

Serum α -hydroxybutyrate (α -HB) predicts elevated 1 h glucose levels and early-phase β -cell dysfunction during OGTT

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ABSTRACT

Objective: Serum α -hydroxybutyrate (α -HB) is elevated in insulin resistance and diabetes. We tested the hypothesis that the α -HB level predicts abnormal 1 h glucose levels and β -cell dysfunction inferred from plasma insulin kinetics during a 75 g oral glucose tolerance test (OGTT).

Research design and methods: This cross-sectional study included 217 patients at increased risk for diabetes. 75 g OGTTs were performed with multiple postload glucose and insulin measurements over a 30–120 min period. OGTT responses were analyzed by repeated measures analysis of variance (ANOVA). Multivariable logistic regression was used to predict 1 h glucose ≥ 155 mg/dL with α -HB added to traditional risk factors.

Results: Mean \pm SD age was 51 \pm 15 years (44% male, 25% with impaired glucose tolerance). Fasting glucose and insulin levels, but not age or body mass index (BMI), were significantly higher in the second/third α -HB tertiles (>3.9 μ g/mL) than in the first tertile. Patients in the second/third α -HB tertiles exhibited a higher glucose area under the receiver operating characteristics curve (AUC) and reduced initial slope of insulin response during OGTT. The AUC for predicting 1 h glucose ≥ 155 mg/dL was 0.82 for a base model that included age, gender, BMI, fasting glucose, glycated hemoglobin (HbA1c), and insulin, and increased to 0.86 with α -HB added ($p=0.015$), with a net reclassification index of 52% ($p<0.0001$).

Conclusions: Fasting serum α -HB levels predicted elevated 1 h glucose during OGTT, potentially due to impaired insulin secretion kinetics. This association persisted even in patients with an otherwise normal insulin–glucose homeostasis. Measuring serum α -HB could thus provide a rapid, inexpensive screening tool for detecting early subclinical hyperglycemia, β -cell dysfunction, and increased risk for diabetes.

INTRODUCTION

The US Centers for Disease Control estimates that approximately 30% of individuals with diabetes are not aware that they have

Key messages

- Fasting serum α -hydroxybutyrate (α -HB) levels predicted elevated 1 h glucose during oral glucose tolerance test, potentially due to impaired insulin secretion kinetics.
- This association persisted even in patients with otherwise normal insulin–glucose secretion kinetics.
- Measuring serum α -HB could thus provide a rapid, inexpensive screening tool for detecting early subclinical hyperglycemia, β -cell dysfunction, and increased risk for diabetes.

the condition.¹ Prediabetes, which confers a 50% lifetime risk for diabetes, affects more than 38% of US adults (>90 million people), and yet less than 10% of those affected have ever received a formal diagnosis.^{2–3} Prediabetes and diabetes (type 2) share the fundamental pathophysiological features of insulin resistance and pancreatic β -cell dysfunction.^{4–6} Even without hyperglycemia, the presence of insulin resistance and β -cell dysfunction increases the risk for cardiovascular disease, retinopathy, neuropathy, microalbuminuria, certain cancers, pulmonary disease, depression, dementia, hospitalization, and early death.^{7–12} It is clear that detection and treatment of these earliest, fundamental stages of the disease process could provide a more effective strategy for prevention. Consequently, efforts are underway to develop convenient, sensitive, and predictive methods for earlier diagnosis of metabolic disease.^{13–15}

Several circulating metabolites identified via an unbiased metabolomics approach have recently been associated with insulin resistance or impaired glucose tolerance (IGT).^{16–17} One of these small molecule organic metabolites, α -hydroxybutyrate (α -HB), was found to have the highest correlation—of 485 screened

metabolites—with the glucose disposal rate as assessed by the hyperinsulinemic-euglycemic clamp, and was elevated in patients without diabetes who had impaired fasting glucose or IGT based on a 2 h oral glucose tolerance test (OGTT).¹⁸ Importantly, α -HB and a second metabolite linoleoylglycerophosphocholine (L-GPC, a putative lipid signaling molecule also associated with insulin sensitivity) have recently been shown to predict progression of dysglycemia and development of type 2 diabetes (T2D).¹⁹ Furthermore, α -HB, but not L-GPC, was associated in these studies with impairments in surrogate indices of pancreatic β -cell function. Consistent with this, in vitro studies of cultured INS1e insulinoma cells showed that preincubation with α -HB tended to dose-dependently decrease insulin secretion in response to stimulation by glucose and arginine.¹⁹ Therefore, elevated α -HB could identify a subset of individuals at greater immediate risk for deterioration of glycemic control and progression to T2D.

The biology of α -HB is not fully understood. This organic ketoacid is produced in the liver as a byproduct during the formation of α -ketobutyrate (a product of either threonine catabolism or methionine metabolism via cystathionine) under conditions of excess glutathione demand resulting from high oxidative stress. In addition, conditions that promote a high ratio of dihydronicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide (NADH/NAD⁺), such as the increased oxidative stress state characteristic of insulin resistance, may increase production of α -HB. Hence, the idea of α -HB as a marker of oxidative stress holds some intuitive appeal as a potential pathophysiological link between increased α -HB and early impairment in pancreatic β -cell function, especially given the proposed role of oxidative injury in β -cell dysfunction (for a recent review, see Montane *et al*.²⁰).

The primary objective of this study was to examine the relationship between the fasting α -HB level and insulin–glucose homeostasis in routine clinical practice patients, and to assess whether α -HB might detect underlying metabolic abnormalities in overtly normal patients. It has been shown that 1 h postload plasma glucose ≥ 8.6 mmol/L is a significant risk factor for progression to diabetes.²¹ We hypothesized that patients with higher fasting α -HB levels would be more likely to have 1 h postload plasma glucose ≥ 8.6 mmol/L and evidence of impaired β -cell function.

RESEARCH DESIGN AND METHODS

Subjects

Between March 2012 and May 2013, 217 patients underwent a 75 g OGTT and fasting blood collection to evaluate glucose tolerance at several outpatient centers across the USA (Madison, Wisconsin; Jackson, Mississippi; Montgomery, Alabama; Charleston, South Carolina; Seattle, Washington; and Salt Lake City, Utah). Clinical indications for testing included obesity, a history of first-degree family members with diabetes, and the presence

of one or more components of the metabolic syndrome, including elevated blood pressure, high serum triglycerides, low high-density lipoprotein cholesterol (HDL-C), and either impaired fasting glucose or elevated glycated hemoglobin (HbA1c) in the non-diabetic range (ie, <48 mmol/mol ($<6.5\%$)). The study protocol was approved by the Copernicus Group Institutional Review Board (IRB), Durham, North Carolina. All analyses involved de-identified data and were covered by a waiver of consent and authorization. Use of discarded clinical samples of patients in Salt Lake City, Utah, was additionally approved under a separate review and waiver process by the University of Utah Human Subjects Institutional Review Board.

Laboratory/assays

Blood samples were sent by overnight courier on dry ice to Health Diagnostic Laboratory, Inc (HDL, Inc, Richmond, Virginia, USA) for measurement of glucose, insulin, C-peptide, lipids, and α -HB. To ensure quality preservation and handling of the samples, blood was collected in BD Vacutainer SST ‘Tiger Top’ tubes with a clot activator and gel, allowed to clot for no more than 30 min, and centrifuged for 15 min at 3000 rpm before shipping. α -HB and L-GPC were determined by electrospray ionization liquid chromatography-mass spectrometry. Fasting glucose (mmol/L) was measured by an ultraviolet method, while HbA1c levels (mmol/mol) were determined by high performance liquid chromatography. Antigliutamic acid decarboxylase (anti-GAD) antibody levels were measured by an ELISA technique. Insulin and C-peptide were assessed by electrochemiluminescence immunoassay. Low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were measured using direct enzymatic assays.

Oral glucose tolerance testing

OGTT was performed according to the standardized protocol. Fasting blood samples were collected before administration of glucola (75 g glucose solution), which was consumed within 5 min. Additional blood samples were collected at either (1) 30, 60, 90, and 120 min, or (2) 60 and 120 min from completion of the glucola. All patients were restricted from eating, drinking, or smoking during the testing period. Two estimates of whole-body insulin sensitivity were derived from the OGTT data—the Matsuda Index²² and the ‘Clamp-like Index’ (CLIX), the latter as previously described ($(\text{serum creatinine} (\times 0.85 \text{ if male}) / (\text{mAUC}_{\text{glucose}} \times \text{mAUC}_{\text{C-peptide}})) \times 6600$), where $\text{mAUC}_{\text{glucose}}$ equals the total $\text{AUC}_{\text{glucose}}/120$ and $\text{mAUC}_{\text{C-peptide}}$ equals the total $\text{AUC}_{\text{C-peptide}}/120$.²³ In a subset of patients where creatinine values were missing (29%), group mean creatinine values were utilized. In patients with no diabetes, CLIX correlates highly with the glucose disposal rate (ie, M value) measured during the euglycemic hyperinsulinemic clamp. An OGTT-derived version of the disposition index ($\text{AUC}_{\text{insulin}/\text{glucose}} \times \text{Matsuda Index}$) was used

to estimate β -cell function, reflecting insulin secretion relative to insulin resistance.²⁴

Statistical methods

The study included 217 patients with suspected diabetes risk. Cross-sectional differences in patient characteristics were tested among fasting α -HB tertiles using one-way analysis of variance (ANOVA), and χ^2 tests for continuous and categorical data, respectively. Repeated measures ANOVA models were used to analyze the glucose and insulin mean response profiles over the 3-time point or 5-time point 2 h OGTT. The insulin response-dependent variable was transformed using the natural transformation to improve the normality and homoscedasticity of the residual errors. Additionally, one-way ANOVA models were used to test the area under the curve (AUC) for the repeated glucose and insulin measurements among α -HB tertiles using the trapezoidal rule and either the 3-time point or 5-time point data as available. Residual diagnostics were conducted to verify model assumptions.

Multivariable logistic regression was used to test the association (ie, odds ratio) and incremental improvement in discrimination (ie, c-statistic) for classifying patients with 1 h glucose ≥ 8.6 mmol/L when α -HB was added to the following list of potential confounding variables: age, gender, body mass index (BMI) categories (normal <25 , ≥ 25 overweight <30 , obese ≥ 30 kg/m²), fasting glucose, log(HbA1c), log(triglycerides), HDL-C, and LDL-C. HbA1c and triglycerides were natural logarithm transformed to reduce leverage of extreme observations. When testing the usefulness of a novel biomarker, the American Heart Association recommends reporting the marker's statistical association, discrimination, calibration, and reclassification performance.²⁵ The Hosmer-Lemeshow test was used as a measure of model calibration.²⁶ In addition, the reclassification of patients with 1 h glucose ≥ 8.6 mmol/L was tested when α -HB was added to the fully adjusted logistic regression model by calculating the integrated discrimination improvement (IDI), which can be described as the average increase in sensitivity given no change in specificity. Lastly, the continuous net reclassification index (NRI), which measures the percentage of patients who had model probabilities changed in the correct direction (ie, increased for those with events and decreased for non-events) due to the addition of α -HB to the fully adjusted model, was also tested.^{27 28} SAS V.9.3 (Cary, North Carolina, USA) was used for all analyses, and the critical level $\alpha=0.05$ was used to prescribe statistical significance.

RESULTS

Table 1 displays patient characteristics according to α -HB tertiles (<37.5 μ mol/L (3.9 μ g/mL), 37–53.8 μ mol/L (3.9–5.6 μ g/mL), >53.8 μ mol/L (>5.6 μ g/mL)). Fasting α -HB levels were not associated with age, gender, BMI, HbA1c, L-GPC, or lipid levels (table 1). However,

mean fasting glucose, insulin, and C-peptide values were higher in the second and third α -HB tertiles compared with the first tertile. The OGTT results also revealed higher mean levels for 1 h and 2 h glucose, and lower scores on the Matsuda Index, CLIX, and an OGTT-derived version of the disposition index ($AUC_{\text{insulin}/\text{glucose}} \times \text{Matsuda Index}$) in the highest two tertiles compared with the lowest ($p<0.05$).

High fasting α -HB levels are associated with impaired insulin secretion and glucose homeostasis

The mean glucose and log(insulin) levels at 30 min time points are shown by α -HB tertiles in figure 1. Since there were no significant differences between the second and third α -HB tertiles at any time point (see online supplementary figure 1A and table S1, minimum $p=0.22$), they were combined for a simpler interpretation. Figure 1A shows that the mean glucose levels were similar at baseline, but then in the group of patients with α -HB ≥ 3.9 μ g/mL were elevated by the 30 min time point and remained higher compared to that in those with α -HB <3.9 μ g/mL. The association between circulating glucose and α -HB was also revealed by the partial AUC_{glucose} values (ie, that part of the $AUC_{\text{glucose}} >2.8$ mmol/L (50 mg/dL)), for which the mean (SD) values were 127 (75), 172 (67), and 179 (92) units for increasing α -HB tertiles. The AUC_{glucose} for patients in the second and third α -HB tertiles was significantly higher than the AUC_{glucose} of patients in the first tertile, minimum $p=0.0008$, and there was no difference between AUC_{glucose} for the second and third α -HB tertiles ($p=0.56$); providing further support for combining these groups (figure 1B).

The mean log(insulin) response during the OGTT by α -HB groups is shown in figure 1C. All α -HB groups exhibited similar peak insulin secretion levels during the OGTT (60 min minimum $p=0.29$; online supplemental figure 1B); however, the response for those with α -HB <3.9 μ g/mL was different than with α -HB ≥ 3.9 μ g/mL. Specifically, insulin levels rose and declined more rapidly in the former (figure 1C). The 30 min geometric rise (95% CI) for insulin was 61.2 (51.6 to 70.8) pmol/L (10.2 (8.6 to 11.8) μ U/mL) and 51 (41.4 to 60) pmol/L (8.5 (6.9 to 10) μ U/mL) for those <3.9 μ g/mL and ≥ 3.9 μ g/mL, respectively ($p=0.0003$; figure 1D).

α -HB predicted elevated OGTT 1 h postload glucose level

We used multivariable logistic regression to test the ability of fasting α -HB to classify patients having elevated 1 h postload glucose level ≥ 8.6 mmol/L during the OGTT. Considering fasting α -HB alone, a patient was 1.38 times as likely for each 1 μ g/mL (9.6 μ mol/L) increase (OR, 95% CI 1.20 to 1.59, $p<0.0001$) to have 1 h glucose ≥ 8.6 mmol/L. This strong relationship remained unchanged after adjusting for age, gender, BMI, fasting glucose, log(insulin), log(triglycerides), HDL-C, and LDL-C, OR=1.38 (95% CI 1.17 to 1.63, $p=0.0002$). The model was well calibrated, that is, the

Table 1 Patient characteristics and clinical factors grouped by α -HB tertiles ($\mu\text{g/mL}$)

| Variable | <3.9 n=73 | 3.9–5.6 n=72 | >5.6 n=72 | p Value* |
|--|--------------|-----------------|---------------|---------------------|
| Age (years) | 52 (16) | 50 (13) | 52 (15) | 0.68 |
| Male (n (%)) | 32 (44) | 31 (43) | 33 (46) | 0.94 |
| BMI (kg/m^2) | 30.5 (7.6) | 32.8 (7.8) | 32.6 (9.2) | 0.18 |
| Glucose (mg/dL) | 88 (11) | 94 (14) † | 94 (18) † | 0.024 |
| 1 h glucose (mg/dL) | 125 (50) | 154 (45) † | 163 (59) † | <0.0001 |
| 1 h glucose ≥ 155 (mg/dL) (n (%)) | 17 (23) | 28 (39) | 43 (60) | <0.0001 |
| 2 h glucose (mg/dL) | 103 (47) | 130 (51) † | 126 (62) † | 0.0057 |
| 2 h glucose ≥ 140 (mg/dL) (n (%)) | 12 (16) | 24 (33) | 20 (28) | 0.0601 |
| HbA1c (%) | 5.3 (0.4) | 5.5 (0.8) | 5.5 (0.7) | 0.12 ‡ |
| Insulin ($\mu\text{U/mL}$) | 11.0 (10.2) | 16.7 (16.1) † | 14.9 (13.2) † | 0.0020 ‡ |
| C-peptide (ng/mL) | 2.8 (1.6) | 3.4 (2.0) † | 3.3 (1.8) | 0.0232 ‡ |
| CLIX | 7.1 (4.7) | 4.8 (2.5) † | 5.9 (7.4) † | 0.0023 ‡ |
| Matsuda Index ‡ | 7.0 (5.4) | 4.1 (2.7) † | 5.5 (5.3) † | 0.0019 ‡ |
| AUC _{glucose} | 227 (75) | 272 (67) † | 279 (92) † | 0.0001 |
| AUC _{insulin} | 146 (131) | 159 (98) | 171 (179) | 0.26 ‡ |
| AUC _{insulin/glucose} | 0.63 (0.47) | 0.60 (0.36) | 0.62 (0.62) | 0.68 ‡ |
| AUC _{insulin/glucose} × Matsuda Index | 3.2 (2.0) | 1.9 (0.9) † | 2.3 (2.9) † | <0.0001 ‡ |
| L-GPC* | 20.0 (8.3) | 19.5 (9.5) | 20.1 (14.5) | 0.61 |
| Anti-GAD Positive (n (%)) | 3 (4.1) | 1 (1.4) | 4 (5.6) | 0.40 |
| Triglycerides (mg/dL) | 136 (159) | 132 (92) | 117 (71) | 0.40 ‡ |
| HDL cholesterol (mg/dL) | 59 (19) | 53 (14) | 55 (17) | 0.19 |
| LDL cholesterol (mg/dL) | 101 (37) | 109 (36) | 103 (40) | 0.42 |

Data are mean (SD) unless stated otherwise, N=217.

*One-way ANOVA and χ^2 test for continuous and categorical data, respectively.

†Dunnett adjusted p value <0.05 compared to the lowest α -HB tertile.

‡Used natural logarithm transformation for improved normality and homoscedasticity of residual errors in ANOVA models.

α -HB, α -hydroxybutyrate; AUC, area under curve; ANOVA, analysis of variance; BMI, body mass index; CLIX, Clamp-like Index; GAD, glutamic acid decarboxylase; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; L-GPC, linoleoylglycerophosphocholine.

Bold highlights the significant p values (<0.05).

predicted counts of patients having elevated 1 h glucose was similar to the actual counts across event probability deciles (Hosmer-Lemeshow $p=0.52$). The c-statistic (area under the receiver operating characteristic (ROC) curve) was 0.82 for the base model adjusted for the multiple potential confounding variables listed above, and increased to 0.86 when α -HB was added (change in c-statistic 95% CI 0.01 to 0.07, $p=0.015$, figure 2). The addition of α -HB also resulted in a significant IDI of 0.078 ($p<0.0001$). This was due to increases in the average sensitivity and average specificity of 0.047 ($p=0.0091$) and 0.032 ($p=0.0001$), respectively. In addition, the continuous NRI was 0.52 ($p=0.0001$), that is, 52% of the patients had their probability of having 1 h glucose ≥ 8.6 mmol/L changed in the correct direction. These findings indicate that α -HB is a useful, independent predictor of elevated OGTT 1 h glucose in at-risk patients from routine clinical practice.

α -HB predicts subclinical hyperglycemia in patients with normoglycemia

Two subgroups were formed from the overall patient cohort. The first (N=137) excluded patients with fasting glucose ≥ 5.6 mmol/L (100 mg/dL) and HbA1c ≥ 39 mmol/mol (5.7%), representing those patients who

would be identified as normoglycemic based on typical fasting glycemic measures. The second subgroup (N=93) further excluded patients with fasting insulin >72 pmol/L (12 $\mu\text{U/mL}$), 2 h glucose ≥ 7.8 mmol/L (140 mg/dL), and an anti-GAD antibody positive in order to create a cohort which would typically be considered normal even if more extensive testing was performed. Figure 3A, B show the ROC curves for classifying patients with 1 h postload glucose ≥ 8.6 mmol/L for these two subgroups of patients using fasting glucose, HbA1c, or α -HB. The area increased by 0.10 ($p=0.059$), 0.09 ($p=0.087$), or 0.17 ($p=0.0044$), respectively, for the subgroup of patients with normal levels of fasting glucose and HbA1c. Similarly, α -HB exhibited strong predictive capability within the second cohort of patients with normoglycemia (figure 3B). In these patients, with low-risk levels for all the above biomarkers, the area increased by 0.15 ($p=0.016$), 0.15 ($p=0.038$), or 0.24 ($p=0.0007$), using only fasting glucose, HbA1c, or α -HB to classify 1 h postload glucose ≥ 8.6 mmol/L.

DISCUSSION

Prediabetes is one of the strongest clinical risk factors for diabetes and vascular disease. In prediabetes and the

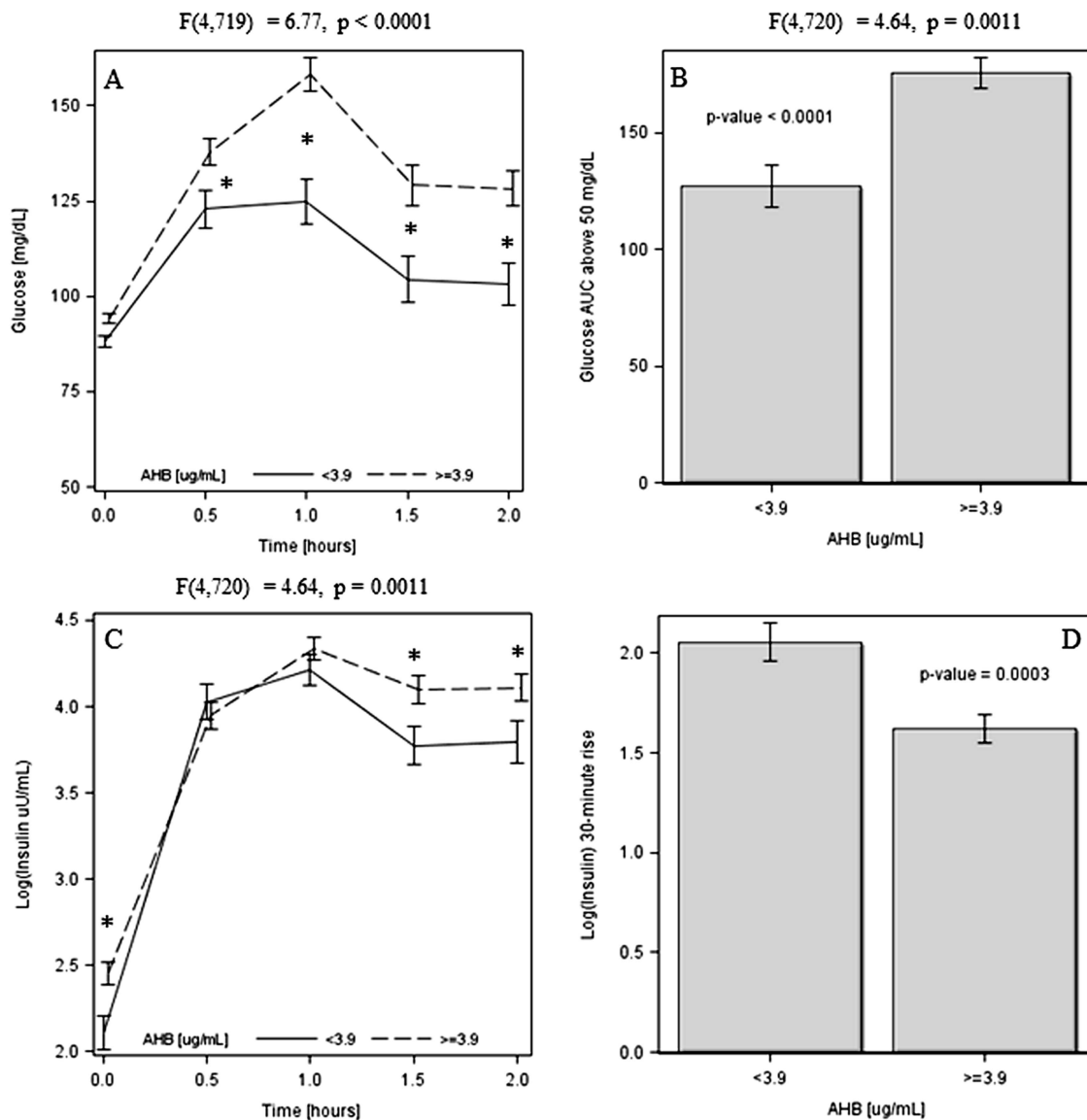


Figure 1 (A) Oral glucose tolerance test (OGTT) glucose and (C) natural logarithm of insulin responses over time with SEM bars comparing the first tertile to the second and third tertiles combined in routine clinical practice patients (N=217); *p value <math><0.05</math>. The F-test for the α -hydroxybutyrate (AHB) group by time interaction is given above the panel. (B) OGTT glucose response area under the curve (AUC) above 50 mg/dL. (D) 30 min rise in log(insulin).

early stages of diabetes, fasting glucose and HbA1c are often highly correlated, reflecting basal hepatic glucose output in the fasting state. The 75 g OGTT provides important, additional, diagnostic information by challenging insulin–glucose homeostasis with conditions that simulate the usual postprandial state, in which there is induction of insulin secretion, insulin-stimulated glucose uptake by skeletal muscle and other tissues, and suppression of hepatic glucose production. As such, the OGTT can uncover defective glucose regulation that is not observed in the fasting state. Consistent with this, up to 40% of individuals with abnormal 2 h OGTT glucose values have normal fasting glucose levels.²⁹ Hence, fasting blood glucose and HbA1c values are not adequate substitutes for the OGTT. Nevertheless, the OGTT is not routinely administered in most clinical

practice settings, in part due to the time and technical effort it requires.

Recent studies are revealing that there is more to be learned from an OGTT than 2 h glucose values, which primarily reflect the rate of glucose clearance as a function of peripheral insulin resistance. Pancreatic β -cell dysfunction, the more proximal cause of diabetes, is directly reflected in impaired early-phase insulin response, and thus steeper early rises in glucose values during the first hour of the OGTT. Consistent with this, it has been demonstrated in two large prospective studies that the 1 h postload plasma glucose value obtained during the 75 g OGTT is a strong, independent predictor of risk for diabetes, even in individuals with normal (ie, not prediabetic or diabetic) fasting or 2 h glucose levels (normal glucose tolerance; NGT).³⁰ In the San Antonio

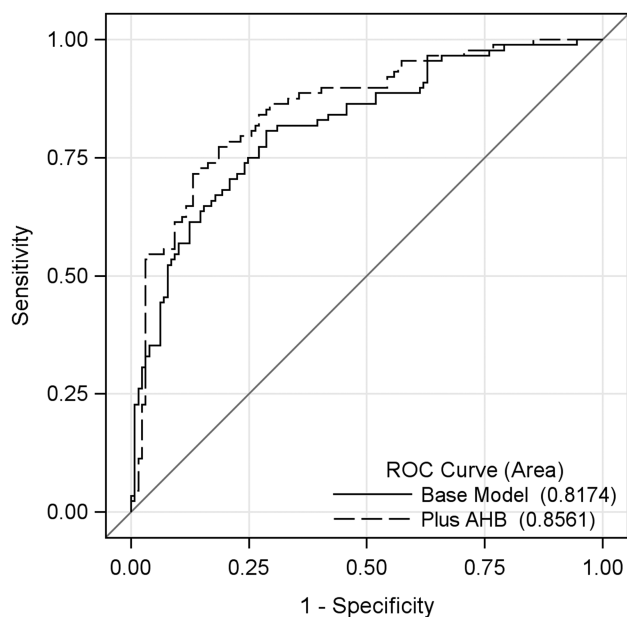


Figure 2 Receiver operating characteristic (ROC) curves for classifying patients having a 1 h glucose ≥ 155 mg/dL during oral glucose tolerance test. The area increased by 0.039 (95% CI 0.008 to 0.070, $p=0.015$) when α -hydroxybutyrate (AHB) was added to age, gender, body mass index, fasting glucose, log(fasting insulin), log(triglycerides), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in the logistic regression model.

Heart Study and the Botnia Study, baseline 1 h glucose levels predicted diabetes incidence better than did either fasting or 2 h glucose, and were strongly

associated with indices of β -cell function and insulin resistance.^{21 31 32}

Cross-sectional data from several recent studies have reinforced the clinical importance of elevated 1 h glucose levels in individuals with otherwise normal glucose regulation. For example, among the 39% of patients with NGT in the GENFIEV study, an elevated 1 h glucose level predicted β -cell dysfunction, insulin resistance, and increased numbers of cardiovascular risk factors such as dyslipidemia and elevated blood pressure.³³ Others have shown that patients with NGT and elevated 1 h glucose levels have features of insulin resistance or impaired β -cell function by diverse measures: acute insulin response and disposition index (calculated from the frequently sampled intravenous glucose tolerance test (IVGTT)), decreased glucose disposal on hyperinsulinemic-euglycemic clamp, elevated markers of chronic inflammation (hsCRP and fibrinogen), and increased levels of hepatic transaminases.^{34–37} Moreover, increased 1 h glucose predicts increased cardiovascular risk. In patients with hypertensive NGT, elevated 1 h glucose levels are associated with higher blood pressure, increased vascular stiffness, and left ventricular hypertrophy.^{38 39} Thus, elevated 1 h postload glucose may represent a unique ‘subclinical’ form of hyperglycemia that is not accounted for in current models for clinical detection of prediabetes or diabetes, but which plays an important role in the pathophysiology of diabetes and diabetes-related cardiovascular complications.

In the present study, we found that fasting serum levels of α -HB predicted insulin resistance, impaired

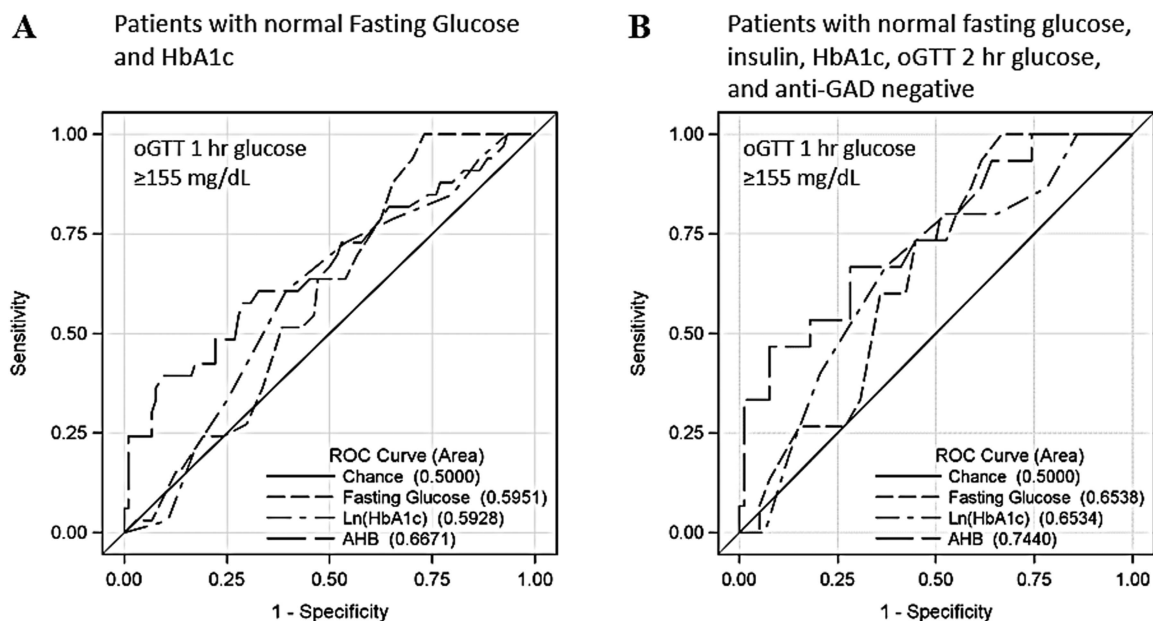


Figure 3 Comparison of receiver operating characteristic (ROC) curves for predicting oral glucose tolerance test (OGTT) 1 h postload glucose ≥ 155 mg/dL in (A) patients with fasting glucose < 100 mg/dL and glycated hemoglobin (HbA1c) $< 5.7\%$ ($N=137$, with 33 events); the area increased by 0.10 ($p=0.059$), 0.09 ($p=0.087$), or 0.17 ($p=0.0044$) when using fasting glucose, log (HbA1c), or α -hydroxybutyrate (AHB) alone, respectively; or in (B) patients additionally with insulin ≤ 12 U/mL, 2 h glucose < 140 mg/dL, and a negative antiglutamic acid decarboxylase (anti-GAD) antibody ($N=93$, with 15 events). The area increased by 0.15 ($p=0.016$), 0.15 ($p=0.038$), or 0.24 ($p=0.0007$), respectively.

insulin secretion kinetics, and abnormal glucose regulation during the 75 g OGTT—in particular, elevated 1 h post-load plasma glucose levels. Importantly, the predictive ability of α -HB for 1 h postload glucose levels is observed even in individuals with ostensibly normal insulin–glucose homeostasis by other standard clinical criteria. These findings indicate that α -HB has clinical utility for predicting subclinical hyperglycemia, even under conditions where other commonly used indicators of impaired insulin–glucose homeostasis are ostensibly normal. This suggests that measurement of serum α -HB could provide a rapid, inexpensive screening tool for detecting early, subclinical stages of the disease process leading to prediabetes/diabetes. In addition, measurement of α -HB in conjunction with fasting plasma glucose and HbA1c could enhance the ability to detect hyperglycemia that might otherwise only be apparent by OGTT testing in diabetic and non-diabetic individuals.

Our findings are consistent with prior observations that elevated serum α -HB levels can identify individuals with impaired insulin–glucose homeostasis, and further indicate that this relationship is independent of BMI and other traditional diabetes risk factors. In the present study, no differences between α -HB tertiles were observed for age, gender, BMI, or HbA1c. α -HB was also shown to be independent of other novel biomarkers of diabetes risk such as L-GPC. The observation that α -HB does not associate with other traditional factors associated with diabetes risk but is associated with elevated 1 h glucose in the OGTT suggests it has truly independent value in identifying patients at risk for developing diabetes.

The results of the present study suggest that elevated serum α -HB is associated with delayed insulin secretory kinetics and insufficient insulin secretion during the OGTT, as differences were observed in the early rise in insulin levels during the OGTT and the OGTT-derived version of the disposition index. These findings are consistent with prior observations that serum α -HB correlates inversely with indices of β -cell function during OGTT in the RISC and Botnia study cohorts.¹⁹ The importance of interpreting estimates of β -cell function within the context of prevailing insulin resistance has been well established, as insulin resistance is normally compensated for by increased insulin secretion.⁴⁰ In the present study, a direct estimate of insulin secretion during the OGTT, $AUC_{\text{insulin}/\text{glucose}}$, trended lower according to α -HB tertile, though did not achieve statistical significance (similar to $AUC_{\text{c-peptide}/\text{glucose}}$, data not shown). However, when adjusted for insulin resistance, the observed deficits were striking. It should be noted that the original formulation of the disposition index as reported by Kahn *et al*⁴¹ utilized an estimate of insulin secretion obtained over only the first 8 min of an IVGTT, which was relatively independent of the estimate of insulin sensitivity obtained over the next few hours. However, when the same insulin excursion is used in the estimate of insulin secretion and insulin resistance, as in

the OGTT-derived version of the disposition index used here, interpretation becomes more complicated. Additional experiments investigating α -HB utilizing an IVGTT protocol will be helpful in this regard.

In conclusion, we report the discovery that elevated α -HB is strongly predictive of abnormal 1 h postload plasma glucose levels and decreased insulin secretion in the spectrum of clinic patients undergoing OGTT for evaluation of suspected metabolic risk. In addition, we confirm prior reports that serum α -HB is associated with insulin resistance based on 2 h OGTT glucose levels. Remarkably, we find that α -HB is highly predictive of OGTT glucose responses, and calculated indices of insulin sensitivity and β -cell function—even among a subset of patients selected for having ostensibly normal indicators of insulin–glucose homeostasis. Hence, α -HB may have broad applications as a tool for detecting states of subclinical hyperglycemia, insulin resistance, and β -cell dysfunction that could indicate increased risk for diabetes and cardiovascular disease.

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