

**Phenotypic Type 2 Diabetes in Obese Youth: Insulin Sensitivity and Secretion  
in Islet-cell Antibody Negative vs. Antibody Positive Patients.**

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## ABSTRACT

*Objective:* Some obese youth with a clinical diagnosis of type 2 diabetes (CDx-T2DM) have evidence of islet-cell autoimmunity with positive autoantibodies. In this study we investigated the differences in insulin sensitivity (IS) and secretion between autoantibody negative ( $Ab^-$ ) and positive ( $Ab^+$ ) youth with a CDx-T2DM in comparison with controls.

*Research Design and Methods:* Sixteen  $Ab^-$ , 26  $Ab^+$  CDx-T2DM and 39 obese control youth underwent evaluation of IS (3-hr hyperinsulinemic–euglycemic clamp), substrate oxidation (indirect calorimetry), 1<sup>st</sup> and 2<sup>nd</sup> phase insulin secretion (1<sup>st</sup>PI, 2<sup>nd</sup>PI) (2-hr hyperglycemic clamp), body composition and abdominal adiposity (DEXA and CT scan respectively), and glucose disposition index (GDI) (1<sup>st</sup>PI x IS).

*Results:* Insulin-stimulated total, oxidative and non oxidative glucose disposal, and suppression of fat oxidation during hyperinsulinemia were significantly lower in  $Ab^-$  compared with  $Ab^+$  CDx-T2DM and control subjects with no difference between the latter two. First and 2<sup>nd</sup> PI and C-peptide were lower in  $Ab^+$  compared with  $Ab^-$  T2DM. GDI was not different between the  $Ab^-$  and  $Ab^+$  CDx-T2DM but both were significantly lower than controls. Systolic blood pressure and ALT were higher in  $Ab^-$  vs  $Ab^+$  CDx-T2DM while the frequency of ketonuria at diagnosis was higher in  $Ab^+$  vs  $Ab^-$  patients.

*Conclusion:* Islet cell  $Ab^-$  CDx-T2DM youth are characterized with severe insulin resistance and relative insulin deficiency, while  $Ab^+$  youth have severe insulin deficiency and  $\beta$ -cell failure. The former group has additional features of insulin resistance. These important metabolic differences could influence the natural history of hyperglycemia, insulin dependence and clinical outcomes in these youth.

The clinical presentation of T2DM in youth is diverse, from minimal symptomatology to severe clinical manifestations with evidence of hyperglycemia with or without ketosis (1). Diabetes in humans is classified into two main types, type1 (T1DM) where the pathophysiology is autoimmune destruction of the pancreatic  $\beta$ -cells, and T2DM where insulin resistance is central to the disease process together with a non immune mediated  $\beta$ -cell failure relative to insulin resistance (2). Limited data in the pediatric literature suggest that the pathophysiology of youth T2DM is a combination of severe insulin resistance and relative insulin deficiency (3-6).

The diagnosis of youth T2DM is typically made using clinical criteria where obesity is the major diagnostic entity (7). However, with the increasing rates of obesity in childhood, particularly in children with T1DM this clinical distinction has become ever more difficult and imperfect (8, 9). A number of youth with a clinical diagnosis of T2DM (CDx-T2DM) have evidence of islet cell autoimmunity with autoantibodies present in 10-75% of patients (10-15). Several theories and terminologies have been proposed, such as hybrid diabetes, double diabetes, diabetes type 1.5, latent autoimmune diabetes of youth (LADY) to refer to and to try to explain the underlying pathophysiology in this subset of young patients with a clinical phenotype consistent with T2DM and evidence of autoimmunity consistent with T1DM (8, 13, 15-18). Efforts to identify distinguishing features between  $Ab^+$  and  $Ab^-$  CDx-T2DM in youth, typically focused on clinical features such as obesity, acanthosis nigricans, symptoms at diagnosis, ketonuria, HbA1c, and insulin requirements, have not revealed any unique distinctive features that would differentiate one from the other (10, 12, 15). To our knowledge, no data exist in the pediatric literature regarding the metabolic

characteristics of youth with a CDx-T2DM with vs without islet cell antibodies. Therefore, the aim of the present investigation was to test the hypothesis that youth with CDx-T2DM and positive islet cell antibodies have more impairment in beta cell function and are less insulin resistant than their peers with CDx-T2DM who are autoantibody negative. Our objectives were: 1) to compare *in vivo* insulin sensitivity and secretion in  $Ab^-$  vs  $Ab^+$  youth with CDx-T2DM and in obese controls, and 2) to assess if differences exist between the two groups of CDx-T2DM patients in clinical and/or laboratory features at the time of diagnosis or during the research evaluation.

## RESEARCH DESIGN AND METHODS

**Study Population.** Forty two obese adolescents with CDx-T2DM, made by the attending endocrinologist, were recruited from the Diabetes Center at Children's Hospital of Pittsburgh. Islet cell antibody screening (details below) revealed 16 with negative antibodies and 26 with positive antibodies. Some of the antibody positive patients were initially screened for the Treatment Options of Type 2 Diabetes in Adolescents and Youth trial (TODAY) (Children's Hospital of Pittsburgh is one of 15 participating centers in TODAY) (19) and found to be ineligible because of the presence of islet cell auto antibodies. Some of the antibody negative patients later participated in TODAY. The control group consisted of 39 age-matched obese otherwise healthy adolescents who were recruited from the community through local newspaper advertisement and flyers posted on bus routes and the medical campus. Thirteen out of the forty two subjects with T2DM and all of the obese controls were reported previously (4, 20). Pubertal development was assessed by physical examination according to Tanner criteria (21). At the time of study participation

nine subjects with CDx-T2DM were receiving lifestyle modification and no medications, six patients were on insulin alone, thirteen were on metformin alone, and fourteen were on insulin together with metformin (Table 1). This type of therapy is typical of pediatric diabetes clinical practices nationwide (22, 23). Besides the T2DM treatment none of the participants were receiving any medications that may impact glucose metabolism and none of the females were on oral contraceptive pills at the time of the study. The characteristics of the study population are summarized in table 1. All studies were approved by the Institutional Review Board of the University of Pittsburgh. Informed consent and assent were obtained from each participant and their legal guardians. Clinical and laboratory characteristics of T2DM youth at the time of diagnosis, including presence of symptoms, ketones, glucose levels, HbA1C, and treatment modality and/or insulin use at diagnosis were obtained from the medical records.

**Autoantibody testing.** Glutamic acid decarboxylase 65-kDa autoantibody (GAD65-Ab) and Insulinoma associated protein-2 autoantibody (IA2 Ab) measurements were performed at the Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington (Seattle, WA). This is the same reference laboratory with identical antibody determination for the NIH funded multi center TODAY study (19). Briefly, both GAD and IA2 were determined by a radioligand-binding assay, using either GAD65 or IA2 proteins produced by an in vitro transcription and translation system and labeled with  $^{35}\text{S}$  methionine. Levels of both GAD65-Ab and IA2 Ab were expressed as indexes, with the cutoff for positivity set at the 99<sup>th</sup> percentile of normal. Subjects with GAD65-Ab index  $>0.085$  and/or IA2 Ab index  $>0.018$  were considered to be GAD65-Ab positive and/or IA2-Ab positive respectively. Patients were considered to be

Ab negative if both antibody indexes were below the cutoff. These are the same cutoffs used in TODAY (19).

**Clamp studies.** Participants were admitted twice within a 1-4 week period (except for 3 controls within 6-8 weeks) to the Pediatric Clinical and Translational Research Center (PCTRC) the day before the clamp studies, once for a hyperinsulinemic-euglycemic clamp the other for a hyperglycemic clamp in random order. Each clamp evaluation was performed after a 10- to 12-h overnight fast. Metformin and long acting insulin were discontinued 48 hrs before the clamp studies as reported previously (4). For each study, two intravenous catheters were inserted after the skin and subcutaneous tissues were anesthetized with EMLA cream (Astra Pharmaceutical Products, Westborough, MA). One catheter was placed in a vein on the forearm for administration of glucose, insulin, and stable isotopes; the second catheter was placed in a vein in the dorsum of the contra-lateral heated hand for sampling of arterialized venous blood (4).

***In vivo* glucose metabolism and insulin sensitivity:** Fasting endogenous hepatic glucose production was measured with a primed (2.2  $\mu\text{mol}/\text{kg}$ ) constant rate infusion of [6, 6- $^2\text{H}_2$ ] glucose ( $0.31 \pm 0.01 \mu\text{mol} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$  in T2DM and  $0.30 \pm 0.01 \mu\text{mol} \cdot \text{Kg}^{-1} \cdot \text{min}^{-1}$  in obese controls) (Isotech, Miamisburg, OH) from 7:30-9:30 am, as described by us previously (4). Blood was sampled at the start of the stable isotope infusion (-120 min) and every 10 minutes from -30 min to 0 min (basal period) for determination of plasma glucose, insulin, and isotopic enrichment of glucose. Calculations for fasting hepatic glucose production were made over the last 30 minutes of the 2 h- isotope infusion (-30 to 0 min).

After the 2-h baseline isotope infusion period, insulin-mediated glucose metabolism and *in vivo* insulin sensitivity were measured during a 3-hr hyperinsulinemic-euglycemic

clamp from 9:30-12:30 hrs, in conjunction with indirect calorimetry. Intravenous crystalline insulin (Humulin Regular; Lilly, Indianapolis, IN) was infused at a constant rate of  $80\text{mu/m}^{-2}/\text{min}^{-1}$  and plasma glucose was clamped at  $5.5\text{mmol/l}$  with a variable rate infusion of 20% dextrose as before (4, 20). Continuous indirect calorimetry by a ventilated hood system (Deltatrac Metabolic Monitor; Sormedics, Anaheim, CA) was performed to measure  $\text{CO}_2$  production,  $\text{O}_2$  consumption, and respiratory quotient for 30 min at baseline and at the end of the clamp (4).

***In vivo* insulin secretion:** First and second phase insulin secretion were assessed during a 2-h hyperglycemic clamp ( $12.5\text{ mmol/l}$ ) performed from 09:00-11:00 hrs as described before (4). Glucose, insulin, and C-peptide concentrations were measured every 2.5 min (at 2.5, 5, 7.5, 10, and 12.5 min, first phase) and then every 5 min for glucose and every 15 min for insulin and C-peptide. Before the start of the clamp a fasting blood sample was obtained for total cholesterol, HDL, LDL, VLDL, Triglycerides, ALT and AST measurements.

**Body composition.** Body composition was determined by dual energy x-ray absorptiometry (DEXA). Subcutaneous abdominal adipose tissue (SAT) and visceral adipose tissue (VAT) were examined by a single slice CT scan at L4-L5 as described by us (4, 20).

**Blood pressure determination.** Blood pressure was measured when the subjects were resting in the supine position in bed. Measurements were performed with an automated sphygmomanometer every 10 minutes for 1 hour between 2200-2300 h before the subject fell asleep, and between 0600-0700 h before awakening. The mean of seven measurements during each hour was the outcome for statistical analysis (24).

**Biochemical measurements.** Plasma glucose was measured with a glucose analyzer

(Yellow Springs Instrument Co., Yellow Springs, Ohio), insulin and C-peptide by radioimmunoassay (RIA) as before (4, 25). 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 (4, 25). 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 as reported by us (20).

**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 (24, 25). Insulin stimulated glucose disposal rate (Rd) 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 Rd by the steady-state clamp insulin level (24,25). Insulin-stimulated carbohydrate and fat oxidation rates were calculated according to the formulas of Frayn (24, 20) from the indirect calorimetry data. Non-oxidative glucose disposal was estimated by subtracting the rate of glucose oxidation from the total Rd. During the hyperglycemic clamp, the first and second phase insulin and C-peptide concentrations were calculated as described previously (4, 25). Glucose disposition index (GDI) was calculated as the product of IS x  $1^{\text{st}}$  phase insulin.

**Statistical analysis.** Differences in continuous variables between  $\text{Ab}^+$  and  $\text{Ab}^-$  CDx-T2DM and control subjects were tested with either a one way ANOVA or the nonparametric Kruskal-Wallis test, based on the nonviolation of statistical assumptions. Differences in categorical variables were tested using the Chi square test or the Fisher's exact test. All statistical assumptions were met. Data are presented as mean  $\pm$  SEM unless otherwise indicated. Statistical significance was set at  $P \leq 0.05$ . All statistical

analyses were run using SPSS software (SPSS Inc, Chicago, IL).

## RESULTS

**Study Participants (Table 1).** The clinical and physical characteristics of the study participants are depicted in Table 1. The three groups were comparable with respect to age, gender, ethnicity, pubertal development, BMI, percent body fat, and abdominal visceral and subcutaneous adipose tissue. As expected, HbA1c was significantly higher in CDx-T2DM patients compared with controls, but not different between Ab<sup>-</sup> and Ab<sup>+</sup>. Similarly duration of diabetes was not different between Ab<sup>-</sup> and Ab<sup>+</sup>.

**Fasting Metabolic Profile (Table 2).** Fasting plasma glucose levels were not significantly different between Ab<sup>-</sup> and Ab<sup>+</sup> groups but were significantly higher than in obese controls. Fasting insulin level was lowest and hepatic glucose production was highest in the Ab<sup>+</sup> group (Table 2). Fasting lipids were not significantly different between Ab<sup>-</sup> and Ab<sup>+</sup> groups. Rates of glucose and fat oxidation were not different between the three groups. Baseline FFA was higher in the diabetic subjects compared with controls but not different between Ab<sup>+</sup> and Ab<sup>-</sup> (Table 2).

**In Vivo Insulin Sensitivity (Figure 1).** Steady-state plasma glucose and insulin concentrations during the hyperinsulinemic-euglycemic clamp were not different between the Ab<sup>-</sup>, Ab<sup>+</sup> and obese control groups (5.6±0.03, 5.6±0.03 and 5.6±0.02 mmol/l and 1677±113, 1536±135 and 1758±73 pmol/l respectively). Insulin-stimulated total, oxidative and non-oxidative glucose disposal was significantly lower in the Ab<sup>-</sup> patients compared with their Ab<sup>+</sup> peers and obese controls, with no difference between the Ab<sup>+</sup> CDx-T2DM and the obese controls (Figure 1, panel A). Insulin sensitivity was lowest in the Ab<sup>-</sup> patients vs Ab<sup>+</sup> vs Controls (1.4±0.2, 2.5±0.3, 2.0±1.1  $\mu$ mol/Kg/min per pmol/l respectively, p=0.048). Fat oxidation during

the euglycemic clamp was significantly higher in Ab<sup>-</sup> patients compared with Ab<sup>+</sup> and controls, with no difference between the latter 2 groups (Figure 1, panel B). Percent suppression in fat oxidation during the euglycemic clamp was significantly lower in Ab<sup>-</sup> patients compared with Ab<sup>+</sup> patients and controls (26.4±0.1 in Ab<sup>-</sup> vs. 55.5±6.6 in Ab<sup>+</sup> and 50.8±4.3% in controls, p=0.008) (Figure 1, panel C). Insulin-stimulated glucose disposal, total, oxidative and non-oxidative, and insulin sensitivity remained significantly lower, and fat oxidation remained significantly higher in the Ab<sup>-</sup> patients compared with their Ab<sup>+</sup> peers after adjusting for race, HbA1c and diabetes duration at the time of the clamp studies.

**In Vivo  $\beta$ -cell function during the hyperglycemic clamp (Figure 2).** First phase insulin and C-peptide as well as second phase insulin and C-peptide levels were lowest in Ab<sup>+</sup> CDx-T2DM compared with the other two groups (Figure 2 panels A and B) during hyperglycemia (1<sup>st</sup> phase glucose: 12.6±0.1, 12.3±0.1, 12.2±0.1 mmol/l, 2<sup>nd</sup> phase glucose: 12.7±0.0, 12.7±0.1, 12.4±0.0 mmol/l, respectively). All of these parameters of *in vivo*  $\beta$ -cell function remained significantly lower in Ab<sup>+</sup> CDx-T2DM compared their Ab<sup>-</sup> peers after adjusting for race, HbA1c and diabetes duration at the time of the clamp studies.

$\beta$ -cell function relative to insulin sensitivity i.e. the glucose disposition index did not differ between the Ab<sup>-</sup> and the Ab<sup>+</sup> CDx-T2DM patients, but were significantly lower than obese controls (Figure 2 panel C).

**Clinical and biochemical characteristics of Ab<sup>-</sup> and Ab<sup>+</sup> CDx-T2DM youth.** At the time of diagnosis there were no differences between Ab<sup>-</sup> vs Ab<sup>+</sup> CDx-T2DM youth in HbA1c (10.1±0.8 and 10.8±0.6%, p=0.4), glucose (15.4±2.3, 19.9±1.2 mmol/l, p=0.13), presence of symptoms, polyuria, polydypsia or weight loss by patient report, and family history of diabetes mellitus. However

ketonuria was more frequent in  $Ab^+$  patients (57.7% vs. 18.8%,  $p = 0.01$ ). Initiation of insulin treatment at diagnosis was comparable between the two groups (62.5% in  $Ab^-$  vs 73.1% in  $Ab^+$  patients,  $p = 0.51$ ), while initiation of metformin therapy was more frequent among the  $Ab^-$  patients (75% in  $Ab^-$  vs 34.6 % in  $Ab^+$ ,  $p = 0.03$ ). Four subjects (2  $Ab^-$  & 2  $Ab^+$ ) were started on lifestyle modification only and no meds (12.5%  $Ab^-$  vs 7.6%  $Ab^+$ ,  $p = 0.63$ ). Ten out of the 29 patients who were started on insulin treatment at diagnosis (4  $Ab^-$  and 6  $Ab^+$ ) became insulin free by the time of the clamp studies (34.5%).

At the time of research evaluation there were no differences between  $Ab^-$  vs  $Ab^+$  CDx-T2DM in HbA1c, insulin use and/or treatment modalities (Table 1). However night-time systolic blood pressure ( $121.9 \pm 2.5$  vs.  $113.0 \pm 1.8$  mmHg,  $p = 0.006$ ) and fasting ALT ( $40.5 \pm 5.0$  and  $28.4 \pm 1.7$  U/L,  $p = 0.03$ ), were significantly higher in the  $Ab^-$  compared with  $Ab^+$  patients. The differences in night-time SBP remained significant after adjusting for race, visceral adipose tissue and insulin sensitivity ( $p = 0.011$ ). HbA1c correlated with GDI (Figure 3) but not with insulin sensitivity or insulin secretion. In a multiple regression analysis with HbA1c as the dependent variable and GDI, age and visceral adipose tissue as the independent variables ( $R^2 = 0.612$ ,  $p < 0.001$ ) all three contributed significantly (partial correlations: GDI  $-0.71$ ,  $p < 0.001$ ; age  $-0.52$ ,  $p = 0.001$ ; VAT  $0.42$ ,  $p = 0.013$ ) and independently to explain 78% of the variance in HbA1c.

**Double  $Ab^+$  ( $2Ab^+$ ) vs. single ( $1Ab^+$ ) positive.** Among the 26  $Ab^+$  CDx-T2DM patients 18 were positive for a single  $Ab$  (14 for GAD and 4 for IA2) and 8 were positive for double antibodies (both GAD and IA2). There was no difference in insulin stimulated glucose disposal between  $1Ab^+$  and  $2Ab^+$  groups ( $32.8 \pm 3.9$  vs  $33.3 \pm 4.4$ ,  $p = ns$ ). First and second phase insulin was higher in the  $1Ab^+$  compared with the  $2Ab^+$  patients

( $314.5 \pm 36.1$  vs.  $165.0 \pm 40.0$  pmol/l,  $p = 0.02$ ;  $482.8 \pm 67.1$  vs  $239.0 \pm 68.6$  pmol/l,  $p = 0.03$  respectively). There were no differences in age, BMI, body composition, HbA1c, fasting lipid profile, BP, as well as glucose level, HbA1c and ketonuria at the time of diagnosis.

## DISCUSSION

This study was undertaken to determine whether or not there are differences in insulin sensitivity and secretion between  $Ab^+$  and  $Ab^-$  obese youth with a clinical diagnosis of T2DM in an effort to shed light on distinguishing pathophysiological mechanisms. Our results demonstrate that despite similar body composition and body fat topography: 1) youth with  $Ab^-$  CDx-T2DM have severe impairment in insulin action while those with  $Ab^+$  CDx-T2DM have severe impairment in  $\beta$ -cell function, 2) youth with  $Ab^-$  T2DM had additional features consistent with insulin resistance/metabolic syndrome including decreased suppression in fat oxidation during hyperinsulinemia, higher systolic blood pressure and higher ALT, and 3) there were no clinical distinguishing features between  $Ab^+$  and  $Ab^-$  youth with CDx-T2DM at the time of diagnosis except for the higher frequency of ketonuria in the former group.

Between 10-75% of youth diagnosed clinically with T2DM are reported to have  $\beta$ -cell auto-antibodies (10-15). The antibodies tested and reported to be positive in these patients include ICA, in 5-8% (10, 12, 13, 15), GAD in 8-30% (10-12, 15), IA-2 in 8-42% (11-13, 15), and insulin antibodies in 5-35% (10-13). In one study 11% of CDx-T2DM youth were positive for all four auto-antibodies (12). These studies however had very limited numbers of patients from different racial groups (10-13, 15). Some of these studies investigated but could not find clinically distinguishing features between patients with or without  $\beta$ -cell antibodies (10, 15). A preliminary report of a much larger

scale of 432 youth with CDx-T2DM screened in TODAY study revealed that 17.4% were positive for one or both antibodies, GAD and IA-2 (14). Antibody positive vs negative subjects did not differ in age, diabetes duration, BMI z-score and lipid levels, however there seemed to be a tendency for C-peptide to be lower and HbA1C to be higher in Ab<sup>+</sup> youth (14).

Studies investigating the pathophysiology of youth T2DM are very limited with conflicting results (3-6, 26). In one of the earliest studies first phase insulin response, during a frequently sampled intravenous glucose tolerance test (FSIGT), was lower in T2DM compared with obese controls with no difference in insulin sensitivity (3). This was in contrast with another study where insulin sensitivity was lower (6) in T2DM. However, despite lower insulin response to IVGTT, integrated insulin and C-peptide levels during the OGTT were not different between T2DM and obese as well as lean control subjects (6). In the former study  $\beta$ -cell antibodies were not measured and in the latter it is not clear which, how many, and how antibodies were measured. In our previous publication GAD and ICA antibodies were measured commercially and only T2DM adolescents with negative antibodies were reported and shown to have ~ 50% lower insulin sensitivity and relative insulin deficiency (4). In another study of six adolescents with T2DM and negative GAD, IA-2 and ICA, besides marked insulin resistance the impairment in  $\beta$ -cell function was highly variable (5). In another study of 10 obese youth with T2DM negative for GAD, ICA and IA2, glucose sensitivity of first and second-phase  $\beta$ -cell secretion was significantly lower in T2DM patients compared with obese youths, while insulin sensitivity was similar between the groups (26). Potential explanations for these variable findings could be differences in study population and ethnicity, differences in diabetes duration and therapeutic modalities,

differences in methodologies and differences in measuring antibodies. In the present investigation we elected to use the same laboratory which was deemed most appropriate for the NIH funded multi center TODAY study. Our current results suggest that youth who are clinically diagnosed with T2DM but negative for the two tested antibodies have severe impairment of insulin action, both in glucose and fat metabolism, which is over and beyond that due to obesity since there were no differences in total and abdominal obesity between them and the other 2 groups. Furthermore, the presence of higher blood pressure and higher ALT would suggest that this group has an inherent insulin resistance that is typical of the metabolic syndrome (27, 28). On the other hand patients clinically diagnosed with T2DM but positive antibodies have severe impairment in  $\beta$ -cell function while their insulin action is significantly better than Ab<sup>-</sup> patients and near identical to obese non diabetic controls. This suggests that the major abnormality is in insulin secretion and not insulin action which is typical of autoimmune T1DM. Thus, it is our proposition that these patients are merely obese children who happen to develop autoimmune T1DM. Moreover, our limited data would suggest that there could be a dose response phenomenon since first and second phase insulin secretion in patients who are positive for double antibodies is almost 50% lower than that of patients who are positive for a single antibody. Previous limited studies from a single group of investigators found that some youth clinically classified with T2DM or indeterminate diabetes (admixture of clinical features of types 1 and 2, T1.5) show T cell reactivity to islet proteins (12) and have T1DM-associated HLA alleles (13). Baseline C-peptide levels in the T1.5 patients were significantly lower than those in patients classified as T2DM and higher than those in patients classified as T1DM (13). The findings in our study provide direct evidence

to the more severe degree of impairment in  $\beta$ -cell function in  $Ab^+$  patients proposed in the aforementioned study. The more frequent ketonuria at diagnosis in  $Ab^+$  patients may be a reflection of the severity of  $\beta$ -cell impairment, and has been previously reported (12).

It is not surprising that despite significantly more severe impairment in  $\beta$ -cell function in the  $Ab^+$  group, there was no difference in the frequency of insulin treatment between the 2 groups of CDx-T2DM patients either at diagnosis or at the time of the study (73%  $Ab^+$  vs. 62%  $Ab^-$  at diagnosis, and 54% vs. 38% at the time of the study). Treatment of patients is frequently practitioner and not pathophysiology-driven and these rates of insulin treatment are consistent with the pediatric literature of T2DM (29,12,15). The change in BMI from diagnosis to the time of research was not different between those patients initially treated with vs without insulin (data not shown).

Despite these important differences in insulin action and secretion between  $Ab^-$  and  $Ab^+$ , the GDI, which is insulin secretion corrected for the degree of insulin sensitivity, is not different. Our present metabolic observations are consistent with findings in GAD positive adults with T2DM or latent autoimmune diabetes (LADA) (30). GAD positive adult patients had decreased early insulin response, however when this response was corrected for the degree of insulin sensitivity, GAD positive and negative patients had similar  $\beta$ -cell function. Thus, in the absence of careful metabolic studies using sensitive tools it will be difficult to discern the underlying pathways, impaired insulin action vs  $\beta$ -cell function, which may result in similar GDI. However, such knowledge is important to help guide pathophysiology-based therapy amidst the multiple terminologies used, double diabetes, hybrid diabetes, diabetes 1.5 etc.(8,13,15-18).

In conclusion, important metabolic differences exist in insulin sensitivity and secretion between obese youth with vs without evidence of pancreatic autoantibodies. These metabolic differences highlight different underlying pathophysiological mechanisms between the two groups despite a clinical diagnosis of T2DM. Finally, it remains to be determined if the natural history of diabetes, and/or response to treatment, and/or micro and macro vascular complications differ between  $Ab^+$  and  $Ab^-$  clinically diagnosed obese youth with T2DM.

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**The authors' responsibilities were as follows:** NG & SA: Study design and development of hypothesis; NG, FB & SA: secure funding; NG, FB & SA: collection of data; HT & SA: interpretation of results,

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statistical analysis and drafting of manuscript; editorial review, proofreading of manuscript.  
SA: critical review of manuscript; FB:

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**Table 1.** Characteristics of islet cell antibody negative ( $Ab^-$ ) and positive ( $Ab^+$ ) obese youth clinically diagnosed with T2DM (CDx-T2DM) and Obese controls

	CDx-T2DM		Obese Controls (n=39)	ANOVA p	Post hoc p
	$Ab^-$ (n=16)	$Ab^+$ (n=26)			
Age (years)	14.7 $\pm$ 0.4 (10.1 - 17.2)	14.9 $\pm$ 0.4 (10.2 - 18.3)	14.1 $\pm$ 0.2 (12.3 - 17.2)	ns	ns
Gender (Male / Female)	7 / 9	11 / 15	23 / 16	ns	
Ethnicity (AA / W)	6 / 10	13 / 13	20 / 19	ns	
Tanner Stages					
II-III	2	3	10	ns	
IV-V	14	23	29		
BMI (Kg/m <sup>2</sup> )	36.0 $\pm$ 1.3 (28.3 - 45.4)	33.4 $\pm$ 1.2 (24.5 - 48.9)	36.0 $\pm$ 0.9 (28.0 - 49.6)	ns	ns
Percent Body Fat (%)	40.6 $\pm$ 1.8 (24.9 – 48.4)	40.8 $\pm$ 1.5 (22.6 – 52.9)	42.3 $\pm$ 0.8 (32.1 – 51.1)	ns	ns
Visceral adipose tissue (cm <sup>2</sup> )	79.0 $\pm$ 6.9 (47.1 – 142.8)	72.8 $\pm$ 8.6 (26.5 – 242.4)	76.1 $\pm$ 5.9 (16.7 – 161.4)	ns	ns
Subcutaneous adipose tissue (cm <sup>2</sup> )	520.1 $\pm$ 40.7 (291.6 – 776.1)	491.5 $\pm$ 29.8 (264.9 – 764.4)	556.1 $\pm$ 23.9 (321.2 – 890.1)	ns	ns
Diabetes duration (months)	4.4 $\pm$ 1.4 (0.05-18.0)	9.0 $\pm$ 2.1 (1.0-39.0)	NA		ns
HbA1c (%)	6.8 $\pm$ 0.2 (4.7-8.3)	6.5 $\pm$ 0.2 (5.1-8.0)	5.3 $\pm$ 0.1 (4.3-6.2)	<0.001	ns
Treatment Modality n(%)			NA		
Lifestyle modification	4 (25%)	5 (19.2%)		ns	
Metformin	6 (37.5%)	7 (26.9%)		ns	
Insulin	2 (12.5%)	4 (15.4%)		ns	
Insulin and Metformin	4 (25%)	10 (38.5%)		ns	

## Youth T2DM, Antibody status and $\beta$ -cell function

AA: African Americans, W: Whites, NA: not applicable .ns: not significant. Post hoc P: Bonferonni correction for Ab<sup>-</sup> vs. Ab<sup>+</sup> CDx-T2DM . X<sup>2</sup> analyses with respect to ethnicity, gender, Tanner stage and treatment modality.

**Table 2.** Fasting metabolic profile of islet cell antibody negative ( $Ab^-$ ) and positive ( $Ab^+$ ) obese youth clinically diagnosed with T2DM (CDx-T2DM) and Obese controls

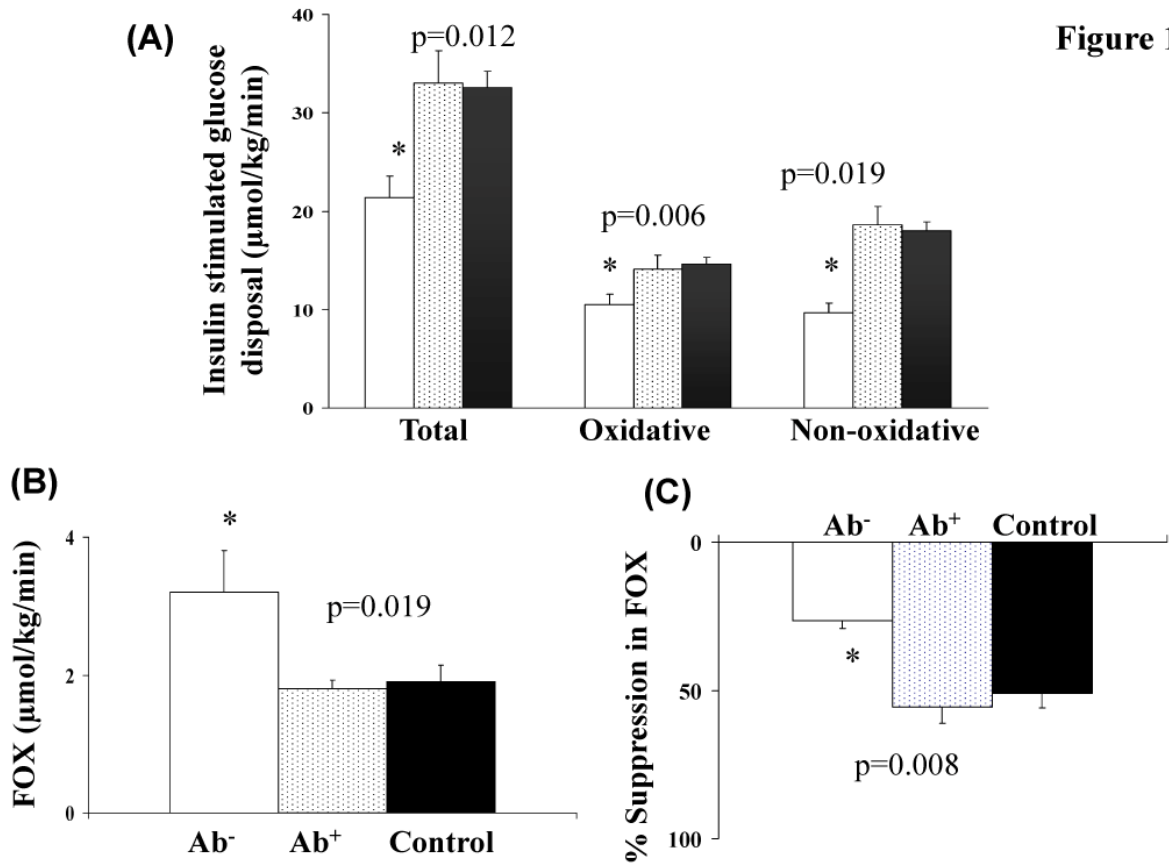
	CDx-T2DM		Obese Controls ( <i>n</i> =39)	ANOVA p	Post hoc p
	$Ab^-$ ( <i>n</i> =16)	$Ab^+$ ( <i>n</i> =26)			
Glucose (mmol/l)	6.6±0.4 (4.6-9.5)	7.4±0.4 (5.0-13.5)	5.4±0.1 (4.8-6.2)	< 0.001	ns
Insulin (pmol/l)	274±37 (77-690)	183±20 (49-472)	249±18 (95-573)	0.03	0.05
HGP ( $\mu$ mol/kg/min)	13.3±0.6 (10.1-18.0)	16.1±1.1 (9.2 – 34.1)	13.1±0.5 (9.2 – 25.5)	0.02	ns
Glucose Oxidation ( $\mu$ mol/kg/min)	6.5±0.7 (3.8 – 11.4)	7.4±0.7 (2.5-17.8)	8.6±0.6 (3.1 – 16.1)	0.09	ns
Fat Oxidation ( $\mu$ mol/kg/min)	4.7±0.4 (2.2 – 7.8)	4.3±0.3 (1.4 – 7.5)	4.1±0.2 (2.1 - 8.8)	ns	ns
FFA baseline ( $\mu$ M)	439±23 (252-598)	427±24 (216-678)	341±18 (161-607)	0.003	ns
Cholesterol (mmol/l)	3.9±0.2 (2.5-5.0)	3.9±0.1 (2.9-5.6)	4.4±0.9 (2.3-6.6)	0.03	ns
HDL (mmol/l)	0.96±0.04 (0.65-1.33)	0.99±0.04 (0.67-1.69)	1.06±0.03 (0.70-1.53)	ns	ns
LDL (mmol/l)	2.3±0.2 (1.3-3.1)	2.4±0.1 (1.4-4.3)	2.7±0.1 (1.1-4.5)	0.04	ns
Triglycerides (mmol/l)	1.5±0.2 (0.6-3.9)	1.3±0.1 (0.6-3.4)	1.3±0.1 (0.6-4.3)	ns	ns
VLDL (mmol/l)	0.31±0.04 (0.11-0.79)	0.26±0.03 (0.11-0.67)	0.28±0.03 (0.11-0.86)	ns	ns

## Youth T2DM, Antibody status and $\beta$ -cell function

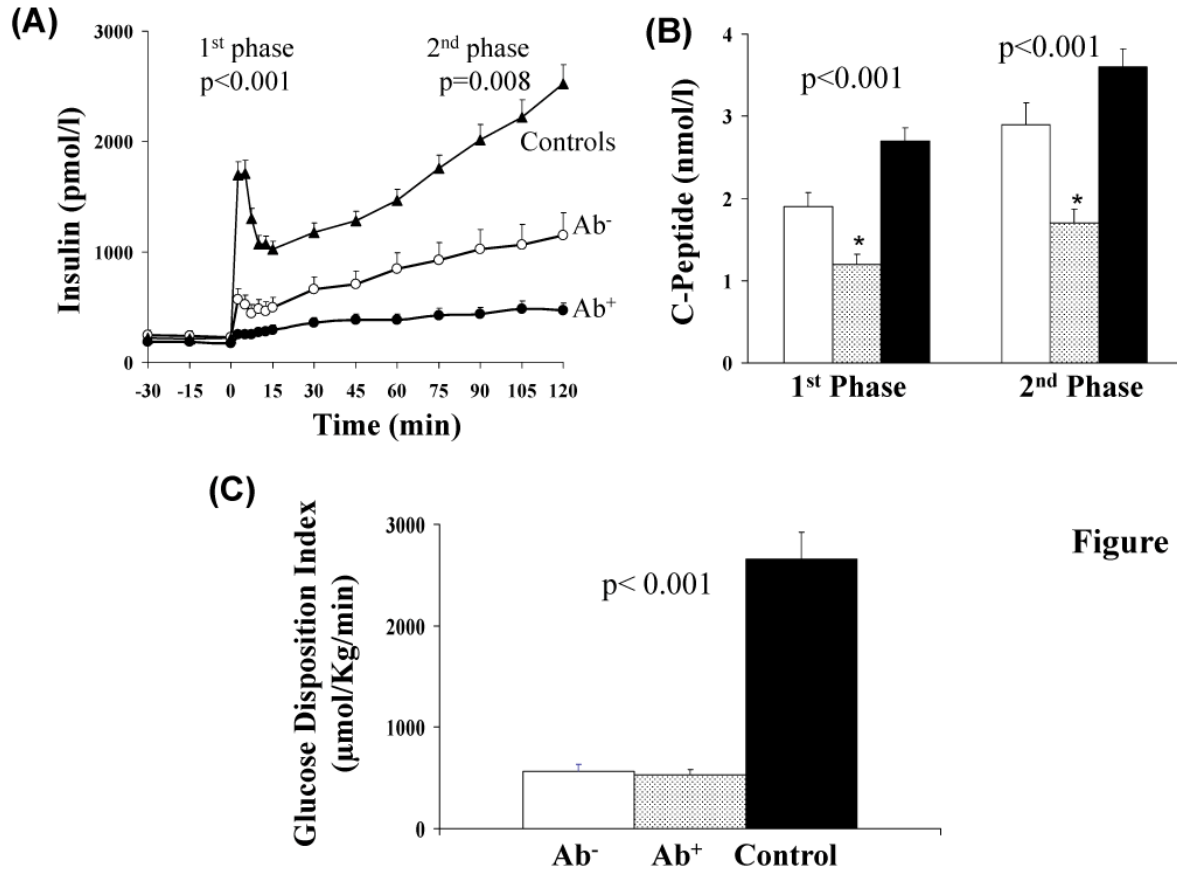
HGP: hepatic glucose production. Post hoc P: Bonferonni correction for Ab<sup>-</sup> vs. Ab<sup>+</sup> CDx-T2DM

. ns: not significant.

**Figure 1.** Panel A: Insulin stimulated total, oxidative and Non-oxidative glucose disposal during the hyperinsulinemic-euglycemic clamp in Ab<sup>-</sup> (empty bars) vs Ab<sup>+</sup> (dotted bars) vs Controls (filled bars). Panel B: Fat oxidation (FOX) during the hyperinsulinemic-euglycemic clamp in the 3 groups. Panel C: percent suppression in fat oxidation during hyperinsulinemia in the 3 groups. P values by ANOVA. \* designates post Hoc Bonferroni correction  $p < 0.05$  Ab<sup>-</sup> vs Ab<sup>+</sup>, Ab<sup>-</sup> vs Controls.



**Figure 2.** Panel A: Insulin secretion during the hyperglycemic clamp in  $Ab^-$  (empty circles) vs  $Ab^+$  (filled circles) vs Controls (filled triangles). Panel B: First and second phase C-peptide levels during the hyperglycemic clamp in  $Ab^-$  (empty bars) vs  $Ab^+$  (dotted bars) vs Controls (filled bars). Panel C: Glucose Disposition index in the 3 groups. P values by ANOVA. \* designates post Hoc Bonferroni correction  $p < 0.05$   $Ab^+$  vs  $Ab^-$ ,  $Ab^+$  vs Controls.



**Figure 2**

**Figure 3.** Relationship between glucose disposition index (GDI) and HbA1c in Ab<sup>-</sup> (empty circles) vs Ab<sup>+</sup> (filled circles) CDx-T2DM.

**Figure 3**

