HORMONES 2015, 14(4):644-650

Research paper

Ketonemia and ketonuria in gestational diabetes mellitus

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ABSTRACT

BACKGROUND: The use of capillary blood 3-β-hydroxybutyrate (3HB) is a more precise method than urine ketones measurement for the diagnosis of diabetic ketoacidosis. Fasting ketonuria is common during normal pregnancy, while there is evidence that it is increased among pregnant women with Gestational Diabetes Mellitus (GDM) who are on a diet. 3HB levels have been related to impaired offspring psychomotor development. Reports with concomitant measurement of blood and urine ketones in women with GDM who followed a balanced diet are lacking. OBJECTIVE: To compare the prevalence of fasting ketonemia and ketonuria in women with GDM following the Institute of Medicine diet instructions and assess their possible relation with metabolic parameters and therapeutic interventions. RESEARCH DESIGN AND METHODS: 180 women with GDM were studied. In each patient, in successive visits, capillary blood and urine ketones were simultaneously measured. The total measurements were 378, while the average number of measurements per patient was 2.1. RESULTS: The prevalence of ketonuria was significantly higher than that of ketonemia (x^2=21.33, p <0.001). Significantly higher mean 3HB levels were observed with respect to ketonuria severity (p=0.001). Bedtime carbohydrate intake was associated with significantly lower 3HB levels (p=0.035). Insulin treatment was associated with significant 3HB levels reduction (p=0.032). Body weight reduction per week between two serial visits was associated with increased 3HB levels (p=0.005). Multiple linear regression analysis showed that weight loss remained the only independent predictor of 3HB levels. CONCLUSIONS: The presence of ketonemia was significantly lower than the presence of ketonuria. Weight loss per week was the only independent factor found to be associated with increased levels of 3HB. The clinical significance of this small increase requires further investigation.

Key words: Gestational diabetes mellitus, Ketonemia, Ketonuria

INTRODUCTION

The ketone bodies, acetoacetate (AcAc), acetone and 3-β-hydroxybutyrate acid (3HB), are catabolic products of free fatty acids, produced in the absence of carbohydrates or insulin.[1]

Quantitative assay of 3HB measurement in blood is a more precise marker of insulin deficiency than urine dipstick detection of AcAc, as blood levels of

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Received 10-02-2015, Accepted16-07-2015

3HB increase rapidly in the case of sudden insulin deficiency, while urinary excretion of AcAc is delayed as it is dependent on glomerular filtration and therefore on renal function and degree of hydration. The measurement of AcAc in urine is a semi-quantitative assay and the result can be influenced by the presence of certain food and drugs.[2] Thus, measurement of 3HB in blood can be used for diagnosis and monitoring of diabetic ketoacidosis (DKA).[1]

Positive urine ketone readings are found in up to 30% of first morning urine samples from pregnant women (with or without diabetes) during starvation and after hypoglycemia.[1] Indeed, ketonuria is not uncommon in normal pregnancy.[3] After an overnight fast, maternal ketone body concentrations are about threefold greater in pregnant than in non-pregnant women.[4] However, it is unclear if starvation ketosis is associated with decreased intelligence in the offspring.[5]

Ketone bodies are probably normally present in the fetal brain at various times during most pregnancies; in addition, fasting ketonemia in poorly controlled diabetic pregnant women has been associated with decreased intelligence and impaired fine motor skills in the offspring.[6]

The reported association of maternal ketonuria during pregnancy with subsequent reduction in children's IQ values[7] has influenced the clinical care of pregnant women. The need to prevent ketonuria has been one further reason to maintain appropriate weight gain during pregnancy, avoid weight-reduction diets and maintain adequate food intake during the nausea and vomiting phase of early gestation.

The aim of this study was to investigate firstly the presence of ketonemia and ketonuria in gestational diabetes mellitus (GDM) women who followed the Institute of Medicine (IOM) diet instructions, in the morning after a night fast, and secondly any possible associations of ketonemia with metabolic parameters and medical interventions.

RESEARCH DESIGN AND METHODS

Study field

This study was undertaken at the Diabetes Mellitus Unit of "Alexandra" Hospital in Athens, Greece. The protocol of this study was approved by the Hospital Scientific and Bioethics Committee.

Inclusion/Exclusion criteria

Women with GDM who consecutively attended the Diabetes Mellitus Unit from May 2011 to November 2011 were considered as potential candidates. Women with Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus or other systematic illnesses and those with Hct values below 25% were excluded. In order to avoid interference with the method of 3HB measurement, women using food supplements with ascorbic acid or such drugs as methyldopa and salicylate were also excluded.

Study subjects

One hundred and eighty (180) women with GDM according to IADPSG 2010 criteria[8] in the third trimester of gestation were included in this study. All women signed a written informed consent before entering the study. Patient's characteristics are shown in Table 1. All patients were in good metabolic control.

Diet and self-monitoring protocol

All women were instructed to follow a diet in order to gain weight during pregnancy according to the IOM 2009 recommendations.[9] Forty-five percent of the diet calories came from carbohydrates and were distributed through six meals (breakfast, morning snack, lunch, afternoon snack, dinner, bedtime snack). The standard monitoring protocol included daily self-measurements of blood glucose (SMBG) using a standardized portable glucose meter four times a day (fasting before breakfast and one hour postprandial after breakfast, lunch, dinner) by women who controlled their GDM

Table1. Characteristics of the study subjects Patients (n=180)

Characteristics	$\bar{x} \pm SD$
Age (years)	33.0±5.4
Height (cm)	162.0±6.9
BMI before pregnancy (kg/m2)	27.1±5.9
Body weight at the first examination (kg)	71.5±17.3
Systolic blood pressure (mmHg)	121.1±15.7
Diastolic blood pressure (mmHg)	77.8±11.9
BMI before pregnancy: Normal: 44.2%, Overweight: 22.7%, Obese: 33.1%	
Patients using insulin therapy: 74/180 (41.1%)	

using diet and six times a day (before and one hour after breakfast, lunch and dinner) by women using insulin therapy to control their glucose levels. The glycemic targets were: fasting glucose 60-95 mg/dl (3.3-5.3 mmol/L), 1h after meals 100-130 mg/dl (5.5-7.2 mmol/L)[10]. Insulin was started when two or more abnormal values in SMBG occurred over 1 or 2 weeks (fasting plasma glucose was >95 mg/dl, 1h post-prandial values >130-140 mg/dl) and/or when there was evidence of macrosomia by fetal ultrasound.

During consecutive visits of the patients to the Diabetes Unit 378 measurements of 3HB in capillary blood were performed simultaneously. Additionally, the same numbers of measurements of AcAc in first morning urine samples were also made (Figure 1). Furthermore, plasma blood glucose and HbA1c were measured.

Ketonemia was defined as: negative 0 – 0.6 mmol/L, moderate 0.6 – 1.5 mmol/L and positive 1.5 – 3.0 mmol/L, according to manufacturer's method.

Ketonuria was defined as: negative 0/±, moderate +/++ and positive +++/++++, according to manufacturer's method.

INSTRUMENTATION

For SMBG measurements, the Accu-Chek reflectance meter (Bayer, Switzerland) was used, while the Glucomen-LX β-ketone sensor (Menarini, Italy) and Ketostix (Bayer, Germany) reagent strips were used for ketonemia and ketonuria assessment, respectively.

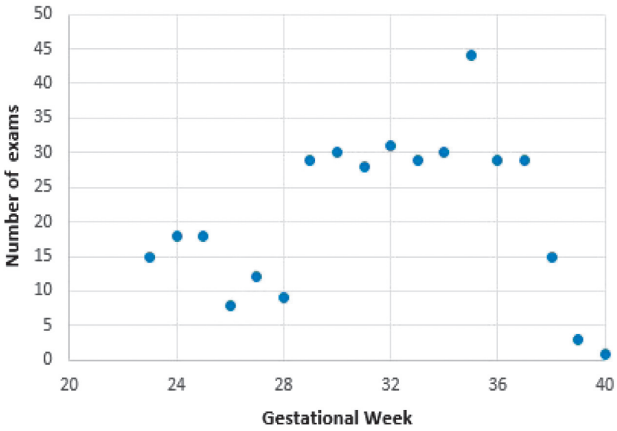

Figure 1. Number of measurements per gestational week.

Blood plasma glucose and HbA1c measurements at the time of the visit to the Diabetes Unit were performed using the hexokinase method (Integra 400, Roche) and the high-performance liquid chromatography method (HA 8160, Menarini), respectively.

STATISTICAL ANALYSIS

In order to reveal statistical significant differences, the chi-square test (χ^2), post hoc pairwise t-tests and one way Analysis of Variance (ANOVA) were applied to the obtained dataset. In order to identify possible associations of 3HB-levels with anthropometric characteristics and clinical variables, Pearson's correlation coefficient was calculated and the linear regression model was applied. The level of significance was considered as equal to 0.05. The SPSS software package was used to carry out the statistical analysis.

RESULTS

Ketonemia was found positive in 2% of the measurements, moderate in 5% and negative in 93% of the measurements. On the other hand, ketonuria was positive in 13% of the measurements, moderate in 22% and negative in 65% (Figure 2). Positive results of ketonemia were significantly lower than the positive results of ketonuria ($x^2 = 21.33$, $p < 0.001$). The contingency Table 2 provides further details regarding the incidence of ketonemia and ketonuria. Mean levels of 3HB increased significantly in relation to the severity of ketonuria (p=0.001). Mean values of 3HB were 0.10±0.01 mmol/L in women with negative ketonuria results, 0.21±0.03 mmol/L in women with moderate ketonuria results and 0.55±0.08 mmol/L in women with positive ketonuria results, respectively (Figure 3). Post hoc pairwise t-tests revealed significant differences ($p < 0.05$) of the mean 3HB levels between the groups of: (i) positive versus negative ketonuria (t=28.57, p=0.001), (ii) positive versus moderate ketonuria (t=39.47, p=0.001) and (iii) negative versus moderate ketonuria (t=33.03, p=0.001). Table 3 shows the distribution of ketonemia measurements in relation to pre-pregnancy body mass index (BMI). The mean values of 3HB were 0.14±0.22 mmol/L in normal pre-pregnancy BMI, 0.27±0.46 mmol/L in the overweight BMI category

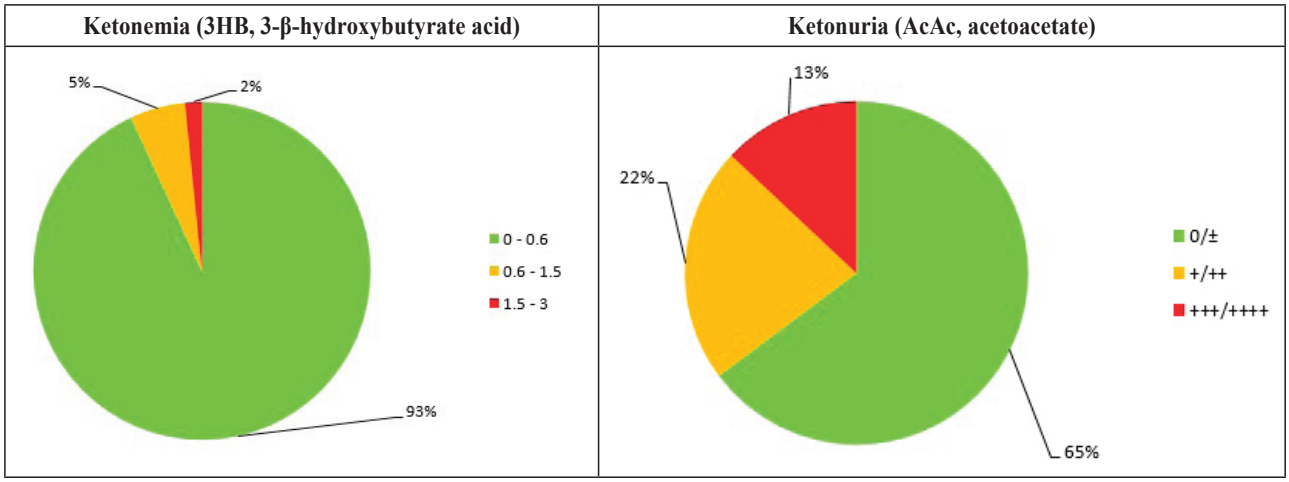

Figure 2. Percentage of ketonemia and ketonuria in the study population.

Table 2. Contingency table with concomitant measurements of ketomenia and Ketonuria

Ketonuria	**Ketonemia**			
	Negative (0-0.6 mmol/L)	**Moderate** (0.6-1.5 mmol/L)	**Positive** (1.5-3.0 mmol/L)	**Sum**
Negative (0/±)	235 (224.2)	5 (12.9)	1 (3.9)	241 (241)
Moderate (+/++)	80 (78.2)	4 (4.5)	0 (1.3)	84 (84)
Positive (+++/++++)	33 (45.6)	11 (2.6)	5 (0.8)	49 (49)

Chi-square 61.59 (>9.488)

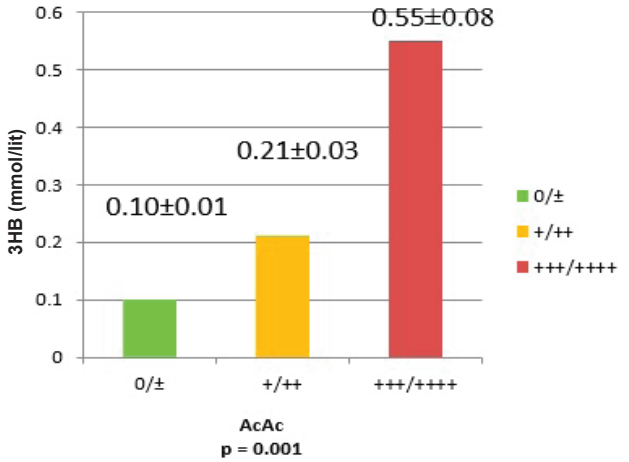

Figure 3. 3-β-hydroxybutyrate acid (3HB) levels depending on ketonuria (AcAc, acetoacetate) severity.

and 0.17±0.28 mmol/L in the obese category. Table 4 shows the distribution of ketonuria measurements in relation to pre-pregnancy BMI.

It is of note that 37.6% of the participants did not comply with the instruction to have the bedtime snack. The omission of the bedtime snack was associated with significantly increased mean levels of 3HB (0.23±0.03 mmol/L vs 0.16±0.02 mmol/L, p=0.035). Insulin therapy was added in order to achieve good glucose control in 74/180 (41.1%) women. The use of insulin administration was associated with significantly lower levels of 3HB (0.15±0.02 mmol/L vs 0.22±0.03 mmol/L, p=0.032). Further, Table 5 shows the distribution of ketonemia measurements regarding increase/decrease in weight between two consecutive visits and in the same manner Table 6 shows the distribution of ketonuria measurements. Weight loss per week, between two consecutive visits, was associated with significantly higher mean levels of 3HB compared to weight gain per week (0.23±0.05 mmol/L vs 0.10±0.02 mmol/L, p=0.005). A positive Pearson's correlation was observed between mother's age and 3HB levels (r=0.16, p=0.002). No statistically significant associations were found between levels of 3HB and BMI before pregnancy (r=0.006, p=0.910),

Table 3. Ketonemia and prepregnancy BMI

Prepregnancy BMI	Ketonemia			
	Negative (0-0.6 mmol/L)	**Moderate** (0.6-1.5 mmol/L)	**Positive** (1.5-3.0 mmol/L)	**Sum**
Normal	139	7	1	147
Overweight	92	6	4	102
Obese	109	6	1	116

Table 4. Ketonuria and prepregnancy BMI

Prepregnancy BMI	Ketonuria			
	Negative (0/±)	**Moderate** (+/++)	**Positive** (+++/++++)	**Sum**
Normal	98	33	14	145
Overweight	60	22	20	102
Obese	75	25	14	114

parity (r=0.039, p=0.456), week of gestation (r=0.008, p=0.881), plasma glucose levels (r=-0.088, p=0.088) and HbA1c (r=-0.079, p=0.140).

When a multiple linear regression model was applied associating the levels of 3HB with the parameters shown in Table 7, the only independent factor found to be associated with increased levels of 3HB was weight reduction per week. Applying linear regression, considering the weight change as the only factor affecting 3HB levels, resulted in beta coefficients equal to -0.096 (p=0.004).

DISCUSSION

Plasma 3HB during the third trimester of pregnancy is negatively associated with the neuropsychological development of the offspring and has led to the recommendation to avoid ketosis during pregnancy.[11]

In recent years a new method has become available to measure capillary levels of 3HB. This test uses the same equipment used for SMBG determination but with specific strips. In comparison to the urine ketone test, it does not produce false negative or positive results, it is highly correlated to standard automated assays and patients find it more acceptable.[1,12] Moreover, in a prospective study capillary 3HB and the urine dipstick were found to be equally sensitive for detection of DKA (98.1%). However, 3HB was more specific (78.6 vs. 35.1%).[13] Also in another study Voulgari C et al reported that capillary ketonemia had a higher performance (sensitivity 99.87%, specificity 92.89%, positive predictive value 92.89%) for the diagnosis of DKA compared with

Table 6. Ketonuria and Weight Change/Week

Weight Change/Week	Ketonuria			
	Negative (0/±)	**Moderate** (+/++)	**Positive** (+++/++++)	**Sum**
Increase	93	28	5	126
Decrease	39	18	12	69

Table 7. Multiple linear regression model associating the levels of 3HB with

	Parameter	beta	p
Multiple linear regression model	Weight change	-0.110	0.003
	Bed time snack	-0.081	0.115
	Week of gestation	0.001	0.795
	Mother's age	0.006	0.134
	Insulin use	-0.044	0.330
	BMI before pregnancy	-0.001	0.670
	Plasma glucose	-0.001	0.602

Table 5. Ketonemia and Weight Change/Week

Weight Change/Week	Ketonemia			
	Negative (0-0.6 mmol/L)	**Moderate** (0.6-1.5 mmol/L)	**Positive** (1.5-3.0 mmol/L)	**Sum**
Increase	127	1	0	128
Decrease	63	4	2	69

ketonuria (sensitivity 89.89%, specificity 52.73%, positive predictive value 41.87%).[14] Hence, it has been suggested that 3HB levels may replace repeat laboratory measurements of arterial blood gases in the management of DKA.[15] To date, only a small scaled study[16] has used this method to compare 3HB levels in normal and GDM women. The authors reported significantly higher mean ketone levels, although within the normal range, in GDM versus controls, but these measurements were not performed in the fasting state. Furthermore, in that study there was no concomitant comparison between the presence of ketonemia and ketonuria.

The purpose of our study was to examine the prevalence of abnormal values of 3HB in blood (using the capillary blood ketone strip method) together with the values of AcAc in urine simultaneously in women with GDM who followed diet instructions according to the IOM 2009[9] in the real life situation of an outpatient clinic. Further, we investigated whether the levels of 3HB, which is believed to be the main ketone body and has been implicated in untoward effects on offspring intellectual development, correlated with age, BMI before pregnancy, gestational age, weight change per week, usage of insulin, fasting blood glucose and HbA1c.

The prevalence of ketonuria (35.2%) found in this study in GDM women was not different from that reported in normal pregnant women.[1] By contrast, the presence of ketonemia was found to be very low (1.6%) and this result is rather reassuring. As far as we know, there are no reports regarding the presence of ketonemia in GDM women following IOM diet instructions. Although there was a weak correlation between the levels of 3HB and AcAc, we should emphasize that the 3HB values were in the normal range. This discrepancy between ketonemia and ketonuria may be explained by the fact that in this study, as in others focusing on pregnancy, single fasting urine samples were used. It is known that AcAc rises and declines much more slowly than 3HB, a finding that was also observed in DKA.[17]

In this study we observed an association of 3HB levels with the omission of the bedtime snack. Starvation ketosis is a well-known physiological adaptation during normal pregnancy, hence, the recommendations for diet management of GDM are three meals and three snacks, with major importance placed on the bedtime snack. It is interesting to note that in this study we strongly recommended to all participants to have the bedtime snack, but 37.6% did not comply.

We adopted as proposed glycemic targets for GDM women glucose values ($\bar{x}$±2SD) that are found in normal pregnant women, as was shown in a meta-analysis by Henderson.[10] Insulin treatment was considered when the abovementioned glycemic targets were not achieved, taking also into account evidence of fetal macrosomia in the ultrasound scan. Additionally, the levels of 3HB were significantly lower in women with GDM who used insulin treatment. This is in agreement with the pathophysiological mechanism of ketone body formation as the ketones are catabolic products of free fatty acids, especially in the absence of carbohydrates or insulin.[1]

Weight loss during pregnancy in overweight and obese women is a matter of debate. The consensus of the IOM guidelines suggests an overall weight gain of 7-11 kg in overweight and 5-9 kg in obese women. Weight loss during pregnancy has been associated with preterm deliveries and small for gestational age neonates.[18] We found that even a small weight loss (200 gr) per week was associated with significant increase in 3HB levels, although within the normal range. This increase remained significant even when age, BMI, insulin treatment and bedtime carbohydrate intake were taken into account (p=0.006). An early experimental study on the same subject[19] with a small number of obese GDM women showed that the decrease in calorie intake from 2500 kcal to 1200 kcal was associated with an increase of 269% of 3HB concentration and a twofold increase in ketonuria.

The strength of this study is that it examines the prevalence of ketonemia and ketonuria in everyday clinical practice in a well-controlled group of GDM women following diet instructions according to IOM 2009. It also shows that despite the recommendations and efforts to educate women with gestational diabetes to follow IOM dietary instructions, a relatively large percentage do not comply. IOM diet instructions seem to be beneficial with respect to ketonemia, as in our study abnormally high blood ketones levels were observed in only a small percentage (1.6%) of

pregnant women with GDM, although we cannot draw a final conclusion since a comparison group was not included in the study.

A limitation of this study is that no data on pregnancy outcomes related with the presence of ketonemia were available. We conclude that the bedtime snack is useful and should not be omitted because it is associated with lower levels of 3HB. Insulin administration, when appropriate, not only has a beneficial effect on good glycemic control but also helps decrease ketonemia. The clinical significance of the independent association of weight loss with increased levels of 3HB in pregnant women, although within normal limits, requires further investigation in larger scale studies which will also include data on pregnancy outcome.

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