Baseline Serum Metabolites Predict Fractures in a subset of Individuals who were Black and had Type 2 Diabetes

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INTRODUCTION: Type 2 Diabetes Mellitus (T2D) is a metabolic disorder with increasing prevalence worldwide. Fractures are increased in people with T2D[1]. Black patients have higher bone mineral density than White individuals[2], suggesting the potential for distinct mechanisms for fracture within specific populations. Changes to circulating metabolites occur in populations with T2D, and are unique in individuals who are Black[3]. We hypothesized that changes to metabolism during T2D may contribute to skeletal fragility in individuals who were Black.

METHODS: We focused exclusively on participants in the Action to Control Cardiovascular Risk in Diabetes trial (ACCORD, ClinicalTrials.gov NCT00000620), a randomized clinical trial of patients with T2D[4], who were Black, an understudied population with regards to fracture risk. All data and biospecimens obtained for this study were de-identified; this work was not considered research involving human subjects, as determined by the MaineHealth Research Compliance Office. 1,791 participants were Black and had records for "frac", indicating their participation in ACCORD BONE. Of these 1,791 identified participants, sufficient sample was available in 571 (32%). We obtained 349 female and 321 male serum samples (average age 62.1 years) from the National Heart, Lung, and Blood Institute (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center. Using ultrahigh-performance liquid chromatography (UHPLC)[5], metabolites were analyzed in baseline serum samples that had been collected at the initial visit. Metabolite identification was performed using in house authentic standards analysis.

Using longitudinal data from ACCORD BONE[6], we compared participants who later fractured with those who did not fracture. Principal component analysis was used to identify sets of metabolites that differed between participants who later fractured versus those with no records of fracture. Metabolites containing no variance in at least one group (fractured or non-fractured individuals) were identified and tested by Mann-Whitney U tests. For metabolites containing variance in both groups, individual t-tests were performed in R to assess differences between participants who later fractured and those who had no recorded fractures. Adjusted p-values were calculated. To determine if the baseline characteristics of the participants affected the association of individual metabolites with fracture, we used generalized linear models. We evaluated the predictive power of each individual metabolite (predictor) on fracture (outcome), using patient characteristics as covariates. Patient characteristics evaluated included sex, the number of years since diabetes diagnosis, age, baseline glycosylated hemoglobin (HbA1c), bodyweight, waist circumference, height, and body mass index (BMI).

RESULTS: Of the 571 participants included in our metabolomics assessments, 7.0% experienced a fracture. Baseline characteristics were not statistically significant between individuals who fractured and those who did not fracture (age, weight, height, waist circumference, BMI, HbA1c, fasting plasma glucose, or diabetes duration). Although not statistically different between fractured and nonfractured individuals, principal components 1 (PC1) and 2 (PC2) explained the greatest amount of variance within the dataset, 7.02% and 5.36%, respectively. Visual assessment of principal components 1 and 2 revealed three distinct clusters of participants, with fractured individuals present in all clusters. Individual metabolite analysis revealed circulating metabolites that were significantly different at baseline in those that fractured versus those who did not (Tab. 1). One metabolite had both adjusted and unadjusted p-values that reached significance: 7,8-dihydrofolate. In participants who later experienced a fracture, baseline metabolite abundance correlated with years since diabetes diagnosis and baseline HbA1c. Using a generalized linear model, significant interactions between the years since diabetes diagnosis and baseline HbA1c were identified for several individual metabolites (Fig 1). Sex, age, bodyweight, waist circumference, height, and BMI did not have interactions with individual metabolites and thus lacked an effect on the predictability of fracture risk in this population. In participants who later experienced a fracture, baseline levels of hydroxyproline, leucine, oxalate, and glutamine were positively correlated with the number of years since the participant's diagnosis of diabetes. Conversely, baseline levels of 3-hydroxysuberoylcarnitine and guanine were negatively correlated the number of years since the participant's diagnosis of diabetes. In participants who later experienced a fracture, baseline HbA1c negatively correlated with baseline measures of 4 metabolites: 2-octandioic carnitine, 3-hydroxysuberoylcarnitine, 2-octendioic carnitine, and 3-methylglutarylcarnitine.

DISCUSSION: In summary, the metabolic differences identified here highlight the role of altered systemic metabolism and its relationship to fracture risk. Using a large sample of individuals who were Black and had T2D, we identified individual serum metabolites that may serve as predictors of future fractures. Importantly, several metabolites identified with significant unadjusted p-values were related to the tricarboxylic acid cycle. The serum metabolomic profiles examined were not specific to products produced in bone and likely influenced by other organ systems as well as diet. Future work should validate the utility of these identified metabolites in other populations.

CLINICAL RELEVANCE: This work is the first to identify specific metabolites that may serve as predictors of fracture in individuals who are Black and have T2D. Our findings highlight the systemic changes in individual metabolites that occur prior to fracture in individuals who are Black and have T2D.

REFERENCES: [1]Hamann+ 2011 [2]Luckey+ 1989 [3]Lee+ 2016 [4]Buse+ 2007 [5]Drolet+ 2017 [6]Schwartz+ 2012

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Table 1. Future fractures were associated with baseline metabolite levels.

Product	Non-fractured *Average ± St.Dev*	Fractured *Average ± St.Dev*	Unadjusted p-value	Adjusted p-value (FDR)
7,8-Dihydrofolate	0 ± 0	0.0003 ± 0.0016	0.0003	0.0030
4-Guanidinobutanoate	0.7878 ± 0.6501	0.6093 ± 0.2518	0.0004	0.1940
Cortisol	0.5113 ± 0.2066	0.4243 ± 0.1532	0.0015	0.3031
Pyruvate	0.1430 ± 0.0422	0.1200 ± 0.0437	0.0024	0.3031
NAD	0.0090 ± 0.0138	0.0055 ± 0.0060	0.0027	0.3031
5-Amino 3-Oxohexanoic Acid	0.9967 ± 1.0788	0.7245 ± 0.4973	0.0040	0.3587
Dihydrouracil	0.1280 ± 0.0853	0.1035 ± 0.0491	0.0061	0.4517
Maltitol	0.0254 ± 0.1073	0.0118 ± 0.0138	0.0083	0.4846
5-Aminovaleric Acid	0.1579 ± 0.1879	0.0943 ± 0.1380	0.0087	0.4846

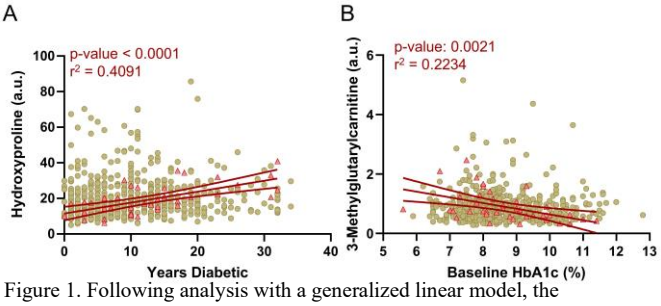

Figure 1. Following analysis with a generalized linear model, the interaction between years since diabetes diagnosis (A) and baseline HbA1c (B) were identified for individual metabolites (hydroxyproline and 3-methylglutarylcarnitine displayed).