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Short-Term Predictors of Near-Normoglycemic Remission in African-American Subjects with KPDM

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By

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An abstract of
A thesis submitted to the Faculty of the Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
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2008

ABSTRACT

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African-American Subjects with KPDM
By
Dawn Smiley, M.D.

Purpose: Patients that present with diabetic ketoacidosis (DKA) but have clinical features of type 2 diabetes are referred to as having ketosis-prone type 2 diabetes (KPDM) and most patients are able to discontinue insulin (near-normoglycemic remission) within 12 weeks of therapy. Most patients, however, experience a hyperglycemic relapse within a year. The aims of this study were 1) to identify clinical, metabolic, and immunogenetic variables that are markers of remission and 2) to correlate measures of pancreatic β-cell function and insulin sensitivity with the ability to achieve remission.
Null Hypotheses: Measured variables, measures of β-cell function *nor* measures of insulin sensitivity correlate with the ability to achieve remission.
Design and Methods**.** In a prospective approach, obese, African-American subjects with new onset diabetes presenting with either DKA (glucose $\geq$ 250 mg/dL, ph < 7.3 and bicarbonate $\leq$ 18 mmol/L) or severe hyperglycemia (HG, glucose $\geq$ 400mg/dL) were enrolled in the study and treated with pre-mixed insulin for up to 12 weeks. Within a week of diagnosis, subjects underwent glutamic acid decarboxylase (GAD) antibody testing, β-cell function (glucagon stimulation test, GST) and insulin sensitivity assessments (IV glucose tolerance test, IVGTT). At 1 week following discontinuation of insulin or at 12 weeks of insulin therapy (no remission), β-cell function and insulin sensitivity assessments were repeated.

Results. Of 39 enrolled subjects, 17 had DKA (12M/5F) and 22 had HG (12M/10F). Nearly 70% went into remission within 12 weeks. Except metabolic acidosis in the DKA cohort, there were no baseline clinical differences between groups. Weight loss at presentation trended with the ability to attain remission. There were no group differences in β-cell function or insulin sensitivity at presentation; however, subjects that achieved remission at $\leq$ 12 weeks of insulin therapy had a greater acute insulin response to glucagon compared to subjects that failed remission.

Our preliminary data did not find significant differences between the study cohorts at presentation nor establish a consistent prognostic model for remission. The initial data suggests that longitudinal studies may be more appropriate to establish a model to predict outcomes, particularly as it relates to response to remission and medical treatment.

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INTRODUCTION

More than half of newly diagnosed obese African-Americans (AA) presenting with unprovoked diabetic ketoacidosis (DKA) display clinical, metabolic, and immunogenetic features of type 2 diabetes during follow-up. Prior studies by our group and other investigators indicate that, at presentation, these patients a) have markedly decreased pancreatic insulin secretion and impaired insulin action, b) have a low prevalence of positive β-cell autoantibodies (1-3), and c) aggressive diabetic management results in significant improvement in β-cell function and insulin sensitivity sufficient to allow discontinuation of insulin therapy within 3 months of follow-up. Upon discontinuation of insulin, the period of near-normoglycemic remission (defined as the ability to discontinue insulin for at least one week and remain in good metabolic control – fasting blood glucose ≤ 130 mg/dL, A1C < 7%) may last for a few months to several years. Despite this initial response, 60% of obese AA patients with DKA who achieved remission relapsed into hyperglycemia within 1 year if treated with diet alone. Patients with ketosis-prone diabetes mellitus (KPDM) are therefore an ideal model to follow throughout their clinical course in order to correlate their response to treatment with the markers and mechanism(s) of short- and long-term remission and to determine the optimal therapeutic approach in order to prevent future glycemic decompensation.

This study attempts to better characterize patients with KPDM and investigate factors that correlate with short-term remission. Responses in obese DKA subjects were compared to obese subjects with nonketotic hyperglycemia.

BACKGROUND AND SIGNIFICANCE

KPDM in Obese African-Americans

Obesity is common in African-American (AA) patients who present with newly diagnosed, decompensated diabetes (2; 4; 5). Previous studies from our research group and other investigators reveal that obesity or overweight body habitus is present in 41-81% of newly diagnosed AA patients presenting with diabetic ketoacidosis (DKA) and/or severe hyperglycemia (1; 2; 4; 6). These results differ from studies in Caucasians with type 2 diabetes in whom the prevalence of obesity in this clinical context is less than 20% (4). In contrast to patients with insulin-dependent type 1 diabetes, most obese patients with a history of DKA and/or severe hyperglycemia frequently experience near-normoglycemic remission within the first few months of treatment (1; 6; 7). These subjects also differ from patients with type 1 or autoimmune diabetes such that they have a strong family history of diabetes, have a low prevalence of autoimmune markers, variable HLA genetic association (1; 3; 6; 8-11). This variant of type 2 diabetes has been referred to as atypical diabetes, Flatbush diabetes, type 1.5 diabetes, idiopathic type 1B diabetes, and more recently ketosis-prone type 2 diabetes (KPDM) (3; 7-9; 12). KPDM is a metabolic disorder consisting of β-cell failure and insulin resistance. At presentation, they have markedly impaired insulin secretion and insulin action, but intensive diabetic management results in significant improvement in β-cell function and peripheral insulin sensitivity sufficient to allow discontinuation of insulin therapy in > 50% of subjects (1; 2; 4; 5). Upon discontinuation of insulin, the period of near-normoglycemia remission may last for a few months to several years. Recently, McFarlane et al (7) and Mauvais-Jarvis et al (3) reported that 42% of mildly obese AA patients followed for > 1 year achieved near-normoglycemic remission. Despite this initial response, however, we reported that 60% of obese AA patients with DKA who achieved remission relapsed into hyperglycemia within 1 year if treated with diet alone

(4). Insulin secretion at presentation and during follow up but not the severity of insulin resistance appears to be the most important predictors of achieving and maintaining near-normoglycemic remission. Basal and stimulated C-peptide levels of >1.0 ng/dL (0.33 nmol/L) and >1.5 ng/dL (0.5 nmol/L shortly after presentation and >1.5 ng/dL (0.5 nmol/L) and >2.25 ng/dL (0.75 nmol/L) during follow up are predictive of remission in subjects with KPDM (1; 3; 9; 13; 14). **In light of the recovery of β-cell function in obese AA patients with KPDM, we hypothesize that markers of continued metabolic dysfunction can be discerned by comparing patients who achieve near-normoglycemic remission with those who remain insulin-dependent.**

Potential Markers of Outcomes

Observational studies have suggested variables associated with long-term remission in KPDM include measurable insulin secretion both at presentation (8) and during follow up (2), the presence of obesity (6), distinct HLA pattern (2), and absence of autoimmune markers of β-cell destruction (2; 3; 14). We reported that obese AA subjects with KPDM experience a three-fold increase in stimulated C-peptide after 8 – 10 weeks of intensive insulin therapy (1; 4; 14). McFarlane et al (7) and Jarvis et al (3) also reported that improvement of β-cell function correlates with near-normoglycemia remission; however, they observed that despite the initial improvement in β-cell function, most patients with KPDM experience a progressive deterioration of insulin secretion overtime. We and other investigators have reported that the presence of obesity is a good predictor of near-normoglycemia remission (5; 15), as well as the absence of autoantibodies (ICA, GAD, ICA512, IAA) (6; 14). During an analysis of patients with and without pancreatic antibodies and pancreatic reserve, Balasubramayam et al found long-term β-cell function and insulin independence in patients with DKA was best predicted by the lack of pancreatic antibodies (ICA and GAD) and finding of adequate basal pancreatic function

(C-peptide:glucagon ratio ≥ 1.5 ng/mL) (16). Since most of the data derives from observational studies, it is not known if phenotypic, metabolic, or autoimmune markers may better predict pancreatic β-cell function recovery in KPDM. Identification of such markers is of great clinical importance in selecting patients who will require more aggressive follow up and perhaps, a more intensive therapy to prevent β-cell exhaustion and future hyperglycemic crises.

Pancreatic insulin reserve in KPDM

Despite the presentation with severe symptoms of insulinopenia and ketoacidosis, clinical and immunogenetic observations indicate that most obese AA patients with DKA have type 2 diabetes. In such patients, we have reported that a) at presentation, obese AA patients with KPDM have markedly decreased insulin secretion, which is significantly greater than in lean patients with DKA but lower than in obese patients with nonketotic hyperglycemia (1), and b) obese AA with DKA and obese AA with hyperglycemia have similar degrees of insulin resistance both at presentation and during follow-up . A surrogate of β-cell function can be assessed using a glucagon c-peptide stimulation test and the FSIVGTT. Previous studies showed that pancreatic insulin reserve can be determined by changes in C-peptide levels after glucagon (1 mg I.V.), both one-day after resolution of DKA and after 10-12 weeks of follow-up (4). One day after resolution of DKA, with glucose averaging 11 mmol/dL (198 mg/dL), basal and stimulated C-peptide levels in the obese DKA patients (1.5 ± 0.1 and 2.5 ± 0.2 ng/mL) were significantly greater than levels in lean DKA patients (0.7 ± 0.1 and 0.8 ± 0.1 ng/mL, both $P < 0.01$) but were lower than in obese patients with hyperglycemia (2.0 ± 0.1 ng/mL and 3.2 ± 0.2 ng/mL). During follow-up, the obese DKA and obese hyperglycemic patients exhibited a significant improvement in basal and stimulated C-peptide levels.

Insulin Sensitivity in KPDM.

Results of a "minimal model" insulin sensitivity analysis from a previous study done by our research group are shown in the Table 4. One day after resolution of hyperglycemia and/or ketoacidosis, insulin sensitivity decreased equally in obese patients with DKA or hyperglycemia (0.3 and 0.4 min^{-1} x μU/mL, respectively, P = NS), compared with non-diabetic obese subjects (1.3 ± 0.3 min^{-1} x μU/mL, both P < 0.01). During follow-up improvement of metabolic control resulted in a marked improvement in insulin action in both diabetic groups. While the rise in insulin sensitivity was greater in the obese ketosis-prone patients, the change was not significantly greater than those in obese patients with hyperglycemia, and both levels were not significantly different from values in obese control subjects. The proposed study will extend these data by linking the degree of insulin sensitivity to short-term outcomes.

Autoimmunity in KPDM

Figure 3 shows that the proportion of obese patients with positive immunologic markers islet cell antibodies (ICA), glutamic acid decarboxylase antibodies (GAD), protein tyrosine phosphatase IA-2/ICA512, and insulin autoantibodies (IAA) is comparable between those with KPDM and those with hyperglycemia, but is significantly lower than lean DKA patients. Therefore, these markers distinguish the obese patients from typical type 1 diabetic patients (lean DKA) (14). We have also shown that patients with positive autoantibodies have lower insulin secretion compared to those with negative antibodies (data not shown). The proposed study will extend these data by potentially linking GAD and ICA antibody status to a subject's ability to attain and remain in near-normoglycemic remission.

METHODS

Specific Aim

Aim 1: Identify clinical, metabolic (GST and FSIVGTT), and immunogenetic (GAD) markers that alone, or in combination, are predictive of short-term near-normoglycemic remission.

Null Hypotheses

Measures of clinical features, GAD antibody status, and β-cell function shortly after presentation, alone or in combination with measures of insulin sensitivity, *do not correlate* with the ability of a patient with KPDM to achieve near-normoglycemic remission. Pancreatic β-cell function or insulin sensitivity at presentation or at 12 weeks *does not* correlate with the ability to achieve short-term remission.

Study Design

This prospective study cohort study enrolled obese, African-American subjects aged 18-65 years presenting with new-onset diabetes and either DKA or severe hyperglycemia. While in the hospital, subjects with DKA were initially treated with a low-dose insulin infusion protocol and upon resolution of ketoacidosis, patients were placed on a pre-mixed or split-mixed subcutaneous insulin combination twice daily at a starting dose of 0.7 units/kg/day. Subjects with severe hyperglycemia were also treated aggressively with the same starting subcutaneous insulin dose twice a day.

All subjects in the study received subcutaneous insulin shortly after diagnosis and were followed closely in the outpatient setting. The subjects were followed for a 12-week study period to adjust insulin doses as needed and to evaluate factors associated with the ability to discontinue insulin therapy (remission) versus factors associated with the lack of remission. The insulin dose in both groups was adjusted to achieve fasting and

premeal glucose levels < 130 mg/dL. Patients were followed in the Grady Memorial Hospital Diabetes Clinic every two-three weeks for the first three months during the insulin tapering period. Insulin therapy was tapered after blood glucose had been at target levels for 2-4 weeks or sooner if a patient experienced hypoglycemia (BG < 70 mg/dL). After 12 weeks of insulin therapy or following 1 week of good metabolic control without exogenous insulin (near-normoglycemic remission with fasting blood glucose < 130 mg/dL, A1c < 7%), all study subjects were readmitted to undergo a second assessment of β-cell function and insulin sensitivity as outlined in the *Detailed Methods* section. Metabolic testing and assessments were carried out in the Emory University satellite General Clinical Research Center (GCRC) at Grady Memorial Hospital. Clinical and metabolic data collection and GAD antibody testing were performed on subjects at the time of diagnosis. Pancreatic β-cell function tests (GST and FSIVGTT) and insulin sensitivity (FSIVGTT) tests were done within 1 week of resolution of DKA and/or hyperglycemia and again at time of remission or at 12 weeks of insulin therapy.

All subjects enrolled continued to receive medical care from their physicians at the Grady Diabetes Clinic or in the community. Patients without remission were maintained on insulin therapy and the subjects that achieved remission were randomized to either a treatment arm of a thiazolidinedione once a day or a placebo tablet once a day. It is anticipated that the results of this arm of the protocol will be reported in 2010.

This study was supported by K12 NIH funds (*1KL2RR025009)* and the Emory University CTSA/GCRC *(M01 RR-00039)*. The Institutional Review Board at Emory University approved the study protocol. Informed consent was obtained from all subjects after explanation and understanding of the nature, purpose, and potential risks of the study.

Patient Selection

All overweight/obese (BMI $\geq$ 28 kg/m^2), African-American patients with new-onset DKA and/or severe hyperglycemia and without apparent precipitating cause were considered for inclusion into the study *between 7/2005-1/2008*. The diagnosis of DKA was established by standard criteria (blood glucose > 250 mg/dL, pH < 7.3, HCO^3 < 18 mmol/L, increased anion gap) (17). The hyperglycemic (HG) group included patients with a plasma glucose $\geq$ 400 mg/dL without the presence of metabolic acidosis. Potential participants were selected at the time of hospitalization or at the initial visit to the diabetes clinic visit for symptoms of uncontrolled diabetes. Subjects were not considered for the study if any of the following existed: 1) significant medical or surgical illness, including but not limited to myocardial ischemia, congestive heart failure, chronic renal insufficiency, liver failure, and infectious processes; 2) recognized or suspected endocrine disorders associated with increased insulin resistance, such as hypercortisolism, acromegaly, or hyperthyroidism; 3) bleeding disorders, thrombocytopenia, or abnormalities in coagulation studies; 4) pregnancy.

A group of 6 obese, nondiabetic African American subjects with a fasting glucose < 100 mg/dL and a 2-hour glucose <140 mg/dL during a 75 g oral glucose tolerance test, and matched for age, gender and BMI were studied as controls.

Study Protocol

EXPERIMENT 1A. Measure organ-specific autoantibodies at presentation, and assess pancreatic insulin secretion within 1 week of resolution of DKA/hyperglycemia and again at 1 week following either discontinuation of insulin therapy or at 12 weeks of insulin therapy (no remission). Responses in subjects that achieved remission were compared to those diabetic subjects that failed to discontinue insulin at 12 weeks. Responses were also compared between obese DKA subjects and obese subjects with nonketotic hyperglycemia.

Protocol (See Detailed Methods section). Determination of glutamic acid decarboxylase (18) antibodies were measured on initial admission or clinic visit. Pancreatic insulin secretion was measured by glucagon (1 mg I.V.) stimulation test within 1 week of DKA/hyperglycemia resolution and at 1 wk following either discontinuation of insulin therapy or 12 weeks of insulin therapy. Nondiabetic control subjects were studied once.

Past studies indicate that at presentation, insulin sensitivity is markedly reduced in obese AA with KPDM, but insulin action improves to levels similar to obese subjects with type 2 diabetes (1). It was anticipated that due to the negative effect of hyperglycemia on insulin action, KPDM patients who remained with hyperglycemia would have lower insulin sensitivity compared to those who achieved remission.

EXPERIMENT 1B. Insulin sensitivity was measured at presentation and following discontinuation of insulin (near-normoglycemia remission) or at 12 weeks of insulin therapy (no remission). Responses in subjects that achieved remission were compared to those diabetic subjects that failed to discontinue insulin at 12 weeks. Responses

were also compared between obese DKA subjects and obese subjects with nonketotic hyperglycemia.

Protocol (see Detailed Methods section). At presentation and near-normoglycemia remission or after 12 weeks of insulin therapy (no remission), glucose, insulin and c-peptide levels were measures during a frequently sampled intravenous glucose tolerance test (FSIVGTT) to assess insulin sensitivity using the Bergman Model indices (19).

Detailed Methods

Organ-specific autoantibodies. Autoantibodies to glutamic acid decarboxylase (20) were measured on admission using standard laboratory assays run by Quest Laboratory Nichols Institute (Madision, NJ) (18).

Assessment of Pancreatic β-cell function. Measurements of β-cell function were performed with an I.V. glucagon stimulation test (1; 14; 15). Studies were performed after a 10-hour fast and the test were done at time of presentation and again at time of remission or 12 weeks of insulin therapy. Blood samples for C-peptide were drawn following the injection of glucagon (1 mg) with samples taken at 0, 3 and 6 minutes. β-cell functional reserve was defined as $\Delta_{C\text{-}peptide}$ where $\Delta_{C\text{-}peptide}$= C-peptide($t_{6min}$) – C-peptide($t_{0min}$).

Assessment of Insulin Sensitivity by Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT). As a surrogate measure of insulin resistance and in the DKA and hyperglycemic subjects, the minimal model of glucose kinetics, developed by Bergman et al was used to calculate the following measures: acute insulin response to glucose (AIR_g), the sensitivity index (SI), and the disposition index (DI). The AIR_g

represents the acute insulin response to a bolus of glucose and is defined as the area under the insulin curve between 0 and 10 minutes. SI indicates the net capacity for insulin to promote glucose disposal and to inhibit the endogenous production of glucose. The DI (AIR_g x SI) is a measure of the ability of the pancreatic islet cells to secrete insulin normalized to the degree of insulin resistance in the periphery. The minimal model using an injection of exogenous insulin has been shown to provide an accurate measurement of insulin sensitivity in diabetic subjects (7) and to provide estimates that correlate well with results obtained from the hyperinsulinemic-euglycemic clamp method (21; 22). The MinMod Millenium software package, version 6.02 was used to calculate the indices according to glucose and insulin values obtained during a frequently sampled intravenous insulin tolerance test (FSIVGTT) (23; 24). In addition to the aforementioned indices, the software was used to calculate the homeostatic model assessment (HOMA) of ß-cell function and HOMA of insulin resistance described by Matthews (25)(Table 5).

After a 10-hour overnight fast, catheters were placed in both antecubital veins. One vein was used for injection of glucose and insulin, and the other for obtaining blood samples for glucose and insulin measurements. After the collection of four baseline samples (-20, -15, -10, -5 minutes), a glucose load of 16.7 mmol x L^{-1} x kg^{-1} (50% dextrose) was injected over 2 minutes, and blood samples for glucose and insulin were collected at 1, 2, 3, 4, 5, 6, 7, 10, 12, 14, 16, and 20 minutes. Twenty 20 minutes following glucose administration, regular insulin was given intravenously at a dose of 0.05 U/kg, and blood samples for glucose and insulin were drawn at 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180 minutes.

Laboratory Assessments.

Glucose, insulin and C-peptide serum samples collected during the glucagon stimulation tests and the FSIVGTTs were stored at -80° C and later batched for processing at the Yerkes National Primate Research Center, Atlanta, GA. Serum glucose levels were measured using the glucose oxidase method (Siemens, Los Angeles, CA) and levels of insulin and C-peptide were measured from serum samples using radioimmunoassay kits commercially prepared by Siemens, Los Angeles, CA. The coefficient of variation of the assays was less than 15 percent. The instrument calibrations for the assays were performed by laboratory staff as recommended by the manufacturers and were within the specifications.

Sample Size Determination and Power

The data obtained in this study was preliminary in nature and thus, the sample size determination was limited by lack of previous data directly comparing a composite of clinical and metabolic factors between KPDM subjects in remission and those with the inability to discontinue insulin. A sample size approximation was performed based on previous investigations noting a ≥ 1.0 ng/mL difference in acute insulin response to glucagon (AIR_g: incremental change in C-peptide over baseline levels), either at presentation and or follow-up, among obese-diabetic subjects who achieved near-normoglycemic remission versus those who failed to achieve remission. A sample size of at least 36 subjects provided at least 80% power to detect any given differences between the groups in Aims 1 and 2. This sample size reflected the estimation based on preliminary data that approximately 80% of the enrolled subjects would be able to discontinue insulin and that another 20% would drop out of the study.

Statistical Analysis

Descriptive analyses were conducted to examine the distribution of measured variables in the cohort. All data are expressed as mean ± standard deviation (SD). Comparisons of continuous data were made between the DKA group, the HG group and the control group using the two-sample t-test The Wilcoxon rank sum test was used for variables not normally distributed. Proportional outcome measures between dichotomous variables, such as GAD antibody status, were analyzed using the Chi-square test. Measured variables were also examined between those in the remission group and the non-remission group in the same fashion.

Continuous outcome measures, such as basal and stimulated C-peptide levels and incremental C-peptide response to glucagon (AIR_g: incremental change in C-peptide over baseline levels), were analyzed by repeated measures analysis of variance (ANOVA) comparing those in remission to those without remission and comparing obese subjects with severe hyperglycemia to obese subjects with DKA across time at presentation and at follow-up.

Selected correlative variables (Table 10) were analyzed for the purpose of doing univariate analyses and multivariate analyses for the purpose of building a valid logistic regression model that could be used to predict the dichotomous outcome in response to aggressive diabetes management. In order to establish cut-off values for logistic model building and determine factors that could be associated with remission, the data was dichotomized based on reports in the literature, correlative analyses and scatter plot separations (Table 11). The data was assessed for interactions between predictor variables placed in the model. The statistical analysis was performed using the SAS 9.1 software package. A p-value of <0.05 was considered significant.

RESULTS

Study population

Subjects were overweight/obese, African Americans (24 M/15 F), recently diagnosed with new-onset diabetes, 17 presenting with DKA and 22 presenting with severe HG. All subjects presented with complaints of days to weeks of polyuria, polydipsia, extreme fatigue and weight loss (DKA: 9.7 ± 8.3, HG: 6.83 ± 3.86; mean ± SD) and those with DKA lacked a precipitating cause for metabolic decompensation (i.e. infection, infarction or trauma). Descriptive data analyses were done for the data set were means, variances, distributions, and skewness were evaluated. At time of enrollment, the subjects (41 ± 8 years of age, body mass index (BMI) 39.45 ± 8.76 kg/m^2) had diabetes for a mean duration of 36 hours and had a mean age was 41 ± 8 years, body mass index (BMI) was 39.45 ± 8.76 kg/m^2. All patients were treated with a twice a day subcutaneous insulin regimen and received an average insulin dose of 0.67 ± 0.25 units/kg/day. The mean A1C and fasting plasma glucose at the time of diagnosis were 12.30 ± 2.07 % and 647.64 ± 248.39 mg/dL, respectively. None of the patients had a precipitating cause of DKA or severe HG. The subjects with DKA and severe HG were of comparable age and BMI; however the subjects with DKA had slightly higher BG levels at presentation, metabolic academia (mean ph 7.2 ±0.08, β-hydroxybutyrate 7.0 ± 3.00 mmol/L), and required higher doses of insulin during the course of the follow-up to achieve glycemic control (Table 2). Of note, 50% of subjects with severe HG without blood ketosis (mean β- hydroxybutyrate < 4.00 mmol/L) had ketones in the urine. The study had a 28% attrition rate which was slightly higher than the 20% anticipated.

The control group consisted of 6 African American volunteers with normal glucose tolerance based on a 2-hour oral glucose tolerance test performed during screening. The controls were comparable in age, BMI and family history of diabetes (Table 1).

Autoantibody Testing

GAD antibody testing was performed in 12 of the 17 subjects (71%) with new-onset obese DKA and 11 of the 22 (50%) with new-onset obese HG. Of the subjects tested, the prevalence of GAD autoantibodies in the groups studied was very low with only one of the obese DKA subjects (8.33%) positive and none of the obese HG subjects affected. The one subject with the positive GAD antibody status belonged to the group that failed to go into remission after 12 weeks of insulin therapy. There was no statistically significant difference in GAD antibody status between the remission group and non-remission group. GAD antibody testing was not performed in the control population during this protocol.

Assessment of Pancreatic β-cell Function by Glucagon Stimulation Testing

Pancreatic insulin reserve was determined in all subjects within 1 week of resolution of DKA and/or hyperglycemia. The effect of 1 mg of I.V. glucagon on acute β-cell function was determined by changes in C-peptide at 0, 3 and 6 minutes. Beta-cell functional reserve was defined as the acute change in C-peptide ($\Delta_{C\text{-}peptide}$) where $\Delta_{C\text{-}peptide}$= C-peptide($t_{6min}$) – C-peptide($t_{0min}$). At time of presentation, obese subjects with a mean blood glucose of 575.90 ± 139.37 had a basal C-peptide of 1.47 ± 0.78, a stimulated 3-minute C-peptide level of 2.62 ± 1.27 ng/dL and a 6-minute C-peptide level 0f 1.14 ± 0.91 ng/dL. Subjects with DKA, on the other hand, had slightly higher mean serum glucose levels of 712.82 ± 323.50 mg/dL (p=0.253) and the presentation glucagon stimulation test revealed a basal C-peptide level of 2.15 ± 1.45 ng/mL, a peak 3-minute C-peptide level of 4.34 ± 2.92 ng/mL and a 6-minute C-peptide level of 4.01± 2.96 ng/mL (Figure 1 and Table 4). The acute C-peptide response to glucagon (incremental changes

in C-peptide above baseline level) was higher in subjects with new onset obese DKA than those with obese severe HG. There were no statistically significant changes between the basal or stimulated C-peptide levels at presentation or at 12 weeks follow-up that differentiated the remission group from the non-remission group.

In obese diabetic subjects that achieved remission, the stimulated C-peptide level at 3 minutes was 5.24 ± 1.77 compared to the stimulated C-peptide level of 7.04 ± 3.46 in subjects that did not achieve remission and in obese hyperglycemia. The acute C-peptide response to glucagon was surprisingly higher in the non-remission group versus the remission group (3.46 ± 1.61 ng/mL vs. 3.19 ± 1.61 ng/mL respectively, $p=0.021$); however the percentage increase from baseline was higher in subjects that went into remission (63.6 % vs. 45.7%, $p > 0.05$). The lack of significance in the percent change of C-peptide between the remission and non-remission groups was likely due to large SD noted in the remission group. The effect of DKA was sustained even in remission where the acute C-peptide response to glucagon was higher in the subjects with DKA-remission (2.23 ± 0.93 ng/mL) than those HG-remission (1.32 ± 1.61 ng/mL).

Assessment of Insulin Sensitivity by FSIVGTT

The FSIVGTT helped us to assess whether the primary defect in the subjects with KPDM (DKA vs. HG) is due to insulin resistance and/or to β-cell dysfunction and whether the improvement in either of these factors influences the ability to attain near-normoglycemic remission within 12 weeks of follow-up.

The frequently sampled IVGTT (FSIVGTT) was done within 1 week of resolution of DKA and/or hyperglycemia (< 250 mg/dL). This assessment was done in order to determine whether the outcome of remission was related to improved insulin sensitivity secretion and/or insulin secretion. Of the 39 subjects initially enrolled in the protocol, 27 were able

to undergo the FSIVGTT at presentation and 21 were able to undergo the repeat FSIVGTT at 12 weeks or sooner. The primary reason for excluding qualified subjects from the FSIVGTT was the inability to gain or maintain IV access. At time of presentation, the fasting glucose (197.86± 62.69 vs. 220.47 ± 66.62 mg/dL), the fasting insulin (12.84 ± 6.48 vs. 10.21 ± 7.22 U/mL) and fasting C-peptide (2.10 ± 1.32 vs. 1.7 ± 0.97 ng/mL) levels were not different in obese subjects with DKA and obese subjects with severe HG. Similarly, at time of near-normoglycemic remission, the fasting glucose (119.19 ± 16.06 vs. 107.56 ± 20.99 mg/dL), insulin (14.14 ± 7.43 vs. 12.72 ± 4.33 U/mL)and fasting C-peptide (3.36 ± 0.82 vs. 3.16 ± 0.98 ng/mL) levels were not different in obese subjects with DKA compared to obese subjects with severe HG. When considering if differences existed between those that achieved remission (FBG < 130, A1c < 7%) at ≤12 weeks of insulin therapy and those subjects that failed to wean insulin, the data showed that the fasting insulin level (13.62 ± 4.73 vs. 63.23 ± 59.13 U/mL, $p < 0.01$) was significantly lower in the remission group but the fasting glucose (112.52 ± 15.44 vs.164.66 ± 102.12 mg/dL, $p = 0.38$) and fasting C-peptide (3.08 ± 0.94 vs. 3.47 ± 1.62 ng/mL, $p=0.66$) levels were not different.

The acute insulin response (AIR_g) to an IV glucose load (16.7 mmol x L^{-1} x kg^{-1}) can be used to assess first-phase insulin secretion during the first 20 minutes of the FSIVGTT. This insulin response is an indicator of acute β-cell function but is also influenced by the degree of insulin sensitivity in the peripheral tissues. Irrespective of a classification of DKA vs. HG or remission vs. non-remission, the first phase insulin response was blunted at the time of diagnosis (-30.37 ± 66.34 mu x L^{-1} x min vs. 0.88 ± 69.24 mu x L^{-1} x min; -12.80 ± 26.54 mu x L^{-1} x min vs. -46.39 ± 88.03 mu x L^{-1} x min, respectively). Mean values for the non-diabetic controls were 1003.29±529.80 mu x L^{-1} x min. The measurement improved appreciably after a ≤ 12 week-course of subcutaneous insulin

therapy such that all subjects regained first-phase insulin response (DKA: 209.44±309.69 mu/Lxmin vs. HG: 80.20±88.88 mu/Lxmin; remission: 74.11±94.83 mu/Lxmin vs. non-remission: 377.33±322.59 mu/Lxmin). The peak insulin response is typically noted within 2 minutes of a given glucose load and during the follow-up phase of this protocol, it was noted that the subjects that achieved remission (DKA and HG) had the most robust insulin increase from baseline when compared to the other groups (Δ increase of 152.45%).

Minimal Model Analysis

Subjects with DKA vs. HG at presentation had comparable SI and HOMA insulin resistance (Table 6) but the DKA cohort had a *significantly* lower acute insulin response to glucose (-28.18±63.71 vs. 0.88 ± 69.24 mu x L^{-1} x min). The HOMA β-cell function at presentation was better in the DKA group; however, this difference was not statistically significant. At follow-up, the SI, DI and β-cell function measurements improved in both groups and had a greater increment in subjects with DKA; however, insulin resistance worsened for subjects with severe HG (12.35 ± 30.28 vs. 6.06 ± 3.79 mM.mu/L^2). Of note, the DKA and the HG subjects still had similar SI values at follow-up but the insulin resistance measurement only improved in the subjects with DKA (Table 8). When comparing those subjects that achieved remission versus those that did not, both groups exhibited improvement of SI, DI and β-cell function (remission > non-remission); however, the non-remission cohort had a significant worsening of the insulin resistance index.

The control group (N=6) had *significantly* higher SI, DI and β-cell function and lower insulin resistance compared to the subjects with DKA or severe HG

Variable Differences between Subjects that Achieve Remission versus Non-remission

Of the 24 subjects enrolled into the protocol at presentation, 71.4 % of DKA cohort and 64.7 % of the severe HG cohort weaned insulin therapy and achieved near-normoglycemic remission after a combined average of 10 weeks of insulin (Tables 3, 7 and 9). Potential factors that may have contributed to the ability to discontinue insulin therapy at ≤ 12 weeks include clinical findings at presentation (i.e. age of onset, BMI, type of glycemic crises) as well as metabolic markers before and after the period of glycemic decompensation (Table 10). Comparisons were made between the subjects that returned for the 12-week GCRC visit that were in remission and those that were not able to discontinue insulin after 3 months of therapy. Each dichotomized variable (Table 11) was then analyzed in a frequency table and using an univariate model. The Chi-square analysis (Table 12) showed that the weight loss on presentation was significantly associated with the outcome of remission. The univariate analysis (Table 13), however, did not show any significant association between the chosen predictors and the outcome of remission. A multivariate logistic regression model was built using a combination of six dichotomized variables that included weight loss @ presentation, BMI, type of diabetic crisis (DKA vs. severe hyperglycemia), gender and GAD antibody status. Regardless of the number of variables placed in the model or the combination, no significant results were found using the multivariate analysis.

Subjects with DKA had significantly lower bicarbonate (HCO_3) levels and required more daily insulin than the subjects with HG; otherwise, comparative analysis of the characteristic data at time of presentation and at ≤ 12 weeks of follow-up did not reveal any differences between subjects that presented with obese DKA versus obese HG.

DISCUSSION

Ketosis-prone type 2 diabetes (KPDM) is a term used to described a subtype of patients with recently diagnosed diabetes, who despite presenting with unprecipitated diabetic ketoacidosis subsequently exhibit clinical and metabolic features of type 2 diabetes (3; 6; 13; 14; 26-30). The subjects with KPDM in this study, similar to cohorts described previously in the literature, were found to have an overweight or obese habitus, have a strong family history of diabetes, a low prevalence of autoimmune markers, and the ability to discontinue insulin after an average treatment period of 10 weeks. It is estimated that this clinical presentation affects 20-50% of newly diagnosed African-American and Latino patients with DKA; thus, better characterization of the metabolic profile and clinical course would help to elucidate the underlying mechanism(s) of glycemic decompensation and direct optimal management of this patient population.

Our hypothesis predicted that measures of β-cell function, alone or in combination with measures of autoantibodies and insulin sensitivity would correlate with the ability of a KPDM patient to achieve near-normoglycemic remission. These measures could in turn be used to determine which patients are at risk of persistent hyperglycemia or recurrence of ketoacidosis. This study showed that subjects with overweight/obese DKA and HG present similarly and do not differ significantly in their *initial* clinical course as it relates to the ability to wean off of insulin. This finding was supported in a recent paper showing that early and intensive insulin therapy (mean 0.74 u/kg/day) in those with new-onset type 2 diabetes without DKA was associated with the increased ability to discontinue insulin therapy, maintain β-cell function and prolong glycemic remission compared with those initially treated with oral antidiabetic agents. (31). Our study did

not reveal any differences in the analyzed predictive factors at time of presentation between subjects with DKA and subjects with HG. This study showed that weight loss at time of presentation was associated with a higher chance of remission compared to those subjects without weight loss at time of diabetes diagnosis. This association was lost, however, when placed in a logistic regression model with other predictive factors. Thus, the results of the logistic regression analysis, controlled for confounding, represent the correct assessment. True differences in clinical outcome may be revealed in future observation studies that will follow the two cohorts over a 3-year interval. It is suspected that subjects with KPDM will remain in near-normoglycemic remission longer if treated with oral antidiabetic agents after the discontinuation of insulin and that subjects that presented with DKA will have a higher risk of and shorter time before experiencing glycemic decompensation developing DKA.

There is evidence that chronic hyperglycemia leads to glucotoxicity or glucose desensitization, a clinical picture of both impaired insulin secretion and action (21; 32; 33). This study revealed that both subjects with DKA and HG had markedly impaired insulin sensitivity/action and insulin secretion compared to control subjects at time of presentation, but intense treatment with high dose subcutaneous insulin resulted in significant improvement in insulin sensitivity and β-cell function sufficient to allow discontinuation of insulin therapy within 12 weeks of follow-up in the majority of subjects. Evidence for glucotoxicity is suggested by the finding that the fasting C-peptide level improved by 1.7-3 fold at time of follow-up where good glycemic control was achieved (BG < 120 mg/dL) and the disposition index greatly improved in both groups at time of follow up.

Limitations

This study had limitations including a relatively small number of patients and the fact that the data applies to an inner-city, overweight/obese, African American population. The attrition rate was slightly larger than anticipated (28%) rather than the estimated 20-25% and thus all enrolled subjects with presentation data did not contribute to the 12-week assessment. Another limitation of the study relates to metabolic assessments performed in this study.

The hyperinsulinemic euglycemic clamp and the hyperglycemic clamp, direct measurements of glucose homeostasis, are the accepted gold standard techniques for assessing insulin sensitivity and assessing pancreatic β-cell function in adults. Using the FSIVGTT data to calculate Minimal Model and HOMA indices for whole body insulin sensitivity have been tested against these standards and have been shown to be scientifically comparable while at the same time less labor intensive, less expensive and less complicated (25; 34-36). The FSIVGTT primarily assesses IS (insulin sensitivity) at the level of the muscle whereas variations of the hyperinsulinemic euglycemic clamp technique can elucidate IS at the level of the muscle and/or hepatic tissue. Unlike the clamp methodology which depends on steady-state conditions, the FSIVGTT/minimal model uses dynamic data and requires more assumptions that include 1) that instantaneous distribution of the glucose bolus occurs in a monocompartmental area, 2) glucose disappearance in response to glucose and/or, insulin occurs at a monoexponential rate, 3) the glucose concentration at the end of the FSIVGTT is identical to the beginning concentration, and 4) insulin works from an extravascular compartment to promote glucose clearance and 5) the total insulin secretion during the FSIVGTT is above a certain threshold. Finally, the minimal model combines the effects

of insulin to promote glucose disposal in skeletal muscle and suppress hepatic glucose output. Due to the dynamic basis of the FSIVGTT and the absolute assumptions, the true S_I and S_G can be nonsensical such that the S_I has a negative value or is underestimated and the S_G is overestimated. In addition, S_I results are less reliable in diabetic subjects that have significant insulin resistance or impaired insulin secretion. A modified FSIVGTT incorporating radiolabled-glucose which would allow a second compartment for glucose can lead to better estimates of S_G and S_I. Of note, the S_I calculation using the minimal model approach is also less reliable in subjects with insulin resistance and this clinical feature is one of the pathophysiologic cornerstones for our study population (37). In the future, clamp assessments will be performed in order to obviate the variability and limitations associated with the FSIVGTT/MinMod approach. This investigator suspects that the calculations from using the HOMA are not exceedingly reliable due to the extreme variability in some of the index measures. This is evidenced in the HOMA β-cell assessment where subjects with DKA had significantly more β-cell function at presentation; a finding that is not congruous with the fact that ketoacidosis is a result of absolute or relative loss of β-cell function.

There were some notable differences between the fasting glucose, insulin and c-peptide levels between subjects that achieved remission and those that failed to achieve remission. The variability of the fasting blood glucose in those subjects with a lack of remission reflects the fact that subjects were maintained on insulin.

In order to rule out the possibility of selection bias in this cohort study, an assessment of the general population seen at Grady Memorial Hospital was performed in this study. The charts of 50 non-participating subjects with qualifying inclusion criteria for enrollment in this study were reviewed and it was found that this sample population cohort was similar to the studied index cohort.

CONCLUSIONS

In summary, ketosis-prone diabetes mellitus is a common occurrence in minority populations and nearly three-fourths of those affected can successfully discontinue insulin therapy. Our study showed that obese/overweight subjects with diabetic ketoacidosis and severe hyperglycemia respond similarly to intensive insulin therapy at time of presentation and that subjects with DKA may have a higher chance of achieving remission. More importantly, our studies provide evidence for the role of hyperglycemia as an important cause of β-cell decompensation in African-American subjects with KPDM and show that patients with KPD have improvement in *both* insulin sensitivity and secretion following the acute metabolic insult. The putative role of glucose-induced β-cell dysfunction has broad implications for the management of patients with decompensated diabetes and implies that hyperglycemia may be a consequence of altered β-cell function. The mathematical assessments of pancreatic β-cell function or peripheral insulin sensitivity at presentation or at 12 weeks *did not*, however, correlate with the ability to achieve short-term remission in this study cohort.

As it relates to patient response to insulin therapy, nearly 70% of the most recent cohort (with DKA and with hyperglycemia) were able to wean off of insulin therapy. Outside of weight loss at presentation as a single prognosticator of the short-term outcome (≤12 weeks) of insulin discontinuation, our study did not find other variables that predict the ability of patients to attain remission. This initial data will grant the opportunity to collect more data needed to 1) better differentiate between obese patients presenting with DKA from those obese patients with nonketotic severe hyperglycemia and 2) establish a strong model to predict long-term outcomes, particularly as it relates to response to remission and medical treatment.

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Tables and Figures

Table 1. Clinical Characteristics of Subjects at Presentation*

	DKA	HG	p value‡	Controls
Number of patients	17	22	---	6
Gender (M/F)	12/5	12/10	0.307_{1}	2/4
Age (years)	41.76 ± 11.96	42.32 ± 8.64	0.877_{2}	37.00 ± 13.9
BMI (kg/m^2)	40.09 ±10.95	35.45 ± 13.21	0.254	
HCO3 (meq/L)	14.71± 4.62	24.64± 3.59	<0.001	**----**
Family History	88.2%	85.7%	1.00_{1F}	83.3.%
Fasting BG (mg/dL)	712.82 ± 323.5	575.90 ± 139.4	0.253_{2}	83.50±14.07
Hemoglobin A1c (%)	12.12 ± 2.12	12.74 ± 2.14	0.399	----
Insulin needs (u/kg/day)	85.12 ± 32.54	64.48 ± 16.05	0.05	----
Weight loss @ presentation	82.4%	95.0%	0.315_{1F}	----
GAD Ab (u/mL)	8.3%	0.0%	0.480_{1F}	----
Fasting C-peptide (ng/mL)	1.81 ± 1.06	1.47 ± 0.78	0.934_{2}	

*Values are mean ± SD **

1=X_2 ± Fisher's(F) 2=Wilcoxon ‡p < 0.05, 2 tailed (DKA vs. HG subjects)

***DKA**, diabetic ketoacidosis; **HG**, severe hyperglycemia*

***GAD Ab**, glutamic acid decarboxylase antibody*

Table 2. Clinical Characteristics of Subjects at ≤ 12 weeks of Follow-up*			
	DKA	**HG**	**p-value‡**
Number of patients	12†	15‡	----
Remission	71.43%	64.71%	1.000_{1F}
Weight gain (kg) @ F/U	7.33 ± 11.36	2.47± 8.04	0.265
F/U weight	267.48 ± 81.78	240.00 ± 46.56	0.554_{2}
F/U FBG (mg/dL)	133.46± 40.00	113.30 ± 21.00	0.164_{2}
Weeks of insulin treatment for those in remission	10.43 ± 1.77	9.61 ± 2.59	0.624_{2}
Follow-up fasting c-peptide (ng/mL)	3.54 ± 0.90	2.96 ± 1.03	0.231
Values are mean ± *SD* *†= reflects 12% attrition ‡= reflects 18% attrition *1=X_2 2=Wilcoxon ‡p < 0.05, 2 tailed (DKA vs. HG subjects)*			

Table 3. Clinical Characteristics in Subjects with Remission versus Subjects without Remission*

	Remission	Non-Remission	p-value
Number of patients	21	10	----
Gender (M/F)	15/6	4/6	0.127_{1F}
Age (years)	43.43 ± 8.81	44.44± 10.26	0.770_{2}
BMI (kg/m^2)	38.53 ± 8.05	36.19 ± 16.4	0.692
DKA/Severe HG	10/11	4/6	1.000_{1F}
Insulin needs (u/kg/day)	70.05 ± 21.60	81.22 ± 41.30	0.460
GAD Antibody* (u/mL)	0%	16.7%	0.316_{1F}
Weight loss @ presentation	65.5%	34.5%	$0.033^{‡}_{1F}$
Fasting C-peptide (ng/mL) @ follow-up	1.47 ± 0.76	2.22 ± 1.23	0.343
3-minute C-peptide (ng/mL) @ follow-up	3.13 ± 0.89	3.47 ± 1.62	0.677
Percent increase C-peptide @ follow-up (ng/dL)	112.9%(164.58 ± 173)	56.3% (48.27 ± 34.7)	0.192_{2}

*Values are mean ± SD **
1=X_2 ± Fisher's(F) 2=Wilcoxon ‡p < 0.05, 2 tailed

Figure 1. Plasma C-peptide levels before and after stimulation with intravenous glucagon (1 mg) in obese subjects with DKA and with HG. Values are means +/- SD.

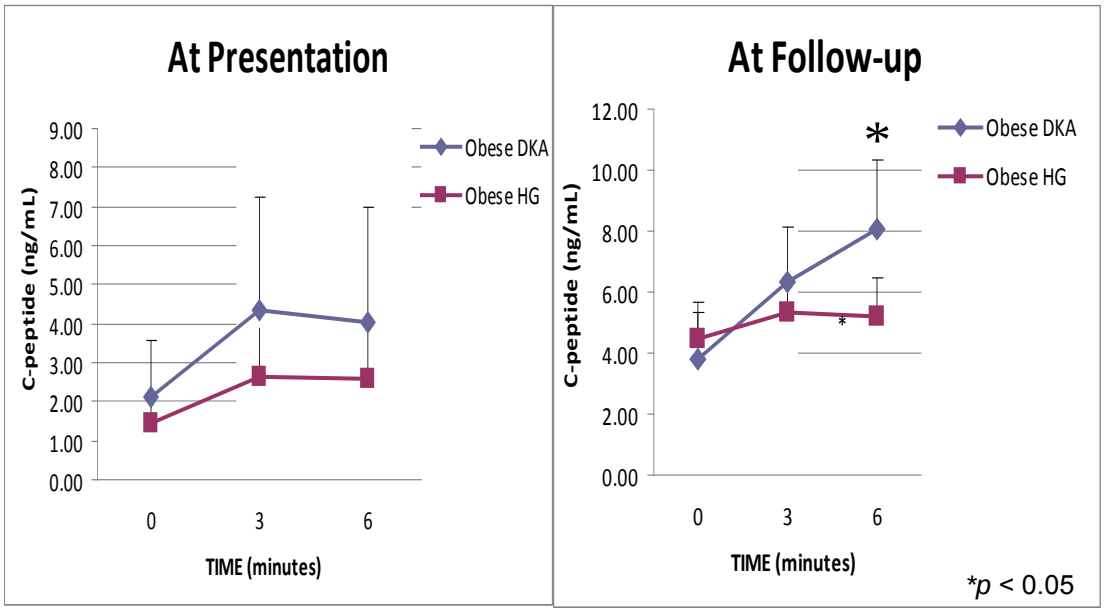

Table 4. Glucagon Stimulation Test C-peptide Response @ 3 minutes between subjects with DKA and subjects with severe hyperglycemia at presentation

	Obese DKA	Obese HG	p-value
Basal C-peptide (ng/mL)	2.15 ± 1.45	1.47 ± 0.78	0.854
Stimulated C-peptide$_{3min}$ (ng/mL)	4.34 ± 2.92	2.62 ± 1.27	0.231
Acute C-peptide $\Delta_{3min\text{-}0min}$ (ng/mL)	1.65 ± 2.17	1.14 ± 0.91	0.361
*Values are mean ± SD * *2-tailed 2=Wilcoxon p < 0.05*			

Table 5. Indices of Insulin Sensitivity and β-cell insulin Secretion @ presentation (Insulin Sensitivity and β-cell function)
Formulas for Insulin Sensitivity
Disposition Index (DI) = AIR_g * SI
Sensitivity Index (SI) = P3 / P2 P2 = removal rate of insulin from the interstitial space P3=movement of circulating insulin to the interstitial space
HOMA Insulin Resistance = FBG mg/dL x Fasting Inuslin (FI) µU/mL / 405
Formulas for β-cell Activity
HOMA ß-cell function = 20 * FI µU/mL / (FBG/18 - 3.5)

Table 6. Minimal Model Indices @ presentation based on group* (Insulin Sensitivity and β-cell function)

	DKA	**HG**	**p-value**	**Controls** ‡
Number of patients	12	15	----	6
Sensitivity Index (SI)* ((mu/L)^-1.min^-1)	1.06 ± 1.33	1.32 ± 1.36	0.467_2	2.45 ± 0.68
Disposition Index (DI)	-33.83 ± 71.99	190 ± 200.10	0.011_2 ‡	2353.77± 1368.96
Insulin Resistance (mM.mu/L^2)	7.40 ± 5.06	5.88 ± 4.51	0.571_2	1.36±0.87
Beta-cell function* (mu/mM)	45.60 ± 37.82	26.78 ± 18.46	0.211_2	133.74 ± 87.9

*Values are mean ± SD **

*p<0.05 *2-tailed 2=Wilcoxon ‡p < 0.05 (DKA vs. HG)*

Table 7. Minimal Model Indices @ presentation based on outcome* (Insulin Sensitivity and β-cell function)

	Remission	Non-remission	p-value	Controls
Number of patients	14	10	----	6
Sensitivity Index (SI)* ((mu/L)^-1.min^-1)	1.61± 2.003	1.66 ± 1.72	0.847_2	N/A
Disposition Index (DI)	-16.95 ± 1.95	-33.7 ± 117.34	0.732_2	N/A
Insulin Resistance (mM.mu/L^2)	5.32 ± 4.25	9.61± 5.80	$0.050_2^{\ddagger}$	N/A
Beta-cell function* (mu/mM)	26.23± 19.4	61.45 ± 41.55	0.126_2	N/A

*Values are mean ± SD **
*p<0.05 *2-tailed 1=X2 ± Fisher's 2=Wilcoxon*

Table 8. Minimal Model Indices @ 12 weeks based on group* (Insulin Sensitivity and β-cell function)

	DKA	HG	p-value	Controls
Number of patients	11	10	----	N/A
Sensitivity Index (SI)* ((mu/L)^-1.min^-1)	1.80 ± 2.43	1.90 ± 2.01	0.654_2	N/A
Disposition Index (DI)	40.35 ± 67.53	192.99 ± 213.72	0.205_2	N/A
Insulin Resistance (mM.mu/L^2)	6.69 ± 4.03	14.16 ± 33.55	0.535_2	N/A
Beta-cell function* (mu/mM)	330.71 ± 69.99	152.07 ± 68.52	0.076_2	N/A

*Values are mean ± SD **
*p<0.05 *2-tailed 2=Wilcoxon*

Table 9. Minimal Model Indices* @ 12 weeks based on outcome (Insulin Sensitivity and β-cell function)

	Remission	Non-remission	p-value	Controls
Number of patients	16	6	----	N/A
Sensitivity Index (SI)* ((mu/L)^-1.min^-1)	2.63 ± 2.04	0.23 ± 0.44	0.298_2	N/A
Disposition Index (DI)	120.91 ± 177.30	46.89 ± 75.98	0.658_2	N/A
Insulin Resistance (mM.mu/L^2)	3.72 ± 1.81	31.86 ± 48.10	0.120_2	N/A
Beta-cell function* (mu/mM)	138.88 ± 72.50	477.02 ± 450.74	0.211_2	N/A

*Values are mean ± SD **
*p<0.05 *2-tailed 1=X2 ± Fisher's 2=Wilcoxon*

Table 10: Putative Factors for Remission in Subjects with New Onset KPDM

• Age	• Length of insulin therapy
• Gender	• Glutamic acid decarboxylase Antibody (GAD)
• Body Mass Index	• Presentation Glucagon Stimulation Test (GST)
• Type of glycemic crisis (DKA or severe HG)	• Presentation Minimal Model Indices
• Fasting Blood Glucose	• Follow-up C-peptide
• Weight loss @ presentation	• Follow-up Minimal Model Indices
• Amount of insulin needed	

Table 11. Dichotomization of Continuous Predictors

Independent variable	Cut off	Remission (1)	Non-remission (0)
Age (years)	39	< 39	≥ 40
Gender (M/F)	1	1	0
DKA or Severe HG	DKA	HG	DKA
BMI	40	< 40	≥ 40
Initial BG	560	< 560	≥ 560
Fasting C-peptide @ presentation	1.5	≥ 1.5	< 1.5
Δ C-peptide $_{(3min)}$	1.4	>1.4	≤ 1.4
GAD Antibody	+	negative	positive
Weight loss at presentation	+	positive	negative

Table 12. Frequency of Predictors for Remission versus Non-remission

Variable	Remission		Non-remission		χ^2	*p* value*
	n		n			
Age (1)	11	28.57 %	3	20.00%	0.260	1.000_F
Gender (1)	15	71.43 %	4	40.00%	2.820	0.127_F
Group (DKA=1)	7	33.33 %	4	40.00%	0.150	1.000_F
BMI (1)	16	76.19 %	9	70.00%	0.034	1.000_F
Initial BG (1)	11	52.38 %	3	30.00%	0.828	0.208_F
C-peptide (1)	10	33.33 %	6	60.00%	0.793	0.247_F
GAD Ab (0)	0	0.00%		16.67%	2.170	0.316_F
Weight loss @ Presentation (1)	19	100.00%	7	70.00%	3.840	0.033_F
F= Fisher's Exact, $p < 0.05$						

Cases: Remission = 1, Controls: Non-remission = 0
Age = Age in years (1 = < 39 years, 0= ≥ 40) Gender (1=M, 0=F)
Group (1= DKA, 0=severe HG) BMI (1= ≤ 39 kg/m^2, 0= >40 kg/m^2)
Initial BG (1= <560, 0= ≥560) C-peptide $_{fasting}$ (1= >1.5, 0= ≤1.5)
GAD Antibody (1 = negative, 0= positive)

Table 13: Measure of Associaion between Potential Predictors and Outcome of Remission

Variable	Crude OR	95% CI
Age	1.60	0.26 - 9.83
Gender	3.75	0.77 - 8.21
Group	0.73	0.16 - 3.38
BMI	1.07	0.21 - 5.58
Initial BG	2.57	0.52 - 2.72
Fasting C-peptide @ presentation	0.33	0.07 - 1.58
GAD Antibody	0.14	0.00 - 3.87
Weight loss	8.57	0.76 - 96.52
SI @ presentation	2.10 4.67	0.92 - 23.78
DI @ presentation	1.68 2.13	0.42 - 10.73

Cases: Remission = 1, Controls: Non-remission = 0
Age = Age in years (1 = < 39 years, 0= ≥ 40) Gender (1=M, 0=F)
Group (1= DKA, 0=severe HG) Initial BG (1= <560, 0= ≥560)
Fasting c-peptide (1= >1.5, 0= ≤1.5)
GAD Antibody (1 = negative, 0= positive)