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REVIEW

Update on biomarkers of glycemic control

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Abstract

Attaining and maintaining good glycemic control is a cornerstone of diabetes care. The monitoring of glycemic control is currently based on the self-monitoring of blood glucose (SMBG) and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate biochemical marker of the average glycemia level over the previous 2-3 mo period. Although hyperglycemia is a key biochemical feature of diabetes, both the level of and exposure to high glucose, as well as glycemic variability, contribute to the pathogenesis of diabetic complications and follow different patterns in type 1 and type 2 diabetes. HbA1c provides a valuable, standardized and evidence-based parameter that is relevant for clinical decision making, but several biological and analytical confounders limit its accuracy in reflecting true glycemia. It has become apparent in recent years that other glycated proteins such as fructosamine, glycated albumin, and the nutritional monosaccharide 1,5-anhydroglucitol, as well as integrated measures from direct glucose testing by an SMBG/continuous glucose monitoring system, may provide valuable complementary data, particularly in circumstances when HbA1c results may be unreliable or are insufficient to assess the risk of adverse outcomes. Long-term associations of these alternative biomarkers of glycemia with the risk of complications need to be investigated in order to provide clinically relevant cut-off values and to validate their utility in diverse populations of diabetes patients.

Key Words: Diabetes mellitus; Hemoglobin A1c; Fructosamine; Glycated albumin; 1,5-anhydroglucitol; Plasma glucose; Glucose variability; Diabetic complications

Core tip: Monitoring of glycemic control is currently based on the self-monitoring of blood glucose and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate marker of the average glycemia level over the past 2-3 mo. The severity of hyperglycemia and glycemic variability contribute to the pathogenesis of complications, but the HbA1c measurement reflects only a piece of these important variables. In this review, we provide a critical update on the use of HbA1c and alternative biomarkers of glycemic control, with particular emphasis on the need for a personalized approach in utilizing and interpreting different tests in a clinically meaningful manner.

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INTRODUCTION

Attaining and maintaining good glycemic control is the cornerstone of diabetes care[1]. The results of the seminal Diabetes Control and Complications Trial (DCCT) clearly evidenced that glycemic control is causatively related to microvascular complications in type 1 diabetes[2]. A long-term follow-up in the Epidemiology of Diabetes Interventions and Complications Study (EDIC) confirmed that keeping glycemia as close as possible to its normal range with intensified insulin therapy ameliorated both microvascular and cardiovascular complications for 30 years in the same cohort of patients[3].

Similar evidence of the beneficial effect of intensive glucose control practices in reducing the risk of diabetic complications, adverse cardiovascular outcomes and mortality were shown in type 2 diabetes patients in both the United Kingdom Prospective Diabetes Study (UKPDS) intervention and in follow-up trials[4,5]. However, although additional intensification of glucose control in type 2 diabetes patients provided some benefits[6,7], it was associated with serious adverse outcomes such as an increased overall mortality[8] that was most likely due to severe hypoglycemia as a side-effect of a more aggressive antihyperglycemic therapy[9]. These data indicated that a personalized approach to glycemic goals that uses clinically validated biomarkers rather than a "one-size-fits-all" concept may provide a valid rationale for optimal diabetes care.

The concept of glycemic control monitoring is currently based on self-monitoring of blood glucose (SMBG) and laboratory testing for hemoglobin A1c (HbA1c), which is a surrogate biochemical marker of the average glycemia level over the previous 2-3 mo period[10]. HbA1c emerged as a key determinant of the risk cut-off for diabetic complications and as a setting point for optimal glycemic control in both DCCT and UKPDS trials, and it is considered to be a gold standard of diabetes care in contemporary clinical practice[11]. HbA1c provides valuable, standardized and evidence-based information that is relevant for clinical decision-making; however, several biological and analytical interferences, as well as clinical conditions, limit its accuracy in reflecting the true glycemia level[12,13]. Recent technological advances in the field of continuous glucose monitoring systems (CGMS) have revealed new insights in short-term glucose dynamics which are not reflected by HbA1c, although it seems to be relevant in assessing the risk of diabetic complications[14,15].

Thus, alternative glycemic markers that provide reliable information about glycemic control in addition to and beyond HbA1c are needed to improve the quality of clinical care across a heterogeneous diabetes population[16,17].

The aim of this narrative review is to provide a critical update on the use of HbA1c and alternative biomarkers of glycemic control, with a particular emphasis given to the need for a personalized approach in utilizing and interpreting different tests in a clinically meaningful manner.

HBA1C

HbA1c results from the posttranslational modification of hemoglobin A by the nonenzymatic covalent binding of glucose to the N-terminal valine of the β-globin chain[10]. This reaction is termed glycation and affects all structural and circulating proteins with free amino-acid residues that are available for binding monosaccharides. The glycation of hemoglobin is a two-step chemical reaction whereby glucose covalently binds to the free amino-groups within globin chains[18]. The first step of this process results in labile aldimine (a Schiff base), which can either dissociate or further convert to a stabile ketoamine by an Amadori rearrangement, depending on the glucose concentration in the blood[10]. HbA1c was first observed as a minor chromatographic fraction of adult hemoglobin in 1958 and was named according to its chromatographic column elution sequence[19], but its relevance in diabetes was revealed in 1969 by Rahbar[20], who observed significantly higher HbA1c values in diabetic patients. Since glycation is a nonenzymatic reaction, it complies with the law of mass action. Thus, assuming normal erythropoiesis

and a stable hemoglobin concentration, HbA1c reflects the average glycemia level during one red blood cell life cycle (2-3 mo)[21].

Considering the high biological variability, the dynamics of glucose, as well as the limitations of blood glucose monitoring technology, at that time, the possibility of obtaining an integrated average glycemia value by the measurement of a single biomarker elicited immense interest and provided a powerful tool in both diabetes research and clinical management. HbA1c testing was soon facilitated by the development of a new analytical methodology that was suitable for use in clinical laboratories.

Various analytical methods for HbA1c determination commonly utilize either of the two principles (Table 1): (1) HbA1c separation from other hemoglobin fractions that is based on charge differences using either chromatography or electrophoresis; or (2) the direct measurement of HbA1c by specific binding (immunochemistry or affinity) or enzymatic cleavage[22]. Due to differences between these analytical methods in their use of different principles and a lack of standardization, HbA1c testing inherently suffers from a significant between-method variability which has seriously affected its clinical accuracy in the longitudinal monitoring of average glycemia with different methods and comparing the results of the DCCT- and UKPDS-derived targets. Heterogeneity of molecular entities that were measured by different methods significantly contributed to the analytical variability, as the glycation reaction involved not only β-N-terminal valine but also other accessible amino groups within the α and β-globin chains, and these results depended on the type of analyte that was captured by a particular method[12]. Thus, the standardization of the HbA1c measurement and reporting that included a uniform definition of the analyte was shortly identified as one of the most important issues in diabetes care[23,24].

Clinical harmonization was accomplished within the National Glycohemoglobin Standardization Program (NGSP), which was established by the American Diabetes Association (ADA) and the American Association of Clinical Chemistry (AACC). The goal of the NGSP was to harmonize the HbA1c results that were obtained by different methods with the highly reproducible but insufficiently specific method (ion-exchange chromatography) that was used in the DCCT and UKPDS trials, thereby enabling the traceability and comparability of results to the evidence-based clinical criteria[25]. Almost simultaneously to the NGSP, the International Federation of Clinical Chemistry (IFCC) set up an HbA1c Standardization Program that was aimed at designing a comprehensive reference system with both reference methods and a primary reference standard for a structurally-defined analyte[23,26,27]. The comparison between the two reference systems revealed an excellent linear correlation between the DCCT- and IFCC-reference systems but significantly lower HbA1c values with the latter, more specific method. This finding raised concerns regarding the risks of deterioration of the glycemic control with the adoption of the new reference system, which had been reported previously[28].

In 2010, a Global Consensus on HbA1c measurement and reporting was issued by an international committee representing the ADA, European Association for the Study of Diabetes (EASD), International Diabetes Federation (IDF), IFCC and International Society for the Pediatric Diabetes (ISPAD)[29]. Briefly, the Global Consensus defined the IFCC reference as the only valid anchor for commercial methods calibration and a dual reporting of the HbA1c results as mmol/mol (IFCC-related units) and % (NGSP/DCCT-related units). A master equation describing the relationship between the two reference systems should be used for the interconversion of the results:

HbA1c NGSP/DCCT (%) = 0.09148 × HbA1c IFCC (mmol/mol) + 2.152

HbA1c IFCC (mmol/mol) = 10.93 × HbA1c NGSP/DCCT (%) - 23.50

Editors of scientific journals were encouraged to require both units of HbA1c reporting to promote the clarity and comparability of results between studies that used HbA1c as an outcome measure and to facilitate the combination of these results in meta-analyses. The Global Consensus definitely enabled the uniform traceability and improved analytical quality of HbA1c measurements[12]; however, it failed to harmonize the reporting of these results, as different countries use different reporting units, which may thus complicate a direct comparison of results across the world[30].

Today, the analytical procedures for HbA1c measurement are harmonized and the between-method/laboratory variabilities have been gradually reduced towards a desirable goal, which is a coefficient of variation (CV) < 3.5%[12]. Regarding the within-laboratory imprecision, current guidelines recommend a CV < 2% for NGSP-HbA1c equivalents[31], and this is achievable with almost all of the commercially available laboratory methods apart from point-of-care systems for HbA1c testing, which still need improvement[22]. However, global harmonization and ongoing efforts to improve the analytical quality[32] cannot obviate the limitations of HbA1c measurement due to the hemoglobin-related interferences.

It has long been recognized that hemoglobin variants interfere with HbA1c synthesis and measurement, and this interference depends on the nature of the congenital disorder afflicting hemoglobin synthesis and the analytical method that is used to measure HbA1c[22]. Thalassemia traits, HbS, HbC, HbE and HbF are among the most abundant hemoglobin-related interferences[33]. Additionally, other posttranslational modifications of hemoglobin such as carbamylation by uremic toxins in end-stage renal disease may significantly interfere with some HbA1c assays[34]. It should be noted that the majority of interferences have been mitigated by improvements of analytical methodologies, and the remaining interferences have been depicted and rigorously scrutinized. A comprehensive list of HbA1c methods that have been characterized for their susceptibility to hemoglobin-

Table 1 Characteristics of the analytical methods for hemoglobin A1c measurement

Method	Advantages	Disadvantages
Ion exchange chromatography	DCCT method, high reproducibility	Lack of specificity; interference from hemoglobinopathies and HbF
Capillary electrophoresis	High reproducibility; specificity	Time-consuming, costly
Boronate affinity chromatography	Minimal interference from hemoglobinopathies	Analyte-related unspecificity (total GHb)
Immunoassay	Specificity	Some interference from HbF

DCCT: Diabetes Control and Complications Trial; HbF: Fetal hemoglobin; GHb: Total glycated hemoglobin.

related interferences is available and is continuously updated on the NGSP website[35].

Biological confounders influencing the accuracy of HbA1c as a glycemic marker have emerged as a significant issue after analytical harmonization, despite the fact that a substantial intraindividual variability in HbA1c values was recognized long ago. Studies on the relationship between HbA1c measurements and average glycemia levels revealed a strong linear correlation with a wide interindividual variability, *e.g.*, an HbA1c of 7% (53 mmol/mol) could correspond to an average glucose concentration ranging from 6.8 to 10.3 mmol/L[36]. Physiological factors such age and ethnicity, as well as genetics, seem to be major determinants of this variability.

Age was found to be associated with a gradual increase of HbA1c levels in nondiabetic individuals independently of sex and level of glycemia, indicating that age-specific reference intervals/clinical cut-off points may improve the clinical accuracy of this test in both the diagnosis and management of diabetes[37]. There are ethnic differences in HbA1c values even when glycemia levels are the same; a recent meta-analysis revealed that Caucasians have slightly lower HbA1c values in comparison to persons of other ethnic groups[38]. While the clinical relevance of this finding needs to be further investigated, the authors concluded that a better understanding of the molecular mechanisms behind this observed between-race variability in HbA1c may improve its clinical applicability.

Recent genetic studies have revealed that multiple genomic loci are associated with HbA1c levels, and this could provide a plausible explanation for the physiological factors determining its variability and clinical utilization towards a more personalized approach[39]. Among the 60 genetic variants that were found to influence HbA1c, 19 variants associated with glycemic pathways were identified, and among the rest of variants that were involved in nonglycemic pathways, 22 erythrocytic variants were found[40]. Among these, a variant on the X chromosome coding for glucose-6-phosphate dehydrogenase (G6PD) was associated with a significantly higher HbA1c variability in populations of African ancestry when compared to other ethnic groups. This highly prevalent variant is associated with a shorter erythrocyte lifespan and, consequently, falsely decreased HbA1c levels, which may have serious impacts for diabetes care in afflicted individuals[40].

Nonglycemic factors affecting HbA1c levels include erythropoiesis, hemoglobin synthesis and conditions influencing red blood cell survival. Deficiency anemias generally elicit falsely increased HbA1c levels due to the increased levels of aged erythrocytes that are found in patients with this disease, whereas falsely decreased HbA1c levels can be observed in hemolytic anemias of any cause [41]. Nonhematological conditions influencing HbA1c values include pregnancy, chronic renal failure and certain medications[22]. Variability in the normal erythrocyte lifespan is another significant confounder of HbA1c accuracy. Malka *et al*[42] recently proposed a mechanistic mathematical model integrating hemoglobin glycation and red blood cell kinetics that provided a personalized insight into average glucose levels and reduced the occurrence of diagnostic errors due to a misinterpretation of average glycemia (as reflected by HbA1c) by more than 50%. The applicability and clinical utility of the proposed model have yet to be determined.

Furthermore, part of the variability in HbA1c is considered to be a consequence of differences in glycation rate, which is a concept that was proposed as the "glycation gap" 15 years ago[43]. The glycation gap hypothesis is based on the differences between the intra- and extracellular surrogate markers of average glycemia, *i.e.*, HbA1c and fructosamine, and it was proposed as an explanation to the commonly encountered clinical problem of discrepancy between various glycemia measures that cannot be attributed to any other confounding factor[44]. In spite of subsequent evidence from a twin study that shows that the glycation gap may be a genetically determined characteristic of an individual [45], this concept has been considered implausible by some authors due to the lack of validating data or supporting evidence of the underlying mechanism[46]. Nevertheless, an accumulating body of evidence indicates that glycemic variability, as assessed by either the glycation gap or another discordance measure called the hemoglobin glycation index[47], is indeed associated with adverse diabetes-related outcomes such as mortality, micro- and macrovascular complications, and hypoglycemic episodes that are associated with intensive treatment[48,49]. Interindividual heterogeneity in glucose transport across the erythrocyte membrane was proposed as a possible explanation for inconsistencies between HbA1c and other measures of glycemia[50]. Genome-wide association studies also support the plausibility of

the glycation gap concept since one of the identified loci, FN3K, encodes fructosamine-3-kinase, which is an enzyme that is involved in deglycation of glycated proteins[39]. Dunmore *et al*[51] recently reported a significant difference in the erythrocyte fructosamine-3-kinase activities between glycation gap categories and pinpointed FN3K both as a novel predictor of the risk for development of and as a potential target for the prevention of diabetic complications.

Current clinical guidelines recommend regular HbA1c testing twice a year in all diabetic patients who achieve their glycemic targets, and they recommend an increased frequency of testing not to exceed four times a year for patients who have changed therapy and/or have not achieved their treatment goals[1]. The general recommendation is to keep the HbA1c levels < 7% (53 mmol/mol); however, the target should be individualized for individual patients depending on the diabetes duration, age or life expectancy, CVD and other comorbidities, hypoglycemia unawareness and psychosocial factors[52]. A reference change value of 0.5% (5 mmol/mol) in the longitudinal monitoring of an individual patient is considered to be clinically significant[22].

The use of HbA1c as a diagnostic test for diabetes with a diagnostic cutoff set at an HbA1c level of 6.5% (48 mmol/mol) has recently been recommended by prominent professional organizations and by the Word Health Organization[53,54]. Low intraindividual biological variability, the stability of the analyte and the independence of results to the prandial status were the most pronounced advantages of HbA1c over plasma glucose, while higher costs and the limited availability of the test were considered as its disadvantages[55]. However, the diagnostic accuracy of HbA1c at a given threshold was found to be poor in many studies[56-58], as well as in a recent global surveillance on the prevalence and diagnosis of diabetes[59], which is at least in part a consequence of numerous biological confounders[38,60]. A comprehensive list of biological, (patho) physiological and pharmacological factors that may influence the synthesis, measurement and/or interpretation of HbA1c is presented in Table 2.

GLYCATED PROTEINS

Fructosamine (1-amino-1-deoxy fructose) is a common term for all glycated plasma proteins. It is a ketoamine that is formed by the irreversible nonenzymatic binding of glucose to plasma proteins in a process called glycation. Glycation is a nonenzymatic process where a labile Schiff base (aldimine) is formed at an early stage and is subsequently rearranged to a stabile Amadori product (ketoamine) due to the covalent binding of glucose to the lysine, arginine and cysteine amino-group residues within protein molecules[61].

Glycated albumin (GA) is formed in a similar reaction as fructosamine and is specific to albumin molecule[62]. In conditions that are associated with high glucose levels, plasma proteins are exposed to greater glycation, which leads to increased fructosamine and GA formation. Fructosamine and GA reflect the average blood glucose concentration during the lifetime of either total plasma proteins or albumin, both of which are within the range of two to three weeks[63].

Despite the fact that albumin is a major constituent of plasma proteins, fructosamine and GA may not be considered as totally equal measures of glycemia due to their differences in analytical procedures and their currently established clinical performance. Fructosamine was identified long ago, but the lack of analytical standardization and problems with the assay's specificity and susceptibility to interference by hyperlipidemia limited its use in diabetes management. Additionally, there was insufficient evidence to correlate fructosamine and GA with long-term outcomes in patients with diabetes[64].

However, over the years, the development and improvement of methods for determining fructosamine and GA have paved the way for many studies that focused on their analytical and clinical significance. Affinity chromatography[65], ion-exchange chromatography[66] and high-performance liquid affinity chromatography[67] were all developed as methods for the direct measurement of GA along with liquid chromatography-tandem mass spectrometry (LC-MS/MS) as a "gold standard"[68]. However, these methods are complicated and expensive and require dedicated equipment and expertise, and this has limited their routine use. Consequently, simpler and more affordable colorimetric and enzymatic methods, applicable on various automated analytical platforms, were developed for use in clinical laboratories[69]. Enzymatic methods showed a better analytical performance and were free of colorimetric interferences (*e.g.*, bilirubin)[70-72]. Various commercial kits are available for GA measurement depending on the type of enzyme that was used in the reaction and the units used to express the results (μmol/L, mmol/L or % GA fraction).

Currently, the method of choice for fructosamine determination is the second generation of the nitroblue tetrazolium colorimetric procedure, in which there is a separation of glycated from nonglycated proteins based on their differences in chemical reactivity [73]. The assay itself is inexpensive, rapid, simple, highly specific and free of interferences from uric acid or polylysine. Nevertheless, despite many improvements, this method is still sensitive to rapid changes in ambient temperature and interferences from extremely high levels of some compounds with reducing properties, such as bilirubin and vitamin C[64]. Still unresolved is the issue of whether the resulting fructosamine measurements should be corrected for either total protein or albumin concentrations. The results are relatively ambiguous[74], but it was recently reported that correcting the fructosamine measurement for

Table 2 Biological, (patho)physiological and pharmacological factors influencing hemoglobin A1c

Factor influencing HbA1c synthesis/measurement/interpretation
Age, ethnicity
Genetic factors (e.g. Glucose-6-phosphate dehydrogenase variants)
Pregnancy
Red blood cell lifespan
Haemolytic anaemia
Iron deficiency anaemia
Haemoglobin variants
Accute haemorrhage
Splenomegaly
Splenectomy
Transfusion
Chronic liver disease
End-stage renal disease
Rheumatoid arthritis
Vitamin C
Drugs (aspirin, erytropoietin, dapsone, antiretroviral agents)
Endogenous interferents (high levels of bilirubin/triglycerides)

HbA1c: Hemoglobin A1c.

proteins may improve its correlation with HbA1c and its overall performance in detecting diabetes[75].

Given the faster protein metabolic turnover, fructosamine and GA values reflect shorter-term glycemia levels rather than HbA1c. Additionally, fructosamine and GA are not influenced by anemia or hemoglobinopathies such as HbA1c is, and they can therefore be used in conditions where HbA1c is not reliable due to analytical or biological interferences[62]. In conditions such as pregnancy[76] and treatment modifications[77] fructosamine and GA can detect changes in average blood glucose earlier than HbA1c and thus provide more timely information about the achievement of glycemic control[62,78,79].

Both fructosamine and GA are the markers of choice when glycemic control needs to be assessed in patients with severe chronic kidney disease (CKD) (stages 4 and 5)[80]. Additionally, in stage 5 CKD patients on hemodialysis, GA can be used as a predictor of overall survival and cardiovascular mortality [81]. Due to the reduced production and lifespan of red blood cells and to erythropoietin treatment in CKD patients, HbA1c cannot be used as reliable marker, as it can significantly underestimate the true glycemic status in these patients[82].

The distribution of GA in healthy subjects has been described in diverse populations[83,84]. The Large Atherosclerosis Risk in Communities (ARIC) study was conducted in a cohort of almost 12000 participants and proved a strong association of fructosamine and GA with the incidence of diabetes and microvascular complications (prevalent retinopathy and risk of CKD)[85]. Together with fructosamine, GA was reported to be strongly associated with HbA1c and fasting glucose[86]. Furthermore, a recent study by Bellia *et al*[87] evaluated the potential clinical usefulness of GA for the diagnosis of diabetes in an asymptomatic Caucasian population (specifically in Europe) with an elevated risk of developing diabetes. At the GA cut-off of 13.5%, a high sensitivity (88.9%; 95%CI: 65.3-98.6) and a good specificity (60.4%; 95%CI: 54.8-65.9), was demonstrated for its possible screening use in similar subjects[87].

It is important to note that fructosamine and GA measurements are not reliable in some physiological and pathological conditions. Every clinical condition that can affect protein and albumin metabolism (nephrotic syndrome, hyperthyroidism, glucocorticoid therapy, liver cirrhosis, *etc*.) may affect these results, where they would also require careful interpretation[14,62]. Additionally, similar to HbA1c, fructosamine and GA are determined by genetic variants that are associated with both glycemic and nonglycemic components, both of which should be considered when putting the results in a clinical context[84].

1,5-ANHYDROGLUCITOL

1,5-Anhydroglucitol (1,5-AG) is a monosaccharide that is structurally identical to D-glucose with the absence of the C-1 hydroxyl group. It is derived mainly through food intake and also absorbed by the intestine at a rate of approximately 4.4 mg/d. The main source of 1,5-AG is soy beans, but small amounts can be found in rice, pasta, fish, fruits, vegetables, tea, milk and cheese. The metabolic role of 1,5-AG is still quite unknown. It circulates in body in its free form and can be found in all organs and tissues (1,5-AG pool) with the total amount several times higher than that in plasma[88]. A negligible amount is presumed to be synthesized *de novo*[89]. 1,5-AG intake is regulated by its urinary excretion, and 99.9% of 1,5-AG is reabsorbed by the kidneys by the specific sodium glucose active cotransporter (SGLT4)[88,90]. Reabsorption is competitively inhibited by glucose. When the plasma glucose level exceeds the renal threshold for glucosuria (approximately 10 mmol/L), 1,5-AG is excreted in the urine, which results in a rapid reduction of its serum levels[91]. Thus, low values of 1,5-AG reflect both high circulating glucose levels and glucose fluctuation, or so-called hyperglycemic excursion[92]. This biomarker may be useful to differentiate between diabetic patients with well-controlled HbA1c but with extensive glucose fluctuations[93]. After normoglycemia is restored, the 1,5-AG concentration returns to its normal value at a rate of 0.3 μg/ml per day, and it can take up to 5 wk for this value to increase up to its normal level[94]. Due to its half-life of approximately 1 to 2 wk, 1,5-AG can be used as a potential marker for short-term glycemia[95]. Additionally, there is evidence that 1,5-AG reflects the 2-h postprandial glucose (PPG) values of the 2 preceding weeks in moderately controlled patients and is more sensitive and specific than HbA1c[96]. PPG values are especially important for clinical decision-making concerning changes in the diet or in changes of the pharmacologic treatment of diabetes and overall glycemic control[97].

1,5-AG can be measured in serum, EDTA-plasma and urine samples. There are two commercially available enzymatic kits for its blood measurement: the Glyco-MarkTM (GlycoMark, Inc) kit that is used in United States and the Determiner-L (Kyowa Medex, Tokyo) kit that is used in Japan. Both of these methods can be applied to automated chemistry analyzers. Recent data has shown a good between-method comparability despite slightly different results that were obtained in the same samples[98]. Another method for the determination of 1,5-AG is chromatography, specifically gas chromatography-mass spectrometry (GC/MS) and high-performance liquid chromatography (HPLC). These methods are sensitive and precise but require sample preparation and are time-consuming and cumbersome[99]. Urine, a sample with lower 1,5-AG levels, requires a more sensitive method such as liquid chromatography/mass spectrometry (LC/MS) or HPLC[100].

Regarding its association with diabetes and microvascular complications, the ARIC study provided evidence that 1,5-AG levels were associated with prevalent retinopathy and incident CKD, particularly in patients who were diagnosed with diabetes. Despite the low association in nondiabetic subjects, there was a good risk prediction of incident diabetes in both groups[86,101].

The results obtained from patients with certain conditions such as kidney disease or pregnancy must be carefully interpreted due to the changes in renal function during these conditions which influences the threshold for glucose excretion. Nevertheless, 1,5-AG can be reliable in subjects with mild to moderate renal insufficiency as a marker for glycemic control[102]. Furthermore, 1,5-AG can be helpful in cases when frequent adjustments in therapy are required and glycemic control has to be maintained [94].

Given the limitations of HbA1c and the recently collected evidence on the clinical utility of nontraditional markers of glycemia, their implementation in clinical practice is expected. The recently published reference intervals provide the most valuable tool in facilitating the translation of these biomarkers into routine clinical practice. In a healthy reference population of almost 1800 individuals, the reference ranges for fructosamine, GA and 1,5-AG were reported as 194.8-258.0 μmol/L, 10.7%-15.1% and 8.4-28.7 μg/mL, respectively[103].

DIRECT MEASURES OF GLYCEMIA

Fasting and postprandial plasma glucose (FPG and PPG, respectively) are obvious measures of glycemia, providing "snapshot" glucose values for primary use in targeting treatment goals, which are currently set at ranges of 4.4-7.2 mmol/L for FPG and < 10.0 mmol/L for PPG[1]. The contributions of these measures to HbA1c have been evaluated[104], and significant association of PPG with cardiovascular risks was evidenced[105]. Daily plasma glucose values are readily available to patients who perform SMBG as a part of their regular diabetes care but reviewing and interpreting the cumulative SMBG results may propose a significant challenge for healthcare professionals[106].

Advances in both the analytical accuracy and software supporting SMBG, the development of continuous glucose monitoring sensors and, most recently, flash-glucose sensing technology, have prompted the development and validation of new, metrics-derived surrogate markers of glycemia which have improved our understanding of the complex glucose dynamics and have provided new tools for patients and healthcare providers in achieving optimal control of diabetes and reducing the

frequency of acute and chronic complications of diabetes[13,14].

Among the integrated SMBG-derived metrics, the glycemic risk assessment diabetes equation (GRADE) and average daily risk range (ADDR) were found to best correspond with the degrees of risk of hypo- and hyperglycemia that were associated with the glucose profile[107], and they showed positive correlations with HbA1c and negative correlations with c-peptide levels[108].

As opposed to the SMBG-derived profiles, which are based on a limited number of static plasma glucose measurements throughout the day, CGMS enable a continuous insight into daily glycemia, thus enabling an individualized approach and offering a powerful tool for patients in achieving their glycemic targets and mitigating glycemic excursion. CGMS has yielded previously unreachable measures of glycemia such as average glucose exposure, time in range, hypo- and hyperglycemia and glycemic variability (glucose excursions). The glycemic variability was considered to be a significant risk factor for developing complications that was not reflected by HbA1c levels[13]. The advantages of using SMBG to improve patient outcomes have been amply evidenced in studies targeting various vulnerable populations of patients with diabetes such as children[109], pregnant women[110], the elderly[111], and the patients suffering from diabetic kidney disease[112] and from hypoglycemic episodes[113]. However, the high costs, insurance-related limitations and patient- and healthcare provider-related attitudes still hinder a wider utilization of CGMS. The recently published International Consensus on Use of Continuous Glucose Monitoring is an encouraging step forward and is aimed at providing technical and clinical recommendations on the use of CGMS in conjunction with HbA1c, and it provides a comprehensive insight into the state-of-the-art evidence supporting CGMS-derived metrics to improve patient care and clinical outcomes[114].

CONCLUSION

Hyperglycemia is a key biochemical feature of diabetes that should be rigorously controlled and maintained in a range as close to normal as possible to mitigate the risk of diabetic complications. Both the level of and exposure to hyperglycemia, as well as glycemic variability, contribute to the pathogenesis of diabetic complications, with different patterns of disease pathogenesis in patients with type 1 or type 2 diabetes. Despite its analytical and biological limitations, HbA1c remains the key biomarker of long-term glycemic control. However, it has become apparent in recent years that other glycated proteins, 1,5-AG, and integrated measures from direct glucose testing by SMBG/CGMS may provide valuable data complementary to HbA1c, particularly in circumstances when the HbA1c results may be unreliable or insufficient to assess the risk of adverse outcomes (Table 3). Long-term associations of these alternative biomarkers of glycemia with the risk of diabetic complications need to be investigated to provide clinically relevant cut-off values and validate their utility in diverse populations of patients with diabetes.

Table 3 Characteristics of glycaemic biomarkers

Markers of hyperglycemia	Assessment period	Advantages	Limitations
HbA1c	2-3 mo	Fasting not necessary; low interindividual variabiliy screening tool for diabetes; association with diabetes complications; standardization	Surrogate biomarker analytical interferences; biological confounders; costs
Fructosamine Glycated albumin	2-3 wk	Fasting not necessary; inexpensive and easily automated; good correlation with HbA1c; association with diabetes complication; marker of choice in severe chronic kidney disease	Surrogate biomarker; higher interindividual variability; unreliable in conditions with altered protein and albumin metabolism (nephrotic disease, severe liver disease), thyroid disfunction; not standardized
1,5-anhydroglucitol	1-2 wk	Fasting not necessary; glycemic excursion detection; good correlation with HbA1c; association with diabetes complications	Surrogate biomarker; unreliable in the setting of chronic kidney disease (stage 4 and 5), dialysis, pregnancy or other conditions with changes in renal threshold (sglt inhibitors); not suitable for diabetes diagnosis
Fasting glucose	8-10 h	Current glycemic status; immediate availability for daily diabetes management SMBG/CGMS	Affected by acute illness and stress; SMBG and CGMS-accuracy
Postprandial glucose	2-4 h		
Indices of glycaemic variabily	24-72 h	Short-term glucose dynamics; improves glycaemic control beyond HbA1c and patient's satisfaction/outcomes	CGMS mandatory; costs education; standardization

HbA1c: Hemoglobin A1c; SMBG: Self-monitoring of blood glucose; CGMS: Continuous glucose monitoring system.

FOOTNOTES

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MINIREVIEWS

Effects of diabetic ketoacidosis in the respiratory system

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Abstract

Diabetes affects approximately 30 million persons in the United States. Diabetes ketoacidosis is one of the most serious and acute complications of diabetes. At the time of presentation and during treatment of diabetic ketoacidosis (DKA), several metabolic and electrolyte derangements can ultimately result in respiratory compromise. Most commonly, hypokalemia, hypomagnesemia and hypophosphatemia can eventually lead to respiratory muscles failure. Furthermore, tachypnea, hyperpnea and more severely, Kussmaul breathing pattern can develop. Also, hydrostatic and non-hydrostatic pulmonary edema can occur secondary to volume shifts into the extracellular space and secondary to increased permeability of the pulmonary capillaries. The presence of respiratory failure in patients with DKA is associated with higher morbidity and mortality. Being familiar with the causes of respiratory compromise in DKA, and how to treat them, may represent better outcomes for patients with DKA.

Key Words: Diabetes ketoacidosis; Respiratory physiology; Mechanical ventilation; metabolic acidosis; Hyperventilation; Kussmaul breathing; Respiratory failure

Core tip: Several electrolyte and metabolic derangements associated with diabetic ketoacidosis (DKA) and its treatment can affect the respiratory system. Since respiratory failure in DKA is associated with increased morbidity and mortality, the recognition and treatment of those derangements have the potential to improve outcomes in DKA.

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INTRODUCTION

Diabetes ketoacidosis (DKA) is one of the most serious and acute complications of diabetes. It is characterized by moderate hyperglycemia (blood glucose usually between 250 mg/dL and 800 mg/dL at presentation), metabolic acidosis, and presence of serum ketones with an elevated anion gap[1]. It represents an extreme in the spectrum of hyperglycemia and presentation of complicated diabetes.

Diabetes affects approximately 30 million persons in the United States[2]. Since 2009, there has been an increase of around 6% of hospitalizations due to DKA (from 19.5 to 30.2 per 1000 persons). However, the in-hospital mortality has declined at an annual average rate of 6.8% (from 1.1% to 0.4%)[2].

The presence of DKA is accompanied by several electrolytes, metabolic and acid-base derangements that affect the respiratory system. Depletion of ions, such as potassium and phosphate, affect the respiratory muscles leading to acute respiratory failure[3]. Reduction in colloid osmotic pressure increases lung water content, leading to noncardiogenic pulmonary edema and decrease in lung compliance[4,5]. As a compensatory mechanism, the presence of metabolic acidosis will cause hyperventilation[6].

Respiratory failure in DKA has been associated with increased morbidity and mortality[3,7]. In this review, we analyze the common electrolytes, metabolic and acid-base abnormalities seen in DKA, their association with respiratory failure and its management.

ELECTROLYTE ABNORMALITIES

Potassium, magnesium and phosphorous are intracellular ions which serum concentrations decrease as a direct consequence of hyperglycemia and ketoacidosis (potassium), or as a consequence of the correction of acidosis with insulin (magnesium and phosphorous). A major goal in the treatment of DKA is to closely monitor these ions concentrations as DKA is corrected. Also, replace them on a timely fashion in order to prevent them from reaching critically low values. The clinical significance of their deficit is discussed below.

Potassium

Patients being admitted for DKA usually have a total body potassium deficit that averages 300 to 600 mEq[8]. Osmotic diuresis is provoked by the hyperglycemia resulting from lack of insulin. In an attempt to maintain osmolality, the kidneys will retain sodium ions at the expense of potassium ions[9]. Furthermore, when acidosis is present, hydrogen ions from the bicarbonate nucleus will be reabsorbed at the expenditure of potassium[10].

The gastrointestinal tract is also responsible for potassium loss in DKA. The body will try to maintain osmotic pressure at the cost of tissue and serum electrolytes. An acute hyperkalemia will happen when potassium shifts into the extracellular fluid (ECF), causing gastric cells to preserve hydrogen ions concentration. Consequently, nausea, vomit and diarrhea will occur, promoting even more potassium loss[11,12]. However, due to a shift of potassium from intracellular fluid into ECF caused by hyper osmolality and insulin deficiency, only 5% of patients with DKA will present with hypokalemia[8,13].

When potassium levels fall below 2.5 mg/dL, severe ascending muscular weakness can occur[14]. The muscular weakness can affect the respiratory muscles causing acute respiratory failure[15], and requirement of mechanical ventilation[16]. Aggressive potassium replacement should start once serum potassium concentration reaches a value of 3.3 mEq/L[13].

Magnesium

At presentation of DKA, the levels of serum magnesium are usually normal. Excessive amounts of magnesium are excreted during acidosis, secondary to insulin deficiency[17]. As the acidosis gets corrected, magnesium levels fall, reaching their nadir within the first 25 h of acidosis correction[18,19].

Hypomagnesemia, defined as having a serum magnesium concentration below 1.6 mg/dL (0.66 mmol/L), usually doesn't lead to clinically significant symptoms until serum levels fall below 1.2 mg/dL (0.5 mmol/L)[20].

Magnesium regulates intracellular calcium levels, influencing smooth muscle tone[21]. Because of its role in regulating smooth muscle tone, magnesium deficiency has been associated with systemic hypertension, neuromuscular excitability, bronchoconstriction, coronary vasospasm and seizures[22].

Muscular weakness and tetany associated with hypomagnesemia can affect the respiratory muscles, impairing ventilation in patients who are spontaneously breathing and delaying extubation of mechanically ventilated patients[22,23]. Empirical magnesium replacement has been associated with improvement of respiratory muscle power in patients with DKA[23].

When treating patients with DKA, clinicians should aim to keep magnesium levels at normal range, since hypomagnesemia is associated with weakness of the respiratory muscles.

Phosphorous

Acidosis causes potassium shifts into the ECF and hyperglycemia causes phosphaturia by osmotic diuresis, which will ultimately lead to hypophosphatemia. However, DKA patients will present with normal phosphorous concentration due to the shift into the ECF associated with ECF volume concentration[24]. The true state of phosphate equilibrium is revealed with volume expansion[24,25].

Severe hypophosphatemia (< 1 mg/dL) is associated to the depletion of high-energy phosphate compounds in muscles, causing muscular weakness and rhabdomyolysis[3,26].

Acute muscular weakness caused by hypophosphatemia in DKA has been associated with hypercapnic respiratory failure and prolonged mechanical ventilation in critically ill patients[26-28].

Routine replacement of phosphorous in patients who presented with DKA is not beneficial and has been associated with worsening hypomagnesemia and causing hypocalcemia[29,30]. However, if serum phosphate concentration falls below 1 mg/dL, or if hypophosphatemia is associated with cardiac dysfunction or respiratory depression, it should be replaced[28,31,32].

HYPERVENTILATION

The presence of metabolic acidosis will normally generate a respiratory response. The reduction of serum bicarbonate and pH will result in hyperventilation and reduction in carbon dioxide (CO_2), partially preventing further fall in pH and bicarbonate concentration. Respiratory compensation for metabolic acidosis will cause the arterial CO_2 to decrease by 1.2 mmHg for each 1 meq/L fall in the serum bicarbonate[33].

The respiratory response usually begins within 30 min of metabolic acidosis onset, and is generally complete within 12-24 h However, a lag in respiratory compensation can occur when respiratory acidosis develops quickly; more than 4 meq/L of bicarbonate decrease in less than 6-12 h[34,35].

There is a limit to the lungs' ability to compensate for metabolic acidosis. Even with serum bicarbonate concentrations below 6 meq/L, CO_2 levels cannot fall lower than 8-12 mmHg[34]. Furthermore, the duration of the respiratory compensation is limited by respiratory muscle fatigue[33,36].

Initially, patients will develop tachypnea, which is increased respiratory rate, leading to decrease in CO_2 concentration. With progression of acidosis, respiratory pattern evolves to hyperpnea, which is increased tidal volume, and ultimately, patients will develop a deep, fast and agonal pattern of breathing, named Kussmaul's respiration (Figure 1A-D)[34,37].

Once patients with DKA develop Kussmaul's respiration, they are reaching the point of respiratory muscles fatigue, and mechanical ventilation should be considered[34,38-40]. Furthermore, patients in DKA are severely "air hungry" prior to intubation, and are at higher risk to develop acute respiratory distress syndrome (ARDS)[3,41] due to hyperpnea. Mechanical ventilation in these patients is particularly delicate, since a lung protective strategy, with low tidal volumes and controlled transpulmonary pressures, should be maintained, while attempting to increase minute-ventilation until metabolic acidosis is completely corrected[42,43].

PULMONARY EDEMA

There are two types of pulmonary edema that have been described in patients with DKA: One associated with elevated pulmonary venous pressure and another associated with increased pulmonary capillary permeability. The diagnosis is made based on clinical findings of dyspnea, an A-a gradient on arterial blood gas and chest image showing bilateral pulmonary infiltrates.

Pulmonary edema due to elevated pulmonary venous pressure

Also known as hydrostatic pulmonary edema, it is usually existent at presentation of DKA, is corrected during the treatment of DKA and is more common in patients with concomitant renal failure[44-47]. The occurrence of circulatory overload and pulmonary edema with elevated pulmonary venous pressure is a result of the acute shift of an abundant volume of fluid into the extracellular compartment. This fluid shift happens as a consequence of solute accumulation in the extracellular compartment secondary to hyperglycemia[44]. Therefore, correction of hyperglycemia shifts fluid back into cells, also correcting hydrostatic pulmonary edema. However, some patients might require hemodialysis and mechanical

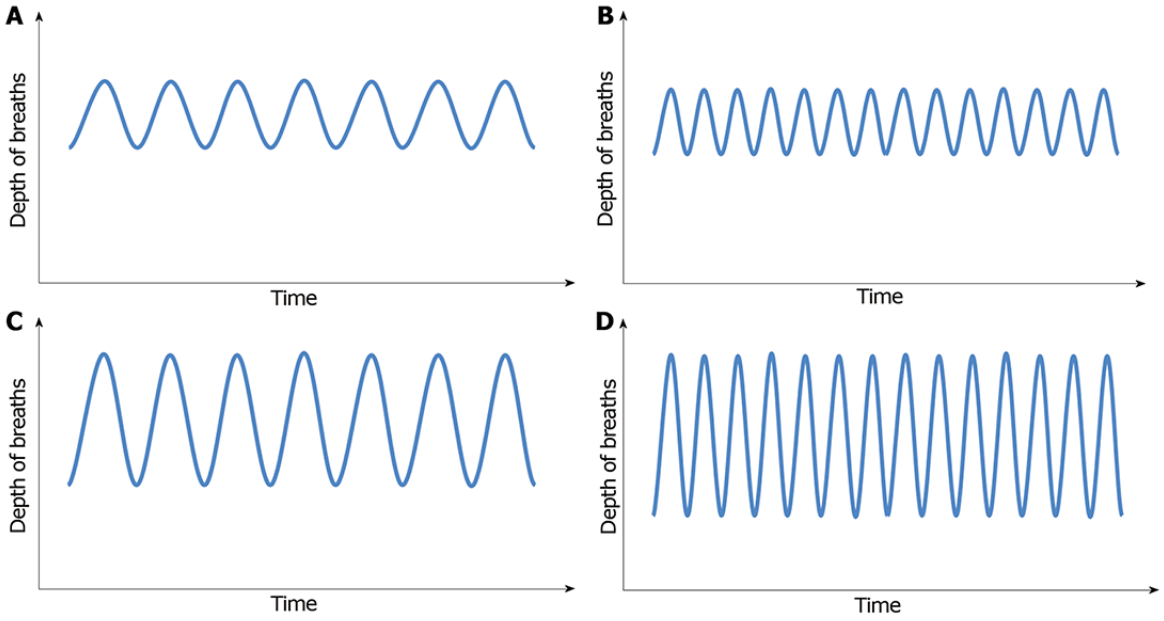

Figure 1 Depth of breaths. A: Normal (eupnea); B: Tachypnea - increased respiratory rate; C: Hyperpnea - normal rate, deep inspirations; D: Kussmaul's - tachypnea and hyperpnea.

ventilation.

The degree of fluid shift and, consequently, the likelihood of developing hydrostatic pulmonary edema during a DKA episode are determined by the severity of hyperglycemia and by the volume status prior to the development of DKA[47]. The amount of fluid transferred from the cells into the extracellular space is directly proportional to the changes in serum glucose concentration[48]. The patients' volume status at the time of hyperglycemia onset is also a determinant of the volume that will shift into the extracellular space. Patients with baseline low extremity edema and/or anasarca have been shown to shift larger amounts of fluid and have a higher incidence of pulmonary edema, than those patients who are euvolemic when becoming hyperglycemic[49].

Even though hydrostatic pulmonary edema has been described more commonly in patients with advanced renal disease, there are several cases reported in patients with DKA who developed pulmonary edema without having renal dysfunction. Several cases have been reported of takotsubo cardiomyopathy happening in the setting of DKA and causing pulmonary edema[50,51]. There are also reports of myocardial dysfunction secondary to severe acidosis and electrolyte abnormalities[52].

Pulmonary edema due to increased pulmonary capillary permeability

Also known as non-hydrostatic pulmonary edema, this type of pulmonary edema is caused by changes at the histological level of the alveolar epithelium. In diabetic patients, there is thickening of the alveolar epithelium and pulmonary capillary basal membrane, corroborating the presence of pulmonary microangiopathy[53,54].

ARDS can develop during the course of DKA or during its treatment[3], and it is more frequent and severe than hydrostatic pulmonary edema[54,55]. The mechanism of ARDS in DKA is not completely understood. The most accepted explanation is activation of lymphocytes and release of cytokines, especially interleukin-1, which serum levels are much higher during treatment of DKA[56-58].

The treatment of non-hydrostatic pulmonary edema in DKA is supportive. Focus should be on treating DKA and its exacerbating factor, early intubation and protective lung ventilation.

CONCLUSION

In DKA, respiratory failure is caused by several electrolytes, metabolic and cardiac and lung end-organ damage. Developing respiratory failure during DKA onset or treatment is associated with high mortality. Early recognition and treatment of the risk factors for the development of respiratory failure have the potential to decrease morbi-mortality of patients with DKA.

FOOTNOTES

Author contributions: All authors have contributed to the conception, design and review of the manuscript; Gallo de Moraes A has been also involved in literature review and drafting of the manuscript.

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MINIREVIEWS

Exploratory metabolomics of metabolic syndrome: A status report

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Abstract

Metabolic syndrome (MetS) is as a cluster of cardio-metabolic factors that greatly increase the risk of chronic diseases such as type II diabetes mellitus and atherosclerotic cardiovascular disease. In the United States, obesity, physical inactivity, aging, and genetics (to a minor extent) have arisen as risk factors for developing MetS. Although 35% of American adults suffer from MetS, its pathogenesis largely remains unknown. Worse, there is a lack of screening and optimum therapy for this disease. Researchers have consequently turned towards metabolomics to identify biomarkers to better understand MetS. The purpose of this review is to characterize various metabolites and their potential connections to MetS. Numerous studies have also characterized MetS as a disease of increased inflammation, and therefore this review also explores how metabolites play a role in various inflammatory pathways. Our review explores a broad range of metabolites including biogenic amines, branched chain amino acids, aromatic amines, phosphatidylcholines, as well as a variety of other molecules. We will explore their biochemical pathways and their potential role in serving as biomarkers.

Key Words: Metabolic syndrome; Syndrome X, Metabolomics; Amino acids; Carnitine; Inflammation; Biomarkers; Diabetes

Core tip: Metabolic syndrome (MetS) is a global epidemic that predisposes to type II diabetes mellitus, atherosclerotic cardiovascular disease and increased mortality. Whilst both insulin resistance and inflammation are advanced as pathogenic mechanisms, much work is needed to identify reliable biomarkers for this common cardio-metabolic disorder. In this mini-review, we provide a status report on the evolving field of metabolomics in MetS and it appears to offer some promising biomarkers such as branched chain amino acids, lysine, carnitine, phosphatidylcholine (PC34:1) and PC34:2. However there is an urgent need to direct greater effort to the metabolome of MetS to unravel its pathopysiology and usher in much needed therapeutics.

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INTRODUCTION

Metabolic syndrome (MetS) describes a cluster of cardiometabolic risk factors that predisposes individuals to type II diabetes mellitus (T2DM) and atherosclerotic cardiovascular disease (ASCVD). MetS is defined by the Adult Treatment Panel (ATP) III criteria as having three of the five following features: increased triglycerides, reduced high-density lipoprotein (HDL)-cholesterol, plasma glucose of 100-125 mg/dL, increased waist circumference (WC), and hypertension. MetS affects approximately 35% of American adults and is increasing by drastic measures globally. Currently there is no optimal treatment for MetS, and consequently, there is a severe need to find new ways of approaching MetS in the hopes of finding better diagnostic and treatment modalities[1]. Recently, studies assessing metabolomics have uncovered some insights into the pathology behind T2DM, CVD, and obesity[2]. In previous studies, our lab demonstrated that MetS is a subclinical pro-inflammatory condition[3]. We also have shown that this inflammation is present even in nascent MetS, which describes patients who meet diagnostic criteria for MetS without having confounding factors such as smoking, ASCVD, or T2DM. These findings suggest a causal role in MetS before the onset of serious sequalae. Various studies have suggested that metabolic diseases changes the levels of many amines, amino acids, and lipids[4-6]. However, few studies have assessed the role of metabolites in MetS, and the biochemical alterations leading to metabolite changes are poorly understood. Therefore, in this review, we assess various metabolites and how they may be playing a role in the development, diagnosis, or management of MetS. We also evaluate if these metabolites may be related to inflammatory pathways, which could help elucidate their potential pathological role in MetS.

BIOGENIC AMINES: TMAO, CHOLINE, AND L-CARNITINE

Several recent studies have suggested that these biogenic amines have a role in the development of ASCVD and T2DM. It's been hypothesized that upon consumption of foods high in L-carnitine (LC) and choline, such as red meats, these amines are digested by gut microbes to produce trimethylamine. Ultimately this compound is converted to trimethylamine N-oxide (TMAO) in the liver[7]. Some studies link TMAO with overall mortality in T2DM patients, predicting that higher circulating levels of TMAO are associated with a 2.1 to 2.7-fold increase in mortality, also seen after researchers adjusted for body mass index (BMI)[8]. Others suggest that TMAO is linked with traits of obesity in mice receiving a high-fat diet, which suggests that the TMAO pathway is linked to obesity. For instance, Schugar *et al*[9] illustrates a positive association between circulating levels of TMAO in mice fed a high fat and high sucrose diet, and body weight, fat mass, mesenteric adiposity, and subcutaneous adiposity. Moreover, a positive association between flavin-containing monooxygenase 3 gene, which encodes a TMAO-producing enzyme, and BMI and waist-to-hip ratio is established in these mice. Interestingly, this association in humans is not provided. Despite new insights into TMAO and its role in metabolic disease, the role of TMAO and its metabolites in the pathogenesis of the disease still remains elusive.

Choline

Choline is a quaternary ammonium compound commonly found in dairy and fish products. It is involved in the synthesis of phospholipids, lipoproteins, and neurotransmitters. Studies have found that choline consumption in healthy adults is related to inflammatory pathways, and subjects who consumed > 310 mg/d had 22% lower C-reactive protein (CRP), 26% lower interleukin (IL)-6, and 6% lower tumor necrosis factor alpha (TNFα) levels[10]. These results support a potential association

between choline and the inflammatory process in healthy adults, but the exact role of choline in inflammatory pathways is unclear since some inflammatory markers were higher, while others were lower in this study. Other studies have shown that it has a role in CVD and is associated with key components of MetS including increased triglycerides, BMI, glucose, and WC. Furthermore, choline may also have some independent effects in metabolic disease. It's also been shown that betaine, formed by oxidized choline in the liver and kidney, is inversely associated with similar factors, suggesting a disruption of this pathway under conditions of mitochondrial dysfunction in MetS. This correlation between blood lipids and choline is in agreement with other studies showing that phosphatidylcholine (PC) supplementation in humans increases triglycerides without affecting cholesterol concentrations[11]. Interestingly, studies show that when choline-deficient mice are fed a high-fat diet they have reduced glucose intolerance, whereas choline-replete mice fed the same diet show increased weight, triglycerides, hyperinsulinemia, and glucose intolerance[12]. This study suggests that choline may have deleterious effects when coupled with fatty foods. Moreover, data from the Newfoundland CODING study illustrated a significant association between high human dietary consumption of choline and betaine and lowered insulin resistance. An inverse correlation was established between dietary choline and betaine intake and fasting glucose and insulin, homeostatic model assessment of insulin resistance (HOMA-IR), and HOMA-B serum levels (r = -0.08 to -0.27 for choline, and r = -0.06 to -0.16 for betaine, P < 0.05). Conversely, increased choline and betaine dietary intakes positively correlated to quantitative insulin-sensitivity check index (r = 0.16 to 0.25 for choline, and r = 0.11 to 0.16 for betaine, P < 0.01). These associations were found in both genders after controlling for parameters such as age, physical activity, and daily caloric intake[13]. Another study also demonstrated an association between high plasma concentrations of choline in human subjects and an adverse cardiometabolic risk-factor profile. More specifically, these high plasma choline concentrations were associated with low HDL-C levels, higher total homocysteine levels, higher BMI, and an greater odds of large-vessel cerebral vascular disease or history of cardiovascular disease[14]. Though this provides further insight on the systemic effects of choline, further studies are needed to evaluate how choline is involved in metabolic disease, particularly MetS.

L-Carnitine

LC is also a quaternary ammonium compound found in meat products. The role of LC in MetS is largely understudied, but research following LC in other metabolic diseases may be predictive of its role in MetS. Interestingly, the deleterious role of LC in metabolic disease remains controversial. One study found that LC attenuates MetS in diet-induced obese rats by modulation of tissue fatty acids including inhibition of stearoyl-CoA desaturase-1 activity[15]. Other studies suggest that LC supplementation at a dose of 1000 mg/d for 12 wk in humans with coronary artery disease resulted in reduced high sensitive CRP (hsCRP), IL-6, TNFα levels, and TNFα negatively correlated with LC levels (r = -0.29, P = 0.02) and antioxidant enzyme activities, superoxide dismutase (r = -0.24, -0.18, and -0.19; P = 0.03, < 0.05, and 0.05 for CRP, IL-6, and TNFα, respectively) and glutathione peroxidase (r = -0.33, -0.31, and -0.19; P < 0.01, < 0.01, and 0.06 for CRP, IL-6, and TNFα, respectively)[16]. However, some have speculated that LC supplementation benefits may be dose dependent[16,17]. While some studies report that LC supplementation reduced inflammatory factors, in the only paper published evaluating LC in a nascent form of MetS, we showed that LC had a 2.5-fold median increase (P < 0.01) and was positively correlated with soluble TNF receptor (sTNFR)-1(r = 0.51, P = 0.02) and leptin (r = 0.39, P = 0.02), and inversely to the important anti-inflammatory adipokine, adiponectin (r = -0.4, P = 0.02)[6].

Some studies also show LC may be involved in metabolic dysfunction. One study indicated that the carnitine palmitoyltransferase *1b166V* gene, coding for an enzyme involved in transferring long-chain fatty acids into the inner mitochondrial space, may have harmful effects in MetS such as increased fasting triglycerides, glucose, higher fatty liver index (FLI), and reduced insulin sensitivity[18]. One of the few studies evaluating carnitine levels in humans showed that serum carnitine levels were increased in MetS patients with bipolar disorder and schizophrenia compared to the same subset of patients without MetS[19]. Together these studies suggest that the role of LC in human metabolic disease may be potentially detrimental, possibly relating to inflammatory pathways. Because of the severe lack of data reporting LC in humans with MetS, future studies will be necessary to confirm the role of LC and its upstream and downstream products in MetS.

Recently we found that nascent MetS patients, without prior progression to CVD and T2DM, have higher levels of LC in urine samples. Since TMAO and choline were not significantly increased in nascent MetS patients, our studies suggest that LC may play a larger role in MetS than previously believed[6]. Furthermore, studies also show that lysine and methionine, two precursors of LC, are decreased in nascent MetS[5], therefore increased LC may be driven by lysine and methionine depletion. Several studies show that the addition of LC in the diet of mice increased TMAO levels leading to worsened aortic lesions[20], suggesting that LC may have a significant role in MetS and CVD. The precise role of LC in MetS remains largely unknown, and more research on this amine will be critical to evaluate if LC has a potential therapeutic or diagnostic role in MetS.

Trimethylamine N-oxide

Multiple studies report that TMAO and its precursors exacerbate glucose intolerance, inhibit hepatic insulin signaling, increase inflammation, and increase atheroma burden in mice and humans[7,21]. It's also been shown that TMAO increases in MetS[2]; however, these studies allowed for multiple confounding variables including smoking and diabetes. Furthermore, if these patients had renal impairment, this could have also skewed the results since TMAO increases as glomerular filtration rate decreases[22]. Complicating the role of TMAO, some researchers have also found that TMAO levels are increased one year after patients undergo laparoscopic Roux-en-Y gastric bypass for morbid obesity, a therapy that reduces cardiovascular disease. Thus, the role of the TMAO and its metabolites remains unclear, especially in MetS[23]. A recent study found that TMAO levels in adults stratified according to BMI had a positive association with adiposity and BMI, with highest TMAO levels in grade III obesity (BMI ≥ 40 kg/m^2). Furthermore, FLI was tightly associated with TMAO levels. Specific cut-offs for circulating levels of TMAO to predict the presence of non-alcoholic fatty liver disease (NAFLD)-FLI and MetS were ≥ 8.02 μM and ≥ 8.74 μM, respectively. This finding suggests that TMAO may be an early biomarker of adipose dysfunction and NAFLD-FLI in circumstances where overt MetS is not present, but specific cut-offs may be needed to identify subjects at high risk for NAFLD-FLI[24].

Studies have also explored the role of inflammation and these metabolites in nascent MetS. *In vivo* research has found that mice injected with TMAO showed an increase in inflammatory markers such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and enhanced uptake of cholesterol in peritoneal macrophages, a critical step in atherosclerosis. The researchers proposed that TMAO promotes recruitment of active leukocytes to endothelial cells[25]. Furthermore, numerous studies have suggested that inflammation is largely related to MetS. For example, in patient with MetS, there are increased levels of IL-1β, IL-6, monocyte-NFκβ, and several macrophage immune receptors[3]. One of the few studies assessing the role of TMAO and inflammation in MetS, found that TMAO significantly correlated with IL-6, endotoxin, and chemerin in patients with nascent MetS[6], which further suggests that TMAO may have a role in ASCVD and metabolic disease *via* inflammatory mechanisms.

AMINO ACIDS

Alanine, Glutamate, and Glutamine

Alanine is a non-essential amino acid that can be synthesized by pyruvate and branched chain amino acids (BCAAs). In mammalian tissues and liver, alanine is vital in the glucose-alanine cycle. Amino acids are broken down to form glutamate by transamination. Through the actions of alanine aminotransferase (ALT), glutamate can then transfer its amino acid group to pyruvate, forming α-ketoglutarate and alanine, respectively. Alanine can then travel to the liver from the bloodstream[26]. Numerous studies have shown that alterations to the alanine cycle, leading to increased levels of ALT may have implications in the development of T2DM and hyperglycemia. For instance, a study assessing if ALT is implicated in the development of MetS evaluated 1097 Caucasian men and women, and found that at follow-up, ALT was associated with fasting plasma glucose[27]. Another study from Western Australian Health Department data linkage system found a strong association between ALT and MetS, independent of insulin resistance[28]. There have been metabolomics studies that have found that alanine is linked to several traits associated with MetS including BMI, WC, triglycerides, hypertension, impaired glucose tolerance, and insulin resistance. The researchers proposed that glutamate likely stimulates glucagon release from pancreatic α cells and increases transamination of pyruvate to alanine, which strongly promotes gluconeogenesis in obesity[26]. Furthermore, a study showed that alanine levels are increased in obesity and correlate with visceral adiposity in a Japanese population[29]. Interestingly, BCAAs seem to boost the conversion of pyruvate to alanine *via* short chain fatty acid production by gut microbiota[30]. This may reflect an intricate role of various amino acids interconnected in metabolic dysfunction, and that liver metabolism likely plays a significant role in metabolic disease. Additionally, one study showed that serum ALT levels were significantly related to plasma CRP and lipid peroxides (LPO), regardless of the presence of underlying MetS, and that the presence of MetS and elevated ALT additively increased CRP and LPO. This study suggests that elevated serum ALT is a marker of active systemic inflammation and increased oxidative stress, independent of its relationship to MetS[31].

Studies assessing metabolites and their relationship to metabolic risk factors found that an increased glutamate-glutamine ratio was associated with insulin resistance in individuals with metabolic risk factors and that glutamine-cycling pathways may have a prominent role in the development of metabolic risk. An increased glutamine-glutamate ratio was associated with lower risk of diabetes and the administration of glutamine in mice led to increased glucose tolerance and lower blood pressures. Additionally, glutamine-fed mice had the lowest plasma glucose levels compared to glutamate-fed and control-fed mice[32]. An increased glutamine-glutamate ratio was also associated with decreased risk of future diabetes in a sample of 601 participants examined over 12 yr[33]. Furthermore, a 2018 study of 563 Chinese adults identified a low glutamine-glutamate ratio as an independent risk factor for

hyperglycemia[34]. Studies have also identified glutamine as reducing pro-inflammatory cytokines, including IL-6, in human intestinal mucosa. Researchers also propose that glutamine could be helpful in modulating inflammatory conditions with imbalanced cytokine production[35], which may prove valuable in treatment of MetS.

While the role of glutamine may be associated with metabolic wellness, glutamate may have an opposite effect. In a study of morbidly obese patients, those with pre-diabetes were found to have higher serum glutamate levels compared to non-diabetic controls. It was speculated that glutamate was elevated in morbidly obese patients due to an increased need for α-ketoglutarate in the tricarboxylic acid cycle (TCA) cycle to compensate for insulin resistance. This same study also found that morbidly obese non-pre-diabetic groups had increased levels of glutamate compared to non-obese and non-pre-diabetic groups, suggesting that obesity plays a role in glutamate metabolism[36]. There have been other studies suggesting that glutamate levels are associated with insulin resistance[37] In a study of women with polycystic ovary syndrome, a disorder that shares features with MetS, investigators found that glutamate levels were down-regulated compared to controls (0.8-fold peak integral change in PCOS/controls). They proposed that glutamate is being used as an alternative energy source in patients with metabolic disorders, leading to its depletion[38].

Additionally, new research suggests that glutamate levels identified by liquid chromatography/mass spectrometry in nascent MetS were significantly decreased compared to controls with median interquartile of 0.4 peak height ratio/creatinine peak height (range of 0.3-0.6) *vs* 2.3 (range of 1.1-3.6) respectively, and $P = 0.0001$. This study also found that gamma-aminobutyric acid (GABA) and D-pyroglutamic acid (PGA) were significantly increased in nascent MetS compared to controls with a 2.8-fold and 2.9-fold median increase and $P < 0.0001$ and $P = 0.004$ respectively. This study also identified a novel metabolite of gut microbiota tryptophan interaction, N-acetyl-D-tryptophan (NAT), was decreased by 90% in nascent MetS patients compared to controls ($P < 0.001$). The authors propose that this decrease in glutamate in nascent MetS could be due to its conversion to both GABA and PGA, which are both increased in this patient population. Researchers also found that GABA correlated significantly with WC, systolic blood pressure (SBP), chemerin, leptin, fetuin A, and endotoxin. PGA correlated positively with IL-6, leptin, fetuin A, and nitrotyrosine. NAT was inversely correlated with WC, SBP, BMI, triglycerides, hsCRP, Toll-like receptor 4 (TLR-4), IL-6 blood glucose, chemerin, and retinol binding protein 4. While GABA and PGA positively correlated with biomediators of inflammation and cardiometabolic risk factors of MetS, the NAT was inversely correlated. This study suggests that GABA and PGA may be contributing to the pro-inflammatory state on MetS while NAT could mitigate the inflammatory response[39]. This new finding could explain decreased glutamate levels in nascent metabolic disease and suggests possible therapeutic or diagnostic opportunity for early stages of MetS. Still, alanine and glutamine pathways offer a complex prospective in metabolic disease. Research in MetS is limited, and studies need to identify if this pathway can be targeted for diagnostic and treatment purposes.

Aspartate and asparagine

Asparagine is required for the development and function of the human brain. It is also known to play a critical role in the synthesis of ammonia. In the human body, oxaloacetate is converted to aspartate using transaminases. An amino group is transferred from glutamate to oxaloacetate making α-ketoglutarate and aspartate. Aspartate can accept an amine group from glutamine to form asparagine. Asparagine and aspartate are associated with numerous medical conditions, but they may also have a role in metabolic dysfunction. While some studies have shown that it is elevated in obesity[29], another study found that asparagine, but not aspartate, is inversely related to numerous metabolic traits including BMI, WC, insulin, HOMA-IR, triglycerides, systolic and diastolic blood pressure (DBP), while directly relating to HDL. Interestingly, in this same study, aspartate but not asparagine, was inversely related to glucose in human subjects[32]. This suggests that aspartate and asparagine levels may both be involved in metabolic disease; however, their exact roles may not necessarily overlap. Moreover, a study evaluating amino acids in a male Mediterranean population with MetS found asparagine to be inversely associated with MetS[2]. Therefore, the current evidence suggests that asparagine and aspartate may have protective roles in MetS or may be depleted as a consequence of disease progression.

Arginine

Arginine is another amino acid that may play a role in metabolic disease. It is most well-known for being the precursor for biosynthesis of nitric oxide and therefore aiding vasodilation, but it also has a role in cell division, wound healing, excretion of ammonia, immune function, and hormone release. The research related to arginine and metabolic disease is limited, though several studies report that it is dramatically increased in obese versus lean individuals[30]. However, there have been other studies suggesting it has a protective role since consumption of low sugar and protein biscuits that were enriched with L-arginine enhanced endothelial function, improved metabolism, insulin sensitivity, and insulin secretion in MetS subjects[40]. Supplementing 4.5 g per day of arginine for four weeks in overweight adults has also been shown to decrease postprandial vasospasm when baseline arginine levels were low[41]. To complicate the role of arginine further, another study in diabetic rats found that supplementation of L-arginine did not improve insulin resistance. but did improve lipid metabolism

where plasma triglyceride levels decreased after oral lipid administration ($P < 0.05$)[42]. Additionally a study assessing metabolite profiling to identify metabolic risks in humans found that arginine significantly correlated with triglyceride levels[32], which further suggests that arginine may play a role in dyslipidemia related to metabolic disease. Research has also shown that in a renal mass reduction (RMR) model of chronic renal failure, a 12-wk treatment of 1.25 g/L of L-arginine in the drinking water of rats improves kidney function by significantly reduced serum creatinine (2.3 to 1.3 mg/dL), serum urea (128.3 to 72.2 mg/dL), urine protein (104.8 to 49.2 ml/24 hr), as well as increased creatinine clearance (0.77 to 1.8 mL/min) ($P < 0.05$ for all factors). After 12 wk of L-arginine treatment in RMR mice, there was improved the systolic blood pressure (from 207.0 to 169.1 mm Hg, $P < 0.05$) and decreased pro-inflammatory cytokines including IL-1α (69.4 to 47.9 pg/mL), IL-1β (86.7 to 51.5 pg/mL), IL-6 (89.3 to 45.8 pg/mL), and TNFα (26.4 to 18.0 pg/mL) ($P < 0.05$ for all cytokines)[43]. Therefore, arginine may play a role in reducing pro inflammatory cytokines and kidney function, both of which may have implications in the development of MetS. Accordingly, there is a potential role of supplemental arginine for the purpose of reducing inflammation. Human studies have identified that L-arginine treatment can ameliorate endothelial dysfunction, inflammation, oxidative stress, adipokine release, and insulin sensitivity in T2DM and coronary artery disease patients[44,45]. Interestingly, a study assessing the relationship between plasma asymmetrical dimethyl L arginine (ADMA) and inflammation found that ADMA was directly correlated with inflammation and soluble adhesion markers in pre-diabetic subjects[46]. Ganz *et al*[47] also recently measured serum levels of arginine and ADMA in 105 persons with T2DM compared to controls and found that arginine was decreased in diabetics (64 ± 28 *vs* 75 ± 31 μmol/L) while ADMA was unchanged. Additionally, low arginine and high ADMA were associated with diabetic microvascular complications. There was no significant difference in BMI between the non-diabetic and T2DM groups (30.5 *vs* 30.6), suggesting that while arginine is increased in obesity, it has a tendency to decrease in an insulin resistant state. These studies suggest a complex interaction between arginine, ADMA and inflammation including mechanisms involved in endothelial dysfunction in a pre-diabetic and diabetic state and further research is needed to determine how arginine is related to metabolic disease, and to evaluate the circumstance under which its effects are beneficial or harmful.

Histidine

Histidine is a semi-essential amino acid with anti-inflammatory functions that it is decreased in T2DM [48], liver injury[49], CVD[50], and chronic kidney disease[51]. Studies have shown that histidine levels are higher in obese patients after bariatric surgery including sleeve gastrectomy, proximal Roux-en Y gastric bypass, and distal Roux-en Y gastric bypass[52]. Another randomized control study in obese women found that histidine supplementation of 4 g/d for 12 wk decreased inflammatory cytokines TNF-α (-28.3%), IL-6 (-29.3%) and improved oxidative stress by measurement of antioxidants superoxide dismutase (16.1%) and glutathione peroxidase (9.0%) in obese women with MetS. Histidine supplementation in this group also significantly decreased the HOMA-IR, BMI, WC, fat mass and non-esterified fatty acids by 18.9%, 2.9%, 3.7%, 6.0% and 18.1% after histidine supplementation, respectively [53]. Recent studies assessing the role of histidine on metabolic changes found that histidine supplementation may alter serum and urine metabolomic and amino acid profiles of obese women. Histidine supplementation of 4 g/d for 12 wk significantly decreased lipids and glucose, thus supporting a practical application of histidine in preventing and treating chronic metabolic diseases, such as MetS. Interestingly, this same study found that histidine supplementation resulted in increased choline, betaine, and TMAO levels, suggesting that these amines may have an interconnected role in metabolic dysfunction[48]. In studies profiling metabolites and how they are associated with metabolic risk factors, histidine was only associated with triglycerides and DBP, but not glucose, BMI, or insulin, which are key features of MetS[32]. This suggests that histidine may play a more complicated role in MetS and may be indirectly associated with the disease pathology.

Methionine/cysteine

Methionine is an essential sulfur-containing amino acid that contributes to both anabolic metabolism and the reduction of free radicals. One study recently observed that methionine levels were elevated in diabetic obese rats with leptin missense mutations[54], which is in line with the model of metabolic dysregulation proposed by Adams in an insulin resistant and obese state. In this model, he proposed that reduction in branched-chain α-keto acid dehydrogenase (BCKD) activity affected metabolism of α-ketobutyrate into propionyl-CoA. Since α-ketobutyrate is a downstream product of methionine, it was theorized that buildup of α-ketobutyrate led to upstream buildup of methionine. This excess of methionine is also thought to increase cysteine and cystine, consequentially leading to a buildup of tyrosine[55]. Interestingly, Reddy *et al*[5] observed that methionine is decreased in nascent MetS despite increases in tyrosine and isoleucine, which are also BCKD substrates. This suggests a fundamental difference in pathway directionality between nascent MetS patients and those with fulminant obesity and diabetes. Reddy *et al*[5] also observed that methionine inversely correlated with LC, which is formed by trimethylation of lysine *via* S-adenosylmethionine. Furthermore, LC was increased in nascent MetS[6], while medium chain acylcarnitine levels were not significantly increased in the same patient population[4]. These findings suggest that LC may be increased as a result of depleting methionine

without expected changes in acylcarnitines, and, as a result, this may indicate a dysregulation of fatty acid metabolism in nascent MetS. Additionally, adiponectin, a regulator of fatty acid oxidation was also decreased in this population. This is also supported by Bene *et al*[56] who observed that a group of 38 MetS patients had elevated total carnitine, comparable free carnitine, and increased C3 and C4 acylcarnitine levels compared to controls, while medium and long chain levels were reduced.

Another possible explanation for the decrease in methionine is the proinflammatory state associated with nascent MetS. Accumulation of visceral fat leads to increased inflammatory cytokines and subsequent generation of intracellular reactive oxygen species[57]. This oxidative stress leads to increased need for the reducing agent glutathione, which is formed by glutamate and cysteine. Since cysteine is a formed from methionine, increased oxidative stress could upregulate this pathway. However, Reddy *et al*[5] observed no correlations between methionine and the following markers of oxidative stress: Oxidized low-density lipoprotein (oxLDL), monocyte superoxide, and nitrotyrosine. Given their exclusion of patients with liver disease, this may indicate that anti-oxidative pathways become more prevalent in more established MetS populations, especially those with NAFLD/Non-alcoholic steatohepatitis. Mohorko *et al*[58] found that cysteine was significantly higher in a group with a single selection criterion for MetS compared to controls and even further increased with two components, but without any increases in methionine or homocysteine. Interestingly, cysteine did not correlate with CRP or TNF-α. In another study with 984 insulin resistant Hispanic children, researchers found no association between cysteine and IL-6, MCP-1, and CRP[59]. A possible explanation for the increases in cysteine could be due to increased dietary intake of methionine causing upregulation of its transsulfuration pathway, rather than a response to oxidative stress[60]. Current evidence suggests that while methionine and cysteine are indeed dysregulated in obesity, T2DM, and nascent MetS, the mechanisms of dysregulation may differ significantly with regards to both inflammatory and metabolic profiles.

Lysine

Lysine is an essential amino acid with basic properties that is synthesized *via* the diaminopimelate and α -aminoadipate pathways. In nascent MetS, Reddy *et al*[5] also observed a substantial decrease (92%) in the basic amino acid lysine, which inversely correlated with LC, like methionine. While HOMA-IR was elevated in their MetS population, this suggests insulin-induced BCKD inhibition leads to rerouting of lysine and methionine to fatty acid oxidation instead of a buildup in nascent MetS. Iida *et al*[61] similarly observed increased levels of α-aminoadipate, a product of lysine degradation, suggesting some catabolic process in MetS. Reddy *et al*[5] also observed that lysine inversely correlated with numerous markers of inflammation including endotoxin, TLR-4, and IL-6. Moreover, acetylation of lysine is seen in states of insulin resistance and is also thought to play a role in immunomodulation[61,62]. This inverse correlation may indicate an attempt to blunt the inflammatory response, leading to a depletion in lysine. Furthermore, diets rich in grain legumes, which are abundant in lysine, are protective against T2DM and salient features of MetS including CVD and increased LDL[63], thus further suggesting that lysine may have potential protective effects, especially in metabolic diseases. Reddy *et al*[5] also support this finding as lysine inversely correlated with WC, SBP, DBP, glucose, while positively correlating with HDL-cholesterol. This data offers promising research for dietary lysine supplementation in mitigating features of MetS.

BRANCHED CHAIN AMINO ACIDS

The branch-chain amino acids include isoleucine, leucine, and valine, all of which are metabolized by BCKD. A 2018 meta-analysis of four cohorts of patients with T2DM showed that all three BCAAs are elevated by approximately 40% in the setting of poor glycemic control[64]. Similarly, Reddy's study saw increases in isoleucine levels in a nascent MetS population compared to controls[5]. These findings are all consistent with insulin-induced impairment of BCKD activity[55]. However, in a study of rats being fed BCAA, the connection between insulin resistance and isoleucine was only observed in the presence of a high fat diet[37]. It was proposed that BCAA buildup was secondary to increased fatty acid oxidation, which increases the NADH/NAD+ ratio. This leads to impairment of BCKD activity, glycolysis, and the TCA cycle. Consistent with this proposition, isoleucine more strongly correlated with markers of adiposity such as leptin, WC, and BMI than HOMA-IR (P = 0.09) in Reddy's study. Isoleucine also inversely correlated with HDL-cholesterol and directly correlated with systolic and DBP [5]. Therefore, isoleucine holds some promise as an early predictor of MetS because it correlates with every risk factor included in the ATP III criteria. Isoleucine has further use as a marker of underlying inflammation, as it positively correlates with IL-6, endotoxin, and oxLDL[65]. Though increased isoleucine seems to be a long-downstream byproduct of insulin and fatty acid oxidation induced metabolic dysregulation, it provides valuable information related to many aspects of the inflammatory and metabolic profile.

Leucine may also provide comparable utility as an inflammatory and metabolic marker, correlating with TNF-α and HOMA-IR, and negatively associating with adiponectin and HDL-cholesterol[48]. TNF-

α is thought to further increase serum BCAA levels by inhibiting its uptake in adipose tissue[66,67]. Valine is perhaps the least well-studied BCAA in the setting of MetS, but is increased in the setting of insulin resistance and adiposity. This was observed in Fiehn's study of obese diabetic African American women and is suggestive of findings in a MetS population[68]. Increases in these BCAAs have similarly been observed in other MetS patient groups. In a population of middle-aged Mediterranean males with MetS, Ntzouvani *et al*[2] observed that isoleucine, valine, and leucine were all significantly increased even after correction for T2DM and liver function. A 2018 study of 563 Chinese adults again showed increased BCAAs in the setting of hyperglycemia and correlations with elevated serum LDL, triglycerides, and decreased HDL[34]. Similarly, other studies have also shown that increases in BCAA correlate significantly with MetS in Chinese, African American and Caucasian population[33,69].

C5 acylcarnitine is formed from breakdown of isoleucine and leucine prior to interaction with BCKD, while C3 acylcarnitine is formed from valine and isoleucine after metabolism by BCKD[70]. Therefore, C5 and C3 acylcarnitine levels may provide additional information about the activity of BCKD in MetS. If BCKD is indeed impaired in MetS, one would expect C5 serum levels to be elevated due to pathway rerouting and possible reduction in C3 levels from upstream inhibition. However, a study recently compared acylcarnitine levels in four groups divided by obesity and metabolic wellness, and the group that most closely aligned with the ATP III criteria showed an increased ratio of C3 and C5 to total acylcarnitines as well as increased levels of C3 carnitine. This indicates that acylcarnitine formation occurs both upstream and downstream of BCKD[71]. Bene *et al*[56] observed similar increases in serum C3 and C5 acylcarnitine[56]. While the findings for C5 acylcarnitine are consistent with BCKD inhibition, C3 acylcarnitine levels are not. C3 acylcarnitine is additionally formed from non BCAAs, including odd-chain fatty acids and threonine, providing us with little reductive information in this regard. Though the BCAAs are universally increased in MetS and evidence suggests ties to both inflammation and fatty acid oxidation, the other pathways leading to C3 acylcarnitine formation must also be explored in order to better assess BCKD activity.

AROMATIC AMINES

Phenylalanine

Phenylalanine is an essential amino acid that has been implicated in the onset of insulin resistance and T2DM. Studies have reported increased serum concentrations of phenylalanine in obese, insulin-resistant, and diabetic subjects[55]. More specifically, Wang *et al*[33] showed that phenylalanine significantly correlated with fasting insulin, HOMA-IR, HOMA-B, and oral glucose tolerance test levels. In addition to BCAAs, aromatic amino acids such as phenylalanine have been shown to be predictors of insulin resistance at 6 year follow-up in normoglycemic young adults. This predictive value is especially pronounced in men. It is theorized that altered aromatic amino acid metabolism precedes insulin resistance in early adulthood before the onset of impaired fasting glucose[72]. Wijekoon *et al*[73] illustrated that phenylalanine levels were 55% higher in young insulin-resistant rats compared to non-obese rats. Despite phenylalanine's significant role in promoting insulin resistance, little is known of how this mechanism occurs. Future studies of the pathogenesis of phenylalanine dysregulation with regards to insulin resistance and its clinical utility of predicting diabetes should be conducted.

Tyrosine

Tyrosine is an aromatic amino formed from the essential amino acid phenylalanine *via* the enzyme phenylalanine hydroxylase. As discussed earlier, some researchers propose that BCKD inhibition leads to buildup of methionine, which is then shunted to cysteine/cystine formation when confronted by states of oxidative stress. Cystine then inhibits tyrosine aminotransferase, leading to a buildup of tyrosine and its precursor, phenylalanine[55]. Additionally, the Framingham Heart Study found that tyrosine was associated with future risk for diabetes[33]. Reddy *et al*[5] and Mohorko *et al*[58] both saw increases in tyrosine in MetS populations without T2DM and CVD. However, Mohorko *et al*[58] saw associations between tyrosine and TNF-α, CRP, HOMA-IR and adiponectin, while Reddy *et al*[5] observed no associations between tyrosine and any of the salient features of MetS. Reddy *et al*[5] proposed that tyrosine may be a bystander in the disease process, but since this study did not record study participant's diet, definitive conclusions are difficult to ascertain. Mohorko's patients had significantly increased protein intake with increasing features of MetS, suggesting that serum tyrosine levels may be partially explained by diet rather than altered metabolism[5,58].

Tryptophan

Tryptophan is an aromatic essential amino acid that must be obtained from dietary sources. Chen *et al*[74] conducted a metabolic profiling study that found circulating tryptophan levels increased in obese subjects compared to healthy lean subjects. These tryptophan levels were lowered after appropriate dietary modifications. Moreover, he found that tryptophan serum levels were independently and positively associated with T2DM risk. In contrast to phenylalanine and tyrosine, more is known about the biochemical pathway of how tryptophan mediates insulin resistance. Significantly increased activity

of the rate limiting enzyme 2,3-dioxygenase was seen in patients with T2DM. Downstream metabolites such as kynurenine and xanthurenic acid were subsequently elevated in patients with T2DM[75]. These metabolites have been shown to play an important role in regulating insulin resistance, pancreatic beta-cell function, and glucose homeostasis. For instance, xanthurenic acid is associated with higher insulin resistance and higher odds of diabetes[76]. Additionally, metabolism of kynurenine is intimately linked to inflammation and immune response. Higher levels of kynurenine metabolites are found in peripheral tissue for inflammatory disorders such as cancer and T2DM[77]. In contrast, some researchers have also failed to establish a link between T2DM and tryptophan levels[55]. Because of its relatively elucidated biochemical pathway and the ability of patients to control intake through diet, tryptophan poses as a potentially powerful clinical marker that could be used to detect and lower risk for T2DM[75].

Phospholipids

Phosphatidylcholines (PC) are major phospholipid components of plasma lipoprotein classes and the only phospholipids known to be required for lipoprotein assembly and secretion. Moreover, PC play a critical role in regulating the quantities of circulating lipoproteins such as very low-density lipoproteins (VLDLs) and HDLs. Studies have shown that increased levels of these PCs in the blood serum of subjects correlated positively with obesity and insulin resistance[65]. Weinberg[78] illustrated significant associations between WC and PC concentrations and a positive association between lysophosphaditylcholine [LPC (14:0)] and diacylphosphatidylcholine [DPC (32.3)]. Another study identifying global lipidomics characterized LPC, PC (32:1), PC (34:2), and PC (34:6) as having significant odds ratios for progression to T2DM[79]. The ADVANCE study also identified PCs, such as PC (34:1), that were associated with future cardiovascular incidents in male T2DM patients[80]. We further investigated the role of PCs in patients with nascent MetS and found that PC34:2 correlated with various features of MetS, including fasting glucose, triglycerides, and WC. This biomarker also correlated with pro-inflammatory markers including IL-1β, IL-8, and hsCRP and identified with features of adipose tissue dysfunction through its positive correlation with leptin and inverse correlation with adiponectin. In contrast to the ADVANCE study, our study did not find significant increases of PC34:1 in patients with MetS[4]. Given their correlation with inflammatory biomarkers, adipose dysregulation, and progression to chronic disease such as T2DM, PCs should be characterized and explored further.

DISCUSSION AND CONCLUSION

Despite the high incidence of MetS and connection to a variety of chronic diseases, there remains limited knowledge about its pathogenesis, treatment, and prevention. Numerous studies have characterized MetS as a pro-inflammatory disease. Accordingly, changes to a variety of metabolite levels have been observed. Analysis of these particular metabolites may help to better characterize MetS and its pathogenesis.

Biogenic amines such as choline, LC, and TMAO are found in red meats. Increased quantities of these amines have been found to induce inflammatory pathways and increase the risk of metabolic diseases. For instance, increased dietary consumption of choline was found to be associated with an adverse cardiometabolic profile and insulin resistance. Additionally, TMAO was observed to be associated with a variety of inflammatory markers such as IL-6, endotoxin, and chemerin in nascent MetS. Other amino acids have been shown to be both protective and risk factors for MetS. For instance, BCAA and alanine have been linked to insulin resistance, while histidine and lysine were observed to decrease inflammation and oxidative stress. Branched chain and aromatic amino acids have also been associated with the pathogenesis of insulin resistance and serve as promising biomarkers for predicting the onset of insulin resistance in normo-glycemic patients. PCs have also emerged as biomarkers that correlated with features of MetS, as well as adipose tissue dysfunction and inflammation.

Although the pathogenesis of MetS remains elusive, metabolomics research offers a promising bridge to understanding the disease from a different perspective. Characterization of many biomarkers gives different avenues through which further research can be conducted. The role of systemic metabolomics for prediction of diseases such as MetS is expanding. For instance, Pujos-Guillot *et al*[81] utilized a combination of untargeted metabolomics and parameters which included clinical, socioeconomic, and dietary subject characteristics to reveal phenotypic changes five years before the onset of MetS. Significant differences between 50 metabolites were found in subjects who would later develop MetS versus control subjects. This integrative approach of systemic metabolomics to characterize MetS on the sub-phenotypic level represents the types of future studies that can be potentially performed in the future in the field of metabolomics[81]. However, the role of many biomarkers, especially tyrosine and phenylalanine, in the pathogenesis of MetS needs to be further clarified. Further research should also be conducted in fields such as lipidomics so that a wider array of biomarkers, such as PC34:2, can be identified. Despite ongoing advances in the field of metabolomics, our review of metabolomics in MetS identifies a critical gap in the current understanding of how metabolites relate to the specific pathogenesis of metabolic disease. More importantly, continued implementation of these biomarkers as predictive or therapeutic tools for MetS should be aggressively pursued.

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FOOTNOTES

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Abstract

BACKGROUND

BKL Walawalkar Hospital is situated near the village of Dervan in the Kokan region of the state of Maharashtra in India. A survey of 2200 surrounding villages showed 51.8% adults had body mass index (BMI) below <18.5 kg/m^2 and only 4.5% were overweight. A survey of 11521 adolescent girls from rural schools showed 64% prevalence of thinness. In the same region, government survey reported the prevalence of diabetes around 7%, and 70% prevalence of leanness. This reinforced the fact that the overall population of Kokan is lean. Hence, we decided to investigate body composition of diabetic people from our hospital clinic by carrying out a clinic-based case control study.

AIM

To study body composition of diabetics in a rural clinic of Kokan.

METHODS

In a case-control study, 168 type 2 diabetic patients (102 men) attending the outpatient department at a rural hospital and 144 non-diabetic controls (68 men) in the Chiplun area of the Kokan region were recruited. History of diabetes (age of onset, duration), anthropometric measurements (height, weight, waist and hip circumference) were recorded. Body composition was measured by bioimpedance using the TANITA analyzer.

RESULTS

More than 45% of diabetic subjects had a 1st degree family history of diabetes, and more than 50% had macrovascular complications. The average BMI in diabetic subjects was 24.3 kg/m^2. According to World Health Organization standards, prevalence of underweight was 8% and that of normal BMI was around 50%. Underweight and normal diabetic subjects (men as well as women) had

significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate when compared to their overweight counterparts.

CONCLUSION

The diabetic population in Kokan has near normal body composition, and BMI has considerable limitations in assessing body composition and it also lacks sensitivity for assessing risk for diabetes in this population. High prevalence of family history of diabetes may point towards genetic predisposition. Leanness is an inherent characteristic of this population and its metabolic significance needs further investigations with a larger sample size.

Key Words: Body composition; Diabetes; Metabolism; Malnutrition; Kokan

Core tip: As per government survey, leanness is widespread in Kokan but diabetes is also on the rise. We studied lean body mass in diabetics in our clinic. Lean individuals had lower body mass index but better percent muscle mass compared to overweight. This could be metabolic response to less caloric intake despite heavy physical activity. This mechanism needs to be clarified. The diabetic population in Kokan has near normal body composition and body mass index has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations on larger scale.

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INTRODUCTION

Recent years have seen a considerable increase in the burden of chronic non-communicable diseases (diabetes, hypertension and coronary heart disease) in clinical practice in urban India. Recent studies in urban populations have shown an unexpectedly high prevalence of diabetes, and the prevalence is rising rapidly[1]. Type 2 diabetes mellitus (T2DM), previously called non-insulin dependent diabetes mellitus (or NIDDM) is a familial disease, on the verge of becoming a pandemic in developing countries like India[2].T2DM is the most prevalent form of diabetes seen in India and constitutes more than 95% of the diabetic population[3]. Migrant Indians in Canada, Britain, the Netherlands, United States and Singapore also have a higher prevalence of diabetes compared to the native populations[4-8].

In contrast, the prevalence of diabetes and coronary heart disease in rural India has been shown to be low. However, there are very few prevalence studies in rural India[9-11] due to remoteness of the villages and lack of health infrastructure for epidemiological studies. Rapid socio-economic developments over the last 2 decades have made the rural population particularly vulnerable to non-communicable diseases. Therefore, there is a need to obtain reliable data on non-communicable diseases in rural India, study the risk factors, and plan effective preventive measures.

In Europe and America, the majority of patients with T2DM are obese. In 1965, Tripathyand Kar[12] highlighted leanness among elderly diabetics in India. Other studies in India have reported a prevalence of low body weight/lean T2DM ranging from 1.6% to 26%[13,14]. A review by Dulloo *et al*[15] highlighted limitations of body mass index (BMI) in assessment of body composition and lack of sensitivity for assessing disease risk, particularly in those who have normal or slightly elevated body weight.

BKL Walawalkar Hospital, situated near the village of Dervan in Ratnagiri district of the state of Maharashtra, has actively promoted rural health care through a variety of education and holistic outreach programs for more than 22 years. The hospital serves the poor socio-economic class populations of the adjoining villages. In 2003-2010 our hospital carried out house-to-house surveys of 2200 villages in the area. In this survey, 51.8% of the subject had BMI < 18.5 kg/m^2 and only 4.5% were overweight, with BMI > 25 kg/m^2. A survey of 11521 adolescent girls from rural schools conducted between 2011 and 2017 showed that 64% of the girls were in grade 1 to 3 of thinness, based upon International Obesity Task Force standards. Stunting was seen in 22% to 28% of the girls. Thus, the overall population of Kokan is lean in their body stature.

The National Family Health Survey[9] conducted by the government of India reported on the prevalence of diabetes in the same region based on random blood sugar as 9% among men and 5.8% among women. The same survey also reported more than 70% prevalence of leanness based on BMI among men as well as women. This again reinforced the leanness of the population of Kokan. Hence, we decided to investigate more about body composition of diabetic people from our hospital clinic by carrying out a clinic-based case control study. The health infrastructure in our hospital provided us the opportunity to study the profile of diabetic as well as non-diabetic subjects.

MATERIALS AND METHODS

This study was carried out in the outpatient department of BKL Walawalkar Hospital and Rural Medical College in the Ratnagiri district.

Diabetic patients from the outpatient department were enrolled in the study. Non-diabetic control subjects were mostly spouses of the patients in the hospital or were from the hospital staff.

The following data was recorded from the clinical history for diabetic subjects: age at diagnosis of diabetes; family history of diabetes; and history of macrovascular complications (hypertension, ischemic heart disease, cardiovascular disease and cerebral vascular disease) and microvascular complications (nephropathy, neuropathy and retinopathy).

The following anthropometric parameters were measured on diabetic as well as non-diabetic subjects: height; weight; waist and hip circumferences. BMI and waist-to-hip ratio (commonly known as WHR) were calculated. Subjects were classified as underweight, normal and overweight using World Health Organization standards for BMI[16]. Those with BMI < 25 kg/m^2, that is those who were underweight or normal, were classified as lean. We used International Diabetes Federation criteria[17] for the waist circumference to classify the subjects as centrally obese.

Body composition was assessed on all the subjects using the Tanita BC 420-MA analyzer (Tanita Corporation, Tokyo, Japan). It measured bioelectrical impedance by passing alternating current through the subject to measure the water content. Body composition measurements (fat mass, lean mass, total body water) were obtained as mass as well as percentage. In addition, we also obtained visceral fat, fat free mass, total body water (TBW) as mass as well as percentage, and basal metabolic rate (BMR).

In total, 201 diabetic subjects reported to the outpatient department of medicine. Those with diabetes with pregnancy, severe chronic illness, pancreatic disease and type-1 diabetes were excluded. After these, 168 diabetic subjects (102 men) were left to form the sample of diabetic subjects. We recruited 144 non-diabetic control subjects (68 men).

Statistical methods

Data is presented as mean ± SD for continuous and as percent for categorical variables. Analysis of variance and χ^2 test was used to compare continuous and categorical variables for differences in groups. Comparison of anthropometric parameters between diabetic and non-diabetic subjects was adjusted for current age. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, United States).

Ethics

Informed and written consent was obtained from all the subjects for use of their data. Ethical approval from the institute's ethics committee was also obtained for the data analysis.

Our institute's ethics committee is registered with the government of India. Earlier, its registration number was EC/755/INST/MH/2015. The registration expired in August 2018 and was subsequently renewed, with the new registration number as EC/755/INST/MH/2015/RR-18.

The study was conducted from January 2018 to June 2018.

RESULTS

Subject characteristics

Table 1 shows clinical characteristics (age, anthropometry and body composition measurements) of subjects. Non-diabetic subjects (men as well as women) were significantly younger (P < 0.001). In diabetic subjects the mean age of diagnosis of diabetes was similar for men and women. Height was similar in both sexes, when comparing diabetic and non-diabetic. Diabetic women were heavier and had higher BMI. Diabetic women also had higher hip circumference. Both diabetic men and women had significantly higher WHR. Body fat percentage as well as mass was similar in diabetic and non-diabetic men but it was significantly higher in diabetic women. In both sexes the muscle mass percentage was similar between diabetics and non-diabetics but diabetic men had higher muscle mass. Diabetic women had higher visceral fat. Diabetic and non-diabetic subjects (men as well as women) had similar TBW and TBW% but BMR was higher in diabetic women compared to non-diabetic women.

Table 1 Anthropometry and body composition

	Diabetic		Non-diabetic		*P* for diabetic *vs* non-diabetic	
	Men, *n* = 102	Women, *n* = 66	Men, *n* = 68	Women, *n* = 76	Men	Women
Age						
Current age	55.2 (13.1)	57.9 (9.5)	41.7 (12.0)	38.2 (10.2)	0.000	0.000
Age at diagnosis	48.7 (12.1)	50.9 (10.5)				
Anthropometry						
Height, cm	164.2 (6.4)	151.1 (6.3)	162.7 (8.4)	152.1 (7.7)	0.17	0.13
Weight, kg	64.7 (11.7)	56.6 (12.1)	59.9 (13.5)	50.4 (11.9)	0.27	0.001
BMI, kg/m^2	24.0 (3.9)	24.7 (4.9)	22.5 (4.3)	21.7 (4.4)	0.45	0.002
Waist circumference,cm	92.0 (9.9)	87.5 (11.4)	81.3 (12.6)	76.0 (9.8)	0.000	0.000
Hip circumference, cm	94.2 (9.2)	97.3 (11.9)	89.8 (10.3)	89.4 (10.5)	0.22	0.000
WHR	0.98 (0.08)	0.90 (0.09)	0.90 (0.09)	0.85 (0.07)	0.000	0.045
Body composition by bio-impedance						
Body fat, %	22.6 (5.7)	35.6 (6.8)	21.3 (6.8)	30.5 (7.2)	0.87	0.026
Body fat, kg	15.2 (6.2)	21.2 (8.4)	14.1 (8.7)	15.7 (6.3)	0.89	0.000
Muscle mass, %	73.2 (5.4)	60.7 (6.4)	72.9 (8.7)	64.4 (10.5)	0.13	0.38
Muscle mass, kg	46.7 (5.9)	33.6 (4.4)	43.4 (8.1)	33.4 (7.1)	0.03	0.19
Visceral fat level	12.1 (4.4)	7.2 (2.6)	8.6 (5.3)	4.6 (2.9)	0.23	0.019
Fat free mass, kg	49.3 (6.3)	35.6 (4.8)	46.1 (8.2)	34.8 (6.3)	0.068	0.057
TBW, %	54.0 (3.6)	46.7 (3.6)	53.5 (3.5)	48.3 (5.7)	0.69	0.077
TBW, kg	34.7 (5.4)	25.6 (4.0)	31.8 (6.0)	24.5 (4.6)	0.12	0.056
BMR, calories/time	1350.2 (188.9)	1059.8 (168.9)	1291.0(231.4)	1043 (185.6)	0.30	0.004

Data are mean (standard deviation). *P* is adjusted for current age for anthropometry; body composition parameters are already age adjusted.BMI: Body mass index; WHR: Waist-to-hip ratio; TBW: Total body water; BMR: Basal metabolic rate.

Table 2 shows the categorical data. In diabetic subjects the proportion of those with 1st and 2nd degree family history of diabetes was similar in both sexes. No control subject had family history of diabetes. A substantial number (around 70%) of diabetic subjects reported macrovascular complications. Diabetics (men as well as women) had higher proportion of those overweight and centrally obese compared to their non-diabetic counterparts.

Body composition and BMI

In diabetic subjects of both sexes, those lean had significantly lower body fat (percentage as well as mass), lower muscle mass but high muscle mass percentage, lower visceral fat, lower fat free mass, higher total TBW%, but lower TBW and BMR compared to their overweight counterparts. Similar differences were observed in non-diabetic subjects between those lean and overweight of both sexes, except for TBW% which was similar in lean and overweight non-diabetic women (Tables 3 and 4).

DISCUSSION

There are many reports on the profile of diabetic subjects in rural regions of India[18-28] but this is the first report from the Ratnagiri district of the Kokan area. Although from a small sample size, our data demonstrated that more than 50% of the men as well as the women subjects with diabetes were lean with a BMI well below the normal BMI cutoff of 25 kg/m^2. Our study is clinic-based and not community-based. The National Family Health Survey[9] conducted by the government of India determined the prevalence of diabetes in the same region based on random blood sugar, being 9% among men and 5.8% among women. The same survey also found more than 70% prevalence of leanness based on BMI among men as well as women. This could be because the population in this region is constitutionally small.

Table 2 Family history, diabetic complications, and anthropometric morbidity

	Diabetic		Non-diabetic		*P* for diabetic *vs* non-diabetic	
	Men, *n* = 102	Women, *n* = 66	Men, *n* = 68	Women, *n* = 76	Men	Women
Diabetes history						
Family history						
1st degree	45 (44)	34 (51)				
2nd degree	7 (7)	9 (14)				
Complications						
Macrovascular	72 (69.9)	47 (71.2)				
Microvascular	13 (12.1)	1 (1.5)				
Underweight	8 (7.8)	7 (10.6)	13 (19.1)	20 (26.3)		
Normal	54 (52.9)	27 (40.9)	35 (51.5)	41 (53.9)	0.07	0.001
Overweight	40 (39.2)	32 (48.5)	20 (29.4)	15 (41.9)		
Centrally obese	59 (58)	49 (74)	19 (28.4)	29 (39.7)	0.000	0.000

Data are *n* (%).

Table 3 Body mass index and body composition in diabetics

	Diabetic, *n* = 168				*P*-value for lean *vs* overweight	
	Men, *n* = 102		Women, *n* = 66		Men	Women
	Lean, *n* = 62	Overweight, *n* = 40	Lean, *n* = 34	Overweight, *n* = 32		
Body fat, %	19.6 (4.4)	27.5 (4.0)	30.6 (4.7)	40.9 (4.1)	0.000	0.000
Body fat, kg	11.5 (3.5)	21.0 (5.1)	15.3 (5.4)	27.2 (6.7)	0.000	0.000
Musclemass, %	76.2 (4.1)	68.5 (3.8)	65.4 (4.4)	55.7 (3.9)	0.000	0.000
Musclemass, kg	43.8 (4.7)	51.3 (4.7)	31.1 (2.9)	36.3 (4.1)	0.000	0.000
Visceral fat level	10.0 (3.9)	15.3 (3.1)	5.2 (1.6)	9.3 (1.7)	0.000	0.000
Fat free mass, kg	46.2 (4.9)	54.1 (4.9)	32.8 (3.2)	38.5 (4.4)	0.000	0.000
TBW, %	55.3 (3.6)	52.0 (2.5)	48.6 (3.5)	44.5 (2.4)	0.000	0.000
TBW, kg	31.9 (4.1)	39.0 (4.4)	23.1 (2.3)	28.6 (3.6)	0.000	0.000
BMR, calories/time	1251.6 (139.1)	1502.3 (152.5)	953.7 (100.8)	1172.7 (153.3)	0.000	0.000

Data are mean (standard deviation). TBW: Total body water; BMR: Basal metabolic rate.

Body composition depends on genetic makeup, dietary habits, physical activities, and susceptibility to chronic illness. Lean diabetes has been described in many populations across the world[29,30], and there have been extensive reports on lean diabetes in India. A prospective study across nine centers in India found about one-fourth of T2DM patients to be lean or with BMI below 19 kg/m^2. Prevalence of type 2 lean DM across the centers varied from 11% to 25%[13]. A recent report[31] showed significantly higher prevalence of T2DM without overweight and obesity in Indians compared to white Caucasians in the United States. Diabetes in lean patients has been described before[32,33]. There are reports on lean diabetes from other regions of South Asia, India, and Africa[29,34,35]. Populations described in these reports were lean, had a history of childhood malnutrition, and had poor socioeconomic status.

Another notable observation in our subjects was the increased muscle mass percentage but low BMR in lean subjects compared to those overweight subjects. Usually, BMR is directly proportional to muscle mass and as a metabolic response to starvation, the the primary concern is to supply energy to the brain [36]. In our study, the BMR was less in spite of better muscle mass. This could be because of less caloric intake despite heavy physical activity. Adverse intrauterine or early postnatal environment with insufficient nutrients has been suggested as a mechanism of beta cell failure in lean diabetics[29]. Another Indian study[37] found that type 2 diabetics had an unfavorable body fat distribution, with an

Table 4 Body mass indexand body composition in non-diabetics

	Non-diabetic, *n* = 144				*P*-value for lean *vs* overweight	
	Men, *n* = 68		Women, *n* = 76		Men	Women
	Lean, *n* = 48	Overweight, *n* = 20	Lean, *n* = 61	Overweight, *n* = 15		
Body fat, %	18.8 (6.3)	27.6 (2.6)	28.4 (5.5)	39.1 (6.9)	0.000	0.000
Body fat, kg	11.0 (6.6)	21.5 (9.1)	13.6 (4.5)	24.7 (5.4)	0.000	0.000
Musclemass, %	75.5 (8.1)	68.7 (7.1)	65.9 (10.8)	58.1 (6.4)	0.000	0.000
Musclemass, kg	40.7 (6.0)	50.1 (8.6)	32.0 (6.2)	38.9 (7.8)	0.000	0.000
Visceral fat level	6.2 (4.3)	14.3 (1.9)	3.6 (1.9)	8.8 (2.2)	0.000	0.000
Bone mass, kg	2.2 (0.4)	2.8 (0.3)	1.8 (0.4)	2.3 (0.5)	0.000	0.000
Fat free mass, kg	42.7 (6.6)	54.1 (5.7)	33.3 (4.7)	41.0 (8.5)	0.000	0.000
TBW, %	54.6 (3.5)	51.0 (1.9)	48.8 (6.1)	46.1 (3.1)	0.000	0.075
TBW, kg	29.0 (4.3)	38.5 (4.1)	23.3 (3.3)	29.4 (5.8)	0.000	0.000
BMR, calories/time	1196.2 (185.2)	1521.5 (159.6)	1001.2 (148.4)	1228.7 (221.8)	0.000	0.000

Data are mean (standard deviation). TBW: Total body water; BMR: Basal metabolic rate.

increase in visceral fat compared to that in non-diabetics. However, there are differing opinions on the causality of this association[38,39].

Visceral fat is more important than total body fat, as excess visceral fat is a risk factor for both pre-diabetes and diabetes, being more so in Indians compared to other Asian populations. In our study, the diabetic subjects had higher visceral fat, and within the diabetics those who are lean had significantly lower visceral fat. Significantly higher muscle mass percentage in the low and normal BMI group of diabetics than in those overweight shows that BMI has limitations pertaining to detailing of body composition. This striking peculiarity in type 2 diabetics in our subjects is bound to influence the natural history of diabetes and it needs further study.

The Kokan region is characterized by mountainous terrain with poor soil quality, hot humid weather, poverty and deep-rooted superstitions which have led to widespread malnutrition amongst people[40]. Our hospital is located in a remote, rural area, and our study population is from a tribal region. A study from our center on 1290 school-going rural adolescents found underweight prevalence of 72%[41]. In a pilot study on adolescent girls from Kokan, a high prevalence of micronutrient deficiencies was also found. More than 65% were deficient in calcium, zinc and folic acid, and were malnourished[42]. In our hospital, 41.9% of the babies delivered were low birth weight (birth weight < 2500 g)[43]. The Tata Memorial Rural Out Reach Program (known as TMCROP) was implemented by our hospital in all 2200 villages in Kokan, and all villagers were screened for cancer by household survey. In that survey, 51.7% population had a BMI less than 18.5 kg/m^{2}and only 4.5% could be classified as obese. These findings highlighted the leanness of the population of Kokan.

In our current study, more than 40% had family history for diabetes, which may suggest genetic predisposition; although, we do not have any genetic data on our subjects. Inadequacy of BMI in distinguishing leanness has suggested future studies should investigate the role of body composition in the development of lean diabetes[44].

Our study has some notable strengths. It has yielded the first report from the Kokan region, where malnutrition is very much prevalent. Unlike many other reports on diabetic subjects from other regions of India[18-28], we have collected the data on body composition. There are, also, many limitations to our study. The sample size is small and it used cross-sectional data from a rural diabetic clinic. No sample size calculations were done. We were only able to recruit a smaller number of controls, making the study prone to bias. We were constrained by the remoteness of the area where the hospital is situated. We could not use Dual-Energy X-Ray Absorptiometry (commonly known as DEXA; the current gold standard for body composition) because of the high equipment costs. We measured the body composition by bioelectrical impedance, using the TANITA body composition analyzer, which is a low cost, convenient and noninvasive technology, but concerns have been raisedin the past about the validity of the analyzer[45,46] and there is an urgent need to develop an ethnicity-specific equation for the Asian Indian population. We could not report on other cardiovascular risk markers (lipids, blood pressure) nor on the socioeconomic status of the participants as very little data were available as a part of patient history. We are aware of the fact that these subjects were diagnosed with diabetes at much earlier age. Recruitment of controls from the hospital setting has induced inherent selection bias. Thus, there is a need for a large community-based prospective study investigating the role of lean mass in

development of diabetes in this region.

To conclude, we attempted to investigate the role of lean body mass in development of diabetes in the predominantly underweight diabetic population of Kokan. The underlying mechanism of lean diabetes has not yet been fully explored and more studies are needed. The diabetic population in Kokan has near-normal body composition, and BMI has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations using larger sample sizes.

ARTICLE HIGHLIGHTS

Research background

Recent years have seen a considerable increase in the burden of diabetes, hypertension and coronary heart disease in clinical practice in urban India. Recent studies in urban populations have shown an unexpectedly high prevalence of diabetes, and the prevalence is rising rapidly.

Research motivation

BKL Walawalkar Hospital carried out house-to-house surveys of 2200 villages in 2003-2010. In that survey, 51.8% of the subjects hadbody mass index (BMI) < 18.5 kg/m^2 and only 4.5% were overweight, with BMI > 25 kg/m^2. Another survey of 11521 adolescent girls from rural schools that was conducted in 2011-2017 showed that 64% of the girls had grade 1 to 3 thinness, based on the International Obesity Task Force standards, and stunting was seen in 22% to 28% of the girls. Thus, the overall population of Kokan is lean in their body stature. The same survey also found a more than 70% prevalence of leanness based on BMI among men as well as women. This, again, reinforced the leanness of the population of Kokan.

Research objectives

In order to investigate body composition of diabetic people from the BKL Walawalkar Hospital Clinic, a clinic-based case control study was carried out.

Research methods

One hundred sixty-eight type 2 diabetic patients (102 men) attending the outpatient department at a rural hospital and 144 non-diabetic controls (68 men) in the Chiplun area of the Kokan region were recruited. History of diabetes and anthropometric measurements were recorded, and body composition was measured by bioimpedance using the TANITA analyzer. All analyses were performed using SPSS 16.0 statistical software.

Research results

In this study, more than 45% of diabetic subjects had a 1st degree family history of diabetes, and more than 50% had macrovascular complications. The average BMI in the diabetic subjects was 24.3 kg/m^2. Underweight and normal diabetic subjects (men as well as women) had significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate compared to their overweight counterparts. Our data pave the way for a new theory of undernutrition as a risk factor in predisposing the Kokan population to diabetes.

Research conclusions

Undernutrition should also be considered as a risk factor for diabetes in lean patients. The molecular basis and physiological adaptations to undernutrition need to be explored.

Research perspectives

Lean diabetics had significantly lower body fat percentage, higher muscle mass percentage, lower visceral fat and lower basal metabolic rate compared to overweight diabetics. This could indicate a metabolic response to less caloric intake despite heavy physical activity, and this mechanism needs to be investigated. The diabetic population in Kokan has near-normal body composition and BMI has considerable limitations. Therefore, the physiological process producing these deviations in body composition and its metabolic significance need further investigations on a larger scale.

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FOOTNOTES

Author contributions: Suvarna P conceptualized the study and also wrote substantial parts of the manuscript; Shruti K extracted the data from records and performed the data entry; Maruti D managed the data and also helped in the analysis; CharudattaJ performed statistical analysis and wrote some parts of the manuscript.

Institutional review board statement: The study was approved by the Institute Ethics Committee of BKL Walawalkar Hospital and Rural Medical College.

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ORIGINAL ARTICLE

Case Control Study

Relationship between sonographically measured median nerve cross-sectional area and presence of peripheral neuropathy in diabetic subjects

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Abstract

BACKGROUND

Neuropathy is a common complication of diabetes mellitus resulting from direct damage by hyperglycemia to the nerves and/or ischemia by microvascular injury to the endoneurial vessels which supply the nerves. Median nerve is one of the peripheral nerves commonly affected in diabetic neuropathy. The median nerve size has been studied in non-Nigerian diabetic populations. In attempt to contribute to existing literature, a study in a Nigerian population is needed.

AIM

To evaluate the cross-sectional area (CSA) of the median nerve using B-mode ultrasonography (USS) and the presence of peripheral neuropathy (PN) in a cohort of adult diabetic Nigerians.

METHODS

Demographic and anthropometric data of 85 adult diabetes mellitus (DM) and 85 age- and sex-matched apparently healthy control (HC) subjects were taken. A complete physical examination was performed on all study subjects to determine

the presence of PN and modified Michigan Neuropathy Screening Instrument (MNSI) was used to grade its severity. Venous blood was taken from the study subjects for fasting lipid profile (FLP), fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) while their MN CSA was evaluated at a point 5 cm proximal to (5cmCATL) and at the carpal tunnel (CATL) by high-resolution B-mode USS. Data was analysed using SPSS version 22.

RESULTS

The mean MN CSA was significantly thicker in DM subjects compared to the HC at 5cmCATL ($P < 0.01$) and at the CATL ($P < 0.01$) on both sides. The presence of diabetic peripheral neuropathy (DPN) further increased the MN CSA at the CATL ($P < 0.05$) but not at 5cmCATL ($P > 0.05$). However, the severity of DPN had no additional effect on MN CSA 5 cm proximal to and at the CATL. There was no significant association between MN CSA and duration of DM and glycemic control.

CONCLUSION

Thickening of the MN CSA at 5cmCATL and CATL is seen in DM. Presence of DPN is associated with worse thickening of the MN CSA at the CATL but not at 5cmCATL. Severity of DPN, duration of DM, and glycemic control had no additional effect on the MN CSA.

Key Words: Median nerve; Cross-sectional area; Sonography; Diabetics; Peripheral neuropathy

Core tip: We report median nerve cross-sectional area findings in diabetics of Nigerian origin. This study demonstrates that the median nerve is thicker at the carpal tunnel and 5 cm proximal to the carpal tunnel in diabetic subjects than age- and sex-matched healthy controls. Further thickening in the median nerve size is seen in the presence of diabetic peripheral neuropathy at the carpal tunnel but not at a point 5 cm proximal to it. Median nerve size has no significant relationship with age, gender, severity of diabetic peripheral neuropathy, duration of diabetes mellitus or glycemic control in diabetic subjects.

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INTRODUCTION

Diabetes mellitus (DM) is used to describe several diseases where there is a persistent increase in blood sugar level due to deficiency in the production and/or action of insulin[1]. Broadly, diabetes mellitus can be classified into two major forms, type 1 or insulin-dependent and type 2 or non-insulin dependent DM according to insulin secretion or action respectively[2]. The prevalence of DM is on the increase worldwide in both developed and developing countries[1,2]. Diabetic peripheral neuropathy (DPN) is the most common complication of DM and is seen in patients with types 1 and 2 DM. DPN and peripheral nerve dysfunction have common signs and symptoms in people with diabetes when other aetiological factors of the defect are not considered[3].

After a long-term persistent hyperglycemia, DPN usually becomes symptomatic in type 1 DM while it is obvious in type 2 DM at detection or after a period of insufficient blood sugar level control[4].

Organ impairment or failure is related with prolonged hyperglycemia of diabetes, and some of the organs that can be affected include the eyes, kidneys, nerves, heart, and blood vessels[5]. Damage to peripheral nerves can occur directly from elevated blood sugar level or indirectly from reduced blood flow to nerves[1].

Diabetic neuropathy is responsible for substantial morbidity, increased mortality and impaired quality of life of diabetic patients[6]. Therefore, early detection of nerve dysfunction is important to appropriately care for patients with diabetic neuropathy[7].

The characteristic signs and symptoms of diabetic neuropathy basically suggest its diagnosis and confirmatory neurophysiological tests are required[8].

Although electroneuromyography and nerve conduction studies (NCS) are the major electro-neurophysiological methods for diagnosing pathology associated with the median nerve and other peripheral nerves, they only allow assessment of peripheral nerve function, but fail to provide any data

on their morphology or the possible visible pathomorphology of the surrounding structures and tissues [9,10].

Magnetic resonance imaging (MRI) of the median nerve provides excellent morphological details of the nerve. Magnetic resonance imaging of peripheral nerves is known as Magnetic Resonance Neurography (MRN)[11,12]. It is used to assess peripheral nerve entrapments and impingements as well as localization and grading of nerve injuries and lesions[11,12]. Magnetic Resonance Neurography could be morphological or functional MRN[11]. The morphological MRN is based on 3D MRI sequences with or without fat suppression while the functional MRN is based on Diffusion-weighted imaging (DWI). DWI, an MR Neurographic technique used to measure the limited random movement of water molecules within tissues depends on the Brownian motion of water[13,14]. Diffusion within nerve fibres or white matter of the brain is usually high and tends to be directed towards the path of minimum opposition to the moving molecules and this is used to generate the final image in DWI. Diffusion tensor imaging (DTI) is an extension of DWI that enables measurement of Brownian movement of water molecules in nerves[11-15]. However, MRI is not widely available, patient selective, expensive, and involves the use of time-consuming techniques.

Ultrasonography (USS) is preferred to MRI as a diagnostic method for a variety of reasons such as noninvasiveness, low cost, accessibility, approach, *etc*[15]. The assessment of extremely small peripheral nerves *via* ultrasound has been made possible by employing Diagnostic high-resolution USS[8]. The median nerve can be examined as it courses from the arm to the hand using high-resolution USS. Ultrasound can be used to evaluate the shape, size, and echo-texture of the MN. The major disadvantage of ultrasound is that it is operator dependent as it requires trained experienced hands with appropriate high resolution equipment[15].

This study aimed to compare the MN cross-sectional area (CSA) measured on USS between DM subjects and age- and sex-matched apparently healthy controls (HC), and evaluate the relationship between MN CSA and presence and/ or severity of DPN.

MATERIALS AND METHODS

Subjects and study area

The study was approved by the Ethics and Research Committee of our hospital. Eighty-five consenting DM patients aged between 18 and 80 years and an equal number of age- and sex-matched HC subjects were randomly recruited from the Endocrinology Unit of the Department of Medicine of our hospital. Hypertensives, current smokers and alcohol consumers, subjects with thyroid disease, liver disease, previous history of carpal tunnel operation, inflammation, malignancy, and elevated total cholesterol after serum fasting lipid profile (FLP) were excluded from the study. Our hospital is a tertiary hospital and one of the major referral centers for diabetes care in the Southwestern zone of Nigeria. It serves a catchment area of about 170000 people.

Clinical parameters

Physical examination was carried out on all prospective study subjects by the managing endocrinologist for the presence and severity of peripheral neuropathy. The modified clinical history part of Michigan Neuropathy Screening Instrument (MNSI) questionnaire[11] was administered to all study participants and scored over 15 to determine and grade peripheral neuropathy (PN). A total "Yes" score of 1-5 represented mild PN, a score of 6-10 moderate and 11-15 represented severe PN as done previously by Moghtaderi *et al*[16].

Phalen test[12] was performed to rule out carpal tunnel syndrome.

In the erect position with the acantho-meatal line set parallel to the floor, subjects had their weight in kilogram (Kg) and height in meters (M) measured without their shoes on. Their body weight and height were measured to the nearest 0.1 kg and 0.1 meter respectively using a mechanical physician weighing scale attached with a height gauge (model ZT-160, China).

Body mass index (BMI) was determined for all study participants using the formula: BMI = Weight/height^2

Laboratory parameters

Venipuncture of the antecubital veins in the left arm was done under sterile conditions for all study participants after an overnight fast of at least 8-12 h. 5 mL of venous blood was taken from each study subject for the assessment of fasting lipid profile (FLP) and glycated hemoglobin (Hb1Ac). The sample was emptied into an EDTA (Ethylene diamine tetra acetic acid) bottle and sent to chemical pathology laboratory for FLP using bioassay systems EnzyChrom cholesterol assay kit (E2CH-100) and Hb1Ac by chromatography using Siemens Hb1Ac machine (model SEMDIA-10311134, United States). Chromatography was either done immediately or sample stored in the refrigerator at a temperature of 2-8 °C and test done within 7 d.

Fasting Blood Glucose (FBG) was determined from finger prick specimen using a glucometer (Accu-check, Roche 365702101104) based on the glucose oxidase method.

Sonographic technique for median nerve assessment

All sonographic examinations were performed using the MINDRAY Real-time ultrasound machine (Model DC-7) equipped with a linear array probe, with a transducer frequency of 6MHz-12MHz. Each participant was seated on examination couch with a pillow on his/her lap. The forearm was placed supine on the pillow with elbow and fingers semi-flexed during the examination of the median nerve. The patient was instructed not to move the fingers during the examination period.

Following adequate positioning, coupling gel was applied to the anterior part of the wrist joint, over the carpal tunnel (CATL) and 5 cm proximal to it (5cmCATL). The volar wrist crease and pisiform bone were used as external reference points and landmarks during scanning. The transducer was positioned at right angles to the distal wrist crease and longitudinal axis of the forearm within the carpal tunnel inlet. The MN was identified and its major and minor axes were taken (Figure 1). Intra-observer variability was minimized by taking three measurements and recording the mean value. The CSA was calculated by the indirect method using the formula: CSA = major axis × minor axis × π × 1/4 (mm^2) [13]; where π is a mathematical constant and is equal to 3.142.

Statistical analysis

All data were entered into the computer spreadsheet using Statistical Package for Scientific Solutions (SPSS) version 22.0 for Windows (SPSS, Chicago, IL, United States). Quantitative variables were indicated as mean ± SD, while qualitative variables were indicated as frequencies and percentages.

Independent Sample *t*-test was used to compare MN CSA between DM and apparently HC subjects. A subgroup analysis amongst the DM subjects was done between those with and those without PN using independent *t*-test. Median nerve CSA was further compared between DM without PN, with mild PN and moderate/severe PN using Analysis of Variance (ANOVA).

Pearson Correlation was done to determine the relationship between MN CSA, clinical and laboratory parameters of the DM and HC subjects.

A level of $P \leq 0.05$ was considered as statistically significant for all tests.

The statistical review of this study was done by a biomedical statistician[17-20].

RESULTS

There were significant differences in mean weight ($P = 0.003$), BMI ($P = 0.010$), DBP ($P = 0.001$), MAP ($P = 0.023$) and FBG ($P = 0.001$) between the diabetic and control groups while mean age ($P = 0.602$), total cholesterol ($P = 0.622$), height ($P = 0.473$) and SBP ($P = 0.557$) were similar in them. Both groups were well matched for Gender (Table 1).

Diabetic subjects had significantly higher median nerve CSA than their age- and sex-matched apparently healthy controls at the CATL level (12.5 ± 2.5 mm^2 *vs* 8.8 ± 1.7 mm^2 ($P < 0.01$) on the right and 12.3 ± 2.5 mm^2 *vs* 8.6 ± 1.7 mm^2 ($P < 0.01$) on the left) and at 5cmCATL (8.0 ± 2.0 mm^2 *vs* 5.3 ± 1.2 mm^2 ($P < 0.01$) on the right and 7.9 ± 1.9 mm^2 *vs* 5.4 ± 1.4 mm^2 ($P < 0.01$) on the left) (Table 2).

The median nerve CSA was significantly higher in diabetics with PN compared to diabetics without PN (12.9 ± 2.5 mm^2 *vs* 11.8 ± 2.4 mm^2, $P = 0.049$) at the level of the CATL but not at 5cmCATL (8.0 ± 2.2 mm^2 *vs* 8.0 ± 2.0 mm^2, $P = 0.856$) (Table 3).

There was no association between median nerve CSA and severity of PN as median nerve CSA did not significantly differ between the absent, mild, and moderate/severe PN categories of diabetic subjects at the CATL ($P = 0.062$) and at 5cmCATL ($P = 0.145$) even after post-hoc Scheffe analysis for intergroup differences (Table 4).

Median nerve CSA at 5cmCATL and at the CATL did not show significant association with age greater than 60 years, duration of DM and glycemic control in both HC and DM subjects. However, MN CSA was significantly more thickened in males compared to females among the HC at both points of measurement. The association of MN CSA with gender was not found among DM subjects even after subgroup analysis of only those with DPN (Table 5).

Duration of DM, FBG and, HbA1c did not show any significant correlation with MN CSA in the diabetic subjects (Table 6).

DISCUSSION

Diabetic neuropathy is a relatively early and common complication affecting approximately 30% of DM patients[14]. The prevalence of DPN from clinical assessment using the MNSI questionnaire was 60% in this study. This is at best an estimate since the gold standard for assessing PN, the nerve conduction test was not employed in our study.

Table 1 Demographic, anthropometric and clinical characteristics of the study subjects *n* (%)

Variables	DM, *n* = 85	HC, *n* = 85	*P*-value
Age (yr)			
mean ± SD[1]	61.7 ± 11.1	60.9 ± 10.3	0.620
< 50 yr	11 (12.9)	11 (12.9)	0.965
50-59 yr	25 (29.4)	27 (31.8)	
60-69 yr	22 (25.9)	23 (27.1)	
≥ 70 yr	27 (31.8)	24 (28.2)	
Gender			
Male	44 (51.8)	44 (51.8)	1.000
Female	41 (48.2)	41 (48.2)	
Height (m)[1]	1.66 ± 0.08	1.65 ± 0.08	0.473
Weight (Kg)[1]	71.3 ± 13.6	65.4 ± 11.5	0.003
BMI (Kg/m^2)[1]			
mean ± SD[1]	26.0 ± 5.3	24.1 ± 4.3	0.010
Underweight	3 (3.5)	4 (4.7)	0.072
Normal BMI	35 (41.2)	51 (60.0)	
Overweight	31 (36.5)	21 (24.7)	
Obese	16 (18.8)	9 (10.6)	
Systolic BP (mmHg)[1]	129.8 ± 20.7	131.9 ± 24.3	0.557
Diastolic BP (mmHg)[1]	84.2 ± 11.5	82.8 ± 13.1	0.404
Total cholesterol (mmHg)[1]	4.90	4.78	0.622
FBG (mmol/L)[1]	7.00 ± 2.16	5.15 ± 0.06	0.001
HbA1C in % (mean ± SD)	8.69 ± 2.46	-	-
Duration of DM in months median (range)	48.0 (0.3-312)	-	-
Peripheral neuropathy	51 (60)	-	-

[1]*P* values < 0.05 are significant. BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DM: Diabetes mellitus; FBG: Fasting blood glucose; HbA1c: Glycated haemoglobin.

Table 2 Comparison of median nerve cross-sectional area between diabetics and healthy controls

Median nerve CSA (mm^2)		DM, *n* = 85	HC, *n* = 85	*P*-value
Right	Carpal tunnel	12.5 ± 2.5	8.8 ± 1.7	< 0.01
	5 cm proximal	8.0 ± 2.0	5.3 ± 1.2	< 0.01
Left	Carpal tunnel	12.3 ± 2.5	8.6 ± 1.7	< 0.01
	5 cm proximal	7.9 ± 1.9	5.4 ± 1.4	< 0.001

CSA: Cross sectional area; DM: Diabetic subjects; HC: Healthy control subjects. *P* values < 0.05 are significant.

We found higher MN CSA in the diabetic cohort which consisted of both types 1 and 2 DM subjects compared to non-diabetic HC at the 2 points of measurement. The finding of increased MN CSA in the diabetics relative to HC in our study agrees with those of 2 other hospital-based studies by Watanabe *et al*[8] in 30 type 2 DM subjects aged 36 to 83 years with mean age of 59.8 ± 10.2 years and 32 healthy volunteers aged 24-72 years with mean age of 53.7 ± 13.9 years in Japan, and Agirman *et al*[21] in 63 Type 2 DM subjects and 14 controls with mean age 47.6 ± 13.1 years in Turkey even though they were conducted in type 2 DM subjects only in racially and geographically different settings.

Table 3 Comparison of median nerve cross-sectional area between diabetics with and diabetics without diabetic peripheral neuropathy

Median nerve CSA (mm²)	Diabetic peripheral neuropathy		*P*-value
	Absent *n* = 34	Present *n* = 51	
Carpal tunnel	11.8 ± 2.4	12.9 ± 2.5	0.049
5cm Proximal	8.0 ± 2.2	8.0 ± 2.0	0.856

CSA: Cross sectional area. *P* values< 0.05 are significant.

Table 4 Comparison of median nerve cross-sectional area between diabetics without diabetic peripheral neuropathy, diabetics with mild diabetic peripheral neuropathy and diabetics with moderate/severe diabetic peripheral neuropathy

Median nerve CSA (mm²)	Peripheral neuropathy			*P*-value
	Absent (*n* = 34)	Mild (*n* = 32)	Moderate/severe (*n* = 19)	
Carpal tunnel	11.8 ± 2.4[a]	13.2 ± 2.6[a]	12.3 ± 2.1[a]	0.062
5 cm Proximal	7.9 ± 2.2[b]	8.3 ± 2.2[b]	7.6 ± 1.4[b]	0.145

CSA: Cross sectional area; DPN: Diabetic peripheral neuropathy; NB: The same alphabets in each row indicate insignificant differences using scheffe post hoc test to evaluate intergroup differences.

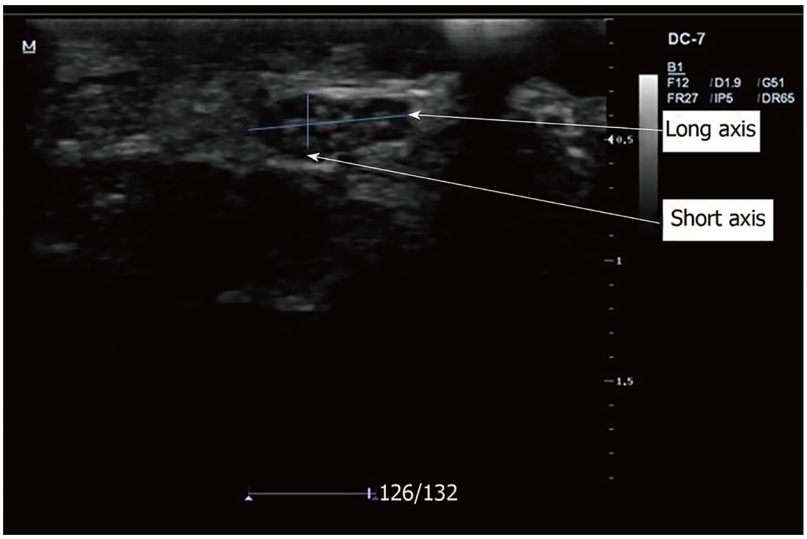

Figure 1 Transverse B-mode sonogram of the median nerve showing its long and short axes.

We also observed a significant additional increase in MN CSA in diabetics in the presence of DPN at the CATL similar to the findings of Watanabe *et al*[8] and Zaidman *et al*[22] which suggests that DPN can cause an increase in MN CSA apart from the hyperglycemic effect of DM. This statistical difference was however low (*P* = 049). The low statistical difference of MN CSA between subjects with and without PN in our study may be due to the fact that we did not diagnose PN using electrophysiological tests like NCS and as such, we may have misclassified some of the subjects with PN as those without PN. The statistically low significant difference between DM subjects with and without PN seen at the CATL was however not significant at 5cmCATL[23].

Furthermore, this study failed to establish a significant association between MN CSA and severity of DPN. It can, therefore, be inferred from the index study that the presence of DPN probably increases MN CSA to a given threshold beyond which no further increase is possible. Therefore, our study shows that the presence and not severity of DPN can give an additional thickening of the MN CSA at the CATL.

Long-term hyperglycemic state has been implicated in the occurrence of DPN[14,18,19]. Both FBG and HbA1c are short-term and long-term monitors of glycemic control respectively. The long-term monitor (HbA1c) gives a good estimate of glycemic control over a period of 3 months duration. Diabetic subjects were dichotomised into those with good and poor glycemic control based on their HbA1c levels

Table 5 Comparison of median nerve cross-sectional area between categories of age, gender, duration of diabetes mellitus, and glycemic control at carpal tunnel and 5 cm proximal to the carpal tunnel

		Median nerve CSA (mm²) at CATL					
		DM_nonDPN	*P*-value	DM_DPN	*P*-value	HC	*P*-value
Duration of DM	≤ 5 yr	11.4 ± 1.8	0.65	12.9 ± 2.4	0.99	-	-
	> 5 yr	11.8 ± 3.3		12.9 ± 2.7		-	
FBG	≤ 7 mmol/L	11.6 ± 2.3	0.79	13.2 ± 2.9	0.34	-	-
	> 7 mmol/L	11.4 ± 2.0		12.5 ± 1.8		-	
HBA1c	≤ 7%	11.0 ± 1.8	0.45	12.3 ± 2.4	0.20	-	-
	> 7%	11.7 ± 2.3		13.2 ± 2.6		-	
Age	≤ 60 yr	11.1 ± 1.9	0.25	12.9 ± 2.3	1.00	8.7 ± 1.5	0.37
	> 60 yr	12.0 ± 2.4		12.9 ± 2.7		8.4 ± 1.9	
Sex	Female	11.3 ± 1.9	0.62	13.2 ± 2.6	0.42	8.1 ± 1.2	0.02
	Male	11.7 ± 2.4		12.6 ± 2.4		9.0 ± 2.0	
		Median nerve CSA (mm2) at 5cmCATL					
		DM_nonDPN	***P*-value**	**DM_DPN**	***P*-value**	**HC**	***P*-value**
Duration of DM	≤ 5 yr	7.6 ± 1.9	0.87	7.7 ± 1.7	0.13	-	-
	> 5 yr	7.5 ± 1.5		8.5 ± 2.2		-	
FBG	≤ 7 mmol/L	7.5 ± 1.3	0.74	8.2 ± 1.9	0.57	-	-
	> 7 mmol/L	7.7 ± 2.5		7.9 ± 2.0		-	
HBA1c	≤ 7%	7.5 ± 1.4	0.86	7.7 ± 2.2	0.29	-	-
	> 7%	7.6 ± 1.9		8.3 ± 1.8		-	
Age	≤ 60 yr	7.3 ± 1.5	0.32	8.4 ± 2.1	0.25	5.5 ± 1.2	0.79
	> 60 yr	7.9 ± 2.2		7.8 ± 1.8		5.4 ± 1.5	
Sex	Female	7.0 ± 1.4	0.10	7.8 ± 1.7	0.37	4.9 ± 0.7	0.00
	Male	8.0 ± 2.0		8.3 ± 2.2		5.9 ± 1.6	

CATL: Carpal tunnel; 5cmCATL: 5 cm proximal to the carpal tunnel; DM_nonDPN: Diabetics without diabetic peripheral neuropathy; DM_DPN: Diabetics without diabetic peripheral neuropathy; HC : Healthy controls. *P* values < 0.05 are significant.

in this study. Glycemic control did not show significant association with the MN CSA at the 2 points of measurements among the DM subjects, even after a subgroup analysis of only those with DPN. There was also no significant correlation between HbA1c levels and MN CSA at both points of measurement (5cmCATL: r = -0.012, P = 0.916 and CATL: r = 0.034, P = 0.758). This observation contrasts the findings of Watanabe *et al*[8] in 32 DM subjects who reported significant correlation between MN CSA and HbA1c levels despite fewer sample size in their study relative to ours. Genetic and racial differences may have contributed to this.

Since long-term hyperglycaemic state has been implicated in the occurrence of DPN[14,18,19], and MN CSA further thickened in the presence of PN in diabetics in this study, it would have been expected that a poor glycaemic state would be associated with a further thickening of the MN CSA in the diabetics with PN and show positive correlation with HbA1c. Factors that were not explored in this study such as impaired insulin signalling, insulin growth factor and C-peptide that mediate DPN as suggested by Dobretsov *et al*[24] and /or genetic factors may have contributed to the contrasting findings in our study.

Duration of DM of more than 5 years had no additional effect on the MN CSA at the 2 points of measurement. The duration of diabetes was estimated from the time of diagnosis in a hospital in this study. This obviously is a conservative estimation as patients would have had the disease before presenting to the hospital. This may be responsible for the insignificant association between MN CSA and duration of DM seen in our study even among DM subjects with PN.

In this study, we used a modified clinical history part of Michigan Neuropathy Screening Instrument (MNSI) questionnaire to identify subjects with peripheral neuropathy. Peripheral neuropathy could be large fiber mono-neuropathy/polyneuropathy or isolated small fiber neuropathy. We could only have

Table 6 Relationship between median nerve cross-sectional area on ultrasound and clinical/laboratory parameters of the diabetic subjects

Variables	CATL		5cmCATL	
	r	*P*-value	*r*	*P*-value
Age	-0.041	0.898	-0.050	0.650
Sex	-0.058[1]	0.601	-0.050[1]	0.651
Weight	0.094	0.390	-0.041	0.708
Height	0.042	0.703	0.025	0.817
BMI	0.091	0.407	-0.056	0.611
SBP	-0.126	0.251	0.050	0.648
DBP	0.043	0.693	0.050	0.648
TC	0.092	0.402	0.092	0.056
Duration of DM	0.110[2]	0.318	0.151[2]	0.167
FBG	-0.059	0.591	0.026	0.811
HbA_{1c}	-0.012	0.916	0.034	0.758

[1]Point-biserial correlation coefficient;
[2]Spearman correlation coefficient. CATL: Carpal tunnel; 5cmCATL: 5 cm proximal to the carpal tunnel; *r*: Pearson correlation coefficient;; CSA: Cross sectional area; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; DM: Diabetes mellitus; FBG: Fasting blood glucose; HbA1c: Glycated haemoglobin *P* values < 0.05 are significant.

been sure of MN neuropathy in our subjects if we had performed neurophysiological study of the MN. Also, we only assessed MN in this study on ultrasound. It is possible that our study participants may have had mono-neuropathy not affecting the MN or have isolated small fibre neuropathy. This could have accounted for the lack of correlation between MN CSA and disease duration, FBG, HBA1c levels in the index study.

DPN symptoms are induced by factors such as total hyperglycemic exposure, high lipid levels, blood pressure, increased height, exposure to high concentrations of ethanol. Also, hereditary factors are considered. In addition to the matching of our DM and HC subjects for age and sex, the results of their anthropometric and laboratory parameters showed that there was no significant differences in their height, BP and TC. Confounders like hypertension, smoking, and alcohol consumption were also eliminated by excluding subjects with a history of these risk factors from the two study groups.

Evidence from this study may have been limited by the selection bias of our hospital-based setting as subjects enrolled were only those that presented in the teaching hospital. We, however, minimised this by recruiting consecutive consenting subjects into the study. Another limitation is the fact that we did not confirm neuropathy using neurophysiological tests like NCS which uses supramaximal stimuli and recruits non-selectively all available fibres (both large and small fibres) and involves the proximal and distal parts of the nerve trunks. NCS would have picked abnormality in the function of the nerves even in the absence of clinical symptoms and as such we would have been able to diagnose subclinical peripheral neuropathy in our study subjects.

However, our findings are unique in that we report findings from diabetics of Nigerian origin. To the best of our knowledge, MN CSA measured on ultrasound in diabetic neuropathy has not been reported in Nigeria prior to this study.

We conclude from our study that DM subjects had thicker MN CSA at 5cmCATL and at CATL compared to their age- and sex-matched HC, Diabetics with PN had thicker MN CSA at the CATL but not at 5cmCATL compared with those without PN and MN CSA had no significant relationship with age, gender, severity of DPN, duration of DM or glycemic control in diabetics.

We recommend the CATL over 5cmCATL as the point of measurement for MN CSA when evaluating the MN in diabetics as both the increase in the MN CSA secondary to DM and additional thickening in presence of DPN seen in this study occurred at the CATL while only the increase secondary to DM occurred at 5cmCATL. Evaluation of the MN CSA in DM subjects is only recommended before DPN sets in as no additional thickening of the MN CSA with a worsening grade of DPN was seen in this study.

ARTICLE HIGHLIGHTS

Research background

Peripheral neuropathy (PN) is a common complication of diabetes mellitus. High-resolution ultrasonography gives good morphological detail in the peripheral nerves.

Research motivation

Sonographic measurement of the Median nerve cross-sectional area may be a valuable tool in addition to clinical examination in identifying subjects with peripheral neuropathy in regions where standard electrophysiological studies like nerve conduction test are not available.

Research objectives

We evaluated the relationship between median nerve cross-sectional area (CSA) and the presence of PN in a cohort of adult diabetic Nigerians.

Research methods

A one-year cross-sectional study carried out in diabetic subjects recruited in the endocrinology unit of a Nigerian tertiary hospital and age- and sex-matched controls.

Research results

This study demonstrates that the median nerve is thicker in CSA at the carpal tunnel (CATL) and 5 cm proximal to the carpal tunnel (5cmCATL) in diabetic subjects than in age- and sex-matched healthy controls. Further thickening in the median nerve CSA is seen in the presence of diabetic peripheral neuropathy at the carpal tunnel but not at a point 5 cm proximal to it. Median nerve size has no significant relationship with age, gender, severity of DPN, duration of DM or glycemic control in our diabetic subjects.

Research conclusions

This study done in Diabetics of Nigerian origin adds to the current literature that Diabetic subjects have thicker MN CSA compared to their age- and sex-matched controls. Median nerve CSA was also thicker at the CATL in Diabetics with PN than in those without PN.

Research perspectives

We suggest the need for longitudinal studies in diabetic subjects who have median nerve neuropathy confirmed with nerve conduction test to elucidate the progressive effect of diabetic peripheral neuropathy on MN CSA.

FOOTNOTES

Author contributions: Attah FA suggested the concept, did the data collection and initial write-up of the manuscript; Asaleye CM was involved in study design, literature search and editing of the manuscript; Omisore AD was involved in study design, data analysis, writing of the final version of the manuscript; Kolawole BA did literature search and editing of the manuscript; Aderibigbe AS did data collation and was involved with analysis; Alo M was part of patient recruitment and manuscript editing.

Institutional review board statement: The study was approved by the Ethical committee of the Obafemi Awolowo University Teaching Hospitals Complex, Ile Ife, Osun State, Nigeria, and a copy is uploaded with the submission.

Informed consent statement: Written informed consent was obtained from all study participants before their inclusion into the study.

Conflict-of-interest statement: The authors have no conflict of interest to report.

Data sharing statement: No additional data are available.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Early *vs* late oral nutrition in patients with diabetic ketoacidosis admitted to a medical intensive care unit

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Abstract

BACKGROUND

Diabetic ketoacidosis (DKA) has an associated mortality of 1% to 5%. Upon admission, patients require insulin infusion and close monitoring of electrolyte and blood sugar levels with subsequent transitioning to subcutaneous insulin and oral nutrition. No recommendations exist regarding the appropriate timing for initiation of oral nutrition.

AIM

To assess short-term outcomes of oral nutrition initiated within 24 h of patients being admitted to a medical intensive care unit (MICU) for DKA.

METHODS

A retrospective observational cohort study was conducted at a single academic medical center. The patient population consisted of adults admitted to the MICU with the diagnosis of DKA. Baseline characteristics and outcomes were compared between patients receiving oral nutrition within (early nutrition group) and after (late nutrition group) the first 24 h of admission. The primary outcome was 28-d mortality. Secondary outcomes included 90-d mortality, MICU and hospital lengths of stay (LOS), and time to resolution of DKA.

RESULTS

There were 128 unique admissions to the MICU for DKA with 67 patients receiving early nutrition and 61 receiving late nutrition. The APACHE (Acute Physiology and Chronic Health Evaluation) IV mortality and LOS scores and DKA severity were similar between the groups. No difference in 28- or 90-d mortality was found. Early nutrition was associated with decreased hospital and MICU LOS but not with prolonged DKA resolution, anion gap closure, or greater rate of DKA complications.

CONCLUSION
In patients with DKA, early nutrition was associated with a shorter MICU and hospital LOS without increasing the rate of DKA complications.

Key Words: Diabetes mellitus; Diabetic ketoacidosis; Diabetic complications; Acidosis; Ketosis; Critical care

Core tip: Considering variability of timing in reinstitution of oral diet in patients with diabetic ketoacidosis and lack of guideline recommendations, we investigated whether early oral nutrition is safe. We found that oral feeding instituted in the first 24 h appeared safe and resulted in shorter intensive care unit and hospital lengths of stay.

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INTRODUCTION

Diabetic ketoacidosis (DKA), a potentially dangerous complication of diabetes, has an associated mortality of 1% to 5%[1]. It causes severe insulin deficiency, electrolyte abnormalities, and dehydration, and often requires admission to an intensive care unit (ICU). Upon admission, patients require insulin infusion and close monitoring of electrolyte and blood sugar levels with subsequent transitioning to subcutaneous insulin and oral nutrition. No recommendations exist regarding the appropriate timing for initiation of oral nutrition. Potential disadvantages of oral nutrition administered within the first 24 h of admission to an ICU (early nutrition) include difficulty in blood sugar monitoring and insulin dosing, altered mental status predisposing to aspiration, and worsening of nausea, vomiting, and abdominal pain.

Due to these concerns and the lack of definitive recommendations, many institutions have varying protocols regarding the initiation of oral nutrition. Our study investigates the safety of early nutrition in adult DKA patients admitted to a medical ICU (MICU).

MATERIALS AND METHODS

A retrospective observational cohort study was conducted at a single academic institution (Baylor Scott and White Heath, Temple, TX, United States) from December 2015 to January 2017. The study was approved by the local institutional review board and granted a waiver of informed consent. The study participants included all patients admitted to the MICU with the diagnosis of DKA. Only the first admission during the specified time frame for each patient was included. Exclusion criteria were age less than 18 years old, pregnancy, admission with DKA to a general ward or other type of ICU and leaving the hospital against medical advice. Data was collected by review of the electronic medical records. The time of first oral intake was labeled as the initiation of oral nutrition. The resolution of DKA was defined as achieving a serum glucose < 200 mg/dL and satisfying two of the following three criteria: pH ≥ 7.3, serum bicarbonate ≥ 15 meq/L, and anion gap ≤ 12. The anion gap was corrected using the value of the closest serum albumin measurement[1]. The severity of DKA was defined by arterial pH, serum bicarbonate, anion gap, and presence of altered mentation according to the American Diabetes Association consensus statement[1]. Early nutrition was defined as the initiation of nutrition within the first 24 h of admission. Late nutrition was defined as the initiation of nutrition after the first 24 h of admission.

Statistical analysis

Characteristics of the study sample were assessed using descriptive statistics. Frequencies and percentages were reported for categorical variables and means and standard deviations (or medians and ranges, if appropriate) were reported for continuous variables. Wilcoxon-Mann-Whitney tests were used to compare non-normally distributed continuous variables between groups. Chi-square and Fisher exact tests were used to compare categorical variables between groups. SAS version 9.4 and StatXact version 11 software was used to perform the statistical analysis. Statistical significance is expressed as $^{a}P < 0.05$, $^{b}P < 0.01$.

RESULTS

There were 330 admissions to the MICU for a diagnosis of DKA. After excluding repeated hospitalizations and those satisfying exclusion criteria, the final cohort consisted of 128 unique patient admissions. Of those patients, 67 received early nutrition and 61 received late nutrition.

Baseline characteristics are described in Table 1. The patient population had a mean age of 47.3 (SD = 17.7) years, 50.8% were female and race was predominately white (65%). The severity of illness scores, Acute Physiology and Chronic Health Evaluation (APACHE) IV mortality and APACHE IV length of stay (LOS) scores, were 9.9 (SD = 18.5) and 4.6 (SD = 1.75), respectively. Comparing the early and late nutrition groups found no statistically significant difference between the groups in age, race, severity of illness based on APACHE IV mortality and LOS indices and DKA severity. A statistically significant difference between the early and late nutrition groups existed in terms of sex (37% *vs* 62% female, $P = 0.0047$).

Outcomes are described in Table 2. The overall 28-d mortality was 3.1 % (4 patients) and 90-d mortality was 3.9% (5 patients). Mean hospital and MICU LOS were 6.16 (SD = 6.54) and 2.21(SD = 3.37) days respectively. There were no differences in the early and late nutrition groups in terms of mortality at 28 d (2.34% vs 0.78%, $P = 0.62$) and at 90 d (2.36% *vs* 1.57%, $P = 1.00$). Early nutrition group was not associated with longer mean time to anion gap closure ($P = 0.1642$) or DKA resolution ($P = 0.1410$). There was a significant decrease in the ICU LOS (1.38 *vs* 3.12, $P = 0.0002$) and overall hospital LOS (4.16 *vs* 8.35 $P = 0.0001$) in the early versus the late nutrition group.

Additionally, no significant difference in mean number of episodes of hyperkalemia (0.56 *vs* 0.43, $P = 0.37$), hypoglycemia (0.97 *vs* 1.54, $P = 0.18$), or severe acidosis (0.04 *vs* 0.20, $P = 0.18$) existed between the early and late nutrition groups. However, fewer episodes of hypokalemia (1.18 *vs* 2.21, $P = 0.0022$) and hypophosphatemia (0.73 *vs* 1.67, $P = 0.0052$) occurred in the early nutrition group.

DISCUSSION

We found that initiating oral nutrition in patients with DKA within the first 24 h of admission to the MICU was safe and decreases hospital and MICU LOS in our cohort of patients. Our 90-d mortality rate is consistent with prior studies[2]. The overall low mortality rate made the comparison between the early and late nutrition groups unlikely to reach statistical significance. Our analysis also demonstrated no difference in secondary outcomes, including time to normalization of the anion gap and resolution of DKA, and mean instances of hypoglycemia, hyperkalemia, and severe acidosis. However, a significant decrease in instances of hypokalemia and hypophosphatemia occurred. Finally, ICU and overall hospital LOS was significantly shorter for the early nutrition group.

DKA results in over 100000 admissions per year in the United States and has significant medical costs [1]. Mortality rates remain low between 1%-2.4%, with the cause of death in DKA patients often stemming from concurrent acute medical conditions and comorbidities[2,3]. The most appropriate location of care delivery for these patients is dictated by local practices, and recent studies report favorable outcomes with management on general hospital wards[4].

The role of nutrition in critical care cannot be overemphasized. The stress of critical illness places an enormous metabolic demand on the body[5]. Adequate nutrition has multiple advantages that include replenishing energy stores and protecting against ICU- and hospital-acquired complications[5]. However, the optimal nutritional components in the ICU remain controversial, and new evidence challenges the intuitive tendency to supplement critically ill patients with high-calorie nutrition[6].

Increasing evidence suggests that ketone bodies play a role in hunger control through a yet an unknown process[7]. This facilitated introduction of the ketogenic diet as an effective modality of weight loss. Additionally, elevated free fatty acid (FFA) levels, which are often observed in starvation states, have been shown to reduce food intake by acting on specific hypothalamic neurons[7]. As it pertains specifically to DKA, a higher degree of ketonemia and elevated circulating FFA could suppress hunger and potentially explains the delay in oral intake when initiated upon the patient's demand. In our study, beta hydroxybutyrate (BHB) and FFA levels were not measured. Varying degrees of of ketonemia in the study groups may have contributed to the difference in LOS and time to resolution of

Table 1 Baseline characteristics

	Entire cohort	Early nutrition	Late nutrition	*P* value
n	128	67	61	
Age, mean (yr)	47.3 (SD = 17.7)	45.7 (SD = 18.4)	49.1 (SD = 16.9)	0.1970
Race (*n*)				
African American	25% (32)	23.9% (16)	26.2% (16)	0.1950
Caucasian	65% (83)	67.2% (45)	62.3% (38)	
Other	10% (13)	8.9% (7)	11.5% (7)	
Female sex (*n*)	50.8% (65)	37.3% (25)	62% (38)	0.0047
DKA severity				
Mild	51	33	28	0.8997
Moderate	36	19	17	
Severe	31	15	16	
Mean APACHE IV Mortality	9.9 (SD = 18.5)	6.0 (SD = 12.7)	14.1 (SD = 22.5)	0.1170
Mean APACHE IV LOS	4.6 (SD = 1.8)	4.2 (SD = 1.5)	4.8 (SD = 2.0)	0.8400

APACHE: Acute Physiology and Chronic Health Evaluation; LOS: Length of stay; SD: Standard deviation.

Table 2 Outcomes

	Early nutrition	Late nutrition	*P* value
Mean time to AG normalization (h)	11.7 (SD = 15.6)	20.0 (SD = 40.7)	0.1642
Mean time to DKA resolution (h)	15.4 (SD = 18.8)	19.6 (SD = 32.6)	0.1410
Mortality at 28 d (*n*)	2.34% (3)	0.78 (1)	0.6300
Mortality at 90 d (*n*)	2.34% (3)	1.57% (2)	1.0000
Hospital LOS (d)	4.16 (SD = 2.63)	8.35 (SD = 8.85)	0.0001
ICU LOS (d)	1.38 (SD = 1.17)	3.12 (SD = 4.58)	0.0002
Mean number of complication occurrences:			
Hypoglycemia	0.97 (SD = 1.49)	1.54 (SD = 2.47)	0.1804
Hypokalemia	1.18 (SD = 1.4)	2.21 (SD = 2.1)	0.0022
Hyperkalemia	0.43 (SD = 0.72)	0.56 (SD = 0.89)	0.3706
Hypophosphatemia	0.73 (SD = 0.9)	1.67 (SD = 2.4)	0.0052
Severe acidosis	0.04 (SD = 0.21)	0.20 (SD = 0.73)	0.1356

DKA: Diabetic ketoacidosis; ICU: Intensive care unit; LOS: Length of stay; SD: Standard deviation.

DKA. However, we found no statistical difference between the groups in either the level of severity of DKA in both groups.

The potential for certain types of food to exacerbate ketosis may lead many physicians to withhold oral nutrition during DKA. Although reducing patients' initial oral intake of a low- carbohydrate diet might promote ketogenesis, the magnitude of its effect is low compared with the ketosis caused by uncontrolled diabetes. The maximum level of ketonemia achieved by a physiologic ketosis due to diet is 7-8 mmol/L as compared with > 25 mmol/L found in DKA[7]. The dietary augmentation of ketosis likely becomes even less significant with the initiation of insulin treatment and carbohydrate delivery to the cells.

In our institution, every patient diagnosed with DKA is admitted to the MICU as a result of level of clinical care related to a continuous insulin infusion. This practice provided the opportunity to assess the safety of early nutrition in all DKA patients. Despite the widespread use of DKA severity for the purposes of deciding the appropriate level of care, the direct link between estimated severity and

outcomes has not been established. Nevertheless, individual components of severity assessment, such as mental status and pH, have been associated with worsened outcomes. Altered mental status in particular could be a manifestation of a more severe underlying condition preventing patients from early nutrition and disproportionately worsening outcomes in the late nutrition group. The DKA severity based on available measurements of initial bicarbonate concentration, pH, and GCS did not differ between the groups in our study. Additionally, there was no statistically significant differences between the groups in the severity of illness represented by APACHE IV mortality and LOS scores.

Patients with DKA often have abdominal pain, nausea, and vomiting, ultimately leading to oral-intake intolerance. Consensus guidelines associate patients' readiness to eat with resolution of ketoacidosis[1]. However, it is possible that when oral nutrition was administered on demand in our study, patients having more severe DKA and worse symptoms on presentation would end up in the late oral nutrition group. This may have implications in further studies investigating any benefit of mandatory early oral nutrition in DKA where randomization would be a key to ensure similar severity of ketoacidosis in the investigation groups.

To control for possible delay in meeting the strict DKA resolution criteria, we separately analyzed the time to normalization of anion gap as this likely represents cessation of ketosis with no change in outcomes. Both of these results were consistent with prior studies[8]. While patients starting oral nutrition after the first 24 h of admission had longer time to DKA resolution and anion gap normalization, neither was statistically significant. Notably, both the time to AG closure and to resolution of acidosis in the late nutrition group were less than 24 h. It is possible that the delay in oral diet resumption may have contributed to delayed transfer of these patients out of the MICU.

Our study confirmed the existing variability among physicians regarding the optimal timing of initiating oral nutrition in patients DKA. Although the study population size was likely too small to demonstrate a significant difference in the mortality, oral nutrition provided to DKA patients on demand appears to be safe. Early reinstitution of oral nutrition did not result in worsening of DKA complications and was associated with improvement in hypokalemia and hypophosphatemia. Finally, on-demand oral nutrition reinitiated within the first 24 h of admission has the potential to shorten ICU and overall hospital LOS.

ARTICLE HIGHLIGHTS

Research background

Diabetic ketoacidosis (DKA) is a common reason for hospitalization in patients with diabetes. It results in significant morbidity, mortality, and financial burden. Research and quality improvement efforts have been put forth to investigate the triggers and risk factors associated with ketoacidosis to prevent initial episode of DKA and minimize recurrence. In the meantime, the standard of care in management of DKA has been more clearly defined attention to serum glucose levels, electrolytes, acidosis and diligent evaluation for and treatment of the underlying etiology. Together, these advances resulted in significant reduction of mortality associated with DKA over the years. Nevertheless, many aspects of care for DKA patients remains unanswered, including severity stratification and appropriate level of care. Many institutions continue to accept patients with DKA to the intensive care unit (ICU) due to frequent electrolyte and glucose monitoring and meticulous insulin titration. Minimizing financial burden and hospital acquired complications associated with frequent and prolonged ICU stay is the subject of current and future investigations.

Research motivation

Tolerance of oral diet is regarded as a marker for resolution of ketoacidosis in DKA patients. Its administration is often postponed until biochemical confirmation of the resolution of ketoacidosis due to fear of unpredictable glucose and electrolyte changes. We hypothesized that allowance of on demand oral nutrition in DKA patients is safe and has a potential to decrease the length of hospitalization.

Research objectives

We aim to compare the mortality, rate of complications, and length of stay between DKA patients receiving oral nutrition before and after the first 24 h of ICU admission.

Research methods

Retrospective data collection was conducted establishing the demographics, initial biochemical characteristics, and outcomes of patients admitted to our single academic medical center. Outcomes included common complications of DKA, 28- and 90-d mortality, and length of ICU and hospital stay. Bivariate analysis was then performed comparing these variables between the two subgroups defined by the timing of their first oral intake.

Research results

The timing of oral nutrition in DKA patients was heterogenous between different care teams with 52.3% of patients restarting oral intake in the first day of admission. This did not result in increased mortality (2.34% *vs* 0.78%, $P = 0.62$) or rate of complications such as hyperkalemia (0.56 *vs* 0.43, $P = 0.37$), hypoglycemia (0.97 *vs* 1.54, $P = 0.18$), or severe acidosis (0.04 *vs* 0.20, $P = 0.18$). Despite having similar overall illness severity and severity of DKA itself, the DKA patients who received oral nutrition in the first 24 h of their admission had a shorter ICU (1.38 *vs* 3.12, $P = 0.0002$) and (4.16 *vs* 8.35 $P = 0.0001$) hospital stay.

Research conclusions

Early oral nutrition (defined as oral intake in the first 24 h) administered on demand in patents admitted to ICU with DKA has a potential to safely reduce the length of stay.

Research perspectives

The study introduces the possibility of early oral nutrition in DKA to improve the length of stay. Further prospective randomized investigation is necessary to validate this finding.

FOOTNOTES

Author contributions: Lipatov K designed research; Lipatov K and Kurian KK performed research; Ghamande S, White HD and Arroliga AC revised methodology, Shaver C analyzed data; Lipatov K and Kurian KK wrote the paper; Ghamande S, White HD, Arroliga AC and Surani S revised and edited the paper.

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