

***In Vivo* Insulin Sensitivity and Secretion in Obese Youth: What are the Differences between NGT, IGT and Type 2 Diabetes?**

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Objective: Impaired glucose tolerance (IGT) represents a prediabetic state. Controversy continues in regards to its pathophysiology. The aim of this study was to investigate the differences in insulin sensitivity (IS) and secretion in obese adolescents with IGT, compared to those with normal glucose tolerance (NGT) and type 2 diabetes (T2DM).

Research Design and Methods: Twelve obese adolescents with NGT, 19 with IGT and 17 with T2DM underwent evaluation of IS (3-hr hyperinsulinemic (80 $\mu\text{u}/\text{m}^2/\text{min}$)–euglycemic clamp), 1st and 2nd phase insulin (1stPI, 2ndPI) secretion (2-hr hyperglycemic clamp); body composition and abdominal adiposity. Glucose disposition index (GDI) was calculated as the product of 1st PI x IS.

Results: Insulin-stimulated glucose disposal was significantly lower in T2DM compared with NGT and IGT with no difference between the latter two. However, compared with NGT, youth with IGT have significantly lower 1st PI and C-peptide levels, and lower GDI ($p=0.012$) while youth with T2DM have an additional defect in 2nd PI. Fasting and 2-hr glucose correlated with GDI ($r=-0.68$, $p<0.001$ and $r=-0.73$, $p<0.001$) and 1st PI but not with IS.

Conclusion: Compared with NGT youth, obese adolescents with IGT have evidence of β -cell defect manifested in impaired 1st PI secretion with a more profound defect in T2DM involving both 1st and 2nd PI. GDI shows a significantly declining pattern, highest in NGT, intermediate in IGT and lowest in T2DM. Such data suggest that measures to prevent progression or conversion from pre-diabetes to T2DM should target improvement in β -cell function.

Impaired glucose tolerance (IGT) is a condition of altered glucose homeostasis associated with a high risk of progression to T2DM in adults (1) and children (2). The prevalence of IGT in children varies depending on the population studied with rates varying from 4.1-4.5% in children recruited from the community (3,4) up to 25% in youth from an obesity clinic (5). Also, 28% of high risk Latino children, with positive family history of T2DM, have IGT (6). Therefore, against the backdrop of the obesity epidemic, IGT constitutes a significant problem in youth especially those from minority ethnic populations and those with family history of T2DM. However, the pathophysiology of IGT in children is not well understood.

In longitudinal studies of adult populations at high risk for T2DM, such as the Pima Indians (7), the progression from normal glucose tolerance (NGT) to IGT and T2DM was associated with an increase in body weight, worsening of insulin sensitivity, and a decrease in the biphasic insulin secretion (7,8). Longitudinal studies are not available in the pediatric age group. Studies in pediatrics using different methodologies have shown conflicting results. Obese children and adolescents with IGT compared with NGT, were reported to have higher BMI, worse fasting indices of insulin resistance but insulin secretion was estimated to be similar between the 2 groups (5). In overweight Latino children with family history of T2DM, insulin sensitivity and acute insulin response were not different but glucose disposition index was lower in IGT (6). In our previous study of obese adolescent girls with polycystic ovary syndrome (PCOS), IGT vs NGT, of similar body composition and abdominal fat distribution, had similar insulin sensitivity, but lower 1st phase insulin secretion and lower glucose disposition index (9). In the present study, we aimed to extend our previous

observation and to investigate the differences in insulin sensitivity and insulin secretion not only between NGT and IGT but also between IGT and T2DM. We hypothesized that 1) insulin sensitivity (IS) is not significantly different between equally obese youth with IGT vs NGT; and 2) insulin secretion is impaired in IGT and T2DM compared with NGT with a severity gradient from IGT to T2DM.

RESEARCH DESIGN AND METHODS

Study Population—Twelve obese adolescents with NGT, 19 with IGT and 17 with T2DM, African American (AA) and American Whites (AW) were studied. All subjects had exogenous obesity and were not involved in any regular physical activity or weight reduction programs. They were recruited through flyers posted in the community and the health center. The NGT and IGT adolescents had normal fasting glucose (<100 mg/dl), with a 2hr glucose value during an oral glucose tolerance test (OGTT) of <140 mg/dl in NGT and 140-199 mg/dl in IGT. They were not on any medications that affect glucose metabolism. The adolescents with T2DM were clinically diagnosed according to ADA and WHO criteria (10), with mean HbA_{1c} (10.1±3.0%) and glucose level (277.2±158.2 mg/dl) at presentation, and negative glutamic acid decarboxylase (GAD) and islet cell (ICA) autoantibodies. They were all in adequate metabolic control with average HbA_{1c} 6.6±0.2% (range: 4.7-8.3%) and average duration of diabetes of 10.3 months (range: 0-18 months). They were on treatment with lifestyle changes alone (n=3), metformin (n=6), metformin+insulin (n=7), or insulin alone (n=1). Metformin and long acting insulin were discontinued 48 hrs before the clamp studies. All studies were approved by the Institutional Review Board of the University of Pittsburgh. Informed consent

was obtained. Characteristics of the study participants are summarized in Table 1.

Clamp Studies—Participants were admitted twice within a 1-3 week period to the Pediatric Clinical and Translational Research Center (PCTRC) the day before the clamp studies, once for a hyperinsulinemic-euglycemic clamp and the other time for a hyperglycemic clamp study in random order. Each participant underwent a 2-hr OGTT (1.75 g/kg of glucola (max 75 g)) the day prior to the first clamp.

In-vivo insulin sensitivity—A fasting blood sample was obtained for determination of cholesterol, LDL, HDL, VLDL, TG and HbA1c. Fasting endogenous glucose production was measured with a primed constant rate infusion of [6, 6-²H₂] glucose (0.306±0.009 μmol/kg/min) (Isotech, Miamisburg, OH) (11, 12). Blood was sampled at the start of the 2-hr stable isotope infusion and every 10 min from -30 to 0 time (basal period) for determination of plasma glucose, insulin, and isotopic enrichment of glucose. Following this basal period, insulin-mediated glucose metabolism and substrate utilization were evaluated during a 3-h hyperinsulinemic-euglycemic clamp (11, 12). Intravenous crystalline insulin (Humulin; Lilly Indianapolis, IN) was infused at a constant rate of 80 μU/m²/min and plasma glucose was clamped at 5.6 mmol/l with a variable rate infusion of 20% dextrose as before (11). Continuous indirect calorimetry (Deltatrac Metabolic Monitor, SensorMedics, Anaheim, CA) was used to measure CO₂ production, O₂ consumption and respiratory quotient for 30 minutes at baseline and at the end of the euglycemic clamp (12).

In-vivo insulin secretion—First and second phase insulin secretion was evaluated during a 2-h hyperglycemic clamp (12.5 mmol/l) as previously described (11).

Body Composition—Body composition was determined by dual energy X-ray absorptiometry (DEXA) scan, and

subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) by a single slice CT scan at L₄-L₅ as before (11).

Biochemical Measurements—Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, Ohio), insulin, C-peptide and adiponectin by radioimmunoassay (RIA) as before (11). HbA1c was measured by high performance liquid chromatography (Tosoh Medics, Inc. 1998) and lipids using the standards of the Centers for Disease Control and Prevention (11). Deuterium enrichment of glucose in the plasma was determined on a Hewlett-Packard Co. 5973 mass spectrometer (Palo Alto, CA) coupled to a 6890 gas chromatograph (11, 12).

Calculations—Fasting hepatic glucose production (HGP) was calculated during the last 30 min of the 2-hr isotope infusion according to steady-state tracer dilution equations (11). Insulin stimulated glucose disposal rate (R_d) was calculated during the last 30 minutes of the euglycemic clamp to be equal to the rate of exogenous glucose infusion. Peripheral IS was calculated by dividing the R_d by the steady-state clamp insulin level (11). Insulin-stimulated carbohydrate oxidation rates were calculated according to the formulas of Frayn (12) from the indirect calorimetry data. Non-oxidative glucose disposal was estimated by subtracting the rate of glucose oxidation from the total R_d. During the hyperglycemic clamp, the first and second phase insulin and c-peptide concentrations were calculated as described previously (11, 12). Glucose disposition index (GDI) was calculated as the product of IS x 1st phase insulin.

Statistics—Statistical analyses were performed using 3 way analysis of variance (ANOVA) followed by post-hoc Bonferroni or Dunnett's correction. Kruskal-Wallis test was used for multiple group comparison of non-parametric variables. Spearman's correlation and multiple regression analyses

were used to evaluate bivariate and multivariate relationships, respectively, and chi-square to evaluate non-parametric variables. Data are presented as mean±SD. Two-tailed p-value ≤ 0.05 was considered statistically significant.

RESULTS

Study Subjects (Table 1): Table 1 depicts the physical characteristics and fasting metabolic profile of the participants (NGT vs IGT vs T2DM). There were no significant differences in age, pubertal stage or ethnic distribution among the 3 groups. There were no significant differences in body composition or abdominal fat distribution among the 3 groups.

Fasting Metabolic Profile (Table 1): There were no significant differences in fasting lipids and insulin levels among the 3 groups. Fasting glucose and HbA1c were significantly higher in T2DM subjects compared with the 2 other groups. HGP was 20% higher in subjects with T2DM compared to the NGT group ($p=0.078$). The HbA1c didn't differ among the 4 groups of T2DM on different treatments (HbA1c $6.5\pm 0.5\%$ in the lifestyle alone, $7.0\pm 0.7\%$ in the Metformin alone, $6.7\pm 1.1\%$ in the Metformin+insulin, and 6.9% in the insulin alone).

Clamp Data (Figure 1 and table 2): During the hyperinsulinemic-euglycemic clamp steady-state glucose and insulin levels were not different among the 3 groups (NGT, IGT, T2DM, Glucose: 5.6 ± 0.08 , 5.6 ± 0.10 , 5.6 ± 0.13 mmol/l; insulin 2020.8 ± 587.2 , 1737.6 ± 508.9 and 1791.6 ± 674.2 pmol/l, respectively). Insulin stimulated total and oxidative glucose disposal were not different between NGT and IGT but were significantly lower in T2DM (Figure 1-A). Non-oxidative glucose disposal tended to be lower in T2DM compared with NGT and IGT ($p=0.08$) (Figure 1-A). The data remained consistent when Rd was expressed per kg fat-free mass ($\mu\text{mol}/\text{min}/\text{kg}$ FFM) ($p=0.006$).

During the hyperglycemic clamp, the IGT adolescents had lower 1st phase insulin and C-peptide levels compared with NGT subjects, with no difference in second phase (Table 2, Figure 1B). T2DM adolescents had lower first and second phase insulin and C-peptide levels compared with NGT and a tendency for lower 2nd PI ($p=0.07$) and lower 2nd phase c-peptide ($p=0.012$) compared with IGT (Table 2, Figure 1-B). GDI was significantly lower in IGT compared with NGT and lowest in T2DM (Figure 1-C). GDI didn't differ in the T2DM subjects in the four treatment groups (0.5 ± 0.3 mmol/kg/min in the lifestyle, 0.6 ± 0.4 mmol/kg/min in the Metformin alone, 0.6 ± 0.3 mmol/kg/min in the Metformin+insulin and 0.6 mmol/kg/min in the insulin alone groups).

Relationship between OGTT indices of glucose tolerance and clamp data: 2-hr glucose level during the OGTT correlated with GDI ($r=-.73$, $p<0.001$), 1st phase ($r=-.69$, $p<0.001$) and second phase ($r=-.59$, $p<0.001$) insulin but not with insulin sensitivity ($r=-.13$, $p=.4$) (Figure 2). In multiple regression analysis with 2 hr glucose post OGTT as the dependent variable and VAT, GDI and 2nd phase insulin as the independent variables, GDI ($\beta=-.54$, $p=0.001$) but not second phase insulin nor VAT contributed significantly to the variance in the 2hr glucose ($R^2= 0.44$, $p<0.001$). Similarly, fasting glucose correlated with GDI ($r=-.68$, $p<0.001$), 1st ($r=-.61$, $p<0.001$) and 2nd phase ($r=-.53$, $p<0.001$) insulin levels but not with insulin sensitivity ($r=-.2$, $p=0.3$).

DISCUSSION

The present investigation demonstrates that IGT in youth is characterized by impaired insulin secretion relative to insulin sensitivity. The GDI is lowest in youth with T2DM, intermediate in IGT and highest in NGT. Compared with NGT, glucose disposition index is ~40% lower in prediabetes and 80% lower in

T2DM. While insulin secretion is impaired in IGT, insulin stimulated glucose disposal is not different from NGT. In youth with T2DM, the impairment in β -cell function is of greater magnitude and involves in addition second phase insulin secretion. The current study adds to the limited existing literature by: a) providing a comparison between three groups of equally obese adolescents (NGT, IGT and T2DM) of similar BMI, pubertal stage, body composition and abdominal fat distribution; and b) providing information on in-vivo insulin sensitivity and secretion measured simultaneously by the clamp methodology.

Impaired glucose tolerance is a well known prediabetic state with a linear relationship between the 2hr post challenge glucose levels and subsequent risk for T2DM in adult prospective studies (1) with observed rates of progression from IGT to T2DM from 20% (13) to 60% (14,15) over an average duration of 2 to 8 years (1). Impaired glucose tolerance seems amenable to intervention with prevention of T2DM reported in many randomized control trials with lifestyle intervention being at least as effective if not more than pharmacotherapy (16). Therefore it is important to identify and characterize the pathophysiological mechanism(s) underlying IGT in youth, in an effort to provide targeted intervention and prevention of progression to T2DM.

Our current findings are consistent with data in adults with IGT where longitudinal studies point to the deterioration in insulin secretion relative to IS in the transition from NGT to IGT to T2DM (8). Pima Indians with isolated IGT had a modest decrease in acute insulin response (AIR), measured by intravenous glucose tolerance test (IVGTT) that was significant given increased insulin resistance in IGT compared with NGT subjects (17). However, in these adult studies, the decrease in IS in subjects with IGT was attributed to aging over the 5 years of the study (8) or to higher BMI in the

subjects with IGT (17). In our study NGT and IGT groups had comparable obesity and body fat distribution. Furthermore, in Pima Indians a greater defect in AIR was found in those who subsequently developed T2DM (7). Similarly, in Mexican Americans, the 7 year risk of progression to T2DM was significantly higher in IGT vs NGT (OR=9.4), and both decreased insulin secretion (determined by $\Delta I_{30}/\Delta 30$) and insulin resistance independently predicted the progression to T2DM (18). These adult longitudinal studies support the role of impaired beta-cell function in the risk of progression from NGT to IGT to T2DM. Studies in pediatrics examining impaired glucose tolerance have been few and somewhat contradictory.

Higher insulin resistance, measured by the hyperinsulinemic-euglycemic clamp method, was reported in adolescents with IGT compared to NGT controls with no significant differences in insulin secretion (19). However, the group with IGT had significantly higher visceral to subcutaneous abdominal fat ($p=0.002$). We previously demonstrated that higher visceral fat is associated with lower insulin sensitivity in obese insulin resistant youth (12). Thus, the lower IS in IGT in the former study could be related to the higher visceral fat. In another study, the same group reported that IGT is characterized by a decline in AIR based on OGTT data (20). In that study, the IGT children were heavier and had significantly higher BMI z-score than NGT children but abdominal adiposity was not evaluated (20). However, when they evaluated NGT, IGT and T2DM subjects of similar BMI and % body fat, using mathematical modeling of the hyperglycemic clamp data, the glucose sensitivity of first phase insulin secretion declined from NGT to IGT and from IGT to type 2 DM (although absolute insulin levels did not) (21). Also, recently they reported decreased glucose sensitivity of 1st phase insulin secretion in IGT vs NGT subjects,

consistent with our findings (22). The different findings in these studies could be attributed to different methodologies used, and the differences in BMI and body composition between the NGT and IGT groups. Data in high risk overweight Latino children were consistent with our present observations. NGT and IGT subjects of similar body composition and abdominal fat distribution had similar IS but subjects with IGT had relative insulin deficiency with significantly lower GDI than NGT (6). Lastly, the current findings confirm our previous observations in PCOS girls (9).

With regards to T2DM, the impairment in first phase insulin secretion is of greater magnitude than that in IGT with the added dysfunction in second phase insulin. In T2DM, first phase insulin is ~70% lower than NGT and ~40% lower than IGT. Second phase insulin is ~60% lower in T2DM than NGT but preserved in IGT. This is consistent with our previous report of decreased 2nd phase insulin levels in T2DM vs obese controls (23) and with the findings of Weiss et al. of decreased glucose sensitivity of 2nd phase insulin in T2DM subjects (20).

Hepatic glucose production was higher in T2DM compared with NGT. This is consistent with our previous report of increased HGP in T2DM youth compared to obese controls (23) and with adult data suggesting that increased endogenous glucose production contributes to fasting hyperglycemia (17).

Finally, our data demonstrate that first phase insulin and GDI are significant determinants of measures of glycemic regulation, including fasting glucose and 2hr glucose during the OGTT. These findings are in agreement with our findings in girls with PCOS (9) and with the adult literature as to the determinants of the glycemic status in subjects with IGT albeit all the variables were not measured simultaneously in these subjects (24,25).

A potential limitation of the current study is that the IGT subjects were compared to T2DM children with different treatment modalities and diabetes duration. However, data analysis in the T2DM subjects separately did not show any significant differences in HbA_{1c} or GDI in the 4 treatment groups. Also, the majority of these adolescents (12 out of 17) were studied within 6 months of diagnosis of T2DM with no significant difference in GDI or HbA_{1c} when evaluated according to duration of DM and no relationship between duration of diabetes and HbA_{1c}, consistent with our previous findings (23). The other limitation of the relatively smaller sample size (NGT=12) is offset by the use of the clamp methodology which allowed us to demonstrate significant differences among the three groups.

In summary, our findings demonstrate that prediabetes or IGT is an intermediate stage in the impairment of β -cell function relative to insulin resistance with T2DM having a more pronounced defect in 1st and 2nd phase insulin secretion. Such data suggest that measures to prevent conversion/progression of IGT to T2DM should target recovery of β -cell function besides improvement in obesity and insulin resistance. The ultimate objective is restoration of glucose homeostasis through improved balance between insulin sensitivity and secretion.

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FIGURE LEGENDS

Figure 1: A) Insulin stimulated total, oxidative and non-oxidative glucose disposal in NGT (white bars), IGT (dashed bars), and T2DM (black bars). B) First and second phase insulin levels in NGT (white triangles), IGT (grey squares), and T2DM (black circles). C) Glucose disposition index in NGT, IGT, and T2DM. Error bars reflect standard error (SE).

Figure 2: Relation of first phase insulin and GDI to the 2-hr glucose during the OGTT in NGT (triangles), IGT (squares), and T2DM (circles).

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Table 1: Physical characteristics and fasting metabolic profile in adolescents with normal glucose tolerance (NGT), impaired glucose tolerant (IGT), and type 2 diabetes (T2DM).

	NGT (n=12)	IGT (n=19)	T2DM (n=17)
Age (years)	14.2±2.2	13.8±1.5	14.7±1.3
Gender (M/F)*	4M / 8 F	6M / 13F	7M / 10F
Ethnicity:* AA	5	5	7
AW	7	14	10
Tanner Stage* II – III	4	3	2
IV – V	8	16	15
Estradiol in females (pmol/l)	232.7±171.1	294.8±211.8	222.8±161.9
DHEAS (nmol/l)			
Females	3669.28±759.2	4108.8±3001.4	3687.0±1902.0
Males	3659.9±1366.0	5415.0±3085.7	4139.9±2332.0
BMI (kg/m ²)	36.0±5.2	35.0±6.6	36.3±5.3
Waist circumference (cm)	108.5±18.9	104.3±14.2	107.9±11.8
% Body Fat	45.4±4.7	44.3±4.3	41.0±6.8
Fat Mass (kg)	40.0±6.9	40.7±10.9	40.1±10.5
Subcutaneous Abdominal Fat (cm ²)	545.7±168.6	501.6±145.7	520.1±152.4
Visceral Fat (cm ²)	75.8±48.3	72.1±25.1	78.7±25.2
HbA1c (%)	5.2±0.5 ^a	5.3±0.4 ^b	6.8±0.8
Fasting glucose (mmol/l)	5.1±0.02 ^a	5.1±0.2 ^b	6.6±1.4
Fasting insulin (pmol/l)	252.6±95.1	240.0±130.7	274.2±142.0
Hepatic glucose production (µmol/kg/min)	10.5±1.9	12.8±3.5	13.3±2.3
Cholesterol (mmol/l)	4.6±0.9	4.3±0.8	3.9±0.8
HDL (mmol/l)	1.1±0.3	1.0±0.3	0.9±0.2
LDL (mmol/l)	2.9±0.9	2.6±0.7	2.3±0.6
Triglycerides (mmol/l)	1.5±0.6	1.8±1.0	1.4±0.8

Superscripts are ANOVA p-values for post-Hoc analysis: a: p<0.05 in NGT vs T2DM, b: p<0.05 in IGT vs T2DM. * The x² analysis revealed no significant differences among groups with respect to ethnicity, gender and tanner stage. Four IGT subjects had VAT and SAT evaluation by abdominal MRI. Excluding these subjects from the analysis does not change the data.

Table 2: Hyperglycemic clamp data in the three groups.

	NGT (n=12)	IGT (n=19)	T2DM (n=17)	ANOVA p-value
1 st phase insulin (pmol/l)	2376.0±1729.9 ^{a,b}	1182.0±625.2	708.0±938.4	0.001
1 st phase c-peptide (nmol/l)	4.3±2.0 ^b	3.0±1.0	2.2±1.4	0.001
2 nd phase insulin (pmol/l)	2563.2±1363.2 ^b	1902.6±1336.2	982.2±797.0	0.003
2 nd phase c-peptide (nmol/l)	5.0±1.5 ^b	4.7±1.9 ^c	3.0±1.1	0.002

Superscripts are ANOVA p-values for post-Hoc analysis (Bonferroni correction): a: p<0.05 in NGT vs IGT, b: p<0.05 in NGT vs T2DM, c: p<0.05 in IGT vs T2DM. C-peptide levels were not available in 3 subjects with IGT.

Figure 1

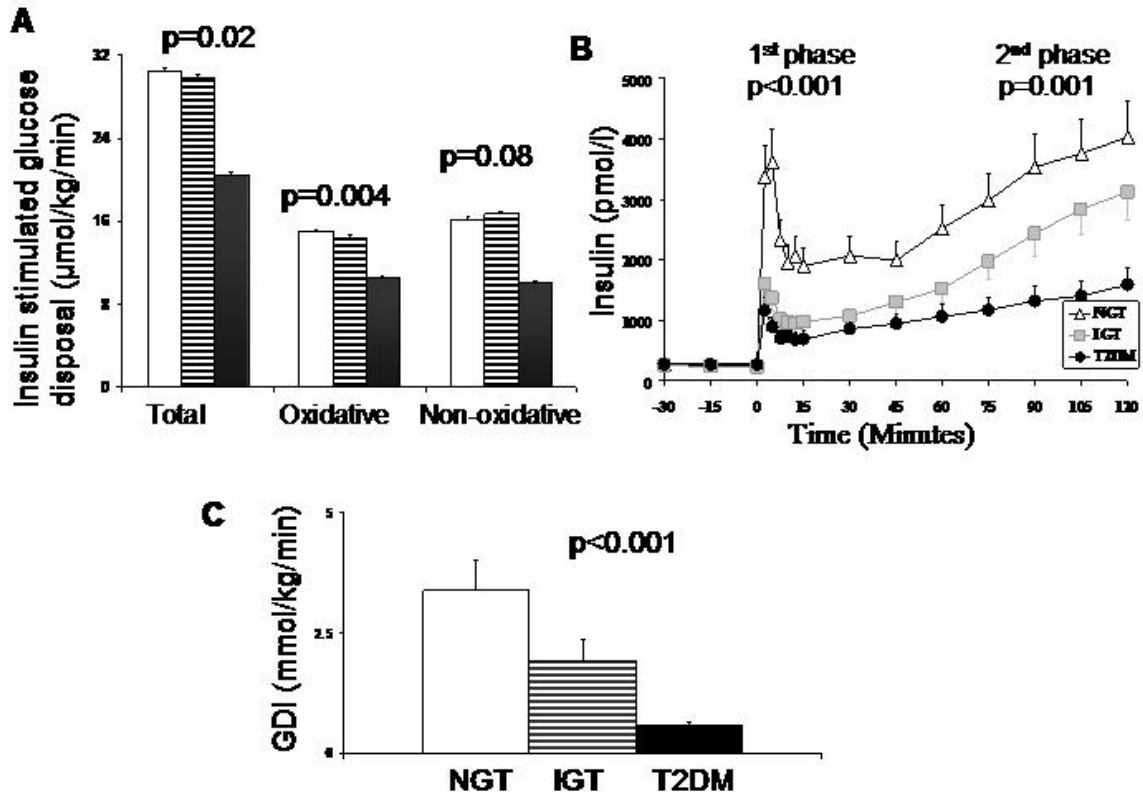


Figure 2

