METABOLISM CLINICAL AND EXPERIMENTAL XX (2013) XXX–XXX

Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness

Bryan C. Batch[a,b,*], Svati H. Shah[b,c,d], Christopher B. Newgard[b], Christy B. Turer[e], Carol Haynes[f], James R. Bain[a,b], Michael Muehlbauer[b], Mahesh J. Patel[c], Robert D. Stevens[a,b], Lawrence J. Appel[g], L. Kristin Newby[c,f], Laura P. Svetkey[b,h]

[a] Division of Endocrinology, Metabolism, and Nutrition, Duke University Medical Center, Durham, NC 27710, USA
[b] Sarah W. Stedman Nutrition and Metabolism Center, Duke University, Durham, NC 27710, USA
[c] Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA
[d] Duke Center for Human Genetics, Duke University Medical Center, Durham, NC 27710, USA
[e] Department of Pediatrics, UTSW Medical Center, Dallas, TX 75390–9063, USA
[f] Duke Clinical Research Institute, Durham, NC 27710, USA
[g] Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore, MD, USA
[h] Division of Nephrology, Department of Medicine, Duke University Medical Center, Durham, NC 27710, USA

ARTICLE INFO

Article history:
Received 21 June 2012
Accepted 7 January 2013

Keywords:
Obesity
Metabolomics
Insulin resistance
Metabolic risk

ABSTRACT

Objective. To identify novel biomarkers through metabolomic profiles that distinguish metabolically well (MW) from metabolically unwell (MUW) individuals, independent of body mass index (BMI).

Materials/Methods. This study was conducted as part of the Measurement to Understand the Reclassification of Disease of Cabarrus/Kannapolis (MURDOCK) project. Individuals from 3 cohorts were classified as lean (BMI<25 kg/m^2), overweight (BMI≥25 kg/m^2, BMI<30 kg/m^2) or obese (BMI≥30 kg/m^2). Cardiometabolic abnormalities were defined as: (1) impaired fasting glucose (≥100 mg/dL and ≤126 mg/dL); (2) hypertension; (3) triglycerides ≥150 mg/dL; (4) HDL-C <40 mg/dL in men, <50 mg/dL in women; and (5) insulin resistance (calculated Homeostatic Model Assessment (HOMA-IR) index of >5.13). MW individuals were defined as having <2 cardiometabolic abnormalities and MUW individuals had≥two cardiometabolic abnormalities. Targeted profiling of 55 metabolites used mass-spectroscopy-based methods. Principal components analysis (PCA) was used to reduce the large number of correlated metabolites into clusters of fewer uncorrelated factors.

Abbreviations: MW, Metabolically Well; MUW, Metabolically UnWell; BMI, Body Mass Index; MURDOCK, Measurement to Understand the Reclassification of Disease of Cabarrus/Kannapolis; HDL-C, High Density Lipoprotein Cholesterol; HOMA-IR, Homeostatic Model Assessment; PCA, Principal Components Analysis; CDC, Centers for Disease Control and Prevention; NHANES, National Health and Nutrition Examination Survey; LDL, Low Density Lipoprotein; hs-CRP, High Sensitivity C-Reactive Protein; CMRF, Cardiometabolic Risk Factor; CVD, CardioVascular Disease; WLM, Weight Loss Maintenance; STEDMAN, Study of the Effect of Diet on Metabolism and Nutrition; MS, Mass Spectrometry; LEU/ILE, Leucine/Isoleucine; NEFA, Non Esterified Fatty Acids; AA, African American; GLX, Glutamate/glutamine; NEFA, Non-Esterified Fatty Acids; ORN, Ornithine; ARG, Arginine; HIS, Histidine; AC, Acylcarnitines; GLY, Glycine; SER, Serine; PRO, Proline; CIT, Citruline; C22, C22 acylcarnitine; ASX, Aspartate/asparagine; HTN, Hypertension; DM, Diabetes Mellitus; HBUT, β-Hydroxybutyrate; KET, Ketone; ALA, Alanine; PHE, Phenylalanine; VAL, Valine; TYR, Tyrosine; MET, Methionine; CATHGEN, CATHeterization GENetics.

* *Corresponding author.* Duke University Medical Center, DUMC Box # 3031, Durham, NC 27710, USA. Tel.: +1 919 668 1219; fax: +1 919 681 9846.
E-mail address: bryan.batch@duke.edu (B.C. Batch).

0026-0495/$ – see front matter
http://dx.doi.org/10.1016/j.metabol.2013.01.007

Results. Of 1872 individuals, 410 were lean, 610 were overweight, and 852 were obese. Of lean individuals, 67% were categorized as MUW, whereas 80% of overweight and 87% of obese individuals were MUW. PCA-derived factors with levels that differed the most between MW and MUW groups were factors 4 (branched chain amino acids [BCAA]) [$p < .0001$], 8 (various metabolites) [$p < .0001$], 9 (C4/Ci4, C3, C5 acylcarnitines) [$p < .0001$] and 10 (amino acids) [$p < .0002$]. Further, Factor 4, distinguishes MW from MUW individuals independent of BMI.

Conclusion. BCAA and related metabolites are promising biomarkers that may aid in understanding cardiometabolic health independent of BMI category.

1. Introduction

Individuals who are overweight/obese are at increased risk for developing a wide range of diseases, including cardiovascular disease, cerebrovascular disease, and type 2 diabetes mellitus [1]. However, not all overweight/obese individuals will develop these diseases or risk factors for disease. A prior study by Wildman et al. [2] examined metabolic "wellness" in a cross sectional sample of the National Health and Nutrition Examination Survey (NHANES) 1999–2004 cohort. The cardiometabolic risk factors (CMRF) of interest in this study included elevated blood pressure, fasting glucose, reduced high density lipoprotein cholesterol (HDL-C), elevated low density lipoprotein cholesterol (LDL-C), elevated triglycerides, high sensitivity C-reactive protein (hs-CRP) and elevated homeostasis model assessment of insulin resistance (HOMA-IR) value. Individuals with one or fewer CMRF were considered metabolically well (MW) and those with two or more were considered metabolically unwell (MUW). In this population-based sample, 23.5% of lean individuals were metabolically unwell (body mass index [BMI] <25 kg/m^2), and 31.7% of obese individuals were metabolically well despite being obese (BMI$\geq$30 kg/m^2).

Novel molecular techniques may help to identify biomarkers that improve discrimination of risk beyond the risk predicted by BMI alone and elucidate the mechanisms underlying this seeming disparity between BMI and presence of metabolic risk factors.

Our group has used high throughput targeted metabolic profiling to identify a biosignature composed of branched chain amino acids (BCAA) and related catabolites that is strongly associated with insulin resistance [3,4], discriminates lean from obese individuals [3], is independently associated with coronary artery disease [5], and predicts who will have improvement in insulin resistance with moderate weight loss [6]. This signature also has been shown by another group to predict incident diabetes [7]. Therefore, we hypothesized that similar methods would identify novel biomarkers that distinguish individuals who are metabolically well (MW) from individuals who are metabolically unwell (MUW), independent of BMI.

2. Methods

The current analysis was conducted as part of the Measurement to Understand the Reclassification of Disease of Cabarrus/Kannapolis (MURDOCK) project, designed to identify novel biomarkers in a range of clinical conditions [8].

2.1. Study population

Individuals included in this analysis were pooled from the Weight Loss Maintenance (WLM) Clinical Trial [9,10], the CATHGEN cardiovascular biorepository [8], and the Study of the Effect of Diet on Metabolism and Nutrition (STEDMAN) project [11], using baseline clinical, demographic, and laboratory data. Stored baseline blood samples were analyzed as part of the MURDOCK project. Descriptions of each source of participants and biological samples are as follows:

2.1.1. Weight Loss Maintenance clinical trial

The WLM trial was a multi-center, randomized, controlled trial (clinicaltrials.gov Identifier: NCT00054925) to determine the effects of two behavioral strategies for maintaining weight loss compared with a usual care control group. The WLM trial methods and main results have been described in detail elsewhere [9,10]. Briefly, the study enrolled overweight and obese individuals (BMI 25–45 kg/m^2) aged 25 years or older who were taking medications for hypertension and/or dyslipidemia. Exclusion criteria were treatment for diabetes mellitus, recent cardiovascular event, weight loss of greater than nine kilograms in the preceding three months, recent use of weight loss medications, or prior weight loss surgery. At entry into the study, venous blood samples were obtained after an overnight fast. Within 1–2 h of phlebotomy, serum and plasma were frozen at −80 °C.

A total of 1035 participants from four United States centers were randomized in WLM. Of these, targeted metabolic profiling was performed on a random sample of 500 individuals [6]. Of these 500 individuals, those enrolled in both WLM and the STEDMAN project (see below) were excluded, leaving 462 unique individuals from WLM available for the current analysis.

2.1.2. The CATHGEN Study

The CATHGEN biorepository consists of over 9000 sequentially-recruited individuals undergoing cardiac catheterization at Duke University Medical Center (Durham, NC) [8]. The indication for catheterization for all subjects was clinical concern for ischemic heart disease. Patients with severe pulmonary hypertension or organ transplant were excluded. All subjects were fasting for a minimum of six hours prior to sample collection. After informed consent, blood was obtained from the femoral artery at the time of arterial access for cardiac catheterization, immediately processed to separate plasma, and frozen at −80 °C until later use. Targeted

Please cite this article as: Batch BC, et al, Branched chain amino acids are novel biomarkers for discrimination of metabolic wellness, Metabolism (2013), http://dx.doi.org/10.1016/j.metabol.2013.01.007