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Metabolic characteristics as phenotypic markers in type 2 diabetic ketoacidosis

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METABOLIC CHARACTERISTICS AS PHENOTYPIC MARKERS IN TYPE 2 DIABETIC KETOACIDOSIS

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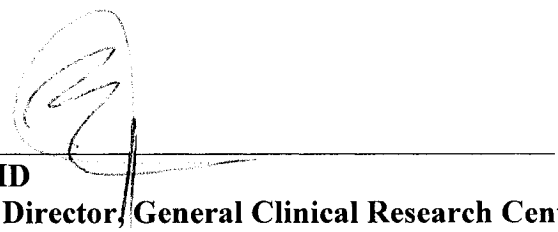

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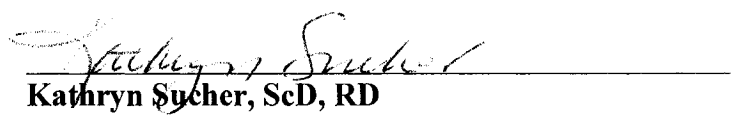

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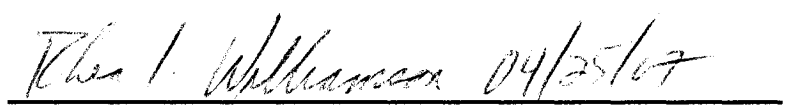

ABSTRACT

METABOLIC CHARACTERISTICS AS PHENOTYPIC MARKERS

IN TYPE 2 DIABETIC KETOACIDOSIS

By Amy Renne Stammreich

To evaluate the possibility of an overlap syndrome between type 1 and type 2 diabetes, we investigated Metabolic Syndrome characteristics as phenotypic markers to determine if these patients fit the phenotype of type 2 diabetes, rather than type 1 diabetes. Data from one hundred eighty-seven patients admitted with either diabetic ketoacidosis or uncontrolled diabetes who met entrance criteria for the study were analyzed for features of the Metabolic Syndrome using NCEP-ATP III criteria. Our results demonstrate that at the time of presentation, patients with ketotic-prone type 2 diabetes (KPDM-2) resemble non-ketotic patients with type 2 diabetes rather than type 1 diabetes. This study also demonstrates that these patients have a higher incidence of extreme elevations in serum triglycerides than patients with either type 1 diabetic ketoacidosis or non-ketotic type 2 diabetes. Lastly, we found that waist circumference was the best predictor for type 2 diabetes when presenting with ketoacidosis.

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PREFACE

The following is a publication style thesis. Chapters I and III are written according to the guidelines outlined in the *Publication Manual of the American Psychological Association, 5th edition, 2001.* Chapter II is written in journal format for submission to *The Journal of Clinical Endocrinology and Metabolism.*

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CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Diabetic ketoacidosis (DKA) is a serious metabolic complication of diabetes mellitus caused by a lack of insulin. The pathogenesis of DKA includes the combination of two distinct factors, the first being the reduction of circulating insulin, and the second a simultaneous increase in counterregulatory hormones, including glucagon, catecholamines, cortisol, and growth hormone. This combination leads to the release of free fatty acids from adipose tissue via lipolysis and the oxidation of hepatic free fatty acids to form ketone bodies, thus leading to the development of ketoacidosis (Ipp & Westhoff, 2002). Since DKA is known to be a result of severe insulin deficiency, it is considered to be a clinical marker of beta cell failure. Therefore, it typically occurs in patients with type 1 diabetes, which is a disease of progressive beta cell destruction with loss of insulin secretion. Ketoacidosis was thought to be a rare occurrence in patients with type 2 diabetes due to adequate beta cell function and insulin secretion, enough to prevent the biochemical cascade into ketoacidosis.

DKA is no longer a complication exclusively seen in persons with type 1 diabetes. Patients with type 2 diabetes are increasingly reported with what is referred to as "ketotis prone type 2 diabetes" (KPDM2). These patients present like patients with type 1 diabetes, requiring insulin in order to treat the ketoacidosis. Yet, many of these patients are able to discontinue use of insulin after a certain period of time and subsequently continue on a clinical course more reminiscent of type 2 diabetes. Human leukocyte antigen (HLA) studies in one report (Banerji, 1994) also reveal that these patients possess features present in type 1 diabetes, yet they also present with clinical features found in

patients with type 2 diabetes, such as obesity, hypertension, negative glutamic acid decarboxylase antibodies, and absence of islet-cell cytoplasmic antibodies. Although, the mechanisms behind this phenomenon are not fully understood, the idea that ketotis prone type 2 diabetes could be an overlap syndrome of type 1 and type 2 diabetes has been suggested. This overlap could explain the extensive insulin deficiency found in this form of diabetes.

Review of Literature

Diabetes Mellitus

Diabetes mellitus can be described as a group of disorders characterized by glucose intolerance and chronic hyperglycemia that results from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2003). Type 1 diabetes mellitus (or insulin dependent diabetes mellitus) is characterized by an absolute insulin deficiency that results from the autoimmune destruction of pancreatic beta-cells in susceptible individuals (Huether, 2000). This destruction is thought to be the result of both genetic and environmental factors that lead to the gradual destruction of beta-cells. Type 2 diabetes mellitus (or non-insulin dependent diabetes mellitus) is characterized by insulin resistance combined with an insulin secretory deficiency. It is also attributed to genetic factors, since it often runs in families, and environmental influences, such as obesity (Huether, 2000). It is important to note that beyond type 1 and type 2 diabetes, other specific types of diabetes exist. These alternate forms of diabetes include genetic defects of the beta-cell, genetic defects in insulin action, and gestational diabetes, to name just a few.

Insulin resistance and type 2 diabetes mellitus

Type 2 diabetes mellitus is the most common form of diabetes, which results from insulin resistance with an impairment of insulin secretion. It is important to state that one can be insulin resistant and not develop diabetes, as for example in obesity, yet when insulin resistance is combined with decreasing amounts of secreted insulin, diabetes will ensue (Leahy, Bonner-Weir, Weir, 1992). The presence of insulin resistance can often be shown during an oral glucose tolerance challenge. During this test, patients with insulin resistance will have high levels of circulating insulin while at the same time may have normal plasma glucose responses. In type 2 diabetes, insulin secretion will diminish to the point where available circulating insulin will fall short of demand and hyperglycemia will prevail. Insulin resistance can result from either environmental causes (diet or obesity-related) or from genetic predisposition, or both. The Pima Indians are a good example of the influential role of obesity and genetic susceptibility on the incidence insulin resistance and type 2 diabetes (Knowler, Bennett, Hamman, and Miller, 1978). Since both obesity and type 2 diabetes have greatly increased in this population over the last century, researchers conclude that cultural and dietary influences have taken their toll on a population genetically susceptible to diabetes.

Metabolic Syndrome

Metabolic Syndrome, a syndrome first described by Gerald Reaven in 1988, reflects the recognition that insulin resistance is often associated with other metabolic and cardiovascular disorders. Reaven suggested that in certain individuals there may be a series of related variables that put them at a greater risk for Coronary Artery Disease

(CAD). These variables include resistance to insulin mediated glucose uptake, hyperglycemia, hyperinsulinemia, increased plasma concentration of VLDL triglyceride, a decreased plasma HDL-cholesterol, and high blood pressure. Reaven suggested that the above variables are consequences of insulin resistance and have been shown to increase CAD risk (Reaven, 1988).

The above mentioned dyslipidemia likely plays a major role in CAD risk. During insulin resistance, adipose cells also become resistant to the effects of insulin, therefore insulin is less effective in maintaining triglyceride storage in adipose cells. This results in the hydrolysis of triglycerides and the release of free fatty acids (Ginsberg, 2000). The physiological consequence of hyperlipidemia on pancreatic beta-cell function should also be noted. There is evidence to suggest that chronic exposure to circulating lipids, either in the form of free fatty acids, triglycerides, or lipoprotein could lead to beta-cell dysfunction (Biden, Robinson, Cordery, Hughes, and Busch, 2004). These same authors suggest that loss of beta-cell mass is a precursory event to the eventual hyperglycemia seen in diabetes. In addition, the involvement of other factors, such as defects in insulin secretion and insulin resistance occurring at the level of non-adipose peripheral tissues could play an integral role.

Diabetic Ketoacidosis

The consequence of a severe deficit in insulin secretion can result in what is called "diabetic ketoacidosis." Diabetic ketoacidosis (DKA) is the most severe metabolic complication of type 1 diabetes. In type 1 diabetes, severe deficiency of insulin, coupled with rising levels of insulin counter-regulatory hormones that increase blood glucose

levels, favor the production of ketones (Ipp & Westhoff, 2002). Under the above conditions lipolysis ensues, and the production of free fatty acids in the liver increases. These fatty acids are then oxidized and converted to ketone bodies, such as acetone and β-hydroxybutyrate. These ketone bodies can be used as fuel for the body for a brief period, but when produced in excess, can lead to ketoacidosis (Williams, 1997).

Ketotic-prone type 2 diabetes mellitus

Recent research verifies that DKA occurs more commonly in patients with type 2 diabetes than previously thought. The earliest evidence of this was presented by Winter in 1987. Winter, et al. (1987) describe an atypical form of diabetes with an unusual clinical course. In that study, African American youths presented with moderate to severe features of acute insulin deficiency, such as hyperglycemia, polyuria, polydipsia, weight loss, and ketoacidosis, and appeared to be insulin dependent at the time of diagnosis. Yet, months to years after diagnosis, these patients were able to stop their insulin regimen. This form of diabetes can be distinguished from insulin-dependent diabetes mellitus and has been appropriately termed "ketosis-prone type 2 diabetes mellitus" or KPDM-2. These patients became non-insulin dependent after a period time and followed a clinical course more like type 2 diabetes than type 1 diabetes.

In 1994, Banerji and colleagues studied a group of African American adults who presented with marked insulin deficiency and ketoacidosis. Like the African American youths studied by Winter, et al.(1987), these patients were treated with insulin at initial diagnosis, and then were able to stop taking insulin months to years later. Again, the authors found that these patients all presented with the same clinical features of

ketoacidosis as a patient with type 1 diabetes, yet followed a clinical course more like type 2 diabetes. Banerji and colleagues found that these patients had measureable C-peptides, which is a protein fragment produced in the formation of insulin, and negative islet cell antibodies and negative glutamic acid decarboxylase antibodies, a picture which is more representative of type 2 diabetes. But, they also presented with genetic features found in type 1 diabetes mellitus; they demonstrated increased frequency of the phenotypes HLA DR3 and DR4, raising the possibility that these patients possess the genes for type 1 diabetes as well. Due to the large number of local patients who presented with DKA, the authors referred to this type of diabetes as "Flatbush Diabetes", after the neighborhood surrounding the hospital in Brooklyn where these patients live. In addition to these patients possessing clinical features of both type 1 and type 2 diabetes, the authors showed that many of these patients were overweight and had a family history of diabetes.

Multiple studies have since shown that a similar phenomenon occurs in various ethnic groups world wide, including Japanese subjects (Aizawa, et al. 1995), Apache Indians (Wilson, Krakoff & Gohdes, 1996), Mexicans (Gomez-Diaz, et al., 1996), South Africans (Zouvanis, Pieterse, Seftel & Joffe, 1997), Chinese (Tan, Mackay, Zimmet, Hawkins & Lam, 2000), Pakistanis (Jabbar, et al., 2004), Africans of Sub-Saharan African origin (Mauvais-Jarvis, et al., 2004), as well as Southeast Asians residing in Canada (Zdravkovic, Daneman & Hamilton, 2004). Within the United States, studies have shown that this form of diabetes affects more people of color than Caucasians, with

the majority still being African American (Balasubramanyam, Zern, Hyman & Pavlik, 1999).

Ketotic-prone type 2 diabetes: a result of severe insulin deficiency

Beta-cell dysfunction leading to severe insulin deficiency is the most likely cause for ketoacidosis in the setting of type 2 diabetes. Linfoot, Bergstrom, and Ipp (2005) evaluate three possible mechanisms that play a part in the development of ketoacidosis: severe insulin deficiency due to suppressed insulin secretion, increased availability of substrate in the form of free fatty acids, and elevated counterregulatory hormones. These three factors are the primary pathogenetic mechanisms in the development of ketoacidosis. That study provided evidence for impaired insulin secretion as the primary event in the development of ketoacidosis in type 2 diabetes. In type 2 diabetes such a severe deficiency is likely due to beta-cell dysfunction rather than the lack of beta cells as is the case in patients with type 1 diabetes, yet why this beta cell dysfunction is so severe is unknown. We cannot say that the mechanism for severe insulin deficiency is the same in both type 1 and type 2 DKA. That is because the mechanism and the underlying etiology for DKA in type 2 diabetes is unknown.

It has been demonstrated that type 2 diabetes is associated with a decrease in beta cell mass, but not to the extent observed in type 1 diabetes, where the autoimmune process eventually destroys all beta cells (Huether, 2000). However, in type 2 diabetes sufficient beta cell mass is retained in general to prevent the development of ketosis (Huether, 2000). Even in KPDM-2, the fact that many patients are later able to manage without insulin treatment confirms the retention of beta cell mass and thus sufficient

insulin secretion to manage without an exogenous source. Because reduction of beta cell mass alone cannot fully explain the ketoacidosis in type 2 diabetes, functional abnormalities are presumed to exist (Linfoot, 2005), which are likely to be at least partly reversible.

KPDM-2- An Overlap Syndrome

Some of the research mentioned previously in this review raises the question whether or not type 2 DKA, or ketotic-prone type 2 diabetes mellitus (KPDM2) can be considered an overlap syndrome, since KPDM-2 patients possess features of both type 1 and type 2 diabetes. The fact that these patients present as overweight, are non-insulin dependent prior to presentation, and are antibody negative suggests that these patients are more reminiscent of patients with type 2 diabetes. Yet, there are features that suggest possible overlap with type 1 diabetes, including the observation that patients are younger at presentation, present with severe insulin deficiency and ketoacidosis, some develop repeat ketoacidosis (Mauvais, et al., 2004), and express an increased frequency of the alleles, HLA DR3 and HLA DR4, all typical of type 1 diabetes. This raises the possibility that KPDM-2 patients may possess some genotypic characteristics of type 1 diabetes, and that KPDM-2 is possibly an overlap syndrome or an intermediate phenotype not yet identified or understood.

Ketotic-prone type 2 diabetes and weight status

Obesity is a key trait in the type 2 diabetes phenotype, including patients with KPDM-2. Indeed, it was the presence of obesity in patients with ketoacidosis that alerted physicians to the fact that these were not typical type 1 diabetic patients, and thus to the

unique KPDM-2 population. Obesity is a common modifier of insulin resistance, yet at least two examples in the literature tie weight gain to insulin deficient syndromes as well, specifically to KPDM-2 and type 1 diabetes.

The first is a study by Mauvais-Jarvis and colleagues (2004) who studied immigrants from North Africa that settled in France, and found that both insulin-dependent and non-insulin dependent ketotic prone subjects had an increase in body weight between immigration to France and onset of diabetes. Overall, they found that 25% of the patients who were ketosis prone remained insulin dependent, were leaner than non-insulin dependent ketosis prone subjects, and displayed an irreversible β-cell failure, which is typical of type 1 diabetes. The remaining 75% became non-insulin dependent after a period of time. Thirty-nine percent of this group suffered relapses of DKA within the ten year follow-up period. Of this 39%, 73% of these patients entered into remission from insulin dependency again. The authors note that a significant increase in body weight, as well as a rise in blood glucose, preceded the reoccurrence of ketoacidosis in the subjects who experienced a relapse. Therefore, Mauvais-Jarvis, et al. (2004) speculated that weight gain may predispose ketotic-prone type 2 diabetics to DKA and relapse, and that obesity may play a role in the acute β-cell failure seen in rising amounts of African American and Hispanic American children in the United States.

The second study that stresses the significance of weight status and insulin deficient diabetes was performed by Kibirige, Metcalfe, Renuka & Wilkin (2003). These authors observed a strong correlation between a child's age at diagnosis of diabetes and their weight status was observed. The authors explain that as weight increases, the age of

diagnosis of type 1 diabetes decreases. They add that increasing weight may accelerate the pathogenesis of type 1 diabetes in these children, since they are already predisposed to develop the disease. The "Accelerator Hypothesis", which Kibirige, et al. (2003) present, argues that these children that develop type 1 diabetes are subject to the same weight gain, insulin resistance, metabolic upregulation, and beta-cell loss as their type 2 counterparts, yet these children are also genetically susceptible to an aggressive immune response on pancreatic beta-cells. The hypothesis goes further, suggesting that in the same way that greater obesity reflects a higher incidence of type 2 diabetes, heavier children will present with type 1 diabetes at a younger age. The mechanism, the authors explain, is that increased insulin resistance could further accelerate beta-cell loss, but they do not provide an explanation for how this may occur.

Conclusions

Ketotic-prone type 2 diabetes is a unique form of diabetes that includes phenotypic characteristics that suggest both type 1 and type 2 diabetes. While these patients seem to be more like type 2 rather than type 1 patients due to their overweight status, negative antibody profile, and remission from insulin dependence, the fact that they develop ketoacidosis, are at least transiently insulin deficient and may have genotypes typically seen in type 1 diabetes, suggest that they have similarities to type 1 diabetes.

This study was designed to evaluate the phenotype of KPDM-2 in more depth; specifically to determine if the clinical phenotype resembles more closely that of type 2 diabetes or type 1, or whether it falls somewhere between the two – an overlapping

phenotype. Because the characteristics of metabolic syndrome are so closely associated with type 2 diabetes, in this study we evaluated these characteristics in patients with KPDM-2 and compared them with patients who have DM-1 or nonketotic DM-2.

CHAPTER II

JOURNAL ARTICLE

Title: Metabolic Syndrome characteristics as phenotypic markers in Type 2 diabetic ketoacidosis.

Abbreviated title for page headings: Metabolic Syndrome in Ketotic Prone Diabetes

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Abstract

Metabolic Syndrome Characteristics as Phenotypic Markers in Type 2 Diabetic Ketoacidosis A. Stammreich, C.B. Hollenbeck, K. Sucher, E. Ipp.

Objective: There is data that suggests the possibility of an overlap between ketosis-prone type 2 diabetes and type 1 diabetes. To evaluate this possibility further, we applied Metabolic Syndrome characteristics as phenotypic markers to determine if these patients fit the phenotype of type 2 diabetes, rather than type 1 diabetes.
Design: Prospective analysis, utilizing data collected between May 2000 and May 2003.
Subjects: One hundred and eighty-seven patients admitted to the Emergency Department at Harbor UCLA Medical Center with either a diagnosis of diabetic ketoacidosis or uncontrolled diabetes without ketoacidosis were enrolled. Patients were classified into three groups: Type 1 diabetes with ketoacidosis (DM-1), Type 2 diabetes with ketoacidosis (KPDM-2), and non-ketotic Type 2 diabetes (DM-2).
Main Outcome Measures: Data was analyzed for the features of the Metabolic Syndrome as defined by the National Cholesterol Education Program's Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (The Adult Treatment Panel III) guidelines.
Results: Our results demonstrate that KPDM-2 patients resemble typical non-ketotic patients with type 2 diabetes rather than type 1 diabetes, using phenotypic variables that characterize the metabolic syndrome. This study also demonstrates that KPDM-2 patients have a higher incidence of extreme elevations of serum triglycerides. Lastly, we found that waist circumference was the best predictor for type 2 diabetes in the setting of ketoacidosis in diabetes.
Conclusions: Evaluation of Metabolic Syndrome Characteristics in patients with ketoacidosis suggests that patients with KPDM-2 are more similar to non-ketotic type 2 diabetes than type 1. The close similarity to type 2 diabetes does not support the contention that KPDM-2 is an overlap syndrome.

Introduction

Diabetic ketoacidosis (DKA) is a serious metabolic complication of diabetes mellitus caused by a relative or absolute lack of insulin and a simultaneous increase in counterregulatory hormones, including glucagon, catecholamines, cortisol, and growth hormone. This combination leads to the release of free fatty acids from adipose tissue via lipolysis and the oxidation of hepatic free fatty acids to form ketone bodies, thus leading to the development of ketoacidosis (1).

Winter, et al. (2) was the first to report type 2 diabetic ketoacidosis in African Americans with adolescent onset type 2 diabetes. These patients required insulin after presentation, but were able to discontinue use soon after. They then continued on a clinical course more typical of type 2 diabetes. Banerji (3) also presented a group of African Americans with type 2 diabetes who presented with DKA. These patients also followed a similar course as Winter's, yet HLA studies revealed that these patients also possessed features seen in patients with type 1 diabetes, although they presented with clinical features seen in typical type 2 diabetes, such as continued C-peptide response, negative glutamic acid decarboxylase antibodies, and absent islet-cell cytoplasmic antibodies. Banerji and colleagues (3) named this atypical form of diabetes "Flatbush Diabetes", after the neighborhood surrounding the New York hospital where these patients lived. Since then, many other studies have described this form of diabetes, and have referred to it by different names, such as "atypical diabetes" (4) or "idiopathic type 1 diabetes" (5). This phenomenon has since been described in various ethnic groups, both in the United States (3) and abroad (6,7).

Development of DKA in type 1 diabetes has been extensively studied and is known to be a result of severe insulin deficiency (1). However, the mechanisms that result in type 1 diabetic ketoacidosis may not be the same mechanisms that occur in type 2 diabetic ketoacidosis. Previous speculation suggested that stress-induced elevation of counterregulatory hormones may play a role (8), while recent data suggest that severe insulin deficiency is the cause (9).

This study was designed to utilize the characteristics of the Metabolic Syndrome in patients with KPDM-2 to address two issues. The first is whether there is an overlap between type 1 and type 2 diabetes that may account for the severe insulin deficiency that leads to ketoacidosis. Metabolic Syndrome characteristics are typically associated with the type 2 diabetes phenotype and thus afford us the opportunity to assess how closely patients with ketotic-prone type 2 diabetes resemble this phenotype as opposed to type 1 patients. Secondly, we evaluated the prevalence of dyslipidemia using the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) definition of the Metabolic Syndrome (10), a syndrome first described by Gerald Reaven in 1988 (11). This was to obtain evidence to support a possible mechanism for hyperlipidemia-induced beta-cell dysfunction in this group of individuals with type 2 diabetic ketoacidosis. Although there is no evidence to suggest that Metabolic Syndrome per se has an effect on pancreatic beta-cell function, there is evidence that associates dyslipidemia, a component in this definition of Metabolic Syndrome, with beta-cell dysfunction (12).

At Harbor-UCLA Medical Center, a county-run hospital located in a metropolitan area of Los Angeles County, many patients who are admitted for DKA have type 2 diabetes. This enabled us to study the phenotypic features of patients with this syndrome and compare them with typical patients with ketoacidosis in type 1 diabetes. The focus of the present study is on the prevalence of Metabolic Syndrome characteristics in ketotic prone type 2 diabetes compared to patients with non-ketotic type 2 diabetes and ketotic type 1 diabetes.

Research Design and Methods

Enrollment criteria

The initial criteria for identifying patients with DKA (with type 1 or type 2 diabetes) included a serum glucose level ≥300 mg/dl and a β-hydroxybutyrate level ≥2.0 mmol/L upon presentation to the emergency department. Criteria for identifying type 2 control subjects who did not have DKA included a serum glucose level ≥300 mg/dl and a β-hydroxybutyrate level ≤0.5 mmol/L upon presentation. Patients with a diagnosis of current or previous pancreatitis were excluded.

Patients

The study population consisted of 187 patients admitted to the emergency department of Harbor-UCLA Medical Center, a county hospital located in Los Angeles County, California who fitted criteria for the study. Patients included in the study were admitted to the hospital between May 2000 and May 2003. The study protocol was approved by the Institutional Review Board of Harbor-UCLA Medical Center, and all participants gave their informed consent. Subjects who agreed to participate in the study

were admitted to the General Clinical Research Center (GCRC) of Harbor-UCLA Medical Center for further study.

We studied three groups of subjects. The first group included patients who presented with ketoacidosis and type 2 diabetes (KPDM-2), and the second group included patients with ketoacidosis due to type 1 diabetes (DM-1) . The third group consisted of patients with typical type 2 diabetes (DM-2) who presented to the emergency department with uncontrolled diabetes, but not in ketosis. Two comparison groups (DM-1 and DM-2) were included in this analysis in order to compare their phenotypic characteristics to patients with KPDM-2.

Laboratory information was used to classify subjects with ketoacidosis into either type 1 diabetes or type 2 diabetes groups. Subjects who had positive islet cell or anti-GAD (glutamic acid decarboxylase) antibodies were classified as having type 1 diabetes. Patients who were antibody negative were classified as having type 2 diabetes if their fasting plasma c-peptide concentrations were >0.1pmol/ml the morning after admission (13).

Procedures

In addition to routine blood samples obtained at the time of presentation with ketoacidosis or decompensated diabetes, fasting blood samples were collected by GCRC nursing staff on the morning after admission. Biochemical analysis at admission included measurement of glucose, acetone, β-hydroxybutyrate, bicarbonate, and anion gap. Fasting blood samples were obtained for glucose, hemoglobin A1c, C-peptide, anti-

islet cell antibodies, anti-glutamic acid decarboxylase (GAD) antibodies, and a lipid panel that included triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol.

Waist circumference at the iliac crest were measured by trained GCRC nutrition staff according to the method outlined in the Third National Health and Nutrition Examination Survey guidelines (14). Height and weight were also obtained, and body mass index was calculated using the formula: weight in kilograms divided by height in meters squared (kg/m^2).

Blood pressure measurements were obtained from the medical record and included measurements taken upon waking at least 24 hours after admission. Other information pertaining to the diagnosis of hypertension, such as prescribed anti-hypertensive medications or previous diagnosis of hypertension were obtained from the patients medical record.

Analytical methods

Data was analyzed for clinical features of the Metabolic Syndrome as defined by the National Cholesterol Education Program's Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (The Adult Treatment Panel III) guidelines (10).

Biochemical analysis

C-peptide was measured by radioimmunassay as previously described (15, 16). β-hydroxybutyrate was measured by enzymatic assays also previously described (17). Islet cell antibodies (ICA) and glutamic acid decarboxylase (GAD) autoantibodies were measured as described by Neufeld (18).

Statistical Analysis

Statistical analyses were carried out using the Statview program by SAS Institute. Data from all three groups studied (KPDM-2, Ketoacidotic DM-1, and DM-2 Control) were analyzed using ANOVA. Logistic regression was used to calculate odds ratios of finding Metabolic Syndrome abnormalities in KPDM-2 and DM-1 patients. We used relative risk for KPDM-2 of each of the four non-glucose Metabolic Syndrome features (increased waist circumference, increased triglycerides, decreased HDL-cholesterol, and hypertension). Logistic regression models were used with type of diabetes as the dependent variable. Unadjusted models include only a single non-glucose Metabolic Syndrome characteristic (previously listed) as a predictor. Age-group adjusted models further add the other three Metabolic Syndrome characteristics as predictors. These models estimate, for each of the Metabolic Syndrome characteristics, the ratio of the odds of type 2 diabetes for subjects with the characteristic, to the odds for subjects without the characteristic, adjusted for the other predictors in the model. Odds ratios for a characteristic were converted to relative risks, standardized to the distributions (pooled over type 1 and type 2 diabetes) for other predictors that were observed in the study (19). BMI's independent effect (over increased waist circumference only, or over all 4 characteristics) on type 2 diabetes was measured in logistic models with age tertile and either increased waist circumference only or all 4 characteristics as predictors.

Results

Table 1 presents the demographics of the three groups. The mean age (± SEM) at presentation was 34.0 ±2.0 years; 41.6 ±1.2 years; and 53.3 ±1.4 years in DM-1, KPDM-2, and DM-2, respectively ($p<0.001$). The groups consisted of 35% female (KPDM-2), 42% female (DM-1) and 41% female (DM-2). Ethnic distribution shows that the majority of patients in each group were Hispanic, followed by African Americans, Caucasians, and lastly other ethnicities. The age of onset of type 1 diabetes was younger than the patients with type 2 diabetes, yet there was no significant difference in age of onset between KPDM-2 and the type 2 DM control group. Duration of diabetes was 7.5 ±1.5 years; 2.5 ±0.5 years; and 9.4 ±1.1 years, respectively ($p<0.001$), which reflects more new onset diabetes in the KPDM-2 group. The average Body Mass Index (BMI) for both type 2 diabetic groups was similar (KPDM-2 = 32.0 ±1.0, DM-2 = 30.1 ±1.0), while type 1 diabetics were significantly leaner (23.8 ±0.87 kg/m^2; ($p<0.001$).

The clinical characteristics of study participants are shown in Table 2. All three groups were in similarly poor diabetes control, with HbA1c of 12.5% ±0.5%; 12.8% ±0.3%; and 12.1 ±0.3%, in DM-1, KPDM-2 and DM-2, respectively. Fasting plasma C-peptide obtained the day following admission was 0.05 ±0.004; 0.3 ±0.02; and 0.9 ±0.1 pmol/mL, respectively . All three groups exhibited severe hyperglycemia. The two ketotic groups were in similar degrees of metabolic acidosis, as evidenced by elevated anion-gap and decreased bicarbonate. Ketones were elevated to similar degrees in both KPDM-2 and DM-1 groups. Metabolic Syndrome Characteristics amongst the three

groups are presented in Table 3. HDL cholesterol was significantly lower in the type 2 diabetic groups compared to the type 1 diabetic group for both males and females ($p<0.001$). Females from all three groups had HDL levels below 50 mg/dL. Serum triglycerides were 133 ±17; 479 ±93 mg/dL; and 166±16 mg/dL, respectively ($p<0.001$). No statistical significant differences were found amongst the three groups with regards to blood pressure.

Table 4 compares ketoacidotic subjects according to diabetes type in reference to defined metabolic syndrome characteristics. Logistic regression was used to calculate odds ratios of finding metabolic syndrome abnormalities in KPDM-2 and ketotic DM-1. Before adjusting for other characteristics listed in the table, increased waist circumference, high triglycerides, and low HDL cholesterol were significantly and independently associated with type 2 diabetes ($p<0.0001$). After adjusting for the other metabolic syndrome characteristics, increased waist circumference ($p<0.001$) and high triglycerides ($p<0.005$) remained independently associated with type 2 diabetes. Hypertension was not a significant predictor of type of diabetes.

Relative risk was calculated for KPDM-2 for each of the four non-glucose metabolic syndrome features (increased waist circumference, high triglycerides, low HDL cholesterol, and hypertension) and is shown in Table 5. Again, increased waist circumference, high triglycerides, and low HDL cholesterol were significantly associated with having type 2 diabetes. But, after adjusting for age and other metabolic syndrome characteristics, both waist circumference and high triglycerides remained independently associated with type 2 diabetes. Table 6 takes into account elevated BMI in

distinguishing between type 2 and type 1 diabetes. After adjusting for age, associated increased waist circumference, and all other metabolic syndrome characteristics, patients with a BMI ≥30 were more likely to have type 2 diabetes rather than type 1 diabetes.

Discussion

Although ketoacidosis is a clinical feature of type 1 diabetes, it is increasingly reported in patients with type 2 diabetes (20). The mechanism for the development of ketoacidosis in type 2 diabetes is thought to be primarily insufficient insulin secretion (9), but the underlying etiology is unclear. There are, however, data that suggest the possibility of an overlap between ketosis-prone type 2 diabetes and type 1 diabetes. In the clinical form of ketosis-prone type 2 diabetes designated as "Flatbush Diabetes", subjects were found to have HLA genotypes that were similar to those found in type 1 diabetes (3). In this study we investigated the phenotype of patients with ketosis prone type 2 diabetes and compared it with the phenotype in patients with typical type 1 and type 2 diabetes. We chose to study the characteristics of the Metabolic Syndrome in all of these patients in order to develop a sense of whether KPDM-2 was more like type 2 diabetes or type 1.

Our data clearly demonstrates that the KPDM-2 patients resemble type 2 patients in all aspects of the Metabolic Syndrome. Since Metabolic Syndrome characteristics are thought to be part of the syndrome of type 2 diabetes, these findings provide support for the hypothesis that ketotic prone type 2 diabetes is a subset of type 2 diabetes rather than type 1 diabetes. It should be noted that this study took place at the time of admission of patients with KPDM-2 with ketoacidosis and thus the comparison groups that were

studied were also obtained at the time of admission for ketoacidosis (type 1 patients) or decompensated diabetes (type 2 patients). This study cannot therefore be used to evaluate the prevalence per se of the diagnosis of Metabolic Syndrome in any of these groups, because it is understood that the syndrome refers to patients who are in typical outpatient steady-state situations. What this study attempts to demonstrate is that when compared with appropriate comparison groups in the same clinical setting, KPDM-2 and DM-2 are very similar when using phenotypic characteristics that are generally associated with type 2 diabetes or the risk for this disease.

The extremely high obesity rate in the United States has been associated with the increasing prevalence of both cardiovascular disease and diabetes. Metabolic Syndrome was first described by Reaven in 1988 (11). He suggests that in certain individuals there may be a series of related variables that put them at a greater risk for Coronary Artery Disease (CAD). These variables include resistance to insulin-stimulated glucose uptake, hyperglycemia, hyperinsulinemia, increased plasma concentration of VLDL triglyceride, a decreased plasma HDL-cholesterol, and high blood pressure. The common feature of this syndrome, Reaven suggested, is insulin resistance, and all five consequences of insulin resistance listed above are shown to increase CAD risk. The fact that all of these disorders may not be seen in the same individual does not lessen their importance in CAD risk.

Various organizations have presented and published their own definitions and interpretations of the Metabolic Syndrome. These groups include the National Cholesterol Education Program (NCEP) (10) and the World Health Organization (WHO)

(21). According to the National Cholesterol Education Program's Adult Treatment Panel III, the clinical characteristics associated with the Metabolic Syndrome include impaired fasting glucose, dyslipidemia represented by high triglycerides and low HDL cholesterol, increased waist circumference, and hypertension (10). While the criteria for Metabolic Syndrome have been questioned (22), the association of each component to CAD risk is not in question. Although obesity, and more specifically central obesity, is not included in Reaven's primary list of consequences caused by insulin resistance, he does note that obesity is associated with greater CAD risk due to the above mentioned variables. Also, although there is no extensive information on the association of Metabolic Syndrome variables, such as hypertension, to beta-cell dysfunction, there is evidence to suggest a link between dyslipidemia and beta-cell dysfunction.

Patients with type 2 diabetes who experience ketoacidosis are phenotypically heterogeneous. The majority is overweight, although a substantial portion tends to be lean. In this analysis, both groups with type 2 diabetes (ketotic-prone and non-ketotic – prone) presented with body mass indexes that indicate Class I obesity, but the ketotic-prone type 2 group was heavier than the non-ketotic type 2 group. Waist circumference, which is a Metabolic Syndrome characteristic, was the best predictor for type 2 diabetes in ketoacidosis. After adjusting for age and other metabolic syndrome characteristics, ketotic type 2 subjects had significantly higher waist circumferences than type 1 ketotic subjects. As expected, this finding coincides with previous data that type 1 diabetes patients are generally lean.

Obesity, specifically android obesity, has been associated with type 2 diabetes as well as elevated serum triglyceride concentrations (23, 24). One of the reasons for diabetic hypertriglyceridemia is the overproduction of very low density lipoprotein (VLDL) triglycerides. The increased flow of glucose and free fatty acids to the liver leads to the overproduction of VLDL-TG. Coupled with the decreased ability to clear VLDL-TG, this leads to the hypertriglyceridemia that is commonly seen in type 2 diabetes (25). In a state of severe hyperglycemia defective clearance of VLDL-TG is extreme, therefore leading to the sometimes severe hypertriglyceridemia seen in type 2 DKA patients. In patients with type 1 DKA, extreme elevations in VLDL are also seen. This is likely due to the lipoprotein lipase deficiency seen in the setting of definite insulin deficiency.

Of particular interest is that nine of our patients with ketotic prone type 2 diabetes presented with severe hypertriglyceridemia. Although a severe elevation in triglycerides may place a patient at risk for acute pancreatitis, none of our subjects were diagnosed with pancreatitis. Studies on the plasma lipids of patients with diabetic ketoacidosis have shown that hyperlipidemia in these patients is a common phenomenon, and these data also shows that after initial hyperlipidemia at presentation, a return to normal lipid concentrations follows after the patient has recovered from ketosis (26, 27). Due to this resolution of hyperlipidemia with the resolution of ketosis, the assumption is that there are very few patients who present with severe hypertriglyceridemia in the setting of DKA that also have a genetic hyperlipidemia. There are multiple studies that have come to this conclusion (28, 29). These studies concluded that although type 2 diabetes and

hypertriglyceridemia often occur together, diabetes and genetic forms of hypertriglyceridemia often do not. Since this extreme elevation in triglycerides may not be related to a genetic anomaly, it is possible that hypertriglyceridemia may play a role in the pathogenesis of ketosis. More research is necessary before any conclusions can be made.

We cannot conclude that the severe hypertriglyceridemia seen in our KPDM-2 patients is due to greater visceral adiposity, since both KPDM-2 subjects and DM-2 subjects presented with roughly the same extent of abdominal obesity. It remains unclear why there is an extreme elevation of triglycerides in KPDM-2 subjects. What we can conclude from this study is that KPDM-2 is more likely to be associated with severe hypertriglyceridemia than either of the other two groups, and may support a possible mechanism for hyperlipidemia-induced beta-cell dysfunction. The relationship between the occurrence of ketoacidosis and degree of obesity will require more research. However, at least from a phenotypic perspective this study does not provide support for the hypothesis that KPDM-2 is an overlap syndrome between type 1 and type 2 diabetes. Using metabolic syndrome characteristics, KPDM-2 patients resemble type 2 diabetes in all respects.

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Table 1. Demographics of Study Groups

	DM-1 (n=42)	KPDM-2 (n=79)	DM-2 (n=66)	*p value*
Age (yrs)	34.0±2.0	41.6±1.2	53.3±1.4	<0.001
Sex:				
Male	25	51	39	NS
Female	17(42%)	28 (35%)	27 (41%)	
Ethnicity				
Hispanic	16	42	39	NS
African American	13	23	13	NS
Caucasian	9	11	11	NS
Other	4	3	3	NS
Age of onset (yrs)	26.2± 2.4	38.8± 1.2	43.8±1.6	<0.001
Diabetes Duration (yrs)	7.5± 1.3	2.5± 0.5	9.4± 1.1	<0.001
Body Mass Index (BMI) (kg/m^2)	23.8±0.87	32.0±1.0	30.1±1.0	NS

Mean ±SEM

Table 2. Presenting Features of Patients with Diabetic Ketoacidosis and Type 2 Controls (Mean ± SEM)

	DM-1 (n=42)	KPDM-2 (n=79)	DM-2 (n=66)	*p value*
Glucose on admission (mg/dl)	552±37.5	534±28.9	478±25.6	NS
β-Hydroxybutyrate (mmol/L)	6.9±0.4	5.6±0.3	0.2±0.0	-
Bicarbonate HCO_{3-} mEq/L)	14.8±0.8	15.4±0.6	24.1±0.5	-
Anion-gap mEq/L)	20.2±0.8	19.2±0.7	9.9±0.3	-
HgA1c (%)	12.5±0.5	12.8±0.3	12.1±0.3	NS
Fasting C-peptide (pmol/ml)	0.05±0.004	0.3±0.02	0.9±0.1	<0.001

Mean ±SEM

Table 3. Metabolic Syndrome Characteristics in Patients with Ketoacidosis

	DM-1	KPDM-2	DM-2	*p value***
Fasting Glucose (after ketoacidosis cleared) (mg/dL)	159.0±12.8	212.7±8.0	202.7±8.0	<0.001
Waist circumference:				
Total	86.35±1.84	106.98±1.81	105.74±2.55	<0.001
Male	86.76±2.14	107.38±2.22	103.02±2.97	<0.001
Female	85.57±3.48	106.16±3.21	110.00±4.55	<0.001
(cm)				
HDL:				
Total	44.38±2.43	34.12±1.16	35.31±1.43	<0.001
Male	44.49±2.49	36.06±1.50	34.39±2.06	<0.02
Female	46.88±4.77	30.64±1.64	36.59±1.86	<0.001
(mg/dL)				
TG:				
Total	133.37±17.32	479.95±93.18	166.95±15.85	<0.001
(>1000)*	133.37±17.32	249.25±22.69	166.95±15.85	<0.001
(mg/dL)				
Systolic BP (mm/Hg)	127±3	134±2	134±3	NS
Diastolic BP (mm/Hg)	74±2	76±1	75±2	NS

*exluding subjects with TG > 1000 (n=9)

** by ANOVA

Table 4. Number of Subjects with Positive Metabolic Syndrome Characteristics in Ketoacidotic Subjects, According to Type of Diabetes

	Type1 (n=42)	Type 2 (n=79)	Unadjusted OR[1] p-value	OR	95% CI	Adjusted OR[2] p-value	OR	95% CI
↑WC	7/42= 17%	51/79=65%	<0.0001	9.1	3.6-23.2	0.001	5.6	20-15.5
↑TG	14/42= 33%	57/79=72%	<0.0001	5.2	2.3-11.6	0.005	3.8	1.5-9.4
↓HDL	21/42= 50%	64/79=81%	<0.0001	4.3	1.9-9.7	0.03	3.1	1.1-8.4
↑HTN	13/42=31%	39/78= 50%	0.04	2.2	1.0-4.9	0.29	1.7	0.6-4.6

1. OR= Odds Ratio: Odds of Type 2 for having characteristic to not having it.
2. Adjustment is for other factors in the table.
WC= waist circumference; TG= triglycerides; HDL= high density lipoprotein;
HTN= hypertension

Table 5. Associations of Metabolic Syndrome Characteristics with Type 2 Diabetes						
	Relative Risk[1]			p-values[2]		
	Unadjusted	Age Group[3] Adjusted	Adjusted[4]	Unadjusted	Age Group[3] Adjusted	Adjusted[4]
↑WC	1.98	1.89	1.57	<0.0001	<0.0001	0.0007
↑TG	1.82	1.72	1.39	<0.0001	0.0002	0.008
↓HDL	1.81	1.74	1.33	<0.0001	0.0006	0.04
HTN	1.3	1.21	1.06	0.04	0.15	0.65

1. Probability of type 2 diabetes for subjects havinga characteristic, relative to other subjects.
2. From logistic regression, for odds ratios.
3. Age group tertiles: <35 (n=39); 35=44 (n=43); and ≥45 (n=39).
4. Standardized to the observed distribution of age group and the other 3 symptom patterns.

Table 6. Probability of DM-2 for Subjects with Elevated BMI, Relative to Other Subjects

	Relative Risk1		p-values[2]	
	WC Adjusted[3]	All Metabolic Syndrome Adjusted[4]	WC Adjusted[3]	All Metabolic Syndrome Adjusted[4]
BMI≥30	1.41 (89% vs 59%	1.36 (81% vs 60%)	0.02	0.04
BMI≥25	1.48 (75% vs 51%)	1.27 (72% vs 57%)	0.02	0.12

1. Probability of of type 2 diabetes for subjects with elevated BMI, relative to other subjects.
2. From logistic regression, for odds ratios.
3. Standardized to the observed distribution of age group and WC.
4. Standardized to the observed distribution of age group and the 4 MS symptoms patterns.

CHAPTER III

SUMMARY AND RECOMMENDATIONS

REFERENCES

Summary and Recommendations

In order to investigate whether patients with KPDM-2 have a mixed clinical phenotype, that might combine traits of type 1 and type 2 diabetes, we applied the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATP III) definition of the Metabolic Syndrome to three groups of patients. We examined waist circumference, triglyceride and HDL concentrations and blood pressure in groups of type 1 or type 2 diabetic patients with ketoacidosis and non-ketotic, but hyperglycemic type 2 controls. Our results demonstrate that patients with ketoacidosis in type 2 diabetes resemble typical non-ketotic patients with type 2 diabetes, rather than patients with type 1 diabetes, using phenotypic variables that characterize the Metabolic Syndrome. Since Metabolic Syndrome characteristics are thought to be part of the syndrome of type 2 diabetes, these findings provide support for the notion that ketotic-prone type 2 diabetes is a subset of type 2 diabetes, not type 1 diabetes. Due to the rising rate of obesity in the United States and its association with both the increase in prevalence of cardiovascular disease and diabetes, it is likely that we will see more patients present with Metabolic Syndrome characteristics, regardless of their diagnoses. When considering Metabolic Syndrome as a tool to distinguish ketotic-prone type 2 diabetes patients from non-ketotic type 2 patients, it cannot be used to differentiate between these two groups.

Yet, when one examines each component of the Metabolic Syndrome, there are significant differences found between ketotic-prone type 2 patients and the other two groups. We found that waist circumference was the best predictor for type 2 diabetes in

the setting of ketoacidosis in diabetes. Patients with type 2 diabetes who experience ketoacidosis are phenotypically heterogeneous. Although the majority is overweight, a significant portion tends to be lean. Our ketotic-prone type 2 group was actually significantly obese as evidenced by both waist circumference and body mass index. This is different from previous studies that have described this group as being more lean than their non-ketotic type 2 counterparts. Yet, ketotic-prone type 2 individuals are significantly more centrally obese, enough so that we can distinguish them from ketotic type 1 diabetes patients.

Lastly, this study demonstrates that patients with ketotic-prone type 2 diabetes have a higher incidence of extreme elevations of serum triglycerides, which places these patients at a higher risk for pancreatitis. None of the patients included in this study had pancreatitis (patients with evidence for pancreatitis were excluded from the study), but nine subjects in the ketotic-prone type 2 diabetes group had triglyceride concentrations that were above 1000 mg/dl. In contrast, in this study none of the other two groups developed this degree of elevation of serum triglycerides. This was of particular interest, because it suggests that this may be a novel manifestation of KPDM-2, not previously described. Furthermore, this finding may be relevant to the pathogenesis of KPDM-2, by providing clues to the metabolic pathways involved in the mechanism that results in insulin deficiency, ketoacidosis and hypertriglyceridemia. For example, it is possible that hypertriglyceridemia, either as a result of chronic obesity or poorly controlled blood glucose, may play a role in the pathogenesis of ketosis. More research is necessary before any conclusions can be made.

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APPENDIX

Appendix A
San Jose State University IRB Human Subjects Approval

San José State
UNIVERSITY

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Graduate Studies & Research

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To: Amy Stammreich
36 Rico Way
San Francisco, CA 94123

From: Pam Stacks, AVP
Graduate Studies & Research

Date: November 28, 2005

The Human Subjects-Institutional Review Board has approved your request for exemption from human subjects review under category "D" in the study entitled:

"Metabolic Syndrome characteristics as phenotypic markers in patients with Type 2 Diabetic Ketoacidosis ."

This approval is contingent upon the subjects participating in your research project or the subject's data collected for the research project being appropriately protected from risk. This includes the protection of the anonymity of the subjects' identity when they participate in your research project and concerning all data that may be collected from the subjects. The Board's approval includes continued monitoring of your research to assure that the subjects are being adequately and properly protected from such risks. If at any time a subject becomes injured or complains of injury, you must immediately notify Pam Stacks, Ph.D. Injury includes but is not limited to bodily harm, psychological trauma, and release of potentially damaging personal information.

Please also be advised that all subjects need to be fully informed and aware that their participation in your research project is voluntary, and that he or she may withdraw from the project at any time. Further, a subject's participation, refusal to participate, or withdrawal will not affect any services that the subject is receiving or will receive at the institution in which the research is being conducted. This approval is granted for a one-year period and data collection beyond November 28, 2006 requires an extension request.

If you have any questions, please contact me at (408) 924-2480.

Cc: Clarie Hollenbeck