Published in final edited form as:
Metabolism. 2013 July ; 62(7): 961–969. doi:10.1016/j.metabol.2013.01.007.

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], and **Laura P Svetkey**[b,h]

[a]Division of Endocrinology, Metabolism, and Nutrition, Duke University Medical Center, Durham, North Carolina 27710

[b]Sarah W. Stedman Nutrition and Metabolism Center, Duke University, Durham NC 27710

[c]Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham NC 27710

[d]Duke Center for Human Genetics, Duke University Medical Center, Durham NC 27710

[e]Department of Pediatrics, UTSW Medical Center, Dallas, TX, 75390-9063

[f]Duke Clinical Research Institute, Durham NC 27710

[g]Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Medical Institutions, Baltimore, MD

[h]Division of Nephrology, Department of Medicine, Duke University Medical Center, Durham, North Carolina 27710

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

Corresponding Author: Bryan C. Batch, M.D., Duke University Medical Center, DUMC Box # 3031, Durham, NC 27710, Phone: 919-668-1219, Fax: 919-681-9846, bryan.batch@duke.edu.

Disclosures: None

Financial Disclosures: No authors reported any relevant financial disclosures or conflicts of interest.

Author Contributions:
Study concept and design: Batch, Shah, Turer, Haynes, Svetkey
Acquisition of data: Shah, Stevens, Muehlbauer, Bain, Svetkey
Analysis and interpretation of data: Batch, Shah, Newgard, Patel, Appel, Svetkey
Drafting of the manuscript: Batch, Shah, Svetkey
Critical revision of the manuscript for important intellectual content: Batch, Shah, Newgard, Turer, Haynes, Bain Muehlbauer, Patel, Stevens, Appel, Newby, Svetkey
Statistical analysis: Batch, Shah, Svetkey

obese (BMI≥30 kg/m^2). Cardiometabolic abnormalities were defined as: (1) impaired fasting glucose (≥100mg/dL and ≤126mg/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.

Results—Of 1,872 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], and 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.

Keywords

Obesity; Metabolomics; Insulin Resistance; Metabolic Risk

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 ≥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.

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].

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:

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 hours 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) 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 metabolic profiling and measurement of insulin and fasting glucose was previously performed on samples from 2023 CATHGEN individuals as part of the MURDOCK Horizon 1 Cardiovascular Disease project [8]; this population was also used for the current analysis. Individuals missing data for key variables necessary for definition of metabolic wellness and those with a clinical diagnosis of type II diabetes mellitus were excluded. Patients with Type II diabetes mellitus were excluded in order to be consistent with the other two cohorts. Further, we considered it appropriate to exclude those with type II diabetes because these individuals have high cardiovascular risk regardless of other known or novel markers of metabolic wellness. A total of 1273 CATHGEN individuals were included in the current analysis.

3) The STEDMAN Project—The STEDMAN project was designed to perform comprehensive metabolic profiling on lean and overweight/obese individuals and to

determine the effect on metabolic profiling of weight loss achieved by various means [11]. Profiling was performed on blood samples obtained after an overnight fast. A total of 137 individuals were included in the current analysis.

Current analysis

The protocols for the WLM trial, the STEDMAN Project and CATHGEN were approved by the Duke Institutional Review Board (IRB), and all participants provided written informed consent for future analyses of stored samples. The specific analyses conducted for the MURDOCK project were approved by the Duke Institutional Review Board under separate IRB review.

Definition of metabolically well and unwell

Individuals were classified into three categories based on baseline BMI: lean (BMI <25 kg/m^2), overweight (BMI ≥25 kg/m^2, <30 kg/m^2) and obese (BMI ≥30 kg/m^2). Cardiometabolic abnormalities were defined as: (1) impaired fasting glucose (≥100mg/dL and ≤126mg/dL); (2) hypertension (defined as a self-reported diagnosis of hypertension or taking a blood pressure medication). Additionally, in a subset of study participants (derived from the CATHGEN study; see below) in whom blood pressure measurements were also available, an individual without a clinical diagnosis of hypertension and taking no blood pressure medication was considered hypertensive if the resting systolic blood pressure was ≥130 or diastolic blood pressure was ≥85 mmHg; (3) triglycerides ≥150 mg/dL; (4) HDL-C <40 mg/dL in men or <50 mg/dL in women; and (5) insulin resistance (calculated Homeostatic Model Assessment [HOMA-IR] index of >5.13). The HOMA Index was calculated using the formula: HOMA-IR = (fasting insulin in uUI/mL * fasting glucose in mM)/22.5 [12]. Although a HOMA-IR of >2.60 is generally accepted as the clinical definition of insulin resistance [13] we chose a higher threshold because of apparent ethnic/racial differences in the clinically significant threshold [14], and to be consistent with previous reports of the relationship between metabolic wellness and BMI [2]. Metabolically well (MW) individuals were defined as having zero or one of these cardiometabolic abnormalities, and metabolically unwell (MUW) individuals were defined as having two or more cardiometabolic abnormalities.

Although other studies have considered hsCRP in the definition of metabolic abnormalities [2], hsCRP was not included in the definition of metabolically well or unwell in this study because the value was not measured for the majority of individuals.

Laboratory measurements

Targeted metabolic and other laboratory analyses were performed on baseline frozen plasma samples. Quantitative determination of metabolite levels was made for 37 acylcarnitines, 15 amino acids, ketones (KET), beta-hydroxybutyrate (HBUT), total free fatty acids (NEFA), glucose and insulin. Sample preparation methodology and coefficients of variation for each assay have been reported [3, 5]. The Sarah W. Stedman Nutrition and Metabolism metabolomics/biomarker core laboratory was blinded to weight loss status, BMI and other clinical variables, and study participants' samples were randomly distributed during analysis.

Standard clinical chemistry methods were used for glucose and insulin with reagents from Roche Diagnostics (Indianapolis, IN), and for free fatty acids (total) and ketones (total and 3-hydroxybutyrate) with reagents from Wako. All assays were performed on a Hitachi 911 clinical chemistry analyzer.

For mass spectrometry (MS)-profiled metabolites (acylcarnitines and amino acids), the following protocol was used, as previously described [15, 16]. Proteins were first removed by precipitation with methanol. Aliquoted supernatants were dried, and then esterified with hot, acidic methanol (acylcarnitines) or *n*-butanol (amino acids). Tandem mass spectroscopy (MS) analysis was done with a Quattro Micro instrument (Waters Corporation, Milford, MA) and employed stable-isotope-dilution. Quantification of the "targeted" intermediary metabolites was facilitated by addition of mixtures of known quantities of stable-isotope internal standards to samples. Leucine/isoleucine (LEU/ILE) are reported as a single analyte because they are not resolved by our MS/MS method, and include contributions from *allo*-isoleucine and hydroxyproline. These isobaric amino acids normally contribute little to the signal attributed to LEU/ILE [16]. The acidic conditions used to form butyl esters results in partial hydrolysis of glutamine to glutamic acid and of asparagine to aspartate. Values reported as GLX or ASX are not meant to signify the molar sum of glutamate and glutamine, or of aspartate and asparagines. They signify the amount of glutamate or aspartate plus the contribution of the partial hydrolysis reactions of glutamine and asparagine, respectively.

Statistical analysis

Given that many metabolites reside in overlapping pathways, correlation of metabolites is expected. We used principal components analysis (PCA) to reduce the large number of correlated variables into clusters of fewer uncorrelated factors, as done in previous metabolic studies [3, 5]. Metabolites that had >25% of values as "0" (i.e., below the lower limits of quantification for that assay) were not analyzed further, leading to exclusion of two acylcarnitine species. PCA was performed on baseline levels of acylcarnitines, amino acids, NEFA, ketones and HBUT. Factors with an eigenvalue ≥1.0 were identified and varimax rotation was performed to produce interpretable factors. Metabolites with a factor load ≥0.4 were reported as composing a given factor. Scoring coefficients were constructed and used to calculate baseline metabolomic factor scores for each individual (weighted sum of the values of the standardized metabolites within that factor, weighted on the factor loading for each individual metabolite).

In our primary analysis, we used unadjusted Wilcoxon rank sum to assess the significance of the difference in mean metabolite factor levels between metabolically unwell and metabolically well individuals, stratified by three BMI categories, and for the overall group (BMI categories combined). To ensure that the significance of the difference in mean metabolic factor levels between the metabolically unwell and well individuals was not confounded by differences in BMI, we also constructed linear regression models adjusted for BMI. Finally, to further assess whether factor levels were predictive of metabolic wellness independent of BMI, we used logistic regression models in the overall group adjusted for BMI.

As all analyses were exploratory in nature and given co-linearity of the metabolites, two-sided p-values unadjusted for multiple comparisons are presented; however, results interpreted in the context of a conservative Bonferroni correction are also reported. Nominal statistical significance was defined as $p \leq 0.05$. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary NC).

RESULTS

Metabolic characteristics of study population

A total of 1872 individuals were included in this analysis. When stratified by BMI, 410 individuals were lean, 610 were overweight, and 852 were obese. Table 1 presents baseline

clinical characteristics of the cohort. Obese individuals were younger (57.0 ± 10.8 years) compared with overweight or lean individuals (61.7 ± 11.0 years and 61.5 ± 13.7 years, respectively). Approximately 50% of obese and lean individuals were female compared with only 38% of overweight individuals. African Americans (AA) were disproportionately represented in the obese category compared with the lean and overweight categories. As expected, obese individuals overall were more insulin resistant. The prevalence of hypertension was similar across BMI groups.

Table 2 shows baseline characteristics of the study population stratified by both BMI and MW and MUW categories. The majority (67%) of the lean individuals were categorized as MUW. The percent of MUW increased with increasing BMI; 80% of the overweight and 87% of the obese groups classified as MUW. In each strata of BMI, MUW was associated with male sex and Non-AA.

Metabolomic profiling

Principal components analysis (PCA) identified 12 metabolic factors (Table 3) composed of correlated metabolites. As expected, and consistent with our prior work [3–6, 17], metabolites generally clustered within factors in biologically plausible pathways. In particular, factors that we previously identified as associated with insulin resistance, type 2 diabetes and cardiovascular disease phenotypes were identified, (factor 4, composed of branched chain amino acids and related catabolites, and factor 9 composed of C4/Ci4, C3, C5 acylcarnitines).

Table 4 shows the association of metabolic factors with MW vs. MUW categories for the overall population and stratified by BMI. When considering the population as a whole, there were significant differences between MW and MUW for factor 3 (composed of ketones and related metabolites) [p =.0058], factor 4 (BCAA) [p <.0001], factor 6 (medium chain acylcarnitines) [p<.0001], factor 8 (various metabolites, glutamate/glutamine, ornithine, arginine and histidine) [p<.0001], factor 9 (C5 acylcarnitines) [p<.0001], factor 10 (glycine, serine and ornithine) [p= .0002], factor 11 (proline, citrulline, and C22 acylcarnitine) [p= .0068], and factor 12 (aspartate/asparagine and arginine) [p=.04]. The four factors with the greatest magnitude of difference between the MW and MUW categories overall were factor 4 (BCAA), factor 8 (various metabolites), factor 9 (C4/Ci4, C3, C5 acylcarnitines) and factor 10 (amino acids) (p<.0001 for factors 4, 8, 9 respectively, p<.0002 factor 10).

The primary goal of stratifying analyses by BMI was to understand the relationship of metabolic factors within clinically relevant BMI groups; in these analyses, only Factor 8 was significantly different overall [p=<.0001] and in each of the individual BMI categories [p<.0001 lean, p= .0008 overweight, p=.0015 obese]. Within BMI categories, some but not all of these differences persisted. For example, Factors 3 and 10 although different overall [p= .0058 and .0002, respectively], were not different in any of the individual BMI categories; Factor 4 and 9 were only significantly different overall [p<.0001, both] and in the obese category [p=.0006 and p=.0003, respectively]. Higher factor 4 levels were seen in the metabolically unwell, and this relationship persisted after adjusting for age (p<0.0001 overall p=0.0038 obese). Factor 4 showed a trend for significance (with a similar direction of effect) in both the overweight and lean categories. Other factors that were significantly different overall showed various patterns within the BMI categories (Table 4). Comparisons within BMI categories must be interpreted in the context of sample size/power: the MW group was fairly small in each BMI category (137, 119, and 114, respectively), limiting power for comparisons with MUW.

To ensure that the difference between metabolic factor levels and metabolic wellness was not due to differences in BMI, we also performed linear regression analyses adjusted for

BMI. These analyses showed that the difference in mean metabolite factor levels for each of these factors remained significantly different in the overall group even after adjusting for BMI (factor 3 p=0.005; factor 4 p=0.0001; factor 5 p=.02; factor 6 p=0.002; factor 8 p<0.0001; factor 9 p=0.0004; factor 10 p=0.007; factor 11 p=0.0003; factor 12 p=0.01) [Table 5]. To further ensure that the association between metabolic factor levels and metabolic wellness was not confounded by differences in BMI, we performed logistic regression analyses adjusted for BMI with metabolic wellness as the dependent (outcome) variable in the overall combined group, which continued to show the same significant relationships between metabolite factors and metabolic wellness (factor 3 p=0.01; factor 4 p=0.0003; factor 5 p=.02; factor 6 p=0.002; factor 8 p<0.0001; factor 9 p=0.0006; factor 10 p=0.01; factor 11 p=0.0004; factor 12 p=0.02) [Table 5].

DISCUSSION

In this study of individuals from three distinct cohorts, we documented that metabolic profiling discriminates metabolic wellness independent of BMI. There results may have potential for translation to clinical applicability; these metabolic markers could be used to identify individuals who are metabolically unwell with a more granular measure of metabolic health that goes beyond BMI alone. Such identification could help target interventions to those at greatest metabolic risk, allowing clinicians to focus on other aspects of health in obese individuals who are metabolically well. Further, these results could lead to the identification of causal pathways serving as potential targets for drug development.

While only one factor (factor 8, composed of NEFA and various amino acids) was significantly different between MW and MUW overall and across all BMI categories, several other factors were significantly different in the overall population, with variable levels of significance in the BMI categories. The relevance of factor 8 and its various components to metabolic health and its role in disease remains to be determined, but the results for factor 4 are of particular interest. Specifically we found levels of factor 4, (composed of BCAA and related metabolites) distinguished metabolically well from unwell individuals overall with a high degree of statistical significance, particularly in obese individuals, with higher levels seen in metabolically unwell individuals. These findings could inform future research to determine causal links between the BCAA metabolic profile and the pathogenesis of obesity-associated adverse health consequences.

In addition to the BCAA factor, a related factor, factor 9 (C3 and C5 acylcarnitines) distinguished between MW and MUW overall and in the obese category. The similar patterns of association of factors 4 and 9 are likely due to the fact that C3 and C5 acylcarnitines are generated in part as byproducts of BCAA catabolism. In fact, these metabolites cluster with the BCAA in a PCA-derived factor in many of our studies, suggesting their biological as well as statistical correlation. The findings for both factors 4 and 9 are particularly provocative given that we have shown previously that the BCAA biosignature is associated with insulin resistance [3], independently discriminates individuals with coronary artery disease [5], and predicts who will have improvements in insulin resistance with weight loss [6]. This group of metabolites also has been shown to decrease drastically after gastric bypass surgery vs. similar amounts of behavioral weight loss [18] and to predict development of incident type 2 diabetes in individuals without baseline diabetes [19].

The potential impact of using metabolic profiling in this way (rather than targeting all obese but no lean individuals with prevention and treatment) depends on the prevalence of metabolic wellness among obese and unwellness among lean individuals. In our population, the proportion of MW obese individuals (13%) was considerably lower than in the study by

Wildman (32%) [2]. The Wildman study used data from 5440 participants of the NHANES (1999–2004) general population survey; thus, may reflect prevalence in the general population. In contrast, a large proportion of our population was derived from a clinical database (i.e., CATHGEN, comprising patients undergoing evaluation for possible ischemic heart disease), and may reflect lower prevalence of metabolic wellness in obese individuals who are older and have cardiovascular risk factors or CVD. Similarly, the number of lean individuals who were metabolically unwell in our analysis was higher than in the NHANES population (67% vs., 23.5%, respectively), again reflecting the difference between a population sample and an older clinical population [2].

These results also may inform understanding of racial/ethnic health disparities with regard to metabolic consequences of obesity. Interestingly, there were fewer AAs than non-AA who were MUW in all BMI categories. These demographic differences are consistent with the results of Wildman et al but are not consistent with epidemiologic data that show that AAs have a higher prevalence of diabetes and hypertension, and AAs have higher rates of complications related to diabetes and hypertension [20]. Many studies have looked beyond BMI for the etiology of these disparities. Studies have shown that BMI alone is not predictive of mortality for AA women in particular [21]. For example, in a study by Stevens et. al, AA women were followed prospectively for up to 15 years. The results show no difference in the strength of association between BMI and mortality across the entire range of BMI [22]. Although investigators have postulated that the racial/ethnic disparities in development of DM and hypertension in AA may be secondary to lower resting metabolic rate, differences in site-specific adiposity and levels of adipocytokines, no theory has successfully defined the underlying cause of the disparity. Our results provide a potential way to assess metabolic wellness in diverse populations where BMI has not reliably predicted metabolic risk.

In the WLM study, dietary BCAA intake was only weakly correlated with peripheral BCAA levels [6], implying some biological/genetic differences in processing of BCAA that may be reflected in peripheral blood levels. Indeed, there is evidence as published by Herman et al that adipose tissue has the potential capacity to regulate circulating BCAA levels in vivo [23].

Understanding how dietary BCAA intake affects insulin resistance is essential as modulation of BCAA in the diet could be utilized as a future target for treatment. A recently published randomized controlled trial designed to study the effects of BCAA supplementation on glycemic control in patients with chronic hepatitis C and insulin resistance showed there were no significant differences between fasting glucose, HbA1c or HOMA-IR in the control or BCAA supplemented groups [24]. Future research in human subjects is needed to further elucidate the effects of dietary BCAA on circulating levels of BCAA and the impact on insulin resistance and glycemic control.

The strengths of the analysis are inclusion of a large, diverse group of men and women, standardized application of the definition of well and unwell to the entire population, use of highly accurate targeted quantitative metabolomic profiling and use of data reduction techniques to minimize the risk of false positive results.

Our analyses do have limitations. First, we pooled datasets from studies with different designs and enrollment criteria rather than a random population sample. Still, the size and diversity of our study populations and the fact that the methods for clinical characterization and metabolic profiling were the same for all datasets enhance the potential generalizability of our results. Second we do not know the impact on results, if any, of varying duration of fasting and source of serum/plasma (venous vs. arterial blood) in the different study

populations. Third, while our observational analyses demonstrate association, we cannot determine cause and effect. Nonetheless, these associations point to future research of potential causal pathways. Fourth, our knowledge of physiologic determinants of BCAA levels is incomplete.

In summary, multiple metabolic factors were able to distinguish metabolic wellness and unwellness in the overall and obese categories, independent of BMI. In particular, a factor comprising BCAA and related metabolites is a promising biomarker that could refine the designation of cardiometabolic risk. Further research is needed to determine the clinical utility of BCAA profiling, the role of nutrient intake, and the extent to which the observed associations reflect causal pathways.

Acknowledgments

We are grateful to all the participants in the WLM clinical trial and the CATHGEN biorepository and to Gayle Meltesen and Alan Bauck at the WLM Coordinating Center, Center for Health Research, Portland, OR.

Funding/Support: This study was supported by UO1 grants HL68734, HL68676, HL68790, HL68920, and HL68955. It was also supported by funding from the Measurement to Understand Re-Classification of Disease of Cabarrus and Kannapolis (MURDOCK) Study and by Grant Number 1UL1 RR024128-01 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research, and its contents are solely the responsibility of the authors and do not necessarily represent the official view of NCRR or NIH. Information on NCRR is available at [http://www.ncrr.nih.gov/]. Information on Re-engineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov/clinicalresearch/overview[-translational.asp

Abbreviations used

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
CATHGEN	
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

References

1. Ogden CL, Carroll MD, Flegal KM. Epidemiologic trends in overweight and obesity. Endocrinol Metab Clin North Am. 2003; 32:741–760. [PubMed: 14711060]
2. Wildman RP, Muntner P, Reynolds K, et al. The Obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering. Arch Int Med. 2008; 168(15):1617–1624. [PubMed: 18695075]
3. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009; 9:311–326. [PubMed: 19356713]
4. Huffman KM, Shah SH, Stevens RD, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. Diabetes Care. 2009; 32:1678–1683. [PubMed: 19502541]
5. Shah SH, Bain JR, Muehlbauer MJ, et al. Association of a Peripheral Blood Metabolic Profile with Coronary Artery Disease and Risk of Subsequent Cardiovascular Events. Circ Cardiovasc Genet. 2010; 3:207–214. [PubMed: 20173117]
6. Shah SH, Crosslin DR, Haynes CS, et al. Branched-chain amino acid levels are associated with improvement in insulin resistance with weight loss. Diabetologia. 2012; 55:321–30. [PubMed: 22065088]

7. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. Nature Medicine. 2011; 17:448–453.
8. Shah SH, Granger CB, Hauser ER, et al. for the MURDOCK Horizon 1 Cardiovascular Disease Investigators. Reclassification of Cardiovascular Risk Using Integrated Clinical and Molecular Biosignatures: Design and Rationale of the MURDOCK Horizon 1 Cardiovascular Disease Study. Am Heart J. 2010; 160:371–379.e2. [PubMed: 20826242]
9. Hollis JF, Gullion CM, Stevens VJ, et al. Weight loss during the initial intensive intervention phase of the Weight Loss Maintenance Trial. Am J Prev Med. 2008; 35:118–126. [PubMed: 18617080]
10. Svetkey LP, Stevens VJ, Brantley PJ, et al. Comparison of strategies for sustaining weight loss: the weight loss maintenance randomized controlled trial. JAMA. 2008; 299:1139–1148. [PubMed: 18334689]
11. Haqq AM, Lien LF, Boan J, Arlotto M, et al. The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) Weight Loss Project: Rationale and Design. Contemp Clin Trials. 2005; 26:616–625. [PubMed: 16239128]
12. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28:412–419. [PubMed: 3899825]
13. Ascaso JF, Pardo S, Real JT, et al. Diagnosing Insulin Resistance by Simple Quantitative Methods in Subjects With Normal Glucose Metabolism. Diabetes Care. 2003; 26:3320–3325. [PubMed: 14633821]
14. Qu H-Q, Li Q, Rentfro AR, et al. The Definition of Insulin Resistance Using HOMA-IR for Americans of Mexican Descent Using Machine Learning. PLoS ONE. 2011; 6(6):1–4.
15. An J, Muoio DM, Shiota M, et al. Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. Nat Med. 2004; 10:268–274. [PubMed: 14770177]
16. Chace DH, Hillman SL, Millington DS, et al. Rapid diagnosis of maple syrup urine disease in blood spots from newborns by tandem mass spectrometry. Clin Chem. 1995; 41:62–6. [PubMed: 7813082]
17. Shah SH, Hauser ER, Bain JR, et al. High heritability of metabolomic profiles in families burdened with premature cardiovascular disease. Molecular Systems Biology. 2009; 5:1–7.
18. Laferrère B, Reilly D, Arias S, et al. Differential Metabolic Impact of Gastric Bypass Surgery Versus Dietary Intervention in Obese Diabetic Subjects Despite Identical Weight Loss. Sci Transl Med. 2011; 3(80):80re2.
19. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. Nature Medicine. 2011; 17:448–453.
20. Bonds DE, Goff DC, Karter AJ, et al. Ethnic and racial differences in diabetes care. Diabetes Care. 2003; 26:1040–1046. [PubMed: 12663570]
21. Fontaine KR, Redden DT, Wang C, et al. Years of life lost due to obesity. JAMA. 2003; 289:187–193. [PubMed: 12517229]
22. Stevens J. Obesity and mortality in African Americans. Nutr Rev. 2000; 58:316–358.
23. Herman MA, Pengxiang She, Peroni OD, et al. Adipose tissue branched chain amino acid (BCAA) metabolism modulates circulating BCAA levels. J Biol Chem. 2010; 285(15):11348–11356. [PubMed: 20093359]
24. Takeshita Y, Takamura T, Kita Y, et al. Beneficial effect of branched chain amino acid supplementation on glycemic control in chronic hepatitis C patients with insulin resistance: Implications for type 2 diabetes. Metabolism. 201210.1016/j.metabol.2012.03.011

Metabolism. Author manuscript; available in PMC 2014 July 01.

Table 1

Baseline characteristics of the study population

	Overall	**Lean (N=410)**	**Overweight (N=*610*)**	**Obese (*N*=*852*)**	**p value[b]**
Age[a]	59.49 ± 11.80	61.52 ± 13.73	61.66 ± 11.02	56.97 ± 10.82	<0.0001
Female (%)	45	45	38	51	<0.0001
Weight (lbs)	193.03 ± 49.93	145.12 ± 22.64	180.59 ± 22.15	225.01 ± 37.50	<0.0001
BMI (kg/m^2)	30.05 ± 6.44	22.28 ± 2.33	27.63 ± 1.45	35.53 ± 4.87	<0.0001
African American (%)	24	18	19	30	<0.0001
HTN (%)	85	74	87	89	<0.0001
Fasting glucose (mg/dL)	94.90 ±13.52	93.29 ± 14.76	93.16 ± 13.10	96.91 ± 12.91	<0.0001
HOMA-IR	2.24 ± 1.87	1.67 ± 1.51	1.81 ± 1.22	2.82 ± 2.21	<0.0001
Triglycerides (mg/dL)	118.56 ± 74.11	91.94 ± 50.71	122.02 ± 81.50	128.90 ± 75.05	<0.0001
HDL-C level (mg/dL)	38.35 ± 14.63	42.44 ± 17.92	36.78 ± 13.95	37.51 ± 12.89	<0.0001
Metabolically unwell (%)		67	80	87	<0.0001

[a] All continuous variables are reported as mean ± SD, all discrete variables are reported as percent (%).

[b] p-value of comparison of distribution of variable between lean/overweight/obese categories

BMI (Body Mass Index)

HTN (Hypertension)

HOMA-IR (Homeostatic Model Assessment-Insulin Resistance)

HDL-C (High Density Lipoprotein)

Table 2

Baseline characteristics of study population stratified by BMI categories and MW and MUW categories

	Lean MW N=137	Lean MUW N=273	p-val	Overweight MW N=119	Overweight MUW N=491	p-val	Obese MW N=114	Obese MUW N=738	p-val
Age[a]	55.62 ± 14.01	64.48 ± 12.61	<0.0001	59.36 ± 10.28	62.22 ± 11.13	0.01	55.84 ± 11.45	57.14 ± 10.72	0.23
% Female/% Male	55/45	40/60	0.004	43/57	37/63	0.23	66/34	48/52	0.0004
Weight (lbs)	143.49 ±21.09	145.93 ± 23.37	0.30	178.44 ± 20.27	181.11 ± 22.57	0.24	213.12 ± 32.46	226.85± 37.90	0.0003
BMI (kg/m2)	22.26 ± 1.95	22.29 ± 2.50	0.88	27.50 ± 1.47	27.66 ± 1.45	0.28	34.63 ± 4.14	36± 4.96	0.03
African American (%)	25	14	0.02	27	17	0.02	36	29	0.02
HTN (%)	38	92	<0.0001	61	94	<0.0001	61	92	<0.0001
Fasting glucose	90.05 ± 11.27	94.92 ± 16.01	0.002	87.11 ± 11.14	94.61 ± 13.13	<0.0001	87.93± 8.30	98.30 ± 12.94	<0.0001

[a]All continuous variables are reported as mean ± SD, all discrete variables are reported as percent (%).

MW (Metabolically Well)

MUW (Metabolically Unwell)

BMI (Body Mass Index)

HTN (Hypertension)

Metabolism. Author manuscript; available in PMC 2014 July 01.

Table 3

Principal Components Analysis. Included in the table below are twelve factors identified from principal components analysis (PCA). Column 2 lists an overall description of the factor, column 3 the individual metabolites comprising that factor, column 4 the eigenvalue for the factor and column 5 the variance for the factor.

Factor	Name	Individual components	Eigenvalue	Variance
1	Medium chain acylcarnitines	C8, C10, C12, C14, C14:1, C10:1, C14:2, C12:1	11.76	.22
2	Long chain dicarboxyl acylcarnitines	C20-OH/C18-DC, C20:1-OH/C18:1-DC, C18-OH/C16-DC, C16-OH/C14-DC, C18:1-OH/C16:1-DC, C20, C14-OH/C12-DC, C12-OH/C10-DC	5.85	.11
3	Ketone related	HBUT, KET, C4-OH, C2, C18:1-OH/C16:1-DC, ALA (−)	4.21	.08
4	BCAA related	PHE, LEU/ILE, VAL, TYR, MET, ALA, HIS	3.64	.07
5	Long chain acylcarnitines	C18:1, C16, C18:2, C18, C14, C14:1-OH/C12:1-DC	2.67	.05
6	Medium chain acylcarnitines	C10:3, C8:1, C10:2, C10:1, Ci4-DC/C4-DC,	2.00	.04
7	Short chain dicarboxyl acylcarnitines	C5-DC, C6-DC, Ci4-DC/C4-DC, C12-OH/C10-DC	1.77	.03
8	Various	C5:1, NEFA, GLX, ORN, ARG (−), HIS (−)	1.62	.03
9	Short chain acylcarnitines	C4/Ci4, C3, C5S	1.38	.03
10	Amino acids	GLY, SER, ORN	1.21	.02
11	Various	PRO, CIT, C22	1.89	.02
12	Various	ASX, ARG (−)	1.06	.02

BCAA (Branch Chain Amino Acids)

HBUT (β-Hydroxybutyrate)

KET (Ketone)

ALA (Alanine)

PHE (Phenylalanine)

LEU/ILE (Leucine/Isoleucine)

VAL (Valine)

TYR (Tyrosine)

MET (Methionine)

HIS (Histidine)

NEFA (Non Esterified Fatty Acids)

GLX (Glutamate/glutamine)

ORN (Ornithine)

ARG (Arginine)

GLY (Glycine)

SER (Serine)

PRO (Proline)

CIT (Citruline)

C22 (C22 acylcarnitine)

ASX (Aspartate/asparagine)

Table 4

Mean metabolite factor levels stratified by metabolic fitness and BMI categories.

Factor	Overall			Lean			Overweight			Obese		
	MW[a]	MUW	p*	MW	MUW	p*	MW	MUW	p*	MW	MUW	p*
1) Medium Chain Acylcarnitines	0.06 (0.96)	−0.01 (1.01)	0.89	0.04 (.94)	0.09 (1.55)	0.50	−0.07 (0.69)	−0.08 (0.76)	0.84	0.21 (1.18)	−0.009 (0.89)	0.07
2) Long chain dicarboxyl acylcarnitines	−0.04 (0.84)	0.007 (1.03)	0.59	0.05 (1.09)	0.11 (0.79)	0.05	−0.03 (0.81)	0.09 (1.14)	0.16	−0.15 (0.45)	−0.08 (1.03)	0.59
3) Ketone related	0.16 (1.20)	−0.04 (0.94)	.0058	0.38 (1.37)	0.18 (1.03)	0.26	0.20 (1.29)	0.02 (1.02)	0.33	−0.13 (0.74)	−0.16 (0.83)	0.52
4) BCAA related	−0.26 (0.95)	0.06 (1.00)	<.0001	−0.46 (1.02)	−0.33 (0.96)	0.18	−0.26 (0.93)	−0.07 (0.96)	0.07	−0.003 (0.82)	0.30 (0.98)	0.0006
5) Long chain acylcarnitines	−0.09 (0.91)	0.02 (1.02)	.09	−0.06 (0.93)	0.13 (1.20)	0.33	−0.01 (0.99)	0.01 (1.03)	0.61	−0.22 (0.78)	−0.01 (0.93)	0.05
6) Medium chain acylcarnitines	−0.18 (0.89)	0.04 (1.01)	<.0001	−0.31 (0.90)	−0.01 (1.17)	0.0004	−0.20 (1.01)	−0.01 (1.07)	0.040	−0.005 (0.72)	0.09 (0.90)	0.41
7) Short chain dicarboxyl acylcarnitines	−0.05 (0.53)	0.01 (1.08)	0.53	−0.07 (0.33)	0.22 (2.16)	0.04	0.06 (0.79)	0.05 (0.74)	0.95	−0.15 (0.34)	−0.09 (0.50)	0.24
8) Various	−0.31 (0.98)	0.07 (0.99)	<.0001	−0.38 (1.0)	0.22 (0.88)	<.0001	−0.13 (0.98)	0.25 (0.90)	0.0008	−0.43 (0.94)	−0.09 (1.05)	0.0015
9) Short chain acylcarnitines	−0.19 (0.89)	0.04 (1.02)	<.0001	−0.27 (0.84)	−0.06 (1.11)	0.14	−0.08 (0.93)	−0.03 (1.08)	0.64	−0.21 (0.91)	0.13 (0.93)	.0003
10) Amino Acids	0.20 (1.05)	−0.05 (0.98)	0.0002	0.43 (1.13)	0.30 (1.05)	0.66	0.23 (1.03)	0.02 (0.97)	0.09	−0.10 (0.88)	−0.23 (0.92)	0.17
11) Various	−0.12 (0.90)	0.03 (1.02)	0.0068	−0.04 (1.02)	0.26 (1.20)	0.0047	0.004 (0.89)	0.11 (1.02)	0.40	−0.33 (0.72)	−0.11 (0.92)	0.02
12) Various	−0.14 (1.79)	0.03 (0.95)	0.04	−0.71 (1.27)	−0.14 (0.94)	<.0001	0.33 0.92)	0.10 (0.75)	0.01	0.05 (1.02)	0.05 (1.06)	0.91

[a] values expressed as mean(SD)

* p-value for the difference in mean metabolite factor levels between MW and MUW groups.

MUW (Metabolically Unwell)

MW (Metabolically Well)

Table 5

Regression model for each factor individually

Factor	Linear Regression Model		Logistic Regression Model		
	Type III SS	P value[a]	OR	95% CI	P value*
1	1.97	0.16	0.94	0.84–1.04	0.20
2	2.21	0.14	1.12	0.96–1.30	0.14
3	7.91	0.005	0.87	0.78–0.97	0.01
4	13.61	0.0001	1.26	1.11–1.43	0.0003
5	5.29	0.02	1.15	1.02–1.30	0.02
6	9.73	0.002	1.22	1.07–1.38	0.002
7	3.06	0.08	1.24	0.99–1.55	0.07
8	57.11	<0.0001	1.64	1.44–1.86	<0.0001
9	12.47	0.0004	1.25	1.10–1.42	0.0006
10	6.91	0.007	0.86	0.77–0.97	0.01
11	12.58	0.0003	1.24	1.10–1.40	0.0004
12	6.03	0.01	1.15	1.02–1.29	0.02

[a]Difference in mean metabolite factor levels between MW and MUW groups adjusted for BMI.

*Factor level as a predictor of metabolic wellness, adjusted for BMI