive declines in insulin sensitivity (**Figure 2C)** and beta cell function (**Table 1**).

The fasting plasma insulin concentration progressively increased from NGT to IGT **(Figure 1C)** to T2DM **(Figure 1D),** while the mean plasma insulin concentration during OGTT showed the typical inverted U-shaped curve (34), increasing from NGT to IFG and then decreasing progressively in T2DM individuals from Group I to II to III (**Figure 2D**).

The progressive impairment in FFA suppression during OGTT was strongly correlated with the progressive decline in beta cell function (Δ AUC-I/Δ AUC-G ÷ IR) (r =-0.52, p<0.0001) (**Figure 3**) and with the progressive decline in insulin sensitivity measured as M value during the insulin clamp (r = -0.42, p< 0.0001). In marked contrast, the rise in fasting plasma glucose and mean plasma glucose during the OGTT only became pronounced when subjects became overtly diabetic (**Figures 1 and 2**).

Thus, as lean NGT subjects become obese (but still maintain NGT) or IGT or T2DM, both the fasting plasma and OGTT FFA (as well as glucose) concentrations increased (**Table 1, Figure 2A and 2B**) and this was explained by the progressive decline in insulin sensitivity **(Figure 2C)**, insulin response during the OGTT (**Figure 2D**), and reduced beta cell function (**Table 1, Figure 3**).

Adipose Tissue IR index

Fasting Adipo-IR increased 2-fold in obese NGT and IGT versus lean NGT (8.0±1.1 and 9.2±0.7 vs 4.1±0.3 both p<0.001) and 3-fold in T2DM (p<0.001, **Figure 2E**). Fasting Adipo-IR was significantly greater in obese T2DM versus lean T2DM (13.8±0.8 vs 8.0±0.7, p<0.001) and inversely correlated with beta cell function indicating that high fasting plasma FFA concentrations and impaired FFA suppression were due mainly to deficiency of insulin secretion. (**Figure 3**). This was confirmed by analysis of the euglycemic hyperinsulinemic clamp data (**Table 1**) that showed that the plasma FFA concentrations at the end of the hyperinsulinemic clamp were similar in all study groups.

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

In a multivariable regression analysis (with age, BMI, gender, presence of diabetes, insulin sensitivity (ln[Matsuda index]), insulin secretion (Δ AUC-I/Δ AUC-G) and beta cell function ((Δ AUC-I/Δ AUC-G ÷ IR) as independent variables) ln(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 gender (males) (p<0.0001). By performing the same analysis separately in males and females we found that both in females (total r=0.82, p<0.0001) and in 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 on whom plasma was available, adiponectin correlated negatively with ln(Adipo-IR) independently of age, gender and BMI (r = -0.49, p = 0.009).

Discussion

We previously have shown that during the transition from "normal" glucose tolerance to IGT to T2DM there is a progressive decline in beta cell function and a progressive increase in peripheral insulin resistance (31; 35). Adipose tissue insulin resistance also is increased in T2DM individuals, but the natural history of development of Adipo-IR, 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, based on tracer turnover (i.e., labeled palmitate or glycerol) or on free fatty acid suppression during insulin infusion (euglycemic hyperinsulinemic clamp) or OGTT. In the present study we used the product of fasting plasma FFA x fasting plasma insulin concentrations as the index of Adipo-IR. Since 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 demostrated 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}C palmitate) during a 5 step hyperinsulinemic euglycemic clamp. Similar results were reported by Bugianesi et al (26) who measured the plasma FFA concentration and rate of lipolysis (by ^{2}H glycerol turnover) during a 2 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 the plasma FFA concentration during the fasting state and during the 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 NGT. The fasting plasma FFA concentration increased markedly and linearly as subjects progressed from lean NGT to obese NGT, to IGT and plateaued without further increase in T2DM subjects, 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, IGT subjects are maximally/near maximally insulin resistant with respect to glucose metabolism and the rise in fasting plasma FFA concentration closely followed 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 there is no further increase in fasting plasma FFA even though Adipo-IR continues to worsen. This most likely is explained by the observation that the fasting plasma insulin concentration increases sufficiently to offset the worsening of 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 T2DM subjects, thus following the Starling's curve of the pancreas (34). BMI and insulin sensitivity (Matsuda index) were strongly related to Adipo-IR and this was true in both males and females although Adipo-R was greater in females compared to males; this finding is in agreement with a previous by Nielsen et al who found increased plasma FFA concentrations in females compared to males (36). It should be noted that our results are cross sectional, but they are consistent with those of two prospective studies, e.g. METSIM and ACT NOW, that showed a significant association between the fasting plasma FFA concentration or Adipo IR and the incidence of T2DM (37; 38).

Therefore, the results of our analysis show that 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 very 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 (due to beta cell dysfunction) during the OGTT as subjects progress from IGT to T2DM (groups I-III). Thus, Adipo-IR during the OGTT does not provide a reliable index of adipocyte insulin resistance in T2DM subjects. Caution should be used when utilizing the Adipo-IR index in subjects 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}C-palmitate turnover (18; 22; 24), i.e. adipocyte insulin resistance in T2DM subjects.

The results displayed in **Figure 2 and 3** emphasize the importance of distinguishing between insulin secretion and beta cell sensitivity to glucose (ie the β-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 clearly evident if one plots the log of the insulin secretion/insulin resistance index against the mean plasma glucose concentration and the mean plasma FFA concentration during the OGTT for the entire subject population (NGT, IGT, T2DM). These variables are strongly and inversely related. A decrease in β-cell secretion of insulin also is associated with an increase in fasting Adipo-IR.

In this analysis we compared lean NGT with obese NGT, IGT and T2DM subjects who were, not only more insulin resistant, but more obese on average. T2DM subjects also were slightly older than NGT and IGT subjects. The difference in weight and age could influence FFA levels. The greater amount of fat in IR subjects is likely to contribute to the higher plasma FFA levels. Older age also influences body composition, since older subjects usually have more fat than younger subjects with the same BMI. However, the effect of ageing on insulin action is small, as shown by Ferrannini et al (41).

In summary, the present results demonstrate that the fasting adipocyte insulin resistance index (fasting FFA x 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 the OGTT rises from NGT to IGT and declines with progression of IGT to T2DM due to the progressive deficiency of insulin secretion in the diabetic group. Thus, in diabetic subjects only the fasting, not the OGTT, adipocyte insulin resistance index provides a reliable measure of fat cell sensitivity to insulin.

In conclusion, the progressive decline in β-cell function that begins in “normal” glucose tolerant individuals is associated with a progressive increase in FFA and fasting Adipo-IR.

AUTHOR CONTRIBUTIONS:

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. A.G. and R.A.D., study design, data analysis and wrote of the manuscript; M.G. data analysis, contributed to discussion and writing of the manuscript. All authors reviewed the manuscript prior to submission.

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DISCLOSURE

A.G., M.G. and R.A.D., report no conflict of interest for the work presented in this manuscript.

FIGURE LEGEND

Figure 1 – Left panel (non-diabetic subjects): Changes in plasma glucose (Panel A), insulin (Panel C), and FFA (Panel E) concentrations during the OGTT in non-diabetic subjects (light grey: lean NGT, dark grey: obese NGT, black: IGT subjects). Right panel (T2DM subjects): Changes in plasma glucose (Panel B), insulin (Panel D), and FFA (Panel F) concentrations during the OGTT in non-diabetic subjects (light grey: lean NGT, dark grey: obese NGT, black: IGT subjects).

Figure 2 – Mean plasma glucose (Panel A) and FFA (Panel B), insulin (Panel D) concentrations during OGTT, Matsuda index of insulin sensitivity (Panel C), and fasting (Panel E) and OGTT (Panel F) Adipo-IR index. The figure demonstrates that as NGT lean subjects become obese but still NGT, IGT and T2DM, not only glucose but also FFA concentrations, increase during both the fasting and OGTT and this was explained by the progressive decline in insulin secretion/insulin resistance index.

Figure 3 – Progressive decline in Insulin secretion/Insulin resistance index was associated with an increase in mean plasma glucose concentration during OGTT (Panel A), mean plasma FFA concentration (Panel B), decline in insulin sensitivity (Panel C), and increase in fasting Adipo-IR (Panel D).

Table 1 – Clinical characteristics of the study subjects

	NGT		IGT	T2DM		
	Lean	Obese		Group I	Group II	Group III
Number	34	30	44	65	64	64
F/M	14/20	24/6	26/18	36/29	28/36	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^2)	25.0±0.4	30.5±0.8*	31.2±0.9*	31.5±0.7*	32.3±0.7*	30.8±0.6*
% Fat	30±1	39±1*	38±1*	38±1*	38±1*	38±1*
Triglyceride (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
HbA1c (%)	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*§
2h 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*§
2h 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
ΔAUC-I/ΔAUC-G	251±47	282±37	162±14*§	48±7*§	22±2*§	10±1*§
ΔI/ΔG÷IR	1500±281	1137±238*	415±25*§	87±7*§	33±2*§	17±1*§

* p<0.05 vs NGT lean subjects, § p<0.05 vs NGT obese subjects. T2DM were grouped according to tertiles of 2h-PG concentrations, i.e., Group I: 2-PG<300 mg/dl; Group II: 2-PG<360 mg/dl; Group III: 2-PG≥360mg/dl

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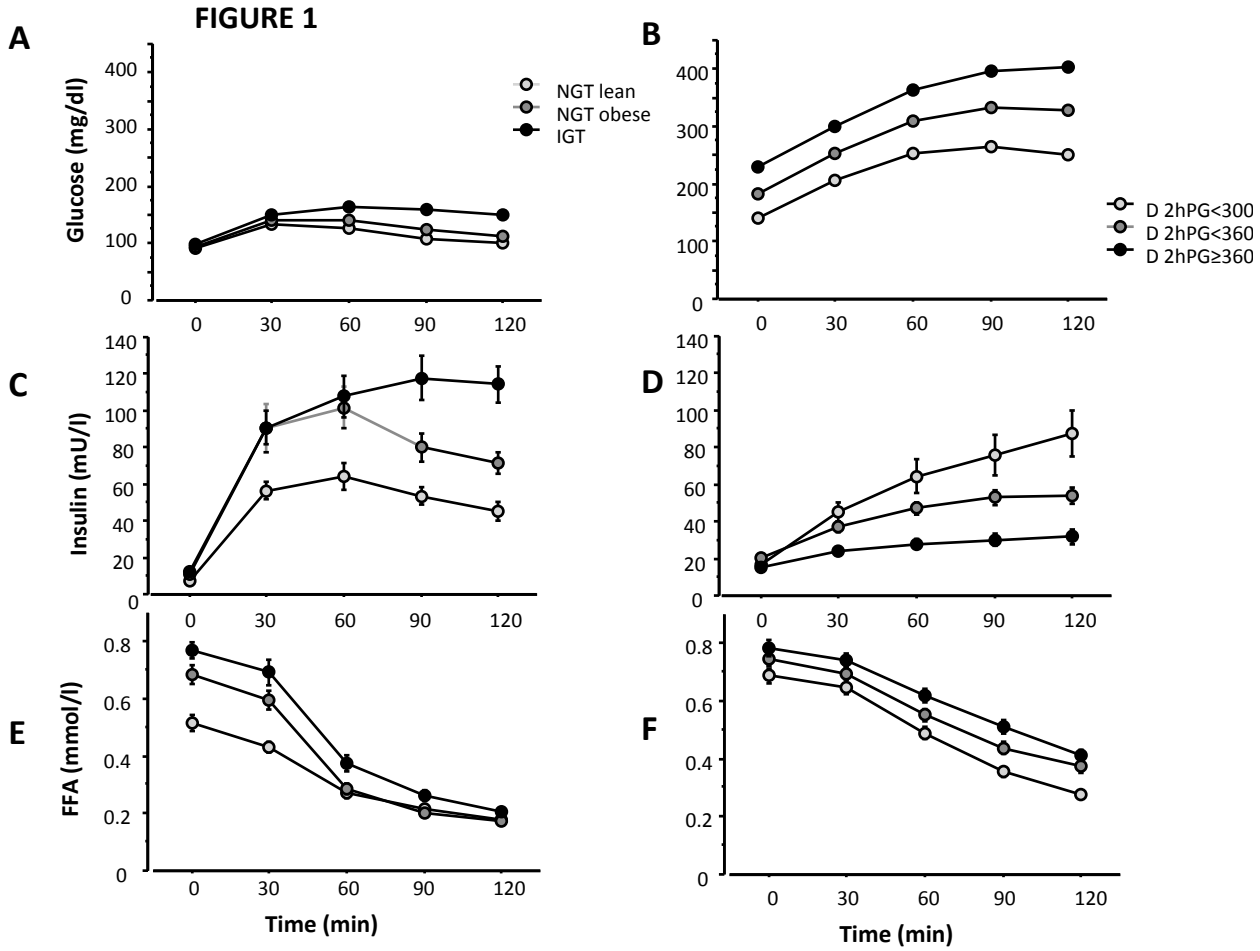

Figure 1 – Left panel (non-diabetic subjects): Changes in plasma glucose (Panel A), insulin (Panel C), and FFA (Panel E) concentrations during the OGTT in non-diabetic subjects (light grey: lean NGT, dark grey: obese NGT, black: IGT subjects). Right panel (T2DM subjects): Changes in plasma glucose (Panel B), insulin (Panel D), and FFA (Panel F) concentrations during the OGTT in non-diabetic subjects (light grey: lean NGT, dark grey: obese NGT, black: IGT subjects).

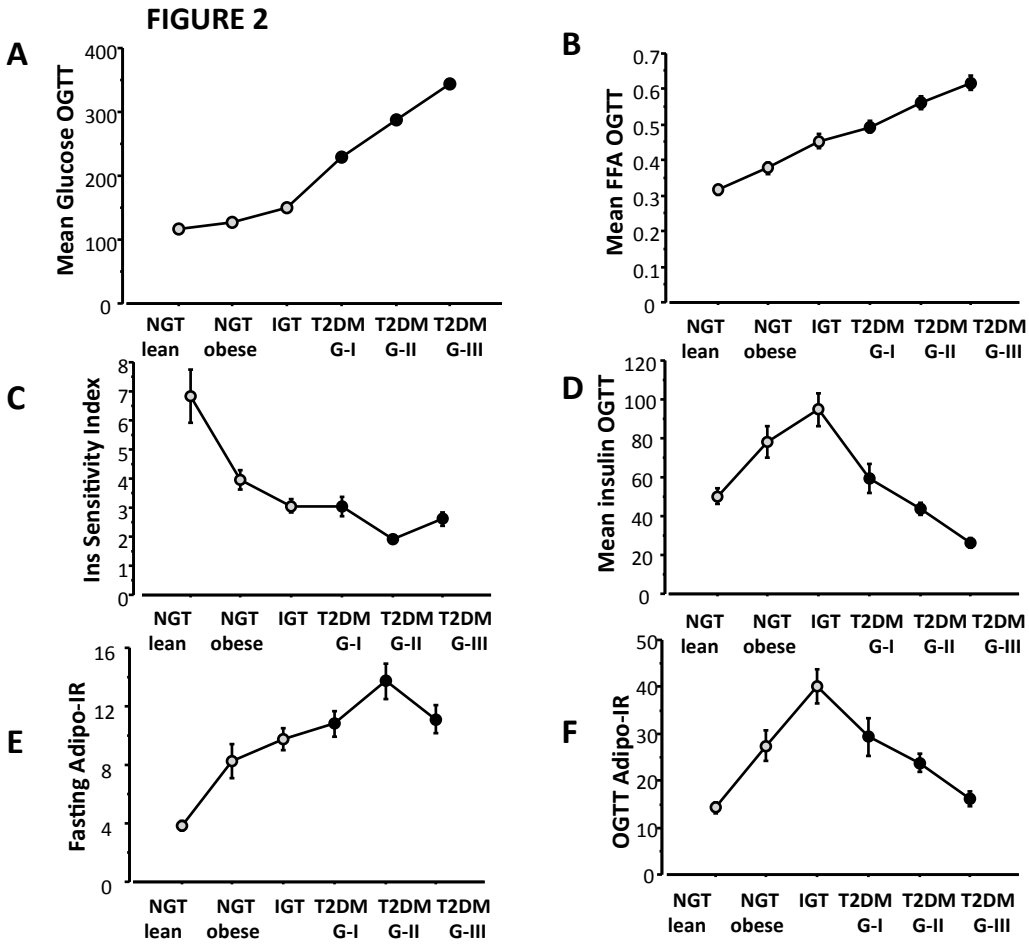

Figure 2 – Mean plasma glucose (Panel A) and FFA (Panel B), insulin (Panel D) concentrations during OGTT, Matsuda index of insulin sensitivity (Panel C), and fasting (Panel E) and OGTT (Panel F) Adipo-IR index. The figure demonstrates that as NGT lean subjects become obese but still NGT, IGT and T2DM, not only glucose but also FFA concentrations, increase during both the fasting and OGTT and this was explained by the progressive decline in insulin secretion/insulin resistance index.

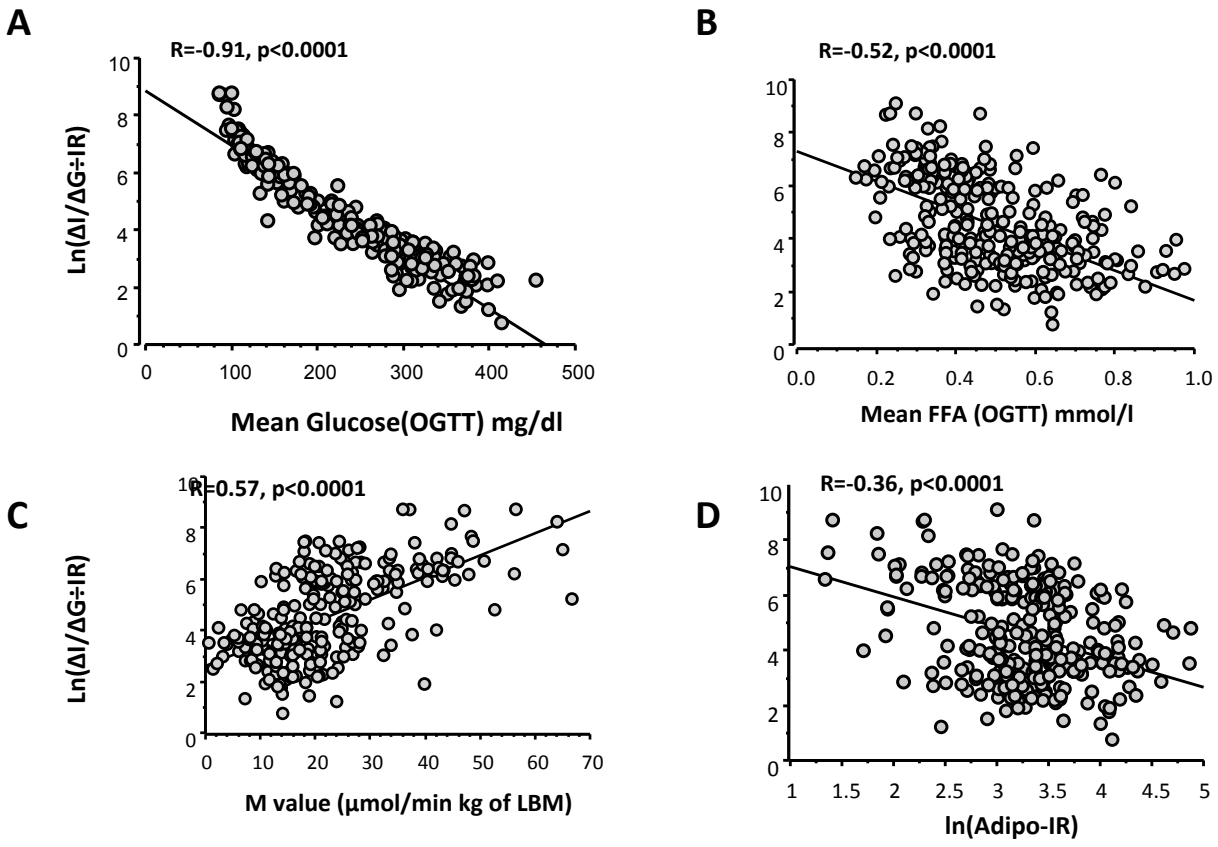

Figure 3 – Progressive decline in Insulin secretion/Insulin resistance index was associated with an increase in mean plasma glucose concentration during OGTT (Panel A), mean plasma FFA concentration (Panel B), decline in insulin sensitivity (Panel C), and increase in fasting Adipo-IR (Panel D).