FRAILTY AND WALKING ABILITY AS INTEGRATED MARKERS OF AGING AND THEIR METABOLOMIC SIGNATURES

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Submitted to the Graduate Faculty of

the Department of Epidemiology

the Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2019

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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March 1, 2019

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University of Pittsburgh, 2019

ABSTRACT

Frailty and slowed gait become more prevalent with advanced age and predict major health outcomes. These complex phenotypes are influenced by multiple aspects of aging and multimorbidity, and may be manifestations of dysregulation in physiologic systems. Metabolomics, the large-scale study of small molecules that are intermediates or end-products of metabolism, can help us better understand aging-related metabolic changes that contribute to frailty and walking ability by capturing global metabolic profiles occurring most closely to the phenotypes. Here, I aimed to 1) identify metabolites and pathways associated with high versus low walking ability using a nested case-control study of 120 older adults matched on age, gender, race, and fasting time, 2) determine metabolites and pathways associated with frailty to vigor among 287 older black men, and 3) develop and validate a metabolite composite score to determine whether it explains the frailty-associated higher mortality. Regarding aim 1, I found 96 metabolites, mostly lipids/lipid-like molecules, especially triacylglycerols, associated with walking ability. Body composition partly explained associations between select metabolites and walking ability, though many remained independently associated. Triaclyglycerols containing mostly polyunsaturated fatty acids were higher, whereas triaclyglycerols containing mostly saturated or monounsaturated fatty acids were lower among those with high walking ability. Arginine and proline metabolism was a top pathway associated with walking ability. In aims 2

and 3, I found 37 metabolites associated with frailty to vigor and used those metabolites to develop a novel composite score. The metabolite composite score significantly predicted mortality and explained 56% of the higher mortality associated with frailty, where organic acids/derivatives (mostly amino acids) and lipids/lipid-like molecules accounted for almost all of the attenuation. The metabolite composite score also predicted mortality in a validation cohort. Differences in patterns of plasma lipids and amino acids were common classes of metabolites associated with these aging-related phenotypes. Knowledge on differences in these metabolites and metabolic pathways associated with frailty to vigor and walking ability is of public health interest because it provides a better characterization of these complex aging-related phenotypes that can inform points in their pathophysiology to intervene on to promote healthy aging and preserve independence throughout late-life.

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PREFACE

I would like to express my sincere gratitude to my PhD advisor, Dr. Anne B. Newman. I am very thankful for her continued mentorship, support, and guidance throughout my academic career and for always taking the time out of her busy schedule to meet. I hope to be discussing science with her for many years to come.

I would also like to thank my committee members: Dr. Robert M. Boudreau for his expert methodological knowledge and support; Dr. George C. Tseng for his guidance in analyzing –omics data; Dr. Stacy G. Wendell for her expertise in metabolomics and biochemistry and her ability to convey that knowledge to me; and Dr. Joseph M. Zmuda for his practical guidance and always asking questions that made me think more deeply into my research. I would like to thank the Center for Aging and Population Health and its wonderful faculty, staff, and students who I have had the pleasure of spending my work days with. I would like to acknowledge my pre-doctoral funding on the Epidemiology of Aging training grant (NIA T32 AG000181-28) at the University of Pittsburgh.

I would like to thank my wonderful husband, Stephen F. Smagula, for his love and support throughout my studies and for always wanting to hear about my research. Last, I would like to thank my mom and dad, sisters, Chelsea and Courtney, and brother, John. Thank you for always being there.

1.0 INTRODUCTION

In the United States, the number of older adults (aged ≥65) is expected to more than double by the year 2060 (1), an estimated 52 million more individuals. Such an increase will result in a financial and societal challenge if the distribution of health among the older adult population is not improved. Currently, only 14% of U.S. older adults are living free of chronic conditions. Whereas, over 60% are living with multimorbidity (2). In other words, 28 million U.S. older adults have two or more chronic conditions, which will increase to 60 million by the year 2060 if the prevalence remains the same. Unhealthy aging negatively impacts quality of life (3) and is extremely costly to both the individual and to society. For example, the majority (71%) of healthcare costs and almost all (90%) Medicare fee-for-service costs are spent caring for individuals with multimorbidity (4-6). In addition, over 60% of deaths among older adults are caused by chronic conditions (7). Thus, there is a need to further our understanding of the biology and physiology of aging to help guide effective interventions focused on improving the health of the older adult population.

1.1 BIOLOGY OF AGING

Aging has been defined as a universal, irreversible, and deleterious biological process, characterized by progressive loss to physiological integrity that manifests as decline in function

and increased vulnerability to disease and death (8, 9). Aging is heterogeneous and influenced by genetics and lifetime behavioral and environmental exposures, such that no individuals age the same exact way across all physiological systems. Even within an individual, aging is heterogeneous, where each physiological system has a different peak in health and capacity, as well as a different rate of decline. The complex, multifactorial nature of aging makes studying its biology difficult. Many researchers instead focus on a single organ system. However, alterations in one physiological system that occur to compensate in the presence of a challenge likely cause changes in other physiological systems, though the relationship between physiological systems and how diminished reserve in one influences the rest is not fully understood (10). To ameliorate negative consequences of aging there is a need for research to be shifted from organ specific to aging as a whole.

1.1.1 Aging versus disease

A misconception is that aging is the same as disease (8). However, an individual can age in the absence of disease. Similarly, disease can be prevented by targeting modifiable risk factors; though, aging is inevitable (8). Aging is also universal, whereas diseases are not. The same disease is not experienced by all individuals, nor do they have similar patterns of multiple diseases (8). In fact, two individuals of the same chronological age can have vastly different biological ages caused by differences in genetics, behavioral, and environmental exposures. The misconception of aging versus disease arises to the fact that biological changes occurring as a result of aging can cause both physiological decline and age-related disease (10). The connection between aging and disease may indicate the same biologic pathways are actually influencing both (10).

1.1.2 Aging phenotypes

To study the biology and physiology of aging, a quantifiable phenotype illustrating the full distribution of aging from the healthy to the unhealthy extreme is needed. Often phenotypes that measure health only differentiate between different levels of unhealthy aging or are often focused on a single physiologic system. However, there is almost unanimous agreement that aging is not a result of a single mechanism (11). Cohen (2016) described aging as the physiologic dysregulation that causes a gradual breakdown in the complex dynamics of regulatory systems that maintain homeostasis, where physiologic dysregulation can begin or accelerate across multiple systems as a result of a single perturbation (11). Thus, in theory an optimal aging phenotype would encompass the integrity of multiple physiologic systems and the interactions across systems. It would also be capable of differentiating between different levels of health by measuring both the healthy in addition to the unhealthy extreme, and lastly it would predict multiple major health outcomes.

Similar to definitions of aging, a decline in walking ability and frailty have been described as influenced by the health of multiple physiologic systems, genetics, and life-long impact of risk factors such as severity and duration of diseases and interaction of multiple chronic conditions. Age-related decline in walking ability or the onset of frailty in late-life can be thought of as manifestations of perturbations in the complex regulatory systems maintaining homeostasis, where examining the biology and pathophysiology of both frailty and walking ability decline may indicate pathways involved in healthy and/or unhealthy aging processes.

1.2 AGE-RELATED DECLINE IN WALKING ABILITY

Five vital signs are used to assess the basic functioning of an individual's body. They include temperature, pulse rate, blood pressure, respiration rate, and weight. Gait speed has been described as the sixth vital sign (12) because of its ability to capture functional capacity, overall health, and predict multiple major adverse health outcomes (13). A healthy gait requires coordination, strength, and sensation. Since the entire body moves while walking, an impairment at any point can result in gait abnormalities and reduced speed (14). Gait speed can be influenced by various measures such as overall health, motor control, musculoskeletal conditions, cognition, physical activity level, sensory and perception, and the environment (12). Maintaining walking ability throughout late life is critical to maintaining health, independence, and quality of life, as well as important financially considering the growing older adult population (15).

1.2.1 Pathophysiology of decline in walking ability

A declining gait speed among older adults may be a manifestation of accumulating age-related changes and chronic conditions. In fact, assessing an individual's gait speed is often the best measure of their overall health, illustrating the severity, duration, and extent of disease, as well as the interaction of multiple chronic conditions (16). Thus, an individual's walking ability, or gait speed, can be thought of as a multimorbidity aging phenotype, where vast age-related molecular changes likely exist when examining individuals of the same age, but with high versus low walking ability.

Age-related changes in gait have similarities to healthy individuals walking on ice, characterized by a widened stance, longer duration with both feet on the floor, shorter step

length, not lifting the feet as high off the ground, and a hunched posture (17). Lower limb pain, neuromuscular and myopathic conditions, and structural abnormalities of bone, joints, or soft tissue can cause changes in gait (18). Abnormalities such as these are broadly classified as neurological, musculoskeletal, or cardiorespiratory etiology (17, 18) and can be thought of as proximal risk factors of age-related decline in gait speed. Figure 1 displays examples of more distal risk factors (obesity, physical inactivity, smoking, and more) of age-related decline in gait speed, which do not have a direct impact on gait speed, but instead have an impact by causing structural, neurologic, and/or cardiorespiratory changes.

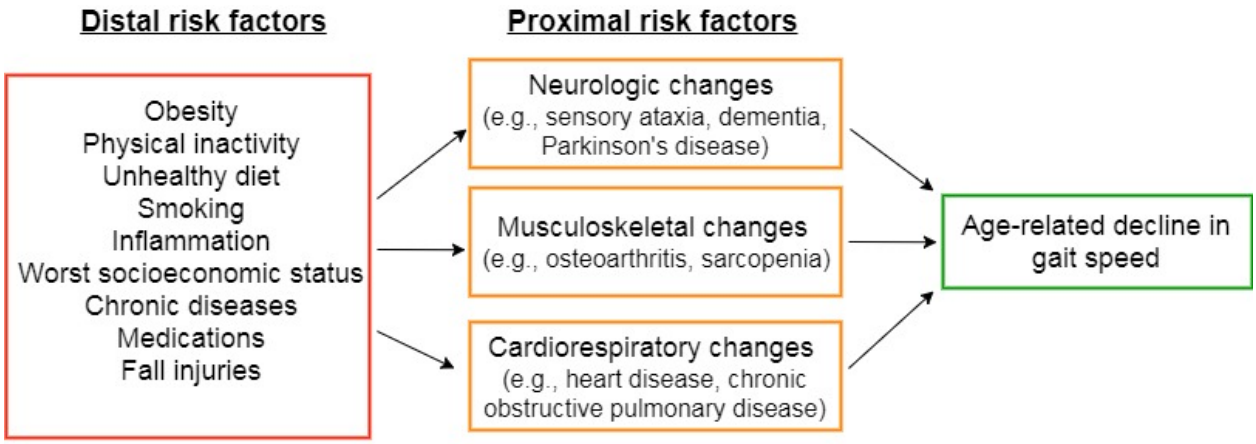


Figure 1. Proximal and distal risk factors of age-related decline in gait speed

The most common musculoskeletal gait abnormality, as well as one of the most common causes of disability among older adults is osteoarthritis (19, 20). The prevalence of osteoarthritis is higher with advanced age and among women (21). Osteoarthritis is characterized as deterioration of articular cartilage, the smooth, lubricated, and highly specialized connective tissue that covers ends of bones to form joints and lacks blood vessels or nerves (22). Once articular cartilage deteriorates to a certain degree, joint tissues with pain fibers become exposed

resulting in osteoarthritic pain, an antalgic gait (limping to avoid placing weight on the painful side), and a reduced gait speed (18). An average gait also requires a healthy brain. Cortical areas of the brain are needed to start walking and to multitask during walking, subcortical areas are needed for spatial navigation, adapting to the surroundings, and for sensory input, and the spinal cord is needed to set the rhythmic pace of walking (23). Lastly, the cardiorespiratory system consists of the heart and lungs working together to deliver oxygenated blood to the muscles and organs throughout the body, where dysregulations in this system can impact walking ability due to diminished aerobic capacity.

1.2.2 Descriptive epidemiology of walking ability

In the United States, one in four adults ages 65 and older have an ambulatory disability defined as serious difficulty walking or climbing stairs (24), where the prevalence is higher with older age. Over half of adults ages 85 and older self-report an ambulatory disability. A significant decline in walking ability begins around the sixth decade of life, where a 10% decrease in gait speed occurs, on average, per each additional decade lived (25, 26). The National Health and Nutrition Examination Survey (NHANES) examined gait speed among 1,923 adults ages ≥50 (27) and found the average (±standard deviation) gait speed of participants ages 50-59 was 1.1±0.2 meters/second (i.e., roughly 24 minutes per mile). Whereas, the average gait speed for participants 70-79 was 0.95±0.2 meters/second and continued to be less among older ages to about 0.80±0.2 meters/second among participants ages 80 and older (i.e., about 33 minutes per mile).

A gait speed of less than 1.0 meter/second has been shown to be a clinically relevant threshold to identify older adults at a higher risk for major health outcomes (28). Among 3,075

older adults from the Health, Aging, and Body Composition (Health ABC) study (mean age: 74±3, 52% women, 42% black), average gait speed at baseline was 1.2±0.2 meters/second, where 26% of the cohort had a gait speed <1.0 meter/second (29). It should be noted that the eligibility criteria for the Health ABC study included self-reporting no difficulty walking ¼ mile, climbing ten steps, or with basic activities of daily living, meaning the Health ABC participants were a relatively healthy older adult cohort. On the other hand, when examining 6,534 initially non-disabled participants from the Established Populations for the Epidemiologic Study of the Elderly (EPESE) aged ≥65, almost all of the men and women (91% and 95%, respectively) had a 4-meter gait speed <1.0 meter/second. In fact, 42% of men and 55% of women from the EPESE had a gait speed <0.6 meters/second, where the prevalence of a worse gait speed was higher among older ages (30). A similar prevalence was observed in the Third National Health and Nutrition Examination Survey (NHANES III), where 92% of older adults had a gait speed <1.0 meters/second (31). Thus, the prevalence of a slow gait speed (<1.0 meters/second) among U.S. older adults is likely around 92%.

1.2.3 Analytic epidemiology of walking ability

1.2.3.1 Walking ability and major health outcomes

Gait speed has been shown to independently predict multiple major health outcomes among older adults, such as incident mobility limitation (32), mobility disability (32-34), activities of daily living disability (34, 35), future falls (36, 37), hospitalization (28, 38), institutionalization (37), incident cardiovascular disease (32), decline in cognitive performance (37, 39), and all-cause mortality (31, 32, 35, 37, 40, 41). Specifically, taking one minute longer to walk 400 meters was associated with a 52% higher risk of incident mobility disability (95%

CI: 1.41, 1.63) among participants from the Health ABC study, while adjusting for multiple confounding variables. In addition, Health ABC participants who attempted the 400 meter walk, but could not complete it, had a 95% higher risk of incident mobility disability (1.56, 2.44) than participants who finished the test (32). When pooling results from nine different cohorts of community-dwelling older adults (31), a 0.10 meter/second faster gait speed was associated with a 12% lower mortality risk (0.87, 0.90). A pooled analysis of incident disability by gender found a 0.10 meter/second faster gait speed was associated with 32% (95% CI: 0.57, 0.81) and 25% (0.68, 0.82) lower risks of bathing or dressing dependence and mobility difficulty, respectively, among men, and was associated with 26% (0.66, 0.82) and 27% (0.67, 0.80) lower risks, respectively, among women (34).

When examining distance walked in 6 minutes among participants from the Cardiovascular Health Study, those in the worst two quintiles walked between 290-338 meters (i.e., 0.81 to 0.94 meters/second) or <290 meters (i.e., <0.81 meters/seconds) in 6 minutes, as well as had a 1.7 (95% CI: 1.2, 2.5) and 2.1 (1.4, 3.0) times the risk of all-cause mortality, respectively, when compared to those in the best quintile (>414 meters in 6 minutes; i.e., >1.1 meters/second), while adjusting for multiple confounders (40). Using this information and Levin's attributable risk equation (42), I can estimate that approximately 30% of deaths among the Cardiovascular Health Study cohort were attributable to low walking speed (≤338 meters in 6 minutes or ≤0.94 meters/second), where reducing the prevalence of low walking speed by half would have prevented 15% of deaths in this cohort (using the equation for preventable fraction from (43)).

Change in gait speed over time also significantly predicts mortality, where a fast decline (i.e., decrease of 0.03 meters/second each year) over 8 years of follow-up was associated with at

least a 70% higher mortality risk when compared to a slow decline (i.e., decrease of 0.02 meters/second each year) among community-dwelling older adults stratified by baseline gait speed (44). Information from these prospective cohorts of older adults indicate that measuring an older adult's gait speed and their change in gait speed over time are important markers describing their risk for major health outcomes. Thus, interventions aimed at improving walking ability and then sustaining that improvement would likely result in reductions in morbidity and mortality, and increase independence among the older adult population.

1.2.3.2 Cross-sectional associations with walking ability

At the time gait speed is assessed, those who have slower measurements tend to be older, more often women, and more often of black race (32, 45). A slower gait speed has also been associated with less than a high school degree (39, 45), current smoking status (32), a more sedentary lifestyle (32), higher body mass index (32, 45), and worse self-reported health (45).

Older adults with slower gait speed or who were not able to participate or complete walking tests had a greater likelihood of presenting with chronic conditions, such as cardiovascular diseases, stroke, diabetes, arthritis (32, 45), peripheral arterial disease, hypertension, pulmonary disease, and depression (32). In addition, a faster gait speed was associated with less subclinical disease, characterized by lower blood pressure, less depressive symptoms (32, 45), better pulmonary functioning, and better cognitive performance (45). Muscle weakness in the leg and arm measured by the isokinetic leg extension and grip strength was also significantly associated with slower gait speed (46).

Gait speed has also been associated with multi-system disease burden (47), measured using the physiologic index of comorbidity. The physiologic index of comorbidity ranges from 0 (healthy) to 10 (unhealthy) and can capture the full range of health from no disease to subclinical

disease to clinically apparent disease across the following five physiologic systems: vasculature, lungs, kidneys, brain, and glucose metabolism (48). Participants from the Cardiovascular Health Study with the worst scores of 7 to 10 on the physiologic index of comorbidity had the slowest average gait speed at baseline of 0.8 meters/second, whereas, participants with the best scores of 0 to 2 had an average baseline gait speed of 1.0 meter/second, where not a single component was driving the association (47).

When examining blood-based markers, higher fasting glucose levels (32), higher levels of interleukin-6 (49), C-reactive protein (45, 49), fibrinogen (45), white blood cell count (45), and cystatin C (50, 51) have been associated with slower gait speed among community-dwelling older adults. In addition, rodent blood exchange models have shown that blood of old mice had a negative effect on physical performance (measured using a four-limb hanging test) when pumped into the circulation of young mice, whereas blood of young mice had a positive effect on muscle regeneration when pumped into the circulation of old mice (52). Taken together, these cross-sectional studies show that gait speed is associated with a plethora of commonly measured variables, such as age, gender, race, lower socioeconomic status, poor health behaviors, chronic conditions, worse markers of disease, and multisystem disease burden, and is thus, likely an important modifiable target to improve health in the older adult population.

1.2.3.3 Risk factors for change in walking ability

Change in gait speed over eight years of follow-up was examined among community-dwelling older adults from the Health, Aging, and Body Composition study (N=2364, mean age: 74±3, 52% women, 37% black) (44). There were three distinct subgroups in the cohort, where each group was characterized by a different pattern of gait speed over time. The most optimal subgroup (27% of the cohort) had a slow decline in gait speed characterized by average baseline

speed of 1.4±0.13 meters/second and average annual decline of 0.02 meters/second. The second subgroup (51% of the cohort) was classified as having a moderate decline in gait speed characterized by a lower average baseline speed of 1.1±0.1 meters/second, but a similar average annual decline of 0.02 meters/second. The worst subgroup (22% of the cohort) had a fast decline in gait speed characterized by an average baseline speed of 0.9±0.2 meters/second and an average annual decline of 0.03 meters/second. The slow, moderate, and fast decline subgroups had a change in gait speed relative to their baseline speed of 11%, 14%, and 22%, respectively. Older age, female gender, black race, less than a high school education, larger number of comorbidities, body mass index ≥30 kg/m^2, knee pain, low knee extensor strength, and low levels of physical activity were all significantly associated with a fast or moderate decline in gait speed when compared to a slow decline. For example, women had 5.7 (95% CI: 4.0, 8.2) times the odds and black participants had 10.0 (6.7, 15.1) times the odds of a fast versus slow decline when compared to men and white participants, respectively.

The physiologic index of comorbidity (48), an index of multi-system disease burden, has also significantly predicted change in gait speed over six years of follow-up in the Cardiovascular Health Study (47). Participants with worse baseline scores (i.e., 7-10) on the physiologic index of comorbidity had an average annual decline in gait speed of 0.03 meters/second, whereas participants with the best scores (i.e., 0-2) had an average annual decline of 0.01 meters/second, while adjusting for baseline confounders. The association remained after additionally adjusting for time-varying covariates. Other studies focused on a single organ system have found similar results. For example, worse baseline measurements of cystatin C, creatinine, and glomerular filtration rate were associated with a greater decline in gait speed over 7 years of follow-up among participants from the Framingham Offspring Study (51). In addition,

changes in adiponectin, interleukin-6, and especially cystatin C have been shown to mirror decline in physical functioning with age (10).

Body composition measures such as thigh intermuscular fat have also been associated with change in gait speed. Among 2306 Health, Aging, and Body Composition participants, higher intermuscular fat was associated with an annual decline in gait speed of 0.01 and 0.02 meters/second among men and women, respectively (53). In addition, an increase in thigh intermuscular fat during follow-up was also associated with greater decline in gait speed, suggesting more fat entering the muscle may contribute negatively toward mobility in late-life. Thus, similar to cross-sectional studies, older age, being a woman, black race, lower socioeconomic status, chronic conditions, markers of disease, and multisystem disease burden were all associated with decline in gait speed among cohorts of community-dwelling older adults. A slower gait speed and a decline in gait speed places individuals at a higher risk for major health outcomes, such as disability and mortality. Secondary and tertiary interventions targeted at improving gait speed will likely reduce or soften the negative impact that chronic conditions and multisystem disease burden have on an older adult, as well as preserve their independence through later ages and reduce their mortality risk.

1.3 FRAILTY IN LATE-LIFE

Frailty is most often defined as the structural and functional decline across multiple physiologic systems causing a decreased reserve and resilience to stressors that ultimately lead to increased vulnerability to major health outcomes (54). There exists many different ways to measure frailty (55), the most popular being the Fried Frailty Phenotype (56) and the frailty index (57, 58).

The Fried Frailty Phenotype was developed and validated in 2001 and was found to be distinct from both disability and comorbidity (56). It was calculated using information from the Cardiovascular Health Study and consists of the following five components: unintentional weight loss in the past year, weakness, poor endurance and energy, slowness, and low levels of physical activity. Using these five components, an individual is classified as positive for frailty if they have at least three components, pre-frail if they have one or two components, and robust if they have none. The Fried Frailty Phenotype was later modified in 2011 by removing the ceiling effect and allowing for measuring the healthy extreme of the frailty-to-vigor spectrum, in addition to the unhealthy extreme (59). This modified Fried Frailty Phenotype is called the Scale of Aging Vigor in Epidemiology (SAVE) (60) and uses the same five components, where each measure is split into tertiles and given a score of 0 for the best tertile, 1 for the mid-tertile, and 2 for the worst tertile. SAVE scores are then calculated as the sum of the five tertile scores, ranging from 0 (least frail) to 10 (most frail). In the applied portion of this dissertation, I use the SAVE to quantify vigor to frailty among older black men from the Health ABC study (Sections 3.0 and 4.0).

The frailty index, on the other hand, is based on an accumulation of deficits (57, 58) by counting signs and symptoms, chronic conditions, and disabilities. For a deficit to be included in the frailty index it must satisfy the following five criteria: 1) associated with health status; 2) prevalence is higher with older age; 3) onset must not occur too early in life; 4) all deficits must cover a range of symptoms; and 5) if longitudinal measurements will be used then the same deficits must be measured at all time points assessed. Once the deficits are decided, they are re-coded to range from 0 (the deficit is not present) to 1 (the deficit is present), where subclinical disease can get a score somewhere in between. The frailty index is then calculated as the

proportion of deficits. A frailty index calculated using the following deficits has been validated against all-cause mortality: vision loss, hearing loss, impaired mobility, vascular problems, gait abnormality, impaired vibration sense, difficulty toileting, cooking, bathing, going out, grooming, and dressing, skin problems, resting tremor, changes in sleep, urinary complaints, gastro-intestinal problems, diabetes, hypertension, and limb tone abnormality (57).

1.3.1 Issues with current frailty measurements

Even though frailty indices have been validated against major health outcomes, there still lacks a consensus on a gold standard for assessing frailty. This is because the biology and pathophysiology of frailty is not well understood. However, examining current measures of frailty, despite their imperfections, can still help to better characterize frailty, and potentially lead to a more objective method of diagnosing frailty.

1.3.2 Pathophysiology of frailty

Structural and functional decline across multiple physiologic systems is thought to play a major role in the development of frailty. Supporting this definition, it has been hypothesized that dysregulated immune, endocrine, stress, and energy responses are involved in the pathophysiology of frailty (55). In addition, aging, genetics, and diseases are thought to cause the molecular changes that lead to alterations in pathways that ultimately cause frailty (55).

Fried and colleagues developed a conceptual model illustrating frailty as a vicious cycle (54, 61). The cycle involves a lower total energy expenditure causing chronic undernutrition, chronic undernutrition causing sarcopenia and weight loss, sarcopenia and weight loss causing a

lower resting metabolic rate, a lower resting metabolic rate causing a lower total energy expenditure, and the cycle continues. It is thought that the frailty cycle can be initiated at any point, though a reduction in total energy expenditure caused by a decrease in physical activity, strength, and/or walking speed is most likely the culprit. Other points of the frailty cycle (e.g., chronic undernutrition or sarcopenia and weight loss) may be initiated or exacerbated by disease, environment, medications, or age-related changes. The presence of sarcopenia and weight loss can further influence reductions in strength, insulin sensitivity, bone density, and VO_2 max, as well as exhaustion. A reduction in strength can lead to impaired balance, falls and injuries, and immobilization, which further leads to a decrease in strength, as well as disability and then eventually dependency in the absence of intervention. Both reduced strength and VO_2 max can negatively influence walking speed, which can result in disability and dependency, as well as lower levels of physical activity and total energy expenditure and thus, further exacerbating the frailty cycle. All points in the frailty cycle are likely influenced by dysregulated energetics (61). Thus, alterations in energy pathways are hypothesized to play a key role in the biology and pathophysiology of frailty in late-life.

1.3.3 Frailty interventions

Most interventions to prevent frailty or reduce frailty severity have not been successful due to focusing on improving only one of the multiple dysregulated physiological systems (61). To date, the most promising frailty intervention has been thought to be one involving physical activity. Fried (2016) proposed the following three mechanisms on why physical activity interventions for frailty should be explored further: 1) lack of physical activity initiates the frailty cycle, where intervening at this point likely slows or halts the deleterious process, 2) physical

activity can maintain or even improve functioning of multiple physiological systems that are thought to be involved in the pathophysiology of frailty, and 3) physical activity may improve aging-related decline in the functioning of mitochondria, causing an increase in adenosine triphosphate (ATP) production (61).

To date, there exist few physical activity interventions to reduce frailty severity. As a post hoc analysis, a randomized controlled trial of older adults (n=424, mean± standard deviation: 77±4) examined the difference in frailty prevalence 12 months post randomization among participants in a physical activity arm versus a successful aging health education arm (62). Adjusting for gender, baseline frailty, and diabetes, there was a decrease in the prevalence of frailty by 14% in the physical activity arm versus only 5% in the educational arm (p=0.01). However, when examining the individual components of the Fried Frailty Phenotype, there was only a significant decrease in the sedentary behavior component by treatment arms. Thus, a more multi-domain intervention, that still includes physical activity, may be more promising in reducing all of the frailty components.

1.3.4 Descriptive epidemiology of frailty

Using various definitions of physical frailty, a systematic review found the weighted average prevalence of frailty and pre-frailty to be 10% and 44%, respectively, among multiple community-dwelling older adult cohorts (63). When examining the Cardiovascular Health Study (CHS) the overall prevalence of frailty using the Fried Frailty Phenotype was 7%, but was higher with older age (n=5317, age range: 65-101, 58% women, 15% non-white race). When examining CHS participants aged ≥85, the prevalence was 25%, as well as the prevalence was twice as high among women and among black participants (54). The Incidence of frailty over four years of

follow-up in the CHS was 7% (54). Pre-frailty was much more prevalent, with almost half (47%) of the CHS cohort having one or two of the Fried Frailty components. Those in the pre-frail state had 2.6 times the risk of developing frailty within the next three years when compared to robust participants (95% confidence interval: 1.9, 3.6) (54). A report of participants ages ≥50 from NHANES examined a version of the Fried frailty phenotype that did not include gait speed and found the prevalence of frailty and pre-frailty to be 4% and 27%, respectively (64). From this we can estimate the prevalence of pre-frailty and frailty in the U.S. older adult population to be at least 31%, but is likely higher since healthier individuals are more likely to participate in research studies.

A prospective longitudinal cohort (n=754, mean age: 78±5, 65% women, 91% white) examined how older adults transitioned to and from a robust, pre-frail, or frail state during follow-up (65). At baseline, 23%, 51%, and 26% of the cohort was robust, pre-frail, and frail, respectively. After 18 months of follow-up, half of the cohort did not transition from their baseline state. Among those who were robust at baseline, 52% remained robust, 40% transitioned to pre-frail, 4% transitioned to frail, and 4% died. Among those who were pre-frail at baseline, 58% remained pre-frail, 25% transitioned to frail, 5% died, and 12% actually transitioned to a robust state. Lastly, among those who were frail at baseline, *64% remained frail*, 13% died, 23% had a healthy transition to a pre-frail state, but none transitioned to robust. Thus, among older adults classified as frail, most will remain frail (61, 66).

1.3.5 Analytic epidemiology of frailty

1.3.5.1 Frailty and major health outcomes

Frailty is associated with multiple major health outcomes, making frailty prevention or reducing frailty severity in the population a public health concern. Among community-dwelling older adults, frailty and pre-frailty was associated with higher risks of incident falls, mobility decline, worsening disability, first hospitalization, and all-cause mortality when compared to robust individuals (54), even after adjusting for demographics, behavioral factors, markers of health status, chronic conditions, and cognitive function. Specifically, frail older adults (using the Fried Frailty Phenotype) had a 98% (95% confidence interval: 1.5, 2.6) higher risk of worsening disability and 2.2 (1.5, 3.3) times the risk of dying when compared to robust individuals, after the same adjustments. Similarly, pre-frail older adults had a 67% (1.4, 2.0) higher risk of worsening disability and a 50% higher risk of dying (1.1, 2.0) when compared to robust older adults. Using this information and Levin's attributable risk equation (42), I can estimate that approximately 30% of worsening disability and 27% of deaths can be attributed to frailty or pre-frailty among this cohort of community dwelling older adults (54). In addition, if the prevalence of frailty and pre-frailty were reduced by half (to 3.5% and 23.5%, respectively), approximately 15% of worsening disability and 13% of deaths among community-dwelling older adults could be prevented (using the equation for preventable fraction from (43)). In addition, vigor to frailty scores measured using the Scale of Aging Vigor in Epidemiology (SAVE) were shown to be heritable among a two-generation cohort of families with a clustering of longevity (h2=0.23, p=1.7x10-13) (60).

1.3.5.2 Cross-sectional associations with frailty

Cross-sectional studies have shown that frailty (defined using the Fried Frailty Phenotype) is more prevalent among older ages, women, and non-white races, and associated with lower levels of income and education. Frail individuals are also more likely to present with chronic conditions, such as hypertension, diabetes, cardiovascular diseases, chronic obstructive pulmonary disease, arthritis, peripheral vascular disease, and osteoporosis. Frailty is also associated with self-reported disability, lower cognitive performance, higher levels of depressive symptoms (54, 55, 67), and oxidative stress (68).

As discussed in Section 1.3.2, a hypothesis of frailty progression involves dysregulated immune, endocrine, and stress responses (55). Evidence for this has come from studies reporting cross-sectional associations between frailty and higher levels of pro-inflammatory cytokines: C-reactive protein and interleukin-6 (69-71), higher levels of circulating clotting markers: factor VII, fibrinogen, and D dimer (70), lower levels insulin-like growth factor-1 and dehydroepiandrosterone (71), higher levels of cortisol with a reduction in the diurnal variation (72), and lower levels of 25-hydroxy vitamin D (69). A pro-inflammatory state may initiate the frailty cycle by promoting skeletal muscle loss (73) and suppressing appetite (55), it also may be causing the elevated circulating clotting markers that are associated with frailty (74). Insulin-like growth factor-1 signals growth and differentiation, specifically it stimulates bone formation, protein synthesis, glucose uptake in muscle, neuronal survival, and myelin synthesis (75), where a decrease likely contributes to frailty progression through sarcopenia and weight loss as well as dysregulated energetics. Dehydroepiandrosterone is thought to influence frailty since it may be directly involved in maintaining muscle mass (76), where lower levels lead to less ability to adequately maintain muscle. Cortisol, a steroid hormone released in response to stress, may be

associated with frailty progression by negatively impacting skeletal muscle and disrupting components of the immune system (55). Lastly, vitamin D is needed to absorb calcium and maintain skeletal strength, where lower levels of 25-hydroxy vitamin D, a product of vitamin D, may indicate less ability to maintain skeletal strength leading to sarcopenia and further exacerbation in the frailty cycle.

1.3.5.3 Risk factors for frailty

In at least two longitudinal studies examining risk factors for frailty (defined using the Fried Frailty Phenotype), older age (77-80), lower education (78-80) prevalent diabetes (67, 77) and arthritis (77, 80), larger total number of chronic conditions (77, 79), past or current smoking (77, 80), self-reported disability (67, 80), and worst cognitive performance (67, 77) significantly predicted incident frailty or worsening frailty severity. Other prospective studies have additionally found being a woman (78), under or overweight (80), prevalent cardiovascular diseases (80), hypertension (80), and chronic obstructive pulmonary disease (80), history of a hip fracture (80) and falls (80), negative affect (77), higher levels of depressive symptoms (80), allostatic load (81), C-reactive protein (82), and insulin resistance (82) significantly associated with incident frailty or increases in severity, whereas higher income (80), living alone (80), higher self-reported health (80), and better leg power at baseline appear to be protective.

1.4 INTEGRATED MEASURES OF AGING

Both frailty severity and gait speed have been described as complex phenotypes influenced by multiple aspects of aging, as well as multimorbidity. These aging phenotypes can be thought of

as manifestations of the dysregulation across and within multiple physiologic systems that occur from age-related molecular changes, genetics, and disease. It is likely that older adults with a fast gait speed or older adults who have low levels of frailty severity will have significantly different profiles of circulating markers than their counterparts. Though, a limitation of the currently known and commonly measured biomarkers associated with these aging phenotypes is that they are also associated with numerous other conditions and health outcomes (83) and thus, do not act as a precise markers of age-related decline.

Metabolomics has the potential to better characterize frailty and walking ability in late-life by simultaneously measuring hundreds of metabolites that can illustrate profiles of metabolic processes specific to both or either frailty and walking ability. Understanding the associated metabolic processes of these aging-related phenotypes can lead to a better understanding of their biology and pathophysiology, which will help inform better interventions to reduce frailty severity and decline in walking ability, and ultimately, preserve physical functioning and independence throughout old age.

1.5 METABOLOMICS

Metabolomics is the large-scale study of endogenous and exogenous metabolites present in an organism (84, 85). Metabolites are small molecules (1500 Da) in cells, tissues, and bodily fluids that help in or are intermediates or end products of cellular metabolism, some examples being sugars, lipids, and amino acids. Even though ‘metabolomics’ is a relatively recent term, the concept of measuring small molecules in bodily fluids to detect disease has been around since ancient times. Around 500-600 AD, the ancient Indians tested for diabetes, then referred to as the

"sweet urine disease," by examining whether ants were attracted to patients' urine due to excessive levels of the metabolite, sugar (86).

Metabolites can be influenced by both genetics and the environment and are thought to best reflect the molecular phenotype of an individual because they illustrate the underlying biochemical activity and the state of cells and tissues through what is left behind from cellular processes (87). Measuring metabolites has the potential to capture how metabolic pathways are functioning within an organism. Metabolic pathways consist of reactions that can be either anabolic or catabolic. Anabolic reactions require energy to build more complex molecules from simpler ones, whereas catabolic reactions release energy by breaking down molecules into simpler ones (87). Thus, differences in metabolite values by the phenotype of interest may indicate certain pathways are altered to undergo catabolism versus anabolism.

Metabolomics is the closest –omics approach to the phenotype of interest and thus, is particularly promising since it may provide information that is more biologically coupled to the phenotype of interest. Information on altered amounts of metabolites and altered metabolic pathways among those with a disease or condition versus their healthy counterparts can be used to better characterize the disease or condition that can be used to identify new biomarkers to detect and intervene at earlier points in its pathogenesis, as well as inform therapeutic targets (88), with the potential for more personalized drugs and improved treatment strategies.

1.5.1 Targeted versus untargeted metabolomics

There are two main approaches to metabolomics: untargeted and targeted. Targeted involves measuring specific metabolites or specific groups of metabolites, whereas untargeted is a global approach to measure as many metabolites present in a sample as possible (87, 89) including

metabolites that may not yet be fully characterized. Untargeted metabolomics can be viewed as a hypothesis generating or exploratory approach using inductive reasoning, where the goal is to detect patterns in the observed metabolites to develop a conceptual model. Targeted, on the other hand, can be viewed as hypothesis testing using deductive reasoning, where an established conceptual model determined what metabolites to examine. Ultimately, results using an untargeted approach can inform the hypothesis for a more targeted approach (89). There are multiple steps involved in measuring metabolites. The first is designing a study, followed by preparing samples, extraction, mass spectrometry, and last acquiring, processing, analyzing, and interpreting the data (87).

1.5.2 Methods

1.5.2.1 Sample preparation

An important step is deciding what type of biological sample will be collected, e.g., plasma, serum, tissue, urine, or saliva, as well as the time of day samples will be collected, what supplies will be used (e.g., glass versus plastic vessel), and how long participants should fast prior to collection (90). It is important to keep collection times similar across participants since metabolic reactions have circadian variation. Other potential confounding factors are likely the participants' activity level, diet, and hydration level. If samples are not analyzed right away then they should be stored at -80°C, preferably in 1 mL aliquots.

1.5.2.2 Extraction

Extraction is the process of separating metabolites based on physical and chemical properties. Metabolites are both physically and chemically diverse (91) and continuously being

absorbed, synthesized, and degraded, and interacting with other molecules within and between metabolic pathways (87). Due to the dynamic nature and chemical/physical diversity, metabolite extraction is difficult (91). The most common metabolite extraction methods are liquid-liquid extraction, solid-liquid extraction, and solid-phase extraction (91). Liquid-liquid extraction is a method of adding two immiscible liquids (usually water and a non-polar organic solvent) to the samples, which causes compounds, or metabolites, to separate based on solubility. An example of an immiscible non-polar organic solvent is chloroform. Chloroform is denser than water, non-reactive, volatile, and when paired with water results in the separation of polar and non-polar metabolites (92), where the polar metabolites dissolve in the water (e.g., glucose, amino acids, ketone bodies, creatinine) and the non-polar metabolites dissolve in the chloroform (cholesterol, fatty acids, phospholipids, triglycerides) (90). Similarly, solid-liquid extraction is a method of extracting compounds from a solid by adding a liquid. The compounds are extracted from the solid and dissolved into the liquid, the liquid extract can then be separated from the solid using a filter (93). An example of solid-liquid extraction is the process of brewing coffee: coffee flavor compounds are extracted by adding water to coffee grounds and then filtering out the coffee grounds (93). Last, solid-phase extraction, also known as liquid-solid phase extraction, is a method that uses solid particles to separate metabolites dissolved or suspended in a liquid.

1.5.2.3 Liquid chromatography-mass spectrometry

There are multiple analytical technologies used to acquire data on metabolites, such as liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, nuclear magnetic resonance, differential mobility, and imaging mass spectrometry. Each method has their advantages and disadvantages. Here, we will focus on liquid chromatography-mass

spectrometry, which is the method used in the applied portion of this dissertation (see Sections 2.3.4 and 3.3.2).

Liquid chromatography is a method of separating metabolites in a liquid based on chemical and physical properties. High-performance liquid chromatography uses pressurized pumps to pass liquid samples through a column containing small particles (94). Metabolites are separated because their physical and chemical diversity causes them to pass through the column at different speeds. The time it took for a component to pass through the column is referred to as the retention time.

Mass spectrometry is a technique that essentially measures the mass of chemical substances within a sample. Mass spectrometry ionizes a chemical substance, which causes molecules to break into charged fragments. The resulting ions are then sorted based on their mass-to-charge ratio. Based on their mass-to-charge ratio, metabolites are detected, identified, and quantitated. Mass spectrometry coupled with liquid chromatography reduces the complexity of a sample by first separating the compounds based on physical and chemical properties before ionization (89). The column used in liquid chromatography allows for specific metabolites to be measured. For example, using polar columns in hydrophilic interaction liquid chromatography separates out polar metabolites, such as sugars, amino acids, carboxylic acids, and nucleotides (89). Whereas, using C18 columns along with liquid chromatography coupled to electrospray-ionization mass spectrometry separates semi-polar metabolites, such as glycosylated steroids, phenolic acids, and alkaloids (89). A main advantage of liquid chromatography-mass spectrometry, over both gas chromatography-mass spectrometry and nuclear magnetic resonance, is that is can analyze almost all compounds with high sensitivity and selectivity (89).

1.5.2.4 Data processing

The result of liquid chromatography-mass spectrometry will be an output of data including peaks with retention time and mass-to-charge ratio (95). Databases are available that can be used to match mass-to-charge ratio (96). Typically an internal reference library is also available in the lab where results were obtained. The reference library includes information on both retention time and mass-to-charge ratio for hundreds of known metabolites, allowing the detected metabolites to be identified by matching them up to the known standards. Restricting analyses to metabolites identified from reference libraries has been referred to as semi-targeted metabolomics (96), where as many metabolites are being measured as possible still, but only the known metabolites available in reference libraries are being identified and analyzed. Once metabolites are identified, the peak areas of each can be used for statistical analysis. In the following sections, metabolomics of aging-related phenotypes and disease will be discussed to get a sense of metabolites that may be involved in aging.

1.5.3 Physical performance measures and incident mobility disability

Variability in physical functioning increases with chronological age, where differences may be a result of chronic exposure to an abundance of metabolites or to low levels of metabolites. For example, a chronic abundance of circulating fatty acids may contribute to poor physical functioning because of excess fat entering muscle. Greater intermuscular fat has shown to predict a faster decline in gait speed among older adults (53). Another example would be that chronic low levels of amino acids may cause a decrease in muscle synthesis, leading to sarcopenia over time which is associated with poorer physical functional status (97). To date, four studies have examined the relationship between metabolites and physical performance measures. Metabolites

associated with gait speed and incident disability were examined in 319 black men from the Health, Aging, and Body Composition study (median age: 73) (98). Among the 350 plasma metabolites examined, seven metabolites were correlated with gait speed (Table 1) at a 0.01 significance level, while adjusting for weight change from the prior year, age, study site, and smoking status. After adjusting for multiple comparisons using a 30% false discovery rate (99), only the following two metabolites remained significantly associated with gait speed: 2-hydroxyglutarate (correlation=-0.17) and salicylurate (correlation=-0.19). The former metabolite has been linked to cancer, and thus classified as an oncometabolite, whereas salicylurate eliminates salicylates (e.g., aspirin) from the body. These two metabolites may reflect more chronic disease and medication use among those with a slower gait speed (98). The authors found 23 metabolites significantly associated with incident mobility disability (Table 1), the majority being markers of kidney function (creatinine, N-carbamoyl beta-alanine, SDMA, quinolinate, inositol, inosine, hypoxanthine, indoxyl sulfate), suggesting a potential causal relationship between kidney health and mobility. The associated metabolites may also indicate pathways of amino acid metabolism and degradation, as well as oxidative stress involved in the pathophysiology of mobility disability. A targeted set of 148 metabolites (amino acids, biogenic amines, and lipids) was examined in 504 adults ages ≥50 from the Baltimore Longitudinal Study of Aging (100). The authors found eight metabolites, all of which were lipids, associated with gait speed after adjusting for age and gender (Table 1). The authors also examined which metabolites were associated with change in gait speed and found lower levels of the lipid, C18:2 LPC, was associated with faster decline in gait speed over a median of 4 years of follow-up, independent of age, gender, and chronic conditions.

Lustgarten et al. (2014) examined 177 metabolites (amino acids, fatty acids, and acyl carnitines) and two physical performance measures among 73 sedentary older adults (mean (SD) age = 77.7 (3.9), 41% men) from a randomized controlled trial of whey protein supplementation in addition to supervised resistance exercise training (101). When examining baseline metabolites (i.e., prior to the intervention), higher levels of two amino acid principal components were significantly associated with worse scores on the Short Physical Performance Battery ($p \leq 0.009$) and five amino acid factors and one acyl carnitine species factor were associated with gait speed ($p \leq 0.005$), suggesting metabolites related to gut bacterial metabolism, peroxisome proliferator-activated receptor-alpha activation, and insulin sensitivity may influence physical performance among sedentary older adults. Similarly, a small cohort of older men from the U.S. Veterans LIFE Study applied a principal components analysis to 45 plasma acyl carnitines to get a single factor score, where higher levels of the acyl carnitine score was significantly associated with worse scores on the Short Physical Performance Battery, gait speed, and the chair stand test (102). Taken together, these studies of metabolites associated with physical performance suggest certain lipids, amino acids, and acyl carnitines, in particular, may be markers to explore further to better understand molecular changes that may contribute to differences in physical functioning among those of the same chronological age.

1.5.4 Frailty

Few studies have examined the metabolomics of frailty. Fazelzadeh et al. (2016) examined differences in muscle metabolites among 43 frail or pre-frail older adults versus 66 healthy older adults. The authors found 26 muscle metabolites differed by frailty status ($p<0.05$), where the majority were amino acids (Table 1). The differences in metabolites among frail versus healthy

older adults reflect mitochondrial functioning, tissue turnover, and fiber-type composition (103). Another study examined the association between plasma metabolites measured in 2530 samples from the TwinsUK BioResource (all volunteers were women; mean age: 60.5, range 17-93) and frailty defined using the Rockwood index (104). The authors found 20 metabolites associated with frailty at a 0.001 significance level, of which most were amino acids and lipids.

Serum metabolites associated with being fit, unfit, or frail among older adults with breast cancer has also been examined (105). Using information from a targeted metabolomics study of 45 amino acids, 40 acylcarnitines, and 150 phsophlipids, the authors found a set of amino acids and acyl carnitines that best differentiated the frail from the non-frail cancer patients. However, after adjusting for differences in age only one amino acid remained significantly associated with frailty status, and no acyl carnitines remained significant. Other metabolites significantly associated with frailty status among the cancer patients after adjusting for age were sphingolipids and glicerophospholipids (Table 1); suggesting perturbations in lipid metabolism may characterize frailty development among breast cancer patients. Perturbations in lipid metabolism were also observed among a lipidomic study of frail versus non-frail HIV+ patients (106). Thus, these few studies of frailty-associated metabolites similarly indicate that alterations in levels of certain lipids and amino acids may play a role in frailty.

1.5.5 Healthy aging index

We previously examined metabolites associated with the healthy aging index among 319 black men from the Health, Aging, and Body Composition study (median age: 73) (107). The Healthy Aging Index is a measure similar to the Physiologic Index of Comorbidities that captures multi-system disease burden (108). Among 341 metabolites, 19 (listed in Table 1) were associated with

the unhealthy versus healthy extremes of the healthy aging index (scores 8- 10 versus 0-3, respectively), while accounting for multiple comparisons using a Benjamini-Hochberg 5% false discovery rate (99). Two of the 19 metabolites associated with the healthy aging index also significantly predicted cardiovascular mortality. One standard deviation higher expression of glucuronate and symmetric dimethylarginine was associated with a 29% (95% CI: 1.05, 1.57) and 54% (1.09, 2.19) higher risk of cardiovascular disease mortality, respectively, while adjusting for age, body mass index, prevalent cardiovascular disease, creatinine, and the healthy aging index, as well as deaths from other causes as competing risks and multiple comparisons. The results suggest pathways of oxidative stress, nitric oxide formation, gut microbial activity, and the citrate cycle involved in aging.

1.5.6 Metabolism

Aging and aging-related disease are likely influenced by common metabolic pathways. It has previously been observed that cohorts of long-lived individuals tend to have healthier metabolic characteristics and a delayed onset of aging-related disease when compared to controls (109, 110). In addition, among families with a clustering of longevity, those who also have a clustering of healthy metabolism may have an even more extreme longevity phenotype (111). Proposed metabolic strategies promoting healthy aging and longevity have involved caloric restriction, lower signaling of the insulin/insulin-like growth factor-1 pathway, and lower signaling of the mammalian target of rapamycin (mTOR) pathway (112). The following sections discuss studies that have examined metabolomics of aging-related metabolic diseases, specifically obesity, diabetes, and cardiovascular disease, to provide further insight into potential metabolomic signatures of healthy versus unhealthy aging.

1.5.6.1 Body composition

Metabolites correlated with lean mass and adiposity was examined among the same cohort of black men from the Health, Aging, and Body Composition study, as in sections 1.5.5 and 1.5.3 (113). Using a strict multiple comparisons Bonferroni adjustment, 92, 48, 96, and 53 metabolites were associated with body mass index, percent fat, percent trunk fat, and appendicular lean mass, respectively, controlling for age, site, and smoking status (Table 1). The majority of associated metabolites were lipids. In addition, branched-chain amino acids and carnitine metabolites were correlated with each of the four body composition measures. Branched-chain amino acids (leucine, valine, and isoleucine) are essential in regulating protein structure and benefit muscle composition, whereas carnitine metabolites are markers of energy production and lipid catabolism (113), suggesting disruptions in lipid homeostasis may be involved in obesity and muscle metabolism.

1.5.6.2 Obesity

Newgard et al. (2009) performed a Principal Components Analysis (PCA) to reduce the number of metabolites compared among obese versus lean participants (114). Among 18 PCA factors, a branched-chain amino acid-related metabolic signature (Table 1) was the most significantly associated with obese versus lean participants ($p<0.0001$), while adjusting for age, race and gender. The same factor was also positively correlated with homeostatic model assessment for insulin resistance (correlation=0.58, $p<0.0001$). Briefly, the authors furthered their understanding by feeding rats either a high-fat diet, high-fat with branched-chain amino acid (BCAA) supplementation, or a standard diet and found mice on the BCAA supplemented high-fat diet were just as insulin resistant as the rats on the high-fat diet, even though they had a reduced food intake and similar weight gain to the rats on a standard diet. Thus, the authors

concluded that branched-chain amino acids may contribute to insulin resistance when in the presence of a poor diet that is high in fat (114).

1.5.6.3 Diabetes

Branched-chain amino acids (leucine, isoleucine, valine) were also significantly associated with incident diabetes among a nested case-control study of 189 participants who developed incident diabetes versus 189 propensity-matched controls sampled from the Framingham Heart Study cohort (115). Phenylalanine and tyrosine (aromatic amino acids), in addition to the branched-chain amino acids, were also significantly associated with incident diabetes. One standard deviation higher log-transformed score on any of the five metabolites was associated with a 1.57 (1.17, 2.09) to 2.02 (1.40, 2.92) times the odds of incident diabetes, while adjusting for age, gender, body mass index, and fasting glucose. Similar associations were found when further adjusting for family history of diabetes, triglycerides, and dietary intake of protein, amino acids, and total calories. All five metabolites remained significantly associated, with a similar magnitude, with incident diabetes when replicated in an independent cohort. Similarly to Newgard et al. (2009), the authors concluded that circulating amino acids may directly promote insulin resistance and cause disruptions in insulin signaling within skeletal muscle by activating the following signaling pathways: mammalian target of rapamycin (mTOR), JUN, and insulin receptor substrate 1 (115). Another hypothesis was that more circulating amino acids may promote diabetes by also causing hyperinsulinemia and pancreatic cell exhaustion.

1.5.6.4 Cardiovascular disease

Branched-chain amino acids have also been positively correlated with cardiovascular disease, suggesting common underlying pathways of cardiovascular disease, obesity, and

diabetes. After prolonged exercise, such as marathon running, metabolite changes occur, where a significant decline in concentrations of most circulating amino acids was observed, as well as increases in lipolysis and products of ketogeneisis (116, 117). Thus, suggesting a pathway at which exercise can improve aging-related molecular changes.

In addition to branched-chain amino acids, higher phenylalanine, monounsaturated fatty acids, unsaturated lipids, and lower levels of omega-6 fatty acids and docosahexaenoic have also been associated with cardiovascular disease (116). The association of higher levels of trimethylamine-N-oxide and cardiovascular disease has been validated in multiple cohorts. Trimethylamine-N-oxide is a metabolite present in higher quantities among those with a meat-based diet since the metabolite is produced by gut bacteria that feed on dietary phosphatidylcholine and carnitine (compounds from dietary meat). Supporting this, trimethylamine-N-oxide supplementation in animal models has shown to promote atherosclerosis (116).

From these studies, it appears that lipids and amino acids are classes of metabolites that were commonly associated with physical performance, frailty, healthy aging, body composition, diabetes, and cardiovascular disease among community-dwelling older adult cohorts. Thus, I hypothesize that in the applied portion of this dissertation, lipids (e.g., sphingomyelins, and triacylglycerol) and amino acids (e.g., leucine, isoleucine, valine, and tyrosine) will be among the metabolite classes that are most significantly associated with walking ability and frailty.

1.6 SPECIFIC AIMS AND CONCEPTUAL MODEL

As discussed in the previous section, most studies of metabolomics focus on a single organ system or disease. However, common pathways of aging and aging-related disease may exist that lead to physiologic dysregulation across multiple organ systems, where metabolomics of integrated measures of aging, rather than a single disease, are needed to identify them. Few studies have examined metabolomics of more integrated measures of aging, such as frailty and physical functioning. Limitations of the existing studies include: conducted in specialized populations (e.g., cancer patients, HIV+ patients), only examined older adults in the unhealthy extreme of aging (e.g., sedentary older adults with low physical functioning), and/or using performance measures that may not well-differentiate between older adults with adequate to healthy aging (e.g., 20m walk alone). Examining both extremes of the aging distribution among a more generalized population of community-dwelling older adults has a greater potential to identify underlying mechanisms of healthy aging since there will be a greater amount of variability to provide more contrast between healthy versus unhealthy aging. The overall aim of my dissertation was to identify metabolites indicating shared mechanisms of aging across multiple physiologic systems using measures of frailty to vigor and walking ability that can capture both extremes of the aging distribution. The specific aims of this project were as follows:

Aim 1: To examine plasma metabolites and metabolic pathways associated with high walking ability (n=60) versus low walking ability (n=60) among participants from the Cardiovascular Health Study (CHS) All Stars study using a nested case-control study design, matched one-to-one on age, gender, race, and fasting time. Walking ability was defined using objectively measured gait speed and self-reported ability to walk both ½ mile and 1 mile (see Section 2.3.5).

Aim 2: To examine plasma metabolites and metabolic pathways associated with frailty to vigor scores among 287 black men from the Health, Aging, and Body Composition (Health ABC) study, while adjusting for age and study site. Frailty to vigor scores were measured using the Scale of Aging Vigor in Epidemiology (SAVE; see Section 3.3.3).

Aim 3: To develop and validate a novel metabolite composite score using metabolites associated with frailty to vigor scores among 287 black men from the Health ABC study, and to determine whether the metabolite composite score explains the higher mortality risk associated with frailty. The metabolite composite score will be validated against all-cause mortality in an independent cohort of 120 community-dwelling older adults from the CHS All Stars study. The metabolite composite score was a tertile-ranked sum of the 37 metabolites associated with frailty to vigor scores (see Section 4.3.4).

My overall hypothesis was that older adults in different extremes of aging phenotypes (i.e., high walking ability or low levels of frailty) would have specific metabolomic signatures reflective of healthy versus unhealthy aging. Figure 2 illustrates that stressful stimuli may be causing metabolic pathways to become altered to maintain homeostasis in the presence of a stressful event, which then contributes to an increase in physiologic dysregulation contributing to poor aging-related phenotypes, such as a decline in walking ability and higher frailty severity, which then leads to an increased vulnerability to major health outcomes. A limitation of this study is the cross-sectional nature, which will only allow me to speculate causal relationships, but will not provide evidence toward temporality. However, information gained from this dissertation will set the foundation needed to inform future studies on metabolic processes to investigate further as potentially involved in the pathophysiology of aging-related phenotypes, frailty and poor walking ability. Metabolomic signatures specific to older adults characterized as

healthy agers may provide points of intervention to improve and preserve physical function in the older adult population.

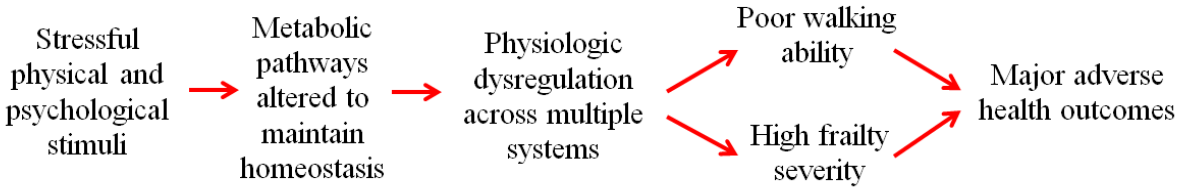


Figure 2. Proposed causal diagram of altered metabolic pathways and aging phenotypes

1.7 TABLES

Table 1. Metabolites associated with aging-related phenotypes

Phenotype (reference)	Cohort	Adjustments	Targeted or untargeted metabolomics	Number of associated metabolites	Metabolites associated with phenotype
Healthy aging index (107)	Health ABC study: subset of 319 black men (mean age: 74)	------	*Untargeted*: 350 plasma metabolites	*19 metabolites*	Kyuneric acid, C5:1 carnitine, Hydroxyphenylacetate, C5:DC carnitine, Trimethylamine N-oxide, Glucuronate, C7 carnitine, C58:8 carnitine, 5-aminolevulinic acid, Alpha-glycerophoshate, N-carbamoyl beta alanine, Symmetric dimethylarginine, Uracil, Creatinine, Citrulline, Isocitrate, Aconitate, Fructose, Urate
Physical performance and disability:					
Gait speed (over 6m) (100)	504 adults ages ≥50 from Baltimore Longitudinal Study of Aging (mean age: 71±10; 49% women)	Age, gender	*Targeted;* 148 plasma metabolites: 25 amino acids, 11 biogenic amines, 1 hexoses, 10 sphingolipids, 7 acylcarnitines, 94 glycerophospholipids	*8 metabolites(FDR<0.05)*	Hexoses, C16:1 SM, C18:0 SM, C18:1 SM, C32:3 PC aa, C17:0 LPC, C18:1 LPC, C18:2 LPC
Change in gait speed over median of 4.2 years (100)	504 adults ages ≥50 from Baltimore Longitudinal Study of Aging (mean age: 71±10; 49% women)	Age, gender, and chronic diseases (hypertension, coronary artery disease, congestive heart failure, peripheral arterial disease, stroke, diabetes, chronic obstructive pulmonary disease, Parkinson's disease, lower extremity joint disease, chronic kidney disease)	*Targeted;* 148 plasma metabolites: 25 amino acids, 11 biogenic amines, 1 hexoses, 10 sphingolipids, 7 acylcarnitines, 94 glycerophospholipids	*4 metabolites after adjusting for age and gender, but only 1 metabolite after further adjusting for chronic diseases*	C18:2 LPC (remained significant after further adjusting for chronic diseases), C32:3 PC aa, C38:3 PC ae, creatinine
Gait speed (over 20m) (98)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking, and weight change from the prior year	*Untargeted*: 350 plasma metabolites	*7 metabolites (p<0.01), but only 2 metabolites remained significant after multiple comparisons adjustment (q<0.30)*	Salicylurate and 2-hydroxyglutarate (remained significant after multiple comparisons), Glucuronate, C54:10 TAG, Asparagine, Tryptophan, C24:1 Ceramide D18:1

Table 1 Continued

Phenotype (reference)	Cohort	Adjustments	Targeted or untargeted metabolomics	Number of associated metabolites	Metabolites associated with phenotype
Gait speed (over 400m) (101)	Sedentary 73 older adults (mean age: 78±4; 59% women)	Age, gender	*Targeted;* 177 serum metabolites: 98 amino acids, 59 fatty acids, and 20 acylcarnitines	*Five amino acid principal component factors* *One acylcarnitines principal component factor*	<u>Amino acid factors:</u> N-acetylglycine Branched-chain amino acid degradation products, indolelactate Phenol sulfate Branched-chain amino acids Trans-urocanate <u>Acylcarnitines factor:</u> Isobutyrylcarnitine
Short physical performance battery (101)	Sedentary 73 older adults (mean age: 78±4; 59% women)	No additional adjustments because age, gender, and BMI were not significant in multivariable model	*Targeted;* 177 serum metabolites: 98 amino acids, 59 fatty acids, and 20 acylcarnitines	*Two principal component factors*	Amino acid factor with Hydrocinnamate, Cinnamoylglycine Amino acid factor with N-methylproline, N,N-dimethylproline
Short physical performance battery and its components: gait speed and chair stands (102)	77 men ages ≥70 from the US Veterans LIFE study (mean age: 79±5)	Age, body mass index, health conditions (arthritis, diabetes, problems with circulation in peripheral limbs)	*Targeted;* 45 plasma acylcarnitines	*One principal component factor*	Factor score consisting of 45 acylcarnitines: C10-OH/C8-DC, C12:1, C14:1-OH/C12:1-DC, C12-OH/C10-DC, C14:1, C12, C14, C14:2, C16:1, C16:2, C8:1-DC, C18:1, C10:1, C2, C18:1-OH/C16:1-DC, C14-OH/C12-DC, C16:1-OH/C14:1-DC, C16, C18:2, C6-DC, C6:1-DC/C8:1-OH, C8:1, C10:2, C4-OH, C10, C10:3, Ci4-DC/C4-DC, C20:1-OH/C18:1-DC, C6, C20, C18, C16-OH/C14-DC, C8, C5-OH/C3-DC, C5-DC, C20-OH/C18-DC, C18:2-OH, C18-OH/C16-DC, C3, C5's, C4/Ci4, C7-DC, C22, C20:4, C5:1
Incident mobility disability (98)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking, and weight change from the prior year	*Untargeted*: 350 plasma metabolites	*23 metabolites:* Lipid and lipid-like metabolites (n=11) Organix acids and derivatives (n=5) Organoheterocyclic (n=3) Organonitrogen compound (n=1) Organooxygen (n=1) Nucleoside, nucleotide, analogues (n=1) Unclassified (n=1)	C36:1 PS plasmalogen, C36:4 PC plasmalogen, C36:4 PC A, C36:4 PE, C38:2 PE, C38:4 PE, C18:2 CE, C5 DC carnitine, C4 carnitine, C3 carnitine, Acetylcholine, Indoxyl sulfate, C4-OH carnitine, N-Carbamoyl beta-alanine, 5-Aminolevulinic acid, Butyrobetaine, Creatinine, Hydroxyproline, Hypoxanthine, Inosine, Inositol, Quinolinate, SDMA
Frailty:					
Frailty, defined using the Fried Frailty Phenotype (103)	43 frail or pre-frail older adults (mean age: 78±8; 42% women; mean BMI: 27.5±4) versus 66 healthy older adults (mean age: 72±5; 29% women; mean BMI: 25.5±3)	Gender and frailty by gender interaction	*Targeted;* 96 muscle metabolites: amine, acylcarnitines, organic acids, oxylipins, and nucleotides	*26 muscle metabolites:* TCA cycle metabolites (n=1) Acylcarnitines (n=4) Intracellular buffering (n=1) Oxylipins (n=3) Polyamine metabolism (n=2) Other amino acids (n=14) Aminobutyric acids (n=1)	TCA cycle: citric acid Acylcarnitines: isovalerylcarnitine (C5), octenoylcarnitine (C8), malonylcarnitine(C3-DC), carnitine (C0) Intracellular buffering: carnosine Oxylipins: LA (CYP450) 12,13DiHOME, DGLA (LOX) 8HETrE, 15SHETrE Polyamine Metabolism: spermidine, spermine Other Amino Acids: histidine, asparagine, taurine, serine, glycine, oacetylserine, homoserine, tyrosine, tryptophan, methionine, glutamine, pyroglutamic acid, glutamic acid, glycylglycine

Table 1 Continued

Phenotype (reference)	Cohort	Adjustments	Targeted or untargeted metabolomics	Number of associated metabolites	Metabolites associated with phenotype
Frailty, defined using the comprehensive geriatric assessment (105)	99 older adults with breast cancer (aged 70-97, median=77) defined as fit (n=49), unfit (n=23), and frailty (n=17)	Age	*Targeted;* 235 serum metabolites: 45 amino acids, 40 acylcarnitines, and 150 phospholipids	*20 metabolites:* Amino acids (n=1) Hydroxysphingomyelins (n=3) Phoshphatidylcholine (n=14) LysoPhoshphatidylcholine (n=2)	Amino acids: 3-Methylhistidine Sphingolipides: SM (OH) C16:1. C22:1, C24:1 Phoshphatidylcholine: PC aa C32:2, C36:3, C36:4, C38:5, PC ae C32:2, C34:0, C34:2, C34:3, C36:2, C36:3, C36:4, C36:5, C38:4, C42:2 LysoPhoshphatidylcholine: lysoPC a C18:1, C20:4
Frailty, defined using the Rockwood frailty index as a proportion of 33 possible deficits (104)	2530 women volunteers from the NIHR BRC TwinsUK BioResource (mean age: 60.5±14; age range: 17-93)	Age	*Untargeted*: 305 plasma metabolites	*20 metabolites (p<0.001)*	Glutamate, urate, N-acetyl glycine, C-glycosyl tryptophan, pseudouridine, docosahexaenoate, mannose, HWESASXX, uridine, epiandrosterone sulphate, proline, indolepropionate, 1-docosahexaenoyl-glycerophosphocholine, gamma-glutamylvaline, gamma-glutamylleucine, gamma-glutamylphenylalanine, N-acetylalanine, butyrylcarnitine, glycerol, 2-linoleoylglycero-phosphocholine
Body composition:					
Percent fat (113)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking	*Untargeted*: 350 plasma metabolites	*48 metabolites (p<0.00014):* Lipids (n=38) Organic acids, including one branched-chain amino acids (n=6) Organoheterocyclic (n=1) Unclassified (n=3)	Acetylglycine, C52:3 TAG, C56:8 TAG, C54:6 TAG, C54:7 TAG, C40:6 PC, C52:6 TAG, C56:7 TAG, C38:3 PC, C56:9 TAG, C52:5 TAG, C56:6 TAG, C50:4 TAG, C34:3 DAG, C50:5 TAG, C54:8 TAG, C52:2 TAG, C52:1 TAG, C5 carnitine, C20:3 CE, C58:10 TAG, Valine, C22:6 CE, C18:1 LPC, C40:9 PC, Kynurenic acid, C46:4 TAG, C52:7 TAG, C58:7 TAG, C36:1 DAG, C38:5 DAG, Beta alanine, Betaine, C36:4 DAG, C50:3 TAG, Glycine, C52:4 TAG, C34:1 PC plasmalogen A, C56:5 TAG, C36:3 PC plasmalogen, C48:4 TAG, C20:4 CE, Tyrosine, C34:2 DAG, C36:3 DAG, C46:3 TAG, C38:6 PC, C20:5 CE
Percent truck fat (113)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking	*Untargeted*: 350 plasma metabolites	*96 metabolites (p<0.00014):* Lipids (n=75) Organic acids, including branched-chain amino acids (n=11) Alkaloids (n=1) Organoheterocyclics (n=3) Unclassified (n=5)	C52:3 TAG, C56:7 TAG, C54:6 TAG, C56:6 TAG, C52:1 TAG, Acetylglycine, C52:2 TAG, C56:8 TAG, C36:1 DAG, C34:3 DAG, C50:4 TAG, C52:6 TAG, C58:7 TAG, C38:5 , DAG, C5 carnitine, C56:9 TAG, C50:5 TAG, C40:6 PC, C58:6 TAG, C54:7 TAG, Valine, C56:5 TAG, C50:1 TAG, C38:3 PC, C46:4 TAG, C36:3 PC plasmalogen, C50:3 TAG, C58:10 TAG, C36:2 PC plasmalogen, C50:2 TAG, C34:2 DAG, C54:8 TAG, C52:5 TAG, C34:1 PC plasmalogen A, C46:3 TAG, C32:1 DAG, C52:7 TAG, C48:3 TAG, C48:4 TAG, C50:0 TAG, C18:1 LPC, C36:2 DAG, C54:2 TAG, Kynurenic acid, C34:1 DAG, Betaine, C54:1 TAG, C36:3 DAG, C58:9 TAG, C38:4 DAG, C18:2 LPC, C48:2 TAG, C32:2 DAG, C52:4 TAG, C48:1 TAG, C58:8 TAG, C34:4 PC, Glutamate, C40:9 PC, C3 carnitine, Xanthurenate, C48:5 TAG, C36:4 DAG, Tyrosine, C40:6 PE, C58:11 TAG, C56:2 TAG, Isoleucine, C50:6 TAG, C52:0 TAG, C20:5 LPC, C34:2 PC plasmalogen, 3 hydroxyanthranilic acid, C5:1 carnitine, C20:3 CE, C46:2 TAG, Beta alanine, C32:0 DAG, C56:4 TAG, C30:0 DAG, C20:5 CE, C34:0 DAG, C44:2 TAG, C56:1 TAG, C38:6 PC, C38:4 PC, Quinolate, C20:4 CE, Phenylalanine, C18:2 LPE, Serine, C54:9 TAG, Urate, Leucine C36:1 PC plasmalogen, Glycine

Table 1 Continued

Phenotype (reference)	Cohort	Adjustments	Targeted or untargeted metabolomics	Number of associated metabolites	Metabolites associated with phenotype
Appendicular lean mass (113)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking	*Untargeted*: 350 plasma metabolites	*53 metabolites ($p<0.00014$):* Lipids (n=39) Organic acids (n=10) Alkaloids (n=1) Intermediates in tryptophan metabolism (n=2) Unclassified (n=1)	Valine, Leucine, Isoleucine, C5 carnitine, Phenylalanine, C56:8 TAG, C56:7 TAG, 2-aminoadipate, C52:3 TAG, C34:0 DAG, Tyrosine, C56:9 TAG, C54:6 TAG, Lysine, C58:10 TAG, C3 carnitine, C36:0 DAG, C34:3 DAG, C18:0 MAG, C40:6 PC, C30:0 DAG, C38:3 PC, C32:0 DAG, C20:5 CE, C54:8 TAG, C58:9 TAG, C54:7 TAG, C52:1 TAG, 4-hydroxymandelate, Methionine, C52:6 TAG, C34:1 PC plasmalogen A, C58:7 TAG, Tryptophan, C52:5 TAG, C52:2 TAG, C52:4 TAG, C34:2 DAG, C38:7 PE plasmalogen, C50:4 TAG, C58:11 TAG, Acetylglycine, Cotinine, C36:2 PC plasmalogen, C20:3 CE, C32:1 DAG, C56:6 TAG, C36:1 PS plasmalogen, C14:1 MAG, C40:9 PC, C50:1 TAG, C50:3 TAG, Xanthurenate
Body mass index (113)	Health ABC study: subset of 319 black men (mean age: 74±3)	Age, site, smoking	*Untargeted*: 350 plasma metabolites	*92 metabolites ($p<0.00014$):* Lipids (n=72) Organic acids, including branched-chain amino acids (n=11) Alkaloids (n=2) Intermediates in tryptophan metabolism (n=3) Organooxygen metabolite (n=1) Unclassified (n=3)	Valine, C5 carnitine, C52:3 TAG, C56:7 TAG, C56:8 TAG, C54:6 TAG, C56:9 TAG, Acetylglycine, C52:6 TAG, C54:7 TAG, C34:3 DAG, Isoleucine, C54:8 TAG, C40:6 PC, C58:10 TAG, C38:3 PC, C50:4 TAG, Leucine, C52:5 TAG, C56:6 TAG, Phenylalanine, C52:2 TAG, C52:1 TAG, C50:5 TAG, C58:7 TAG, C52:7 TAG, C38:5 DAG, C58:9 TAG, C52:4 TAG, C18:1 LPC, C3 carnitine, C50:3 TAG, Tyrosine, C36:2 PC plasmalogen, C34:0 DAG, C34:2 DAG, C34:1 PC plasmalogen A, C36:1 DAG, Betaine, C20:5 CE, C58:11 TAG, C32:1 DAG, C50:1 TAG, C36:4 DAG, C40:9 PC, C50:2 TAG, C30:0 DAG, C20:3 CE, C32:0 DAG, C50:0 TAG, C36:3 DAG, Kynurenic acid, C48:3 TAG, C58:6 TAG, 2-aminoadipate, C48:4 TAG, C46:3 TAG, C34:1 DAG, C36:3 PC plasmalogen, C32:2 DAG, C56:5 TAG, C46:4 TAG, Xanthurenate, C54:9 TAG, Hexose, C38:7 PE plasmalogen, C56:10 TAG, Cotinine, C22:6 CE, C58:8 TAG, C48:2 TAG, C54:2 TAG, C48:1 TAG, Glutamate, C36:1 PC plasmalogen, C38:4, DAG, C36:2 DAG, C38:6 PC, C50:6 TAG, C40:6 PE, C54:1 TAG, Urate, Beta alanine, 3-hydroxyanthranilic acid, C34:4 PC, C52:0 TAG, C36:0 DAG, C46:2 TAG, Glycine, C18:0 LPE, C18:2 LPC, C18:1 LPE
Obesity (114)	N=140 participants: Median age: 52; 57% women, 45% black n=73 obese: median age and BMI: 52 years and 37 kg/m^2 n=67 lean: median age and BMI: 50 years and 23.2 kg/m^2	Age, race, gender,	*Targeted*: 98 metabolites: serum acylcarnitines, amino acids, and free fatty acids, plasma total fatty acids, and urinary organic acids	*One principal component*	Branched-chain amino acid-related metabolic signature consisting of: branched-chain amino acids (leucine, isoleucine, valine), methionine, glutamate/glutamine, aromatic amino acids phenlalanine and tyrosine and C3 and C5 acylcarnitines

Table 1 Continued

Phenotype (reference)	Cohort	Adjustments	Targeted or untargeted metabolomics	Number of associated metabolites	Metabolites associated with phenotype
Diabetes:					
Incident diabetes (115)	Framingham Heart Study: nested case-control study of 189 incident diabetes cases (mean age: 56, 42% women, mean BMI: 30.5 kg/m^2) vs. 189 propensity-matched controls	Matching variables: Age, gender, body mass index, fasting glucose	*Targeted*: >60 plasma metabolites: amino acids, biogenic amines, and other polar plasma metabolites	*5 metabolites ($p<0.001$):* Branched-chain amino acids (n=3) Aromatic amino acids (n=2)	Leucine, isoleucine, valine, phenylalanine, tyrosine
Cardiovascular disease:					
Cardiovascular diseases, blood pressure, hypertension (116)	A review on metabolites associated with cardiovascular disease	Traditional risk factors of cardiovascular disease	-------	-------	- Unsaturated lipids associated with **cardiovascular disease**: lysophosphatidylcholine 18:1, lysophosphatidylcholine 18:2, monoglyceride 18:2, and sphingomyelin 28:1 - Trimethylamini-N-oxide associated with **coronary artery disease** - Metabolites correlated with **blood pressure and hypertension**: alanine, hyppuric acid derivatives of gut microbial activity, hexadecanedioate (dicarboxylic acid) - Branched-chain amino acids associated with **cardiovascular disease**, insulin resistance, and type 2 diabetes

SM = sphingomyelin
PC = phosphatidylcholine
PE= phosphatidylethanolamine
LPC = lipophosphatidylcholine
LPE= lysophosphatidylethanolamine
TAG= triacylglycerol
DAG = diacylglycerol

2.0 METABOLITES ASSOCIATED WITH HIGH VERSUS LOW WALKING ABILITY AMONG THE OLDEST OLD FROM THE CHS ALL STARS STUDY

2.1 ABSTRACT

Slow gait speed becomes more prevalent in late-life and is potentially a manifestation of accumulating chronic conditions and age-related molecular changes. Using metabolomics to characterize metabolic differences in older adults of the same age with different walking abilities may provide insight into altered metabolic processes occurring with aging that contribute to decline in physical functioning. In this section, I sought to identify metabolites associated with high versus low walking ability using a nested case-control study of 120 community-dwelling adults ages 79-95 from the CHS All Stars study, matching on age, gender, race, and fasting time. Using liquid chromatography-mass spectrometry, 569 metabolites were measured in overnight-fasting plasma. High versus low walking ability was defined as gait speed *and* Walking Ability Index scores in the best versus worst tertiles (≥0.9 versus <0.7 meters/second and 7-9 versus 0-1, respectively). Using a paired t-test, 96 metabolites were associated with walking ability extremes ($p<0.05$, false discover rate<0.30), where 24% were triacylglycerols. Triacylglycerols containing mostly polyunsaturated fatty acids were higher among those with high walking ability, whereas triacylglycerols containing mostly saturated or monounsaturated fatty acids were lower among those with high walking ability. Arginine and proline metabolism was a top pathway associated

with walking ability extremes. Using conditional logistic regression, body mass index or waist circumference partly explained the association between a subset of metabolites and walking ability extremes. The reproducibility and generalizability of these results need to be determined to understand whether differences in these metabolites truly characterize differences in walking ability among the older adult population. A better characterization of age-related differences in walking ability may provide insight into altered biologic mechanisms that can be targeted to promote healthy aging.

2.2 INTRODUCTION

As discussed in Section 1.2, gait speed is a marker of overall health and wellbeing (16), influenced by musculoskeletal conditions, cognitive status, physical activity, sensory, and perception, as well as the environment (12). A decline in gait speed is thought of as a manifestation of accumulating chronic conditions and age-related changes, such as damage and repair in cells and cellular senescence. Given this information, the ability to walk can be viewed as a multimorbidity aging phenotype, where vast aging-related molecular differences likely exist among those of the same chronological age, but with exceptional versus poor walking ability.

In the United States, approximately 92% of older adults have a gait speed <1.0 meters/second (31), which has been shown to be a clinically relevant threshold for detecting individuals at higher risk for multiple adverse health outcomes (28). Using metabolomics can further the understanding of the age-related molecular changes that contribute to low walking ability and ultimately inform interventions aimed at preserving physical function and independence throughout life. In this section, I sought to identify metabolites and metabolic

pathways associated with high versus low walking ability using a nested case-control study design of 120 older adults matched one-to-one on age, gender, race, and fasting time from the Cardiovascular Health Study (CHS) All Stars study.

2.3 METHODS

2.3.1 Cardiovascular Health Study (CHS)

The Cardiovascular Health Study (CHS) was a population-based prospective longitudinal cohort of 5888 men and women ages 65 and older during study recruitment (118). During 1988 to 1989, 5201 participants were recruited, of which most (95%) were white. During years 1992 to 1993, an additional 687 non-white participants (99% black) were recruited. The study was designed to determine risk factors, consequences, and the natural history of cardiovascular disease among older adults. Participants were recruited from a random sample of older adults ages ≥65 from a Medicare-eligible list. All age-eligible household members of the randomly sampled participants were also recruited. Participants were recruited from four counties across the United States: Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania. Eligible participants were at least 65 years old during recruitment. Ineligibility included wheelchair bound, unable to participate in a clinic examination, undergoing active cancer treatment, or planning to move out of the study area during the next three years. Participants returned annually until 1999 for a clinic examination and were contacted semi-annually for a telephone interview until 2016.

2.3.2 CHS All Stars study

The CHS All Stars study was an ancillary, prospective, longitudinal study of 1862 men and women alive at year 18 (2005 to 2006) of the CHS (119). All participants from the CHS who were still alive and willing to participate were eligible. The study was designed to examine healthy aging and longevity. An in-person examination was performed for 1135 participants in the clinic or in the participant's home. A fasting blood sample was taken and stored for future use. There were 727 participants who enrolled in the CHS All Stars study, but only had a telephone interview. The CHS and the CHS All Stars study were both approved by the Human Research Protection Office at each participating university and all participants provided informed consent.

2.3.3 Nested case-control design

A nested case-control study design was used to identify metabolites associated with high versus low walking ability among older adults. Metabolites were measured in a subset of 120 participants from the CHS All Stars study who had available fasting plasma samples from the year 18 visit (2005-2006) and had either high walking ability (n=60) or low walking ability (n=60), which is defined in Section 2.3.5.

A total of 1135 participants enrolled in the CHS All Stars study and had an in-person visit. Figure 3 illustrates the breakdown of CHS All Stars who were eligible for our nested case-control study design. Among the 1135 participants, 1045 (92%) had a stored plasma sample. Among the 1045 participants with available plasma, 287 (27%) were excluded because they fasted for less than eight hours prior to phlebotomy and 5 (0.5%) were excluded because they

were missing information on fasting time. Among the remaining 753 participants, 648 (86%) had a gait speed measurement and a score on the walking ability index from the same visit that phlebotomy was performed. Since race was a matching variable in our study design, we excluded three participants who had a race/ethnicity that was rare in our study (e.g., American Indian/Alaskan native and Asian/Pacific Islander). Among the remaining 645 participants, there were 81 (13%) with low walking ability and 154 (24%) with high walking ability (see Section 2.3.5 for walking ability definition).

Next, we randomly sampled 60 participants with low walking ability and matched them to a randomly selected participant with high walking ability, resulting in a final sample size of 120 participants. Participants with low versus high walking ability were matched one-to-one on: age (±1 year), gender, race, and number of hours fasted (±1 hour). All plasma samples had never been thawed and there were no significant differences in the time of day phlebotomy was performed or in the number of days between sample storage and use among the matched pairs. Our sample size of 120 participants (60 pairs) was a realistic and feasible (based on available funds) sample size for exploratory untargeted metabolomics. A nested-case control study design was chosen to increase power, with limited funds, by randomly selecting individuals from both extremes of the walking ability distribution while matching on important confounders: age, gender, race, and fasting time.

2.3.4 Metabolites

Metabolites were measured in EDTA plasma extracts collected from the 120 CHS All Stars after an overnight fast of at least eight hours (mean fasting time: 14 hours, range: 11, 20). Plasma samples had never been thawed and were stored at -80ºC from the time of collection (2005-2006)

until 2018 when metabolites were measured. Plasma extracts were placed on platforms in a random, specified order with a sample from a participant with high walking ability and their matched participant with low walking ability in every other position to limit confounding by batch effects. Metabolites were measured using four complimentary liquid chromatography-mass spectrometry (LC-MS) methods. Metabolite profiling platforms measured: 1) amines and polar metabolites (e.g., amino acids, dipeptides), 2) central metabolites and polar metabolites (e.g., sugars, organic acids, purine and pyrimidines), 3) lipids (e.g., triglycerides), and 4) metabolites of intermediate polarity (e.g., fatty acids). Metabolite values used for this report are LC-MS peak areas, analyzed using TraceFinder (ThermoFisher Scientific, US) and Progenesis QI (Nonlinear Dynamics, UK). Peaks were confirmed manually using known standards. Metabolites below the limit of quantitation (signal/noise<10) were classified as unquantifiable (120).

Positive ion mode detection used a 4000 QTRAP triple quadrupole mass spectrometer (SCIEX) coupled to an 1100 Series pump (Agilent) and an HTS PAL autosampler (Leap Technologies) with a 4.5kV ion spray voltage and at 450ºC source temperature. Using protein precipitation, plasma samples (10µL) were prepared with the addition of nine volumes of 74.9:24.9:0.2 (v/v/v) acetonitrile/methanol/formic acid containing stable isotope-labeled internal standards (0.2ng/µL valine-d8, Isotec; and 0.2ng/µL phenylalanine-d8; Cambridge Isotope Laboratories). Samples were centrifuged for 10 minutes (9,000g, 4°C). Resulting supernatants were injected onto a 150×2mm Atlantis HILIC column that was eluted at a 250µL/min flow rate. Initial conditions were set at 5% mobile phase A (10mM ammonium formate and 0.1% formic acid in water) for one minute and then altered linearly over ten minutes to 40% mobile phase B (acetonitrile with 0.1% formic acid) (120, 121).

Negative ion mode detection used a 5500 QTRAP triple quadrupole mass spectrometer (SCIEX) coupled to an ACQUITY UPLC (Waters) with a modified hydrophilic interaction chromatography method and −4.5kV ion spray voltage and at 500ºC source temperature. Using protein precipitation, plasma samples (30µL) were prepared with the addition of 120µL of 80% methanol containing 0.05 ng/µL inosine-15N4, 0.05 ng/µL thymine-d4, and 0.1ng/µL glycocholate-d4 as internal standards. Samples were centrifuged (10 min, 9,000×g, 4°C) and 10µL of supernatants were injected onto a 150×2.0mm Luna NH2 column (Phenomenex) that underwent elution at a 400µL/min flow rate. Initial conditions were set at 10% mobile phase A (20 mM ammonium acetate and 20 mM ammonium hydroxide; Sigma-Aldrich) in water (VMR) along with 90% mobile phase B (10 mM ammonium hydroxide in 75:25 v/v acetonitrile/methanol (VWR)) and then altered linearly over ten minutes to 100% mobile phase A (120, 121).

Lipids were detected using an Exactive Plus orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled to a Nexera X2 UHPLC (Shimadzu, Marlborough, MA) with electrospray ionization and positive ion mode Q1 scans. The ion spray voltage was 5.0 kV with 400ºC source temperature. Plasma samples (10µL) were extracted using 190µL of isopropanol containing 0.25ng/µL 1-dodecanoyl-2-tridecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids). Samples were centrifuged and 10µL of supernatants were injected onto a 150×3.0 mm Prosphere HP C4 column (Grace). The column was eluted with initial conditions set at 80% mobile phase A (95:5:0.1 vol/vol/vol 10mM ammonium acetate/methanol/acetic acid), then after two minutes, changed linearly over one minute to 80% mobile phase B (99.9:0.1 vol/vol methanol/acetic acid), followed by a linear change over 12 minutes to 100% mobile phase B. Conditions remained at 100% mobile phase B for 10 minutes (120, 121).

Metabolites of intermediate polarity were detected using a Shimadzu Nexera X2 U-HPLC system coupled to a Q Exactive hybrid quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific). Plasma samples (30µL) were extracted using 90µL of methanol containing PGE2-d4 (Cayman Chemical Co). Samples were centrifuged, injected onto 150x2.0 mm ACQUITY T3 column (Waters; Milford, MA), and then eluted with 400µL/minute flow rate. Initial conditions were set at 60% mobile phase A (0.1% formic acid in water) for four minutes, then changed linearly over 8 minutes to 100% mobile phase B (acetonitrile with 0.1% formic acid) (121).

2.3.5 Walking ability

Our walking ability phenotype was defined using both gait speed and the Walking Ability Index. The Walking Ability Index was developed in the Health, Aging, and Body Composition (Health ABC) study (122) using self-report information on the level of difficulty or ease walking ¼ mile and walking 1 mile (Figure 4). Participants in the CHS All Stars study were asked similar questions, except difficulty or ease walking ½ mile was determined instead of ¼ mile, as well as the answer 'a little difficulty' walking ½ mile was not included. The answers for the level of difficulty or ease walking ½ mile and walking 1 mile were scored according to Table 2 and the Walking Ability Index was calculated as the sum of the two sub scores. The Walking Ability Index ranges from 0 to 9, where 0 indicates a participant self-reported they were unable to walk ½ mile and 9 indicates a participant self-reported it was very easy to walk 1 mile. Scores on the Walking Ability Index were split into tertiles based on information from all CHS All Stars who had a score from the in-person visit (n=1077). The worst, middle, and best tertile for the Walking Ability Index ranged from 0-1; 2-6; and 7-9, respectively.

Gait speed (meters/second) was calculated from the time it took to walk 15 feet. Gait speed scores were split into tertiles using information from all available participants in the CHS All Stars study (n=981). The slowest, middle, and fastest gait speed tertiles were <0.7; ≥0.7 to <0.9; and ≥0.90 meters/second, respectively. It should be noted that a gait speed of 0.9 meters/second is not a fast gait speed; however, this was the threshold for the fastest gait speed tertile among the CHS All Stars who were 85 years old, on average.

2.3.5.1 Defining high versus low walking ability

High walking ability was defined as participants who were in the fastest gait speed tertile (≥0.9 meters/second) and scored in the best tertile of the Walking Ability Index (scores 7-9; Figure 3). Low walking ability was defined as participants who were in the slowest gait speed tertile (<0.7 meters/second) *and* scored in the worst tertile of the Walking Ability Index (scores 0-1). Both gait speed and the Walking Ability Index were chosen to develop the extremes of the walking ability phenotype because gait speed provided us with an objective measure of walking ability across 15 feet and the Walking Ability Index provided us with further information on more strenuous activities: walking ½ mile and walking 1 mile. Tertiles of gait speed were used instead of established thresholds because the majority (64%) of participants in the CHS All Stars study (ages 77-102) had a gait speed <0.8 meters/second (average gait speed=0.75 meters/second). Thus, in order to have enough participants in our low and high walking ability groups we used sample-specific tertiles.

2.3.5.2 CHS All Stars with information on metabolites

There were 605 known metabolites observed in our nested case-control study of 120 CHS All Stars. Among those 605 metabolites, 497 (82%) were measured in all 120 participants. There

were 72 (12%) metabolites measured in ≥80% of the participants, of which missing values were assumed to be due to the true values being below the detectable limit and were replaced with half the minimum recorded value for that respective metabolite (123). Thirty-six (6%) metabolites were excluded from the current analysis because they were measured in less than 80% of participants (124). Thus, we examined differences in 569 metabolites among 120 CHS All Stars with high versus low walking ability.

2.3.6 Examination

At the CHS All Stars baseline visit (year 18 of CHS; 2005-2006), participants provided an update on their age, medical history, medications, education, current health, smoking status, alcohol consumption, and difficulty with activities of daily living by self-report questionnaires. Participants had previously self-reported their gender and race. History or presence of heart disease, stroke, cancer, arthritis, asthma, chronic bronchitis, emphysema, and osteoporosis was determined by a self-report of a physician diagnosis. Heart disease included myocardial infarction or congestive heart failure, stroke included transient ischemic attacks, and arthritis included that of the back, hip, or knee. Hypertension was defined as either a systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or taking anti-hypertensive medication. Diabetes was defined as either a self-report of a physician's diagnosis confirmed by medication use or as a fasting glucose ≥126 mg/dL. Cancer included all types except for non-melanoma skin cancer. Depression symptoms were measured using the Center for Epidemiologic Studies Depression Scale (125). All medications used in the past two weeks were assessed in their original containers for a medication inventory.

Systolic and diastolic blood pressure was an average of two seated measurements. Height was measured with no shoes using a calibrated stadiometer (118). Weight was measured with no shoes and light clothing using a calibrated scale. Body mass index (kilograms/meters^2) was weight divided by height^2. Waist circumference was measured at the umbilicus with an anthropometric tape while the participant was standing. Participants fasted for at least eight hours prior to the CHS All Stars visit and self-reported the number of hours since they last ate. Phlebotomy was performed and total, high-density, and low-density cholesterol, and triglycerides were measured in fasting EDTA plasma samples and glucose, interlukin-6, C-reactive protein, cystatin C, and creatinine were measured in fasting serum samples at the CHS central laboratory using standard methods (126).

Overall cognitive performance was assessed using the mini-mental state examination (127). Physical performance was assessed using the short physical performance battery, which is a composite based on gait speed, three balance tests, and ability to stand from a chair five times without the help of your arms (128). Isometric grip strength was measured three times in the dominant hand (Jamar, Sammons Preston Rolyan, Bolingbrook, IL) and averaged.

At year 8 of the CHS (1995-1996), dietary intake was assessed using an interviewer-administered food frequency questionnaire developed by the Willet Group at the Harvard School of Medicine and converted to nutrient information (using Harvard.sffq.062795 database). Total calories, calories from fat, daily protein, and daily caffeine intake were examined.

2.3.7 Potential confounders of metabolites and walking ability extremes

A variable is a confounder if it is causally associated with the outcome and associated with the exposure (in either direction), but is not on the causal pathway between the exposure and the

outcome. Potential confounders of metabolites and walking ability included: medication use, dietary history, smoking status, prevalent chronic conditions, and obesity. Depending on the metabolite, these more commonly measured variables may instead be involved in the causal pathway by either 1) the more commonly measured variable causes certain metabolite values which then contribute to high or low walking ability or 2) the metabolites lead to certain values of the more commonly measured variable which then leads to high/low walking ability; the former being more likely.

2.3.8 Statistical analysis

Mean (standard deviation) or frequency (percent) described differences by walking ability extremes and were tested using a paired t-test for continuous measures and a McNemar's test for categorical measures. Metabolites were log-transformed and standardized. A paired t-test adjusting for the matched aspect of the study design was used to determine metabolites associated with high versus low walking ability. To account for multiple comparisons, a Benjamini-Hochberg correction was used to obtain a false discovery rate for each metabolite (99). Since this was a hypothesis-generating report, we used an a priori determined 30% false discovery rate (124).

Metabolites that were significantly associated with high versus low walking ability ($p<0.05$, false discovery rate<0.30) were further examined in a pathway analysis using MetaboAnalyst (129), which compared the set of metabolites associated with walking ability extremes against established sets of metabolites involved in metabolic pathways. A Fisher's exact test determined whether the number of metabolites that were associated with walking ability extremes and involved in a biologic pathway was more than expected by chance. Impact

scores indicated how centrally located the metabolites that were associated with walking ability extremes were on a particular pathway.

Illustrated in Figure 5, I hypothesized that more commonly measured risk factors, such as body mass index would be an upstream factor causing differences in metabolite values which then contribute to walking ability differences with aging. Specifically, these more commonly measured risk factors would have both a direct and indirect effect on walking ability, where the indirect effect of body mass index and walking ability would be mediated by certain metabolites. It should be noted that Figure 5 is a simplified causal diagram, which does not depict the likely feedback loops or the relationships between more commonly measured risk factors. It should also be noted that our data is from a cross-sectional study, so I cannot formally assess mediation or temporality. Thus, I informally examined whether the association between body mass index and walking ability extremes was mediated by metabolites using the following three steps: 1) I determined whether body mass index was associated with walking ability extremes at a $p<0.05$ using conditional logistic regression of high versus low walking ability on body mass index, adjusting for matched pairs; 2) among the metabolites associated with walking ability, I determined which were also associated with body mass index at a $p<0.05$ using a random intercept model of metabolite values on body mass index, adjusting for matched pairs; and 3) I determined the percent attenuation in the association between body mass index and walking ability extremes after adjusting for a metabolite using conditional logistic regression of walking ability extremes on body mass index and a metabolite, adjusting for matched pairs. In step three, we also examined the extent to which body mass index attenuated the association between a metabolite and walking ability extremes. Using information from step 3, which included both body mass index and a metabolite as the independent variables, I plotted the percent attenuation

in the association between a metabolite and walking ability after adjusting for body mass index *versus* the percent attenuation in the association between body mass index and walking ability after adjusting for the respective metabolite, to get an idea of which variables were becoming most attenuated. Percent attenuation was calculated as: 100*(beta coefficient from unadjusted model – beta coefficient from adjusted model)/beta coefficient from unadjusted model. We repeated those same steps for other more commonly measured variables associated with walking ability: waist circumference, arthritis, total number of prescription medications, and interleukin-6.

2.4 RESULTS

2.4.1 Characteristics by walking ability extremes among 120 CHS All Stars

Characteristics between the 120 CHS All Stars with high versus low walking ability are displayed in Table 3. Participants were 85 years old, on average, 40% were men, 10% were black, and it had been about 14 hours, on average, since participants last ate. Consistent with the matched study design, we found no differences in age, gender, race, or fasting time between those with high versus low walking ability.

Participants in the high walking ability group were more likely to be recruited from the site at Wake Forest University School of Medicine and less likely to be recruited from the site at University of California, Davis and Johns Hopkins University. Those with high walking ability were also more likely to have more than a high school degree and consumed about an average of one additional alcohol beverage per week, and had a higher proportion with an excellent or very

good self-reported health than the low walking ability group. The average body mass index and waist circumference among the high versus low walking ability group was 3 kg/m^2 and 11 cm lower, respectively. There were significantly lower proportions of participants who have had a stroke and who had asthma, arthritis, and difficulty with at least one activity of daily living among those with high versus low walking ability. The high walking ability group performed better cognitively and had fewer depression symptoms.

When examining physical performance, the high versus low walking ability group scored an average of 4 kg and 4 points higher for grip strength and the short physical performance battery, respectively. Consistent with the definition of our walking ability phenotype, those with high walking ability had a significantly faster gait speed than those with low walking ability.

When examining blood-based markers, we found that individuals in the high versus low walking ability group had significantly lower fasting glucose, interleukin-6, and C-reactive protein. Dietary information was assessed 10 years prior to the CHS All Stars exam. Those with high versus low walking ability consumed somewhat less caffeine ($p=0.09$) and slightly fewer total calories ($p=0.15$) and calories from fat ($p=0.15$) at year 8 of the CHS, though differences were not significant at a 0.05 level.

2.4.2 Metabolites associated with high versus low walking ability

Among the 569 metabolites examined, 96 were associated with walking ability extremes ($p<0.05$, false discovery rate<0.30; Table 4). The paired difference between the 96 metabolites among those with high versus low walking ability is listed in Table 4. Lower values for 45 metabolites and higher values for 51 metabolites were associated with high versus low walking ability, with absolute mean standardized paired differences ranging from 0.32 to 0.69.

Table 5 includes the taxonomy classes of the 96 metabolites associated with walking ability extremes. Slightly more than half of the metabolites associated with walking ability extremes were lipids and lipid-like molecules (m=54), such as glycerolipids, glycerophospholipids, sphingoplipids, and more. The remaining 44% of metabolites associated with walking ability extremes were organic acids and derivatives (m=16; mostly amino acids, peprides, and anologues), organoheterocyclic compounds (m=14), nucleosides, nucleotides, and analogues (m=3), organic oxygen compounds (m=3), benzenoids (m=2), phenylpropanoids and polyketides (m=1), alkaloids and derivatives (m=1), and unclassified metabolites (m=2).

2.4.3 Pathway analysis

Among the 96 metabolites associated with walking ability extremes, 89 had an identification number in the Human Metabolome Database Version 4.0 (84) and were included in a pathway analysis. Table 6 includes the top ten pathways among 28 that involved at least one of the metabolites associated with walking ability extremes. The most significant pathways with the largest impact scores were caffeine metabolism and arginine and proline metabolism. The match status for caffeine metabolism was 4/21, indicating 21 known metabolites are involved in caffeine metabolism, of which four were among the 96 metabolites associated with walking ability extremes (caffeine, 3-methylxanthine, 7-methylxanthine, and theophylline). The match status was 6/77 for arginine and proline metabolism (ornithine, L-arginine, L-proline, N-acetylputrescine, 4-acetamidobutanoic acid, and sarcosine).

2.4.4 Attenuation

When examining characteristics of participants with high versus low walking ability in Table 3, we observed a highly significant difference in body mass index ($p<0.0001$), waist circumference ($p<0.0001$), arthritis ($p=0.0005$), and interleukin-6 ($p=0.0003$) between walking ability extremes that may be contributing to metabolite differences. Though, total number of prescription medications was not as significantly different between the walking ability extremes ($p=0.01$) when compared to body mass index, waist circumference, arthritis, and interleukin-6, we still hypothesized that this would be an important variable that may be contributing to differences in metabolite values between the two groups.

Next, we examined which metabolites were correlated with these five variables. Among the 96 metabolites associated with walking ability extremes, 62 were associated with either body mass index, waist circumference, arthritis, number of prescription medications, and/or interleukin-6 ($p<0.05$; Table 7). Specifically, 32, 40, 14, 9, and 32 metabolites were associated with body mass index, waist circumference, arthritis, number of prescription medications, and interleukin-6, respectively. Among the five variables considered, body mass index and waist circumference had the most overlap in correlated metabolites, which was not surprising since body mass index and waist circumference are highly related (correlation coefficient = 0.88, $p<0.0001$). Almost all (91%) of the metabolites associated with body mass index were also associated with waist circumference, but less metabolites that were associated with waist circumference were also associated with body mass index (73%).

Body mass index: The 32 metabolites associated with body mass index at a 0.05 significance level are listed in Table 8 by taxonomy super class. One standard deviation (=4.8 kg/m^2) higher body mass index was associated with a 65% lower odds of being in the high

walking ability group (95% confidence interval: 0.19, 0.67). When adjusting for one of the 32 metabolites, the associations between body mass index and walking ability extremes were attenuated by ≤24%. We also examined the percent attenuation in the association between a metabolite and walking ability after adjusting for body mass index (Table 8), and found attenuations of more than 40% for 18 of the 32 metabolites. In previous reports, an attenuation of at least 10% has been suggested as a relevant threshold (130).

Waist circumference: The 40 metabolites associated with waist circumference are listed in Table 9. One standard deviation (=14.3 cm) higher waist circumference was associated with a 66% lower odds of being in the high walking ability extreme (95% confidence interval: 0.18, 0.64). When adjusting for one of the 40 metabolites, the associations between waist circumference and walking ability extremes were attenuated by ≤26%, whereas the attenuations in the association between a metabolite and walking ability after adjusting for waist circumference were more than 40% for 17 of the 40 metabolites (Table 9).

Arthritis: Table 10 includes the 14 metabolites associated with prevalent arthritis. Participants with arthritis had a 77% lower odds of being in the high walking ability extreme (95% confidence interval: 0.10, 0.53). When adjusting for one of the 14 metabolites, the associations between arthritis and walking ability extremes were attenuated by ≤13%, whereas the attenuation in the association between a metabolite and walking ability after adjusting for arthritis was more than 25% for 7 of the metabolites (Table 10).

Total number of prescription medications: Table 11 includes information on the nine metabolites associated with total number of prescription medications. One standard deviation higher (=4.6) total number of prescription medications was associated with a 37% lower odds of being in the high walking ability extreme (95% confidence interval: 0.42, 0.93). Adjusting for

one of the 9 metabolites, resulted in attenuations in the association between total number of medications and walking ability extremes ranging from 10% to 21%, with one metabolite (glycerate) resulting in a reverse attenuation of 66%, meaning adjusting for glycerate made the association between total number of medications and walking ability extremes stronger. Adjusting for total number of medications also resulted in a similar amount of attenuation in the associations between a metabolite and walking ability extreme, with attenuations ranging from 13% to 29%, with reverse attenuation of 75% for glycerate.

Interleukin-6: The 32 metabolites associated with interleukin-6 are listed in Table 12. One standard deviation (=0.58) higher log-transformed interleukin-6 was associated with a 57% lower odds of being in the high walking ability extreme (95% confidence interval: 0.24, 0.75). Adjusting for one of the 32 metabolites attenuated the association between interleukin-6 and walking ability extremes by ≤26%, whereas adjusting for interleukin-6 attenuated the association between a metabolite and walking ability extremes by more than 30% for 13 of the 32 metabolites.

The first subplot in Figure 6 illustrates the percent attenuation in the association between metabolites and walking ability extremes after adjusting for body mass index (on the y-axis) versus the percent attenuation in the association between body mass index and walking ability extremes after adjusting for a single metabolite (on the x-axis). Each data point represents a single metabolite, which is color-coded according to its taxonomy super class, and corresponds to information in Table 8. The same figure was also produced for waist circumference, arthritis, total number of prescription medications, and interleukin-6 (subplots of Figure 6). There appears to be more attenuation in the associations between a metabolite and walking ability extremes after adjusting for either body mass index or waist circumference, when compared to the

attenuation in the association between either body mass index or waist circumference after adjusting for a single metabolite. This is illustrated in Figure 6 by more data points being spread further up the y-axis, but not very far across the x-axis. This relationship is also apparent when examining the subplots for interleukin-6 and arthritis (Figure 6). Whereas, adjusting for total number of prescription medications appeared to result in the lowest amount of attenuation in the association between select metabolites and walking ability extremes.

2.5 DISCUSSION

Differences in patterns of plasma metabolites were observed by walking ability extremes among a subset of older adults ages 79-95 from the CHS All Stars study. Specifically, I identified 96 metabolites associated with high versus low walking ability using a nested case-control study design matching one-to-one on age, gender, race, and fasting time. Arginine and proline metabolism were among the top pathways involving more of the 96 metabolites associated with walking ability extremes than expected by chance. We found body mass index, waist circumference, arthritis, or interleukin-6 partly explained the association between a subset of metabolites and walking ability.

There was little overlap in the metabolites found to be associated with walking ability in this report when compared to previous publications examining metabolites associated with gait speed. Among adults ages 50 and older from the Baltimore Longitudinal Study of Aging, only eight, out of 148 metabolites examined, were significantly associated with gait speed, while controlling for age and gender (100). The eight metabolites were all lipids and lipid-like molecules, mostly sphingolipids and glycerophospholipids. Among those eight metabolites

associated with gait speed in the Baltimore Longitudinal Study of Aging, three sphingolipids (C18:0-1 and C16:0) were also associated with walking ability extremes in our study of CHS All Stars. Among a subset of older black men from the Health, Aging, and Body Composition Study, seven metabolites out of 350 (salicylurate, 2-hydroxyglutarae, asparagine, tryptophan, C24:1 ceramide d18:1, glucoronate, and C54:10 TAG) were associated with gait speed at a 0.01 significance level (98), of which none were found to be associated with walking ability extremes among the CHS All Stars. Last, a principal component factor score of multiple acylcarnitines was found to be associated with gait speed among a cohort of 77 men ages 70 and older from the U.S. Veterans LIFE study (102). In the CHS All Stars, we found higher levels of C5:1 carnitine associated with high walking ability.

The minimal overlap in metabolites found to be associated with walking ability in our cohort and gait speed in previous reports could be due to differences in the functional status of the older adults in each of the studies. In our subset of 120 participants from the CHS All Stars study, the average (standard deviation) gait speed was 0.5 (0.1) meters/second among those classified as having low walking ability (range: 0.2 to 0.7 meters/second), whereas, those classified as having "high" walking ability had an average gait speed of 1.0 (0.1) meters/second (range: 0.9 to 1.1 m/sec). Thus, even our 60 CHS All Stars with "high" walking ability did not actually walk that fast when compared to younger populations. The previous reports on metabolites associated with gait speed had a cohort of older adult cohorts that were much healthier with faster average gait speeds that had less variability across participants (98, 100, 102). For example, the Health ABC study recruited participants to be non-disabled at year 1, so only 26% of the older black men with metabolomics at year 2 had a gait speed <1.0 meters/second (98). There were also demographic differences between the studies, with the past

reports being restricted to either one gender (102) or one gender/race category (98) and being younger older adult cohorts (98, 100, 102) when compared to the CHS All Stars. In addition, we were more likely to find more associations between metabolites and walking ability since we sampled the extremes of walking ability, providing more power to detect differences, whereas previous reports looked at continuous gait speed with lower variability (98, 100, 102).

Among the 96 metabolites associated with high versus low walking ability, half were lipids and lipid-like molecules. Specifically, almost a quarter was a type of glycerolipid known as triacylglycerols or more commonly known as triglycerides. Higher levels of 12 and lower levels of 11 triacylglycerols were associated with high versus low walking ability. Triacylglycerols are made up of a glycerol bonded to three fatty acids, which can be a combination of monounsaturated, polyunsaturated, and/or saturated fatty acids. Interestingly, all 12 triacylglycerols that were higher among those with high walking ability in our cohort contained mostly polyunsaturated fatty acids (linoleic acid, alpha-linolenic acid, arachidonic acid, and/or docosahexaenoic acid), of which four of those triacylglycerols consisted of only polyunsaturated fatty acids. Whereas, all 11 triacylglycerols that were lower among those with high walking ability contained mostly saturated fatty acids (e.g., palmitic acid) and/or monounsaturated fatty acids (e.g., oleic acid). Similarly, triacylglycerols consisting of polyunsaturated fatty acids have been previously shown to be inversely correlated with insulin resistance, waist circumference (131), and diabetes (132). Many polyunsaturated fatty acids have anti-inflammatory effects. Omega-3 fatty acids, specifically, have reduced inflammation in animal models causing improvements in insulin sensitivity (133). Consistent with our findings, the CHS All Stars with high walking ability also had higher levels of docosahexaenoate, an omega-3 fatty acid found most often in fish oil. Differences in triacylglycerols associated with

walking ability extremes likely reflect differences in diet between the two groups, and potentially reflect differences in energy expenditure. Triacylglycerols composed primarily of polyunsaturated fatty acids may be a protective set of lipids against aging-related dysregulation.

Among the 23 triacylglycerols associated with walking ability extremes, seven were also significantly associated with body mass index or waist circumference in our cohort, of which all but one was also associated with body mass index in the Health ABC study as well (113). Higher levels of all seven triacylglycerols were associated with a *higher* odds of being in the *low* walking ability group, where adjusting for body mass index or waist circumference attenuated at least 40% of the association between those 7 triacylglycerols and walking ability and 100% of their statistical significance. Among the other 29 lipid and lipid-like molecules associated with walking ability extremes, 12 were also associated with body composition in our cohort, where adjusting for body composition resulted in attenuations ranging from 30-60% in the association between those metabolites and walking ability. This suggests the associations between select triacylglycerols and other lipids and lipid-like molecules with walking ability were partly explained by differences in body composition. However, it should be noted that there remained 16 triacylglycerols and 19 other lipids and lipid-like molecules that were associated with walking ability extremes, but not significantly associated with body mass index or waist circumference, suggesting a profile of lipids and lipid-like molecules associated with walking ability independent of body composition.

Triacylglycerols are used mainly to store fats, making them a major energy reservoir in the human body (134). Triacylglycerols also prevent fatty-acid induced lipotoxicity by removing excess fat from cells. In a healthy individual, dietary triacylglycerols are roughly equal to the amount used for energy (135). When the body has more triacylglycerols than needed for energy

expenditure, adipose tissue expands to store excess triacylglycerols, contributing to obesity over time if the balance of dietary lipids to energy expenditure is not altered (133). This triacylglycerol overload can cause adipocytes to secrete monocyte chemotaxis protein-1, which attracts macrophages that then promote tumor necrosis factor-alpha, a protein involved in systemic inflammation (135). As a result, stored lipids in the adipose tissue begin to break down and are released into circulation at an increased rate (133, 135). Chronic high levels of free fatty acids in the circulation can cause ectopic accumulation of fatty acids in myocytes causing insulin resistance, as well as excess storage in the liver causing fatty liver disease (133, 135). In our study, we found seven triacylglycerols associated with low walking ability, which was partly explained by a higher body mass index. These specific triacylglycerols may be markers of adverse aging-related changes in body composition.

Obesity likely contributes to decline in walking ability through two main mechanisms: 1) through a biomechanical burden of excess weight on lower extremities (136) and 2) through biochemical differences in an obese state with adverse effects on metabolism. The latter mechanism involving a potential causal pathway of worse body composition causing altered metabolite values that then cause lower walking ability with aging. In this dissertation, I informally examined whether a metabolite was a mediator of the relationship between body composition and walking ability, but found the association between body composition and walking ability was minimally attenuated by a metabolite (all attenuations ≤26%). This is likely because body mass index and waist circumference are more global measures impacted by multiple factors. Since body composition likely impacts values for multiple metabolites simultaneously, future studies will need to assess the mediation of multiple metabolites, simultaneously, by either having enough power to include all relevant metabolites in the same

mediation model or by computing a metabolite composite score to determine the direct and indirect effects between body composition and walking ability. In addition, future longitudinal studies will be needed to truly test mediation between body composition, metabolites, and walking ability.

Proline was the most strongly associated metabolite with walking ability extremes. One standard deviation higher value of proline was associated with 3.5 times the odds of having low walking ability in our study. Proline is an amino acid that can be synthesized in the human body from glutamate. Higher levels of proline have also been associated with abnormal fasting glucose (137) and sarcopenia (138), and modestly associated with Alzheimer's disease (139). Applying a pathway analysis, arginine and proline metabolism was a top pathway associated with walking ability extremes. There were six metabolites associated with walking ability extremes and involved in arginine and proline metabolism: ornithine, arginine, proline, N-acetylputrescine, 4-acetamidobutanoic acid, and sarcosine, where all, but one (arginine) were higher in those with low walking ability. It has been suggested that conditions that cause high levels of lactate in the blood can cause high levels of proline since lactate inhibits the breakdown of proline (140). Consistent with this, higher levels of both proline and lactate were associated with low walking ability in our study. Proline was also positively associated with body mass, waist circumference, and interleukin-6 in our study, though these three risk factors did not explain away the association between proline and walking ability.

A limitation of our study was the unit-less LC-MS peak areas for metabolite values. If we instead had concentrations of metabolites then we could assess whether values for metabolites were outside a healthy range, though a healthy range for many metabolites is unknown. Another limitation was that we did not have dietary information at the time that metabolites were

measured, though we did have dietary information from ten years prior. Last, it should be noted that participants included in this study were ages 79 to 95, where those classified as having "high" walking ability did not actually have a fast gait speed. The average gait speed for those considered in the "high" walking ability group was only 1.0 meters/second, with 65% below 1.0 meters/second, the clinically relevant threshold for detecting older adults at risk for multiple major health outcomes (28). Thus, in this report of community-dwelling older adults (mean age: 85), we are comparing those who walk slowly versus those who walk extremely slowly. Strengths of our study included our well-characterized cohort of older community-dwelling adults ages 79 to 95 and the carefully collected and stored EDTA plasma samples that had never been thawed prior to metabolomics. Sampling extremes using a nested case-control study design was also a strength of this study, which allowed for more variability and power to detect differences, while matching on important confounders.

Several metabolites, particularly lipids, were associated with high versus low walking ability using a nested case-control study design of 120 CHS All Stars matched one-to-one on age, gender, race, and fasting time. The association between a subset of lipids found to be associated with walking ability extremes appeared to be partly explained by differences in body composition. Triacylglycerols with mostly polyunsaturated fatty acids were positively associated with walking ability and appeared to be a protective set of lipids, whereas triacylglycerols with mostly saturated or monounsaturated fatty acids were inversely associated with walking ability. The reproducibility and generalizability of these results need to be determined to understand whether differences in these metabolites truly characterize differences in walking ability among older adults. Understanding these metabolic differences will provide insight into biologic mechanisms that possibly become altered with aging and disease that contribute to a decline in

walking ability, where these biologic mechanisms can then be targeted in interventions to promote independence throughout life.

2.6 TABLES

Table 2. Scoring for the Walking Ability Index* in older adults

	Score
Part I: Level of difficulty or ease walking ½ mile: Unable to walk ½ mile	0
A lot of difficulty walking ½ mile	1
Some difficulty walking ½ mile	2
Not that easy walking ½ mile	4
Somewhat easy walking ½ mile	5
Very easy walking ½ mile	6
Part II: Level of difficulty or ease walking 1 mile: Difficulty walking 1 mile	0
Not that easy walking 1 mile	1
Somewhat easy walking 1 mile	2
Very easy walking 1 mile	3

*Walking Ability Index = Part I score + Part II score

Table 3. Descriptive statistics of 120 randomly selected CHS All Stars with high versus low walking ability matched one-to-one on age, gender, race, and fasting time

Mean (standard deviation) or Frequency (percent) n = if reduced sample size	High walking ability n=60	Low walking ability n=60	Paired test p-value
Matching variables:			
Age	85 (2.9) Range: 79, 94	85 (2.9) Range: 80, 95	.27
Men	24 (40%)	24 (40%)	.99
Black race	6 (10%)	6 (10%)	.99
Number of hours since last meal	14 (1.6) Range: 12, 20	14 (1.7) Range: 11, 20	.17
Personal history:			
Clinic site:			
Wake Forest University School of Medicine	19 (32%)	7 (12%)	.02
University of California, Davis	21 (35%)	30 (50%)	
Johns Hopkins University	3 (5%)	10 (17%)	
University of Pittsburgh	17 (28%)	13 (22%)	
Original cohort (recruited in 1989-1990)	55 (92%)	56 (93%)	.57
More than a high school education	32 (54%) n=59	17 (28%)	.01
Smoking status:			
Never smoked	43 (72%)	34 (57%)	.10*
Former smoker, quit >1 year ago	17 (28%)	23 (38%)	
Current smoker	0	3 (5%)	
Weekly alcohol consumption	1.8 (3.6)	0.7 (2.4)	.03
Self-reported health:			
Excellent or very good	38 (63%)	7 (12%)	.0003
Good	20 (33%)	22 (37%)	
Fair or poor	2 (3%)	31 (52%)	
Physical measures:			
Height (cm)	162 (10)	161 (8.8) n=57	.36
Weight (lbs)	145 (27)	164 (37) n=59	.0008
Body mass index (kg/m^2)	25 (3.2)	28 (5.6) n=57	<.0001
Waist circumference (cm)	92 (11)	103 (15) n=59	<.0001
Systolic Blood Pressure (mmHg)	134 (21)	135 (21)	.77
Diastolic Blood Pressure (mmHg)	69 (10)	68 (11)	.97
Chronic conditions:			
Heart disease	10 (17%)	18 (30%)	.08
Stroke	5 (8%)	14 (23%)	.04
Hypertension	48 (80%)	51 (85%)	.49
Diabetes	5 (8%)	12 (20%)	.07
Cancer	17 (28%)	16 (27%)	.82
Asthma	3 (5%)	14 (23%)	.01
Emphysema or chronic bronchitis	6 (10%)	13 (22%)	.10
Arthritis	17 (28%)	40 (67%)	.0005
Osteoporosis	14 (23%)	16 (27%)	.62
Kidney disease	2 (3%)	5 (8%)	.27
Difficulty with ≥1 Activities of Daily Living	2 (4%) n=56	21 (40%) n=53	.005
Center for Epidemiologic Studies Depression Scale	4.8 (4.0)	7.8 (5.4) n=59	.005
Modified mini-mental state examination	93 (6.6)	86 (14)	<.0001
Physical performance:			
Gait speed (m/sec)	1.0 (0.1) Range: 0.9, 1.1	0.5 (0.1) Range: 0.2, 0.7	<.0001
Gait speed <1.0 m/sec	39 (65%)	60 (100%)	-----
Short physical performance battery	7.3 (2.0)	3.2 (1.7)	<.0001
Grip strength (kg)	25 (9.0) n=58	21 (7.5)	.002
Blood-based markers:			
Total cholesterol (mg/dL)	182 (36)	186 (40)	.57
High-density lipoprotein cholesterol (mg/dL)	58 (16)	56 (16)	.50
Low-density lipoprotein cholesterol (mg/dL)	99.9 (30)	103 (31) n=59	.69
Triglycerides (mg/dL)	120 (65)	131 (75)	.39
Fasting glucose (mg/dL)	96 (17)	106 (37)	.04

Table 3 Continued

Mean (standard deviation) or Frequency (percent) n = if reduced sample size	High walking ability n=60	Low walking ability n=60	Paired test p-value
Interleukin-6 (pg/mL)	3.2 (2.2) n=54	5.0 (2.9) n=55	.0003
C-reactive protein (ug/mL)	2.9 (4.6)	5.5 (5.9) n=59	.01
Creatinine (mg/dL)	1.1 (0.4) n=59	1.2 (1.0)	.72
Cystatin C (mg/L)	1.1 (0.3) n=59	1.3 (0.8) n=52	.11
Dietary intake at year 8:			
Calories (kcal)	1909 (604) n=58	2103 (834) n=57	.15
Calories from fat (kcal)	1898 (605) n=58	2092 (835) n=57	.15
Protein (gm)	80 (28) n=58	83 (30) n=57	.66
Caffeine (mg)	140 (159) n=58	214 (225) n=57	.09
Medications:			
Total number of prescription medications	7.7 (3.3)	10.1 (5.4)	.01
Anti-hypertensive medications	44 (73%)	49 (82%)	.32
Lipid-lowering medications	26 (43%)	25 (42%)	.85
Medication for diabetes	4 (7%)	11 (18%)	.05
Any diuretic	25 (42%)	32 (53%)	.20
Any ACE inhibitor	19 (32%)	15 (25%)	.40
Any vasodilators	4 (7%)	10 (17%)	.12
Any Beta blocker	21 (35%)	20 (33%)	.82

*Combined current and former smoker for statistical comparison

Table 4. Mean paired difference of 96 metabolites associated with high versus low walking ability (false discovery rate<0.30) among 120 CHS All Stars

Log transformed and standardized Metabolites	HMDB ID number	Taxonomy sub class	Difference between matched pairs (High – Low)	p-value	FDR
Proline	00162	Amino acids, peptides, and analogues	-0.67 (1.1) Med=-0.52	<.0001	0.006
C38:7 PE plasmalogen	11420	Glycerophosphoethanolamines	0.69 (1.2) Med=0.71	<.0001	0.01
1-methylguanine	03282	Purines and purine derivatives	-0.66 (1.2) Med=-0.80	<.0001	0.01
C58:10 TAG	05476	Triradylcglycerols	0.63 (1.3) Med=0.67	.0003	0.04
Imidazole propionate	02271	Imidazoles	-0.64 (1.3) Med=-0.63	.0004	0.04
C36:5 PE plasmalogen	11410	Glycerophosphoethanolamines	0.58 (1.2) Med=0.54	.0005	0.04
C40:7 PE plasmalogen	11394	Glycerophosphoethanolamines	0.58 (1.2) Med=0.89	.0005	0.04
C60:12 TAG	05478	Triradylcglycerols	0.56 (1.2) Med=0.42	.0008	0.048
C56:8 TAG	05392	Triradylcglycerols	0.58 (1.3) Med=0.65	.0008	0.048
C38:7 PC plasmalogen	11229	Glycerophosphocholines	0.59 (1.3) Med=0.76	.0009	0.048
C58:9 TAG	05463	Triradylcglycerols	0.60 (1.3) Med=0.49	.0009	0.048
Diacetylspermine	1876	Carboxylic acid derivatives	-0.59 (1.3) Med=-0.41	.001	0.05
C18:1 SM	12101	Phosphosphingolipids	-0.52 (1.2) Med=-0.57	.001	0.05
N2,N2-dimethylguanosine	04824	Not available	-0.53 (1.2) Med=-0.57	.001	0.05
C56:7 TAG	05462	Triradylcglycerols	0.52 (1.2) Med=0.38	.002	0.05
C58:11 TAG	10531	Triradylcglycerols	0.57 (1.3) Med=0.77	.002	0.05
C36:3 PS plasmalogen	----	unclassified	-0.57 (1.3) Med=-0.46	.002	0.05
C56:9 TAG	05448	Triradylcglycerols	0.56 (1.3) Med=0.58	.002	0.05
Hydrocinnamate	00764	Not available	0.56 (1.3) Med=0.45	.002	0.05
1-methylnicotinamide	00699	Pyridinecarboxylic acids and derivatives	0.57 (1.4) Med=0.58	.002	0.05
C38:6 PE plasmalogen	11387	Glycerophosphoethanolamines	0.52 (1.3) Med=0.70	.002	0.06
C18:2 SM	----	Phosphosphingolipids	-0.53 (1.3) Med=-0.76	.002	0.06
Saccharin	29723	Not available	0.55 (1.4) Med=0.63	.003	0.06
C34:5 PC plasmalogen	11214	Not classified	0.51 (1.3) Med=0.62	.003	0.07
Lactate	00190	Alpha hydroxy acids and derivatives	-0.49 (1.2) Med=-0.39	.003	0.07
C24:0 LPC	10405	Glycerophosphocholines	0.52 (1.3) Med=0.28	.004	0.08
3-methylxanthine	01886	Purines and purine derivatives	-0.51 (1.4) Med=-0.54	.005	0.10
3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)	61112	Fatty acids and conjugates	0.43 (1.1) Med=0.48	.005	0.11
C-glycosyltryptophan	----	unclassified	-0.47 (1.3) Med=-0.32	.006	0.11
C56:10 TAG	10513	Triradylcglycerols	0.50 (1.4) Med=0.52	.006	0.11
p-hydroxyphenylacetate	00020	Phenylacetic acid derivatives	-0.48 (1.3) Med=-0.47	.006	0.11
Serotonin	00259	Tryptamines and derivatives	0.50 (1.5) Med=0.28	.006	0.11
Dimethylguanidino valeric acid	0240212	Short-chain keto acids and derivatives	-0.46 (1.3) Med=-0.53	.007	0.11
C38:6 PC plasmalogen	11319	Glycerophosphocholines	0.50 (1.4) Med=0.57	.007	0.12
C50:3 TAG	05433	Triradylcglycerols	-0.48 (1.4) Med=-0.25	.009	0.13
Cinnamoylglycine	11621	Amino acids, peptides, and analogues	0.44 (1.2) Med=0.36	.009	0.13
C34:3 PC plasmalogen	11211	Glycerophosphocholines	0.42 (1.2) Med=0.49	.009	0.13
7-methylguanine	00897	Purines and purine derivatives	-0.39 (1.1) Med=-0.32	.009	0.14
3-hydroxydecanoate	02203	Medium-chain hydroxy acids and derivatives	0.41 (1.2) Med=0.51	.011	0.15
Bilirubin	00054	Bilirubins	0.46 (1.3) Med=0.56	.011	0.15
C54:9 TAG	10498	Triradylcglycerols	0.47 (1.4) Med=0.55	.011	0.15
Cystine	00192	Amino acids, peptides, and analogues	-0.40 (1.2) Med=-0.35	.013	0.17
C48:2 TAG	05376	Triradylcglycerols	-0.45 (1.5) Med=-0.32	.014	0.18
Indole-3-propionate	02302	Indolyl carboxylic acids and derivatives	0.45 (1.5) Med=0.22	.014	0.18
Docosahexaenoate	02183	Fatty acids and conjugates	0.42 (1.3) Med=0.45	.014	0.18
C38:6 PC	07991	Glycerophosphocholines	0.44 (1.4) Med=0.30	.015	0.18
C54:7 TAG	05447	Triradylcglycerols	0.43 (1.3) Med=0.39	.015	0.18
C22:0 LPE	11520	Glycerophosphoethanolamines	0.43 (1.4) Med=0.44	.016	0.18
C40:9 PC	08731	Glycerophosphocholines	0.44 (1.4) Med=0.30	.017	0.18
Arginine	00517	Amino acids, peptides, and analogues	0.43 (1.3) Med=0.56	.017	0.18
Acetyl-galactosamine	00212	Carbohydrates and carbohydrate conjugates	-0.44 (1.4) Med=-0.35	.017	0.18
C36:5 PC plasmalogen-A	11221	Glycerophosphocholines	0.44 (1.5) Med=0.23	.017	0.18
C54:8 TAG	10518	Triradylcglycerols	0.43 (1.4) Med=0.41	.017	0.18

Table 4 Continued

Log transformed and standardized Metabolites	HMDB ID number	Taxonomy sub class	Difference between matched pairs (High – Low)	p-value	FDR
C50:2 TAG	05377	Triradylcglycerols	-0.44 (1.5) Med=-0.17	.017	0.18
Glycerate	00139	Carbohydrates and carbohydrate conjugates	0.44 (1.4) Med=0.31	.017	0.18
C34:3 PE plasmalogen	11343	Glycerophosphoethanolamines	0.41 (1.3) Med=0.30	.018	0.19
1-methyladenosine	03331	Not available	-0.37 (1.2) Med=-0.22	.019	0.19
Homocitrulline	00679	Amino acids, peptides, and analogues	-0.38 (1.2) Med=-0.37	.020	0.20
Pseudouridine	00767	Not available	-0.36 (1.2) Med=-0.34	.021	0.20
Ethyl glucuronide	10325	Carbohydrates and carbohydrate conjugates	0.36 (1.2) Med=0.12	.021	0.20
C52:1 TAG	05367	Triradylcglycerols	-0.42 (1.5) Med=-0.38	.022	0.20
C50:1 TAG	05360	Triradylcglycerols	-0.42 (1.6) Med=-0.18	.022	0.20
C16:1 SM	29216	Phosphosphingolipids	-0.38 (1.3) Med=-0.44	.023	0.21
C38:5 PE plasmalogen	11386	Glycerophosphoethanolamines	0.37 (1.3) Med=0.41	.023	0.21
N-acetylputrescine	02064	Carboximidic acids	-0.42 (1.5) Med=-0.32	.024	0.21
Malonate	00691	Dicarboxylic acids and derivatives	0.41 (1.6) Med=0.32	.024	0.21
C34:2 PE plasmalogen	08952	Glycerophosphoethanolamines	0.38 (1.3) Med=0.52	.025	0.21
Cys-gly-oxidized	----	unclassified	0.41 (1.4) Med=0.54	.026	0.21
C38:3 PE plasmalogen	11384	Glycerophosphoethanolamines	0.39 (1.3) Med=0.38	.027	0.21
C5:1 carnitine	02366	Fatty acid esters	0.37 (1.3) Med=0.44	.027	0.21
N6-acetyllysine	00206	Amino acids, peptides, and analogues	-0.41 (1.4) Med=-0.32	.027	0.21
C18:0 SM	01348	Phosphosphingolipids	-0.40 (1.4) Med=-0.48	.028	0.21
Dimethylurate	01857	Purines and purine derivatives	-0.40 (1.4) Med=-0.39	.028	0.21
Caffeine	01847	Purines and purine derivatives	-0.40 (1.5) Med=-0.45	.028	0.21
C54:6 TAG	05391	Triradylcglycerols	0.40 (1.4) Med=0.41	.028	0.21
Caproate	00535	Fatty acids and conjugates	0.40 (1.5) Med=0.22	.031	0.23
C48:1 TAG	05359	Triradylcglycerols	-0.40 (1.5) Med=-0.23	.031	0.23
4-acetamidobutanoate	03681	Amino acids, peptides, and analogues	-0.36 (1.3) Med=-0.30	.032	0.23
7-methylxanthine	01991	Purines and purine derivatives	-0.38 (1.4) Med=-0.35	.032	0.23
C52:2 TAG	05369	Triradylcglycerols	-0.39 (1.4) Med=-0.40	.033	0.23
Sarcosine	00271	Amino acids, peptides, and analogues	-0.37 (1.3) Med=-0.29	.033	0.23
Mandelate	00703	Not available	-0.34 (1.2) Med=-0.29	.034	0.24
C22:6 LPC	10404	Glycerophosphocholines	0.36 (1.3) Med=0.21	.035	0.24
C49:2 TAG	11706	Triradylcglycerols	-0.39 (1.5) Med=-0.43	.035	0.24
C51:2 TAG	05362	Triradylcglycerols	-0.39 (1.6) Med=-0.45	.036	0.24
1,7-dimethyluric acid	11103	Purines and purine derivatives	-0.38 (1.4) Med=-0.39	.038	0.25
C46:1 TAG	10412	Triradylcglycerols	-0.38 (1.5) Med=-0.34	.039	0.26
Trigonelline	00875	Not available	-0.36 (1.3) Med=-0.25	.041	0.26
Glycoursodeoxycholate	00708	Bile acids, alcohols and derivatives	-0.38 (1.5) Med=-0.49	.041	0.26
N-mono-methylarginine (NMMA)	29416	Amino acids, peptides, and analogues	0.32 (1.2) Med=0.20	.043	0.27
C36:5 PC plasmalogen-B	11220	Glycerophosphocholines	0.35 (1.3) Med=0.38	.043	0.27
C49:3 TAG	42103	Triradylcglycerols	-0.37 (1.4) Med=-0.28	.043	0.27
C34:2 PC-A	07973	Glycerophosphocholines	0.37 (1.4) Med=0.21	.046	0.28
Heptanoate	00666	Fatty acids and conjugates	0.35 (1.3) Med=0.15	.047	0.28
Ornithine	00214	Amino acids, peptides, and analogues	-0.34 (1.3) Med=-0.37	.048	0.29
Theophylline	01889	Purines and purine derivatives	-0.36 (1.4) Med=-0.17	.0496	0.29

HMDB= Human Metabolome Database (84)
FDR= False discovery rate
LPC= lysophosphatidylcholine
LPE= lysophosphatidylethanolamine
PC= phosphatidylcholine
PE= phosphatidylethanolamine
PS= phosphatidylserine
SM= sphingomyelin
TAG= triacylglycerol

Table 5. Taxonomy classification of 96 metabolites associated with walking ability extremes among 120 CHS All Stars

Taxonomy super class*	Taxonomy class*	Taxonomy sub class*
Lipids and lipid-like molecules (m=54)	Fatty Acyls (m=5)	Fatty acid esters (m=1)
		Fatty acids and conjugates (m=4)
	Glycerolipids (m=23)	Triradylcglycerols (m=23)
	Glycerophospholipids (m=19)	Glycerophosphocholines (m=10)
		Glycerophosphoethanolamines (m=9)
	Sphingolipids (m=4)	Phosphosphingolipids (m=4)
	Steroids and steroid derivatives (m=1)	Bile acids, alcohols and derivatives (m=1)
	Not classified (m=2)	Not classified (m=2)
Organic acids and derivatives (m=16)	Carboximidic acids and derivatives (m=1)	Carboximidic acids (m=1)
	Carboxylic acids and derivatives (m=12)	Amino acids, peptides, and analogues (m=10)
		Carboxylic acid derivatives (m=1)
		Dicarboxylic acids and derivatives (m=1)
	Hydroxy acids and derivatives (m=2)	Alpha hydroxy acids and derivatives (m=1)
		Medium-chain hydroxy acids and derivatives (m=1)
	Keto acids and derivatives (m=1)	Short-chain keto acids and derivatives (m=1)
Organoheterocyclic compounds (m=14)	Azoles (m=1)	Imidazoles (m=1)
	Benzothiazoles (m=1)	Not available (m=1)
	Imidazopyrimidines (m=8)	Purines and purine derivatives (m=8)
	Indoles and derivatives (m=2)	Indolyl carboxylic acids and derivatives (m=1)
		Tryptamines and derivatives (m=1)
	Pyridines and derivatives (m=1)	Pyridinecarboxylic acids and derivatives (m=1)
	Tetrapyrroles and derivatives (m=1)	Bilirubins (m=1)
Nucleosides, nucleotides, and analogues (m=3)	Nucleoside and nucleotide analogues (m=1)	Not available (m=1)
	Purine nucleosides (m=2)	Not available (m=2)
Organic oxygen compounds (m=3)	Organooxygen compounds (m=3)	Carbohydrates and carbohydrate conjugates (m=3)
Benzenoids (m=2)	Benzene and substituted derivatives (m=2)	Phenylacetic acid derivatives (m=1)
		Not available (m=1)
Phenylpropanoids and polyketides (m=1)	Phenylpropanoic acids (m=1)	Not available (m=1)
Alkaloids and derivatives (m=1)	Not available (m=1)	Not available (m=1)
Not classified (m=2)	Not classified (m=2)	Not classified (m=2)

*According to the Human Metabolome Database

Table 6. Top pathways involving at least one of the metabolites* associated with walking ability extremes among 120 CHS All Stars

Pathway name	Match Status	Fisher's exact test p-value	False discovery rate	Impact
Caffeine metabolism	4/21	0.0003	0.02	0.38
Arginine and proline metabolism	6/77	0.001	0.05	0.37
D-Arginine and D-ornithine metabolism	2/8	0.007	0.18	0
Glycerophospholipid metabolism	3/39	0.02	0.48	0.23
Glycerolipid metabolism	2/32	0.09	1.00	0.07
Nicotinate and nicotinamide metabolism	2/44	0.16	1.00	0.02
Phenylalanine metabolism	2/45	0.16	1.00	0.04
Glycine, serine and threonine metabolism	2/48	0.18	1.00	0.05
Glycosylphosphatidylinositol(GPI)-anchor biosynthesis	1/14	0.20	1.00	0.04
Linoleic acid metabolism	1/15	0.22	1.00	0

*88 metabolites with Human Metabolome Database identification number and in MetaboAnalyst database:
proline, C38:7 PE plasmalogen, 1-methylguanine, C58:10 TAG, imidazole propionate, C36:5 PE plasmalogen, C40:7 PE plasmalogen, C60:12 TAG, C56:8 TAG, C38:7 PC plasmalogen, C58:9 TAG, C18:1 SM, N2,N2-dimethylguanosine, C56:7 TAG, C58:11 TAG, C56:9 TAG, hydrocinnamate, 1-methylnicotinamide, C38:6 PE plasmalogen, saccharin, C34:5 PC plasmalogen, lactate, C24:0 LPC, 3-methylxanthine, ibuprofen, C56:10 TAG, p-hydroxyphenylacetate, serotonin, C38:6 PC plasmalogen, anserine, C50:3 TAG, cinnamoylglycine, C34:3 PC plasmalogen, 7-methylguanine, 3-hydroxydecanoate, bilirubin, C54:9 TAG, cysteine, C48:2 TAG, indole-3-propionate, docosahexaenoate, C38:6 PC, C54:7 TAG, C22:0 LPE, C40:9 PC, arginine, acetyl-galactosamine, C36:5 PC plasmalogen-A, C54:8 TAG, C50:2 TAG, glycerate, C34:3 PE plasmalogen, 1-methyladenosine, homocitrulline, pseudouridine, ethyl glucuronide, C52:1 TAG, C50:1 TAG, C38:5 PE plasmalogen, N-acetylputrescine, malonate, C34:2 PE plasmalogen, C38:3 PE plasmalogen, C5:1 carnitine, N6-acetyllysine, C18:0 SM, dimethylurate, caffeine, C54:6 TAG, caproate, C48:1 TAG, 4-acetamidobutanoate, 7-methylxanthine, C52:2 TAG, sarcosine, mandelate, C22:6 LPC, C49:2 TAG, C51:2 TAG, 1,7-dimethyluric acid, C46:1 TAG, trigonelline, glycoursodeoxycholate, NMMA, C36:5 PC plasmalogen-B, C34:2 PC-A, heptanoate, ornithine, theophylline

Note: The following four metabolites did not have a Human Metabolome Database identification number:
C36:3 PS plasmalogen, C18:2 SM, C-glycosyltryptophan, , cys-gly-oxidized.
The following four metabolites had a Human Metabolome Database identification number, but were not in the MetaboAnalyst database: diacetylspermine (HMDB41876), 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF; HMDB61112), C49:3 TAG (HMDB42103), Dimethylguanidino valeric acid (DMGV; HMDB0240212).

Table 7. Associations between 96 metabolites and body mass index, waist circumference, arthritis, total number of prescription medications, and interleukin-6 using a random intercept model adjusting for the matched design among 120 CHS All Stars

Taxonomy super class Log-transformed and standardized metabolites	**Estimate (standard error), p-value**					Number of significant associations*
	Body mass index**	Waist circumference**	Arthritis	Number of medications*	Interleukin-6***	
Lipids and lipid-like molecules:						
C5:1 carnitine	-0.01 (0.08), p=0.87	-0.02 (0.08), p=0.78	-0.23 (0.18), p=0.21	-0.11 (0.09), p=0.21	-0.03 (0.1), p=0.73	0
Caproate	0.07 (0.09), p=0.43	0.11 (0.09), p=0.23	0.11 (0.18), p=0.54	0.006 (0.09), p=0.95	0.003 (0.1), p=0.97	0
Heptanoate	0.13 (0.09), p=0.16	0.12 (0.09), p=0.20	0.14 (0.18), p=0.45	-0.04 (0.09), p=0.67	0.008 (0.09), p=0.93	0
CMPF	-0.09 (0.09), p=0.30	-0.10 (0.09), p=0.26	-0.15 (0.17), p=0.40	0.09 (0.09), p=0.31	0.11 (0.09), p=0.27	0
Docosahexaenoate	-0.23 (0.09), p=0.02	-0.26 (0.09), p=0.004	-0.47 (0.17), p=0.009	0.04 (0.09), p=0.68	-0.19 (0.1), p=0.06	3
C58:10 TAG	-0.12 (0.09), p=0.21	-0.14 (0.09), p=0.12	-0.34 (0.18), p=0.07	0.08 (0.09), p=0.40	-0.22 (0.09), p=0.02	1
C56:8 TAG	-0.11 (0.09), p=0.25	-0.10 (0.09), p=0.26	-0.30 (0.18), p=0.10	0.06 (0.09), p=0.50	-0.17 (0.09), p=0.07	0
C58:9 TAG	-0.11 (0.09), p=0.25	-0.17 (0.09), p=0.07	-0.37 (0.18), p=0.04	0.05 (0.09), p=0.56	-0.10 (0.09), p=0.29	1
C56:9 TAG	-0.12 (0.09), p=0.22	-0.10 (0.09), p=0.28	-0.35 (0.18), p=0.06	0.11 (0.09), p=0.21	-0.23 (0.09), p=0.01	1
C58:11 TAG	-0.09 (0.09), p=0.33	-0.10 (0.09), p=0.31	-0.37 (0.18), p=0.04	0.12 (0.09), p=0.18	-0.25 (0.09), p=0.008	2
C56:7 TAG	-0.09 (0.09), p=0.34	-0.08 (0.09), p=0.38	-0.26 (0.18), p=0.15	0.09 (0.09), p=0.33	-0.15 (0.09), p=0.10	0
C56:10 TAG	-0.11 (0.09), p=0.24	-0.10 (0.09), p=0.30	-0.34 (0.18), p=0.07	0.14 (0.09), p=0.12	-0.26 (0.09), p=0.005	1
C50:3 TAG	0.16 (0.09), p=0.08	0.20 (0.09), p=0.03	0.25 (0.18), p=0.18	0.10 (0.09), p=0.27	0.06 (0.09), p=0.52	1
C54:7 TAG	-0.13 (0.09), p=0.16	-0.03 (0.09), p=0.72	-0.26 (0.18), p=0.15	0.08 (0.09), p=0.40	-0.10 (0.1), p=0.31	0
C54:9 TAG	-0.11 (0.09), p=0.25	-0.08 (0.09), p=0.40	-0.28 (0.18), p=0.12	0.14 (0.09), p=0.13	-0.28 (0.09), p=0.003	1
C54:6 TAG	-0.13 (0.09), p=0.19	-0.01 (0.09), p=0.87	-0.21 (0.18), p=0.25	0.004 (0.09), p=0.97	0.01 (0.1), p=0.88	0
C48:2 TAG	0.18 (0.09), p=0.06	0.17 (0.09), p=0.07	0.26 (0.18), p=0.16	0.09 (0.09), p=0.36	0.02 (0.1), p=0.80	0
C50:2 TAG	0.31 (0.09), p=0.001	0.29 (0.09), p=0.002	0.20 (0.18), p=0.27	0.14 (0.09), p=0.14	0.13 (0.1), p=0.18	2
C50:1 TAG	0.35 (0.09), p=0.0002	0.33 (0.09), p=0.0003	0.19 (0.18), p=0.29	0.10 (0.09), p=0.28	0.17 (0.1), p=0.08	2
C48:1 TAG	0.23 (0.09), p=0.02	0.22 (0.09), p=0.02	0.20 (0.18), p=0.28	0.11 (0.09), p=0.23	0.07 (0.1), p=0.46	2
C54:8 TAG	-0.12 (0.09), p=0.21	-0.05 (0.09), p=0.57	-0.28 (0.18), p=0.13	0.14 (0.09), p=0.12	-0.18 (0.09), p=0.05	0
C52:1 TAG	0.34 (0.09), p=0.0003	0.34 (0.09), p=0.0002	0.16 (0.18), p=0.39	0.15 (0.09), p=0.11	0.23 (0.09), p=0.02	3
C46:1 TAG	0.14 (0.09), p=0.14	0.13 (0.09), p=0.16	0.22 (0.18), p=0.24	0.10 (0.09), p=0.27	0.04 (0.1), p=0.69	0
C52:2 TAG	0.29 (0.09), p=0.002	0.30 (0.09), p=0.001	0.25 (0.18), p=0.18	0.17 (0.09), p=0.07	0.23 (0.1), p=0.02	3
C60:12 TAG	-0.09 (0.09), p=0.35	-0.16 (0.09), p=0.08	-0.32 (0.18), p=0.08	0.06 (0.09), p=0.49	-0.17 (0.09), p=0.08	0
C51:2 TAG	0.21 (0.09), p=0.03	0.17 (0.09), p=0.07	0.20 (0.18), p=0.28	0.03 (0.09), p=0.74	0.03 (0.1), p=0.75	1
C49:2 TAG	0.17 (0.09), p=0.08	0.12 (0.09), p=0.20	0.24 (0.18), p=0.19	-0.02 (0.09), p=0.84	-0.02 (0.1), p=0.85	0
C49:3 TAG	0.11 (0.09), p=0.23	0.11 (0.09), p=0.25	0.26 (0.18), p=0.16	-0.003 (0.09), p=0.98	-0.03 (0.1), p=0.78	0
C38:7 PC plasmalogen	-0.21 (0.09), p=0.03	-0.27 (0.09), p=0.004	-0.29 (0.18), p=0.11	-0.01 (0.09), p=0.87	-0.05 (0.09), p=0.57	2
C24:0 LPC	-0.31 (0.09), p=0.001	-0.23 (0.09), p=0.01	0.08 (0.18), p=0.68	-0.09 (0.09), p=0.31	-0.23 (0.09), p=0.02	3
C34:3 PC plasmalogen	-0.27 (0.09), p=0.003	-0.30 (0.09), p=0.001	-0.17 (0.17), p=0.33	-0.18 (0.09), p=0.04	-0.20 (0.09), p=0.04	4
C38:6 PC plasmalogen	-0.22 (0.09), p=0.02	-0.25 (0.09), p=0.006	-0.36 (0.18), p=0.05	-0.03 (0.09), p=0.75	-0.03 (0.1), p=0.77	2
C38:6 PC	-0.21 (0.09), p=0.03	-0.23 (0.09), p=0.01	-0.37 (0.18), p=0.04	0.14 (0.09), p=0.12	-0.11 (0.09), p=0.25	3
C36:5 PC plasmalogen-A	-0.15 (0.09), p=0.12	-0.15 (0.09), p=0.10	-0.21 (0.18), p=0.26	0.007 (0.09), p=0.94	-0.13 (0.1), p=0.19	0
C40:9 PC	-0.15 (0.09), p=0.12	-0.18 (0.09), p=0.05	-0.40 (0.18), p=0.03	0.17 (0.09), p=0.07	-0.10 (0.09), p=0.27	1
C36:5 PC plasmalogen-B	-0.05 (0.09), p=0.58	-0.15 (0.09), p=0.11	0.009 (0.18), p=0.96	-0.22 (0.09), p=0.01	-0.11 (0.09), p=0.23	1
C22:6 LPC	-0.20 (0.09), p=0.03	-0.17 (0.09), p=0.07	-0.48 (0.17), p=0.007	0.14 (0.09), p=0.12	-0.05 (0.1), p=0.62	2
C34:2 PC-A	-0.05 (0.09), p=0.59	-0.02 (0.09), p=0.85	0.03 (0.18), p=0.88	-0.11 (0.09), p=0.24	-0.07 (0.09), p=0.44	0
C34:5 PC plasmalogen	0.004 (0.09), p=0.96	-0.09 (0.09), p=0.35	-0.13 (0.18), p=0.46	-0.26 (0.09), p=0.005	0.002 (0.1), p=0.98	1
C38:7 PE plasmalogen	-0.18 (0.09), p=0.05	-0.19 (0.09), p=0.04	-0.29 (0.18), p=0.11	0.002 (0.09), p=0.99	-0.06 (0.1), p=0.54	1
C36:5 PE plasmalogen	0.06 (0.09), p=0.55	-0.03 (0.09), p=0.76	-0.05 (0.18), p=0.76	-0.12 (0.09), p=0.19	-0.12 (0.09), p=0.22	0
C40:7 PE plasmalogen	-0.27 (0.09), p=0.003	-0.29 (0.09), p=0.002	-0.17 (0.18), p=0.33	-0.05 (0.09), p=0.60	-0.01 (0.1), p=0.88	2
C38:6 PE plasmalogen	-0.05 (0.09), p=0.60	-0.11 (0.09), p=0.22	-0.06 (0.18), p=0.74	-0.15 (0.09), p=0.09	-0.16 (0.1), p=0.10	0
C34:3 PE plasmalogen	-0.13 (0.09), p=0.16	-0.09 (0.09), p=0.33	-0.008 (0.18), p=0.97	-0.12 (0.09), p=0.20	-0.14 (0.1), p=0.15	0
C38:5 PE plasmalogen	-0.03 (0.09), p=0.78	-0.11 (0.09), p=0.25	0.03 (0.18), p=0.88	-0.16 (0.09), p=0.06	-0.08 (0.09), p=0.40	0
C34:2 PE plasmalogen	-0.09 (0.09), p=0.33	-0.13 (0.09), p=0.16	0.006 (0.18), p=0.97	-0.14 (0.09), p=0.13	-0.16 (0.1), p=0.11	0
C38:3 PE plasmalogen	-0.25 (0.09), p=0.009	-0.29 (0.09), p=0.002	-0.12 (0.18), p=0.53	-0.26 (0.09), p=0.004	-0.23 (0.1), p=0.02	4
C22:0 LPE	-0.33 (0.09), p=0.0005	-0.26 (0.09), p=0.004	-0.34 (0.18), p=0.07	-0.15 (0.09), p=0.11	-0.30 (0.09), p=0.001	3
C18:1 SM	0.09 (0.09), p=0.34	0.03 (0.09), p=0.74	0.22 (0.18), p=0.22	-0.05 (0.09), p=0.58	0.04 (0.09), p=0.66	0
C18:0 SM	0.06 (0.09), p=0.50	-0.004 (0.09), p=0.96	0.06 (0.18), p=0.76	-0.09 (0.09), p=0.33	0.03 (0.09), p=0.74	0
C16:1 SM	0.05 (0.09), p=0.55	0.04 (0.09), p=0.65	0.22 (0.18), p=0.21	-0.06 (0.09), p=0.54	-0.03 (0.09), p=0.73	0
C18:2 SM	-0.009 (0.09), p=0.92	-0.04 (0.09), p=0.66	0.22 (0.18), p=0.22	-0.02 (0.09), p=0.84	-0.05 (0.09), p=0.62	0
C36:3 PS plasmalogen	0.12 (0.09), p=0.22	0.04 (0.09), p=0.69	0.63 (0.17), p=0.0006	-0.06 (0.09), p=0.53	0.22 (0.1), p=0.03	2
Glycoursodeoxycholate	0.21 (0.09), p=0.02	0.16 (0.09), p=0.09	0.21 (0.18), p=0.26	0.008 (0.09), p=0.93	0.13 (0.1), p=0.19	1
Organic acids and derivatives:						
N-acetylputrescine	-0.10 (0.09), p=0.26	-0.09 (0.09), p=0.31	0.12 (0.18), p=0.50	0.14 (0.09), p=0.13	0.06 (0.1), p=0.51	0
Sarcosine	-0.09 (0.09), p=0.34	-0.02 (0.09), p=0.79	-0.04 (0.18), p=0.84	0.10 (0.09), p=0.26	-0.02 (0.09), p=0.79	0

Table 7 Continued

Taxonomy super class Log-transformed and standardized metabolites	**Estimate (standard error), p-value**					Number of significant associations*
	Body mass index**	Waist circumference**	Arthritis	Number of medications*	Interleukin-6***	
Ornithine	0.14 (0.09), p=0.14	0.16 (0.09), p=0.09	0.38 (0.18), p=0.04	0.14 (0.09), p=0.13	0.09 (0.09), p=0.34	1
N-mono-methylarginine	0.08 (0.09), p=0.38	0.05 (0.09), p=0.60	-0.05 (0.17), p=0.78	-0.02 (0.09), p=0.83	-0.07 (0.09), p=0.47	0
Proline	0.23 (0.09), p=0.01	0.26 (0.09), p=0.003	0.17 (0.18), p=0.33	0.03 (0.09), p=0.73	0.34 (0.09), p=0.0006	3
Homocitrulline	0.16 (0.09), p=0.08	0.18 (0.09), p=0.04	0.22 (0.17), p=0.21	0.08 (0.09), p=0.40	0.34 (0.09), p=0.0005	2
N6-acetyllysine	0.10 (0.09), p=0.30	0.11 (0.09), p=0.25	0.29 (0.18), p=0.11	0.006 (0.09), p=0.95	0.28 (0.1), p=0.006	1
4-acetamidobutanoate	0.04 (0.09), p=0.69	0.11 (0.09), p=0.25	0.11 (0.18), p=0.55	0.03 (0.09), p=0.74	0.31 (0.09), p=0.002	1
Cinnamoylglycine	-0.32 (0.09), p=0.0006	-0.29 (0.09), p=0.002	-0.56 (0.17), p=0.002	-0.27 (0.09), p=0.004	-0.09 (0.09), p=0.34	4
Cystine	0.18 (0.09), p=0.04	0.22 (0.09), p=0.01	0.31 (0.17), p=0.08	0.11 (0.09), p=0.22	0.24 (0.09), p=0.01	3
Arginine	-0.24 (0.09), p=0.009	-0.26 (0.09), p=0.006	0.16 (0.18), p=0.39	-0.18 (0.09), p=0.05	-0.09 (0.09), p=0.31	2
Diacetylspermine	0.17 (0.09), p=0.07	0.16 (0.09), p=0.08	0.27 (0.18), p=0.14	0.02 (0.09), p=0.79	0.37 (0.09), p=0.0001	1
Malonate	-0.23 (0.09), p=0.01	-0.19 (0.09), p=0.04	-0.23 (0.18), p=0.21	0.05 (0.09), p=0.57	-0.23 (0.09), p=0.009	3
Lactate	0.14 (0.09), p=0.12	0.18 (0.09), p=0.048	-0.01 (0.18), p=0.95	0.11 (0.09), p=0.24	0.18 (0.09), p=0.05	1
3-hydroxydecanoate	-0.10 (0.09), p=0.28	-0.07 (0.09), p=0.45	-0.34 (0.17), p=0.05	-0.11 (0.09), p=0.23	-0.06 (0.09), p=0.56	0
DMGV	0.25 (0.09), p=0.007	0.27 (0.09), p=0.003	0.19 (0.18), p=0.28	0.09 (0.09), p=0.30	0.38 (0.09), p<0.0001	3
Organoheterocyclic compounds:						
Imidazole propionate	0.22 (0.09), p=0.02	0.24 (0.09), p=0.01	0.42 (0.18), p=0.02	0.16 (0.09), p=0.09	0.22 (0.09), p=0.03	4
Saccharin	-0.13 (0.09), p=0.16	-0.15 (0.09), p=0.12	-0.12 (0.18), p=0.52	-0.04 (0.09), p=0.64	-0.04 (0.1), p=0.69	0
1-methylguanine	0.23 (0.09), p=0.01	0.18 (0.09), p=0.048	0.14 (0.18), p=0.45	-0.002 (0.09), p=0.98	0.37 (0.09), p<0.0001	3
3-methylxanthine	0.09 (0.09), p=0.32	0.19 (0.09), p=0.04	0.14 (0.18), p=0.45	0.21 (0.09), p=0.02	0.19 (0.09), p=0.05	2
Theophylline	0.12 (0.09), p=0.22	0.23 (0.09), p=0.01	-0.04 (0.18), p=0.83	0.16 (0.09), p=0.09	0.07 (0.09), p=0.48	1
7-methylguanine	0.05 (0.09), p=0.60	0.09 (0.09), p=0.28	0.06 (0.17), p=0.71	-0.01 (0.08), p=0.89	0.26 (0.09), p=0.005	1
7-methylxanthine	0.08 (0.09), p=0.40	0.18 (0.09), p=0.05	0.15 (0.18), p=0.41	0.19 (0.09), p=0.03	0.14 (0.1), p=0.14	1
1,7-dimethyluric acid	0.20 (0.09), p=0.04	0.31 (0.09), p=0.0007	-0.06 (0.18), p=0.73	0.11 (0.09), p=0.21	0.18 (0.1), p=0.06	2
Dimethylurate	0.17 (0.09), p=0.06	0.28 (0.09), p=0.003	-0.07 (0.18), p=0.71	0.12 (0.09), p=0.18	0.20 (0.1), p=0.04	2
Caffeine	0.23 (0.09), p=0.01	0.30 (0.09), p=0.001	-0.02 (0.18), p=0.90	0.15 (0.09), p=0.09	0.14 (0.1), p=0.17	2
Indole-3-propionate	-0.44 (0.08), p<0.0001	-0.35 (0.08), p<0.0001	-0.56 (0.18), p=0.002	0.001 (0.09), p=0.99	-0.17 (0.1), p=0.08	3
Serotonin	-0.11 (0.09), p=0.23	-0.20 (0.09), p=0.03	-0.12 (0.18), p=0.51	-0.14 (0.09), p=0.13	0.02 (0.1), p=0.82	1
1-methylnicotinamide	-0.17 (0.09), p=0.08	-0.19 (0.09), p=0.04	-0.51 (0.18), p=0.006	-0.09 (0.09), p=0.31	-0.14 (0.09), p=0.13	2
Bilirubin	-0.16 (0.09), p=0.09	-0.08 (0.09), p=0.40	-0.41 (0.18), p=0.03	-0.28 (0.09), p=0.002	-0.16 (0.09), p=0.09	2
Nucleosides, nucleotides, and analogues:						
N2,N2-dimethylguanosine	0.14 (0.09), p=0.14	0.18 (0.09), p=0.06	-0.03 (0.18), p=0.86	0.004 (0.09), p=0.96	0.39 (0.09), p<.0001	1
1-methyladenosine	0.16 (0.09), p=0.08	0.15 (0.09), p=0.09	0.22 (0.17), p=0.21	-0.02 (0.09), p=0.84	0.33 (0.09), p=0.0005	1
Pseudouridine	0.15 (0.09), p=0.10	0.20 (0.09), p=0.03	0.07 (0.17), p=0.69	0.06 (0.09), p=0.48	0.39 (0.09), p<0.0001	2
Organic oxygen compounds:						
Acetyl-galactosamine	0.02 (0.09), p=0.84	-0.009 (0.09), p=0.92	0.12 (0.18), p=0.52	0.04 (0.09), p=0.64	0.12 (0.1), p=0.24	0
Ethyl glucuronide	-0.13 (0.09), p=0.14	-0.04 (0.09), p=0.62	-0.23 (0.17), p=0.19	0.09 (0.09), p=0.32	0.04 (0.09), p=0.71	0
Glycerate	-0.11 (0.09), p=0.24	-0.07 (0.09), p=0.45	-0.08 (0.18), p=0.67	0.19 (0.09), p=0.04	-0.23 (0.1), p=0.02	2
Benzenoids:						
p-hydroxyphenylacetate	0.27 (0.09), p=0.004	0.27 (0.09), p=0.004	0.14 (0.18), p=0.45	0.12 (0.09), p=0.19	0.27 (0.09), p=0.007	3
Mandelate	0.23 (0.09), p=0.01	0.23 (0.09), p=0.01	0.10 (0.17), p=0.56	-0.03 (0.09), p=0.71	0.40 (0.09), p<0.0001	3
Phenylpropanoids and polyketides:						
Hydrocinnamate	-0.21 (0.09), p=0.02	-0.23 (0.09), p=0.01	-0.47 (0.18), p=0.01	-0.18 (0.09), p=0.06	-0.08 (0.09), p=0.38	3
Alkaloids and derivatives:						
Trigonelline	0.05 (0.09), p=0.58	0.16 (0.09), p=0.08	-0.04 (0.18), p=0.84	0.16 (0.09), p=0.09	0.29 (0.09), p=0.003	1
Class not available:						
C-glycosyltryptophan	0.16 (0.09), p=0.09	0.22 (0.09), p=0.02	0.14 (0.18), p=0.44	-0.02 (0.09), p=0.79	0.42 (0.08), p<0.0001	2
Cys-gly-oxidized	-0.20 (0.09), p=0.03	-0.19 (0.09), p=0.04	-0.19 (0.18), p=0.32	0.01 (0.09), p=0.91	-0.12 (0.09), p=0.20	2

*Number of associations (p<0.05) between a metabolite and body mass index, waist circumference, arthritis, number of medications, and interleukin-6
**Standardized to a mean of zero and standard deviation of one
***Log-transformed and standardized to a mean of zero and standard deviation of one
LPC= lysophosphatidylcholine
LPE= lysophosphatidylethanolamine
PC= phosphatidylcholine
PE= phosphatidylethanolamine
PS= phosphatidylserine
SM= sphingomyelin
TAG= triacylglycerol
DMGV= Dimethylguanidino valeric acid
Purple shading indicates significant association at a 0.05 significance level

Table 8. Body mass index-adjusted associations between walking ability extremes and 32 metabolites that were significantly associated with body mass index using conditional logistic regression adjusting for matched design among 120 CHS All Stars

HMDB Taxonomy super class Log-transformed/standardized metabolites	**Model 1: Unadjusted**	**Model 2: Metabolite + BMI**			
		Metabolite*		BMI*	
	Odds ratio** (95% CI), p-value	Odds ratio** (95% CI), p-value	Attenuation	Odds ratio** (95% CI), p-value	Attenuation
Body mass index*	0.35 (0.19, 0.67), p=0.001	-----			
Lipids and lipid-like molecules:					
Docosahexaenoate	1.60 (1.03, 2.48), p=0.04	1.31 (0.81, 2.15), p=0.28	42%	0.38 (0.20, 0.73), p=0.003	8%
C50:2 TAG	0.67 (0.46, 0.96), p=0.03	0.85 (0.55, 1.32), p=0.47	60%	0.39 (0.20, 0.74), p=0.004	9%
C50:1 TAG	0.69 (0.49, 0.98), p=0.04	0.90 (0.59, 1.37), p=0.62	72%	0.38 (0.19, 0.74), p=0.005	7%
C48:1 TAG	0.67 (0.46, 0.97), p=0.04	0.82 (0.52, 1.27), p=0.37	49%	0.39 (0.21, 0.74), p=0.004	10%
C52:1 TAG	0.68 (0.47, 0.98), p=0.04	0.84 (0.55, 1.28), p=0.41	54%	0.38 (0.20, 0.73), p=0.004	9%
C52:2 TAG	0.67 (0.46, 0.98), p=0.04	0.83 (0.53, 1.30), p=0.42	55%	0.38 (0.20, 0.73), p=0.003	8%
C51:2 TAG	0.68 (0.47, 0.98), p=0.04	0.80 (0.53, 1.20), p=0.28	42%	0.39 (0.21, 0.73), p=0.003	10%
C38:7 PC plasmalogen	1.89 (1.22, 2.93), p=0.005	1.40 (0.88, 2.22), p=0.16	48%	0.42 (0.22, 0.81), p=0.01	17%
C24:0 LPC	1.82 (1.15, 2.90), p=0.01	1.35 (0.83, 2.19), p=0.22	50%	0.40 (0.20, 0.77), p=0.006	11%
C34:3 PC plasmalogen	1.92 (1.16, 3.15), p=0.01	1.43 (0.82, 2.49), p=0.20	45%	0.40 (0.21, 0.76), p=0.006	12%
C38:6 PC plasmalogen	1.63 (1.09, 2.44), p=0.02	1.22 (0.79, 1.88), p=0.37	60%	0.39 (0.20, 0.75), p=0.005	9%
C38:6 PC	1.60 (1.04, 2.46), p=0.03	1.27 (0.81, 1.98), p=0.29	50%	0.38 (0.20, 0.73), p=0.004	8%
C22:6 LPC	1.56 (1.01, 2.39), p=0.04	1.27 (0.79, 2.03), p=0.33	47%	0.38 (0.20, 0.72), p=0.003	7%
C40:7 PE plasmalogen	2.10 (1.28, 3.42), p=0.003	1.49 (0.88, 2.51), p=0.14	47%	0.43 (0.22, 0.83), p=0.01	19%
C38:3 PE plasmalogen	1.57 (1.03, 2.39), p=0.04	1.27 (0.79, 2.05), p=0.33	47%	0.37 (0.19, 0.71), p=0.003	5%
C22:0 LPE	1.72 (1.12, 2.63), p=0.01	1.32 (0.85, 2.06), p=0.21	48%	0.38 (0.20, 0.75), p=0.005	9%
Glycoursodeoxycholate	0.74 (0.51, 1.06), p=0.10	0.86 (0.57, 1.31), p=0.49	51%	0.37 (0.19, 0.69), p=0.002	4%
Organic acids and derivatives:					
Proline	0.30 (0.15, 0.61), p=0.0009	0.39 (0.19, 0.82), p=0.01	21%	0.45 (0.24, 0.85), p=0.01	24%
Cinnamoylglycine	1.87 (1.15, 3.05), p=0.01	1.56 (0.87, 2.79), p=0.13	29%	0.40 (0.21, 0.76), p=0.005	13%
Cystine	0.61 (0.38, 0.98), p=0.04	0.59 (0.33, 1.04), p=0.07	-9%	0.34 (0.17, 0.66), p=0.002	-4%
Arginine	1.77 (1.13, 2.77), p=0.01	1.85 (1.06, 3.23), p=0.03	-8%	0.34 (0.17, 0.66), p=0.001	-4%
Malonate	1.53 (1.00, 2.33), p=0.0498	1.33 (0.82, 2.15), p=0.25	33%	0.37 (0.20, 0.71), p=0.003	6%
Dimethylguanidino valeric acid	0.56 (0.34, 0.90), p=0.02	0.62 (0.36, 1.08), p=0.09	19%	0.37 (0.20, 0.72), p=0.003	6%
Organoheterocyclic compounds:					
Imidazole propionate	0.42 (0.24, 0.74), p=0.003	0.38 (0.19, 0.77), p=0.007	-10%	0.32 (0.16, 0.67), p=0.002	-8%
1-methylguanine	0.41 (0.24, 0.71), p=0.001	0.50 (0.28, 0.89), p=0.02	22%	0.43 (0.23, 0.81), p=0.009	20%
1,7-dimethyluric acid	0.69 (0.45, 1.04), p=0.07	0.78 (0.49, 1.25), p=0.31	36%	0.38 (0.20, 0.71), p=0.002	7%
Caffeine	0.72 (0.49, 1.04), p=0.08	0.80 (0.51, 1.24), p=0.32	32%	0.38 (0.20, 0.70), p=0.002	7%
Indole-3-propionate	1.57 (0.99, 2.49), p=0.05	1.19 (0.69, 2.06), p=0.52	61%	0.37 (0.19, 0.72), p=0.003	6%
Benzenoids:					
p-hydroxyphenylacetate	0.58 (0.37, 0.91), p=0.02	0.57 (0.33, 0.98), p=0.04	-5%	0.33 (0.17, 0.67), p=0.002	-5%
Mandelate	0.63 (0.41, 0.99), p=0.045	0.67 (0.40, 1.12), p=0.12	12%	0.36 (0.19, 0.69), p=0.002	3%
Phenylpropanoids and polyketides:					
Hydrocinnamate	1.90 (1.19, 3.04), p=0.008	2.03 (1.13, 3.65), p=0.02	-10%	0.36 (0.19, 0.68), p=0.002	3%
Taxonomy class not available:					
Cys-gly-oxidized	1.44 (0.94, 2.21), p=0.09	1.52 (0.88, 2.63), p=0.14	-14%	0.37 (0.20, 0.68), p=0.002	4%

*All continuous variables were standardized to a mean of zero and standard deviation of one

**Modeling the probability of high walking ability versus low walking ability

Attenuation=100*(beta coefficient from unadjusted model – beta coefficient from adjusted model) / beta coefficient from unadjusted model

Table 9. Waist circumference-adjusted associations between walking ability extremes and 40 metabolites that were significantly associated with waist circumference using conditional logistic regression adjusting for matched design among 120 CHS All Stars

HMDB Taxonomy super class Log-transformed/standardized metabolites	**Model 1: Unadjusted**	**Model 2: Metabolite + Waist circumference**			
		Metabolite*		**Waist circumference***	
	Odds ratio** (95% CI), p-value	Odds ratio** (95% CI), p-value	Attenuation	Odds ratio** (95% CI), p-value	Attenuation
Waist circumference*	0.34 (0.18, 0.64), p=0.0007	-----			
Lipids and lipid-like molecules:					
Docosahexaenoate	1.65 (1.06, 2.58), p=0.03	1.31 (0.79, 2.18), p=0.29	46%	0.38 (0.20, 0.70), p=0.002	9%
C50:3 TAG	0.57 (0.37, 0.88), p=0.01	0.72 (0.43, 1.20), p=0.20	40%	0.38 (0.20, 0.71), p=0.003	9%
C50:2 TAG	0.67 (0.46, 0.97), p=0.03	0.88 (0.57, 1.37), p=0.57	68%	0.36 (0.19, 0.69), p=0.002	6%
C50:1 TAG	0.71 (0.50, 1.00), p=0.048	0.95 (0.62, 1.44), p=0.80	84%	0.35 (0.18, 0.68), p=0.002	3%
C48:1 TAG	0.69 (0.48, 0.99), p=0.04	0.89 (0.57, 1.39), p=0.61	69%	0.36 (0.19, 0.69), p=0.002	5%
C52:1 TAG	0.69 (0.49, 0.99), p=0.04	0.92 (0.59, 1.42), p=0.70	76%	0.36 (0.18, 0.69), p=0.002	4%
C52:2 TAG	0.67 (0.46, 0.98), p=0.04	0.87 (0.55, 1.37), p=0.54	65%	0.36 (0.19, 0.68), p=0.002	5%
C38:7 PC plasmalogen	1.95 (1.25, 3.03), p=0.003	1.40 (0.86, 2.26), p=0.18	50%	0.41 (0.21, 0.78), p=0.007	17%
C24:0 LPC	1.82 (1.14, 2.89), p=0.01	1.51 (0.92, 2.49), p=0.10	31%	0.37 (0.19, 0.70), p=0.002	7%
C34:3 PC plasmalogen	1.82 (1.14, 2.93), p=0.01	1.47 (0.86, 2.50), p=0.16	36%	0.37 (0.19, 0.70), p=0.002	7%
C38:6 PC plasmalogen	1.69 (1.13, 2.52), p=0.01	1.26 (0.81, 1.96), p=0.31	56%	0.38 (0.20, 0.72), p=0.003	10%
C38:6 PC	1.63 (1.06, 2.50), p=0.03	1.27 (0.81, 1.99), p=0.29	51%	0.37 (0.20, 0.70), p=0.002	9%
C38:7 PE plasmalogen	2.40 (1.45, 3.98), p=0.0007	1.91 (1.11, 3.27), p=0.02	26%	0.43 (0.23, 0.80), p=0.008	22%
C40:7 PE plasmalogen	2.18 (1.34, 3.57), p=0.002	1.55 (0.91, 2.62), p=0.11	44%	0.42 (0.22, 0.80), p=0.009	19%
C38:3 PE plasmalogen	1.54 (1.03, 2.30), p=0.04	1.28 (0.81, 2.02), p=0.30	44%	0.35 (0.19, 0.67), p=0.002	4%
C22:0 LPE	1.60 (1.07, 2.39), p=0.02	1.30 (0.85, 2.01), p=0.23	43%	0.36 (0.19, 0.68), p=0.002	5%
Organic acids and derivatives:					
Proline	0.29 (0.14, 0.59), p=0.0007	0.39 (0.19, 0.81), p=0.01	23%	0.45 (0.24, 0.84), p=0.01	26%
Homocitrulline	0.59 (0.38, 0.93), p=0.02	0.71 (0.43, 1.16), p=0.17	34%	0.36 (0.19, 0.68), p=0.002	6%
Cinnamoylglycine	1.82 (1.13, 2.93), p=0.01	1.54 (0.87, 2.73), p=0.14	28%	0.38 (0.20, 0.72), p=0.003	10%
Cystine	0.58 (0.36, 0.93), p=0.02	0.62 (0.35, 1.10), p=0.10	12%	0.36 (0.19, 0.67), p=0.001	4%
Arginine	1.60 (1.05, 2.42), p=0.03	1.67 (1.01, 2.75), p=0.046	-9%	0.32 (0.17, 0.62), p=0.0008	-5%
Malonate	1.40 (0.96, 2.05), p=0.08	1.30 (0.83, 2.03), p=0.26	24%	0.35 (0.19, 0.66), p=0.001	3%
Lactate	0.52 (0.32, 0.84), p=0.008	0.59 (0.36, 0.98), p=0.04	20%	0.38 (0.20, 0.70), p=0.002	9%
Dimethylguanidino valeric acid	0.53 (0.33, 0.85), p=0.008	0.57 (0.33, 0.98), p=0.04	12%	0.35 (0.18, 0.67), p=0.002	3%
Organoheterocyclic compounds:					
Imidazole propionate	0.41 (0.23, 0.72), p=0.002	0.38 (0.19, 0.76), p=0.006	-9%	0.33 (0.17, 0.65), p=0.001	-3%
1-methylguanine	0.40 (0.23, 0.67), p=0.0006	0.45 (0.25, 0.80), p=0.007	13%	0.40 (0.22, 0.75), p=0.004	15%
3-methylxanthine	0.57 (0.37, 0.88), p=0.01	0.56 (0.34, 0.94), p=0.03	-3%	0.36 (0.20, 0.65), p=0.0008	5%
Theophylline	0.69 (0.45, 1.06), p=0.09	0.82 (0.50, 1.36), p=0.44	47%	0.37 (0.20, 0.68), p=0.002	7%
1,7-dimethyluric acid	0.68 (0.45, 1.02), p=0.06	0.85 (0.53, 1.37), p=0.51	59%	0.36 (0.19, 0.69), p=0.002	7%
Dimethylurate	0.67 (0.44, 1.01), p=0.06	0.80 (0.49, 1.31), p=0.37	43%	0.37 (0.20, 0.68), p=0.002	7%
Caffeine	0.71 (0.49, 1.02), p=0.06	0.83 (0.53, 1.30), p=0.42	48%	0.36 (0.20, 0.68), p=0.002	6%
Indole-3-propionate	1.51 (1.00, 2.30), p=0.05	1.30 (0.81, 2.09), p=0.28	37%	0.36 (0.19, 0.68), p=0.002	5%
Serotonin	1.59 (1.07, 2.38), p=0.02	1.24 (0.80, 1.93), p=0.34	53%	0.37 (0.20, 0.71), p=0.003	9%
1-methylnicotinamide	1.77 (1.15, 2.70), p=0.009	1.98 (1.16, 3.38), p=0.01	-20%	0.31 (0.16, 0.61), p=0.0006	-8%
Nucleosides, nucleotides, and analogues:					
Pseudouridine	0.56 (0.33, 0.94), p=0.03	0.64 (0.36, 1.14), p=0.13	25%	0.36 (0.20, 0.67), p=0.001	6%
Benzenoids:					
p-hydroxyphenylacetate	0.57 (0.36, 0.89), p=0.01	0.52 (0.29, 0.90), p=0.02	-17%	0.31 (0.16, 0.61), p=0.0007	-9%
Mandelate	0.63 (0.41, 0.99), p=0.045	0.63 (0.37, 1.08), p=0.09	-1%	0.34 (0.18, 0.64), p=0.0009	0%
Phenylpropanoids and polyketides:					
Hydrocinnamate	1.93 (1.20, 3.10), p=0.007	1.88 (1.07, 3.30), p=0.03	4%	0.37 (0.20, 0.69), p=0.002	8%
Alkaloids and derivatives:					
C-glycosyltryptophan	0.56 (0.35, 0.90), p=0.02	0.65 (0.38, 1.13), p=0.12	26%	0.37 (0.20, 0.69), p=0.002	8%
Cys-gly-oxidized	1.51 (1.00, 2.28), p=0.05	1.73 (1.02, 2.95), p=0.04	-33%	0.31 (0.16, 0.61), p=0.0007	-7%

*All continuous variables were standardized to a mean of zero and standard deviation of one

**Modeling the probability of high walking ability versus low walking ability

Attenuation=100*(beta coefficient from unadjusted model – beta coefficient from adjusted model) / beta coefficient from unadjusted model

Table 10. Arthritis-adjusted associations between walking ability extremes and 14 metabolites that were significantly associated with arthritis using conditional logistic regression adjusting for matched design among 120 CHS All Stars

HMDB Taxonomy super class Log-transformed/standardized metabolites	**Model 1: Unadjusted**	**Model 2: Metabolite + Arthritis**			
		Metabolite		Arthritis	
	Odds ratio* (95% CI), p-value	Odds ratio* (95% CI), p-value	Attenuation	Odds ratio* (95% CI), p-value	Attenuation
Arthritis	0.23 (0.10, 0.53), p=0.0005	-----			
Lipids and lipid-like molecules:					
Docosahexaenoate	1.69 (1.08, 2.64), p=0.02	1.47 (0.92, 2.37), p=0.11	26%	0.26 (0.11, 0.60), p=0.002	8%
C58:9 TAG	2.08 (1.27, 3.42), p=0.004	1.93 (1.16, 3.22), p=0.01	10%	0.25 (0.11, 0.60), p=0.002	5%
C58:11 TAG	1.94 (1.23, 3.07), p=0.005	1.69 (1.04, 2.76), p=0.03	21%	0.28 (0.12, 0.65), p=0.003	12%
C38:6 PC	1.65 (1.07, 2.53), p=0.02	1.33 (0.83, 2.15), p=0.24	42%	0.27 (0.12, 0.64), p=0.003	11%
C40:9 PC	1.63 (1.06, 2.49), p=0.03	1.34 (0.83, 2.14), p=0.23	41%	0.27 (0.12, 0.63), p=0.003	10%
C22:6 LPC	1.55 (1.01, 2.39), p=0.04	1.21 (0.74, 1.96), p=0.45	58%	0.26 (0.11, 0.61), p=0.002	7%
C36:3 PS plasmalogen	0.50 (0.31, 0.82), p=0.006	0.59 (0.35, 0.97), p=0.04	22%	0.28 (0.12, 0.66), p=0.004	12%
Organic acids and derivatives:					
Ornithine	0.67 (0.44, 1.01), p=0.05	0.75 (0.47, 1.18), p=0.21	29%	0.25 (0.11, 0.58), p=0.001	5%
Cinnamoylglycine	1.79 (1.12, 2.88), p=0.02	1.63 (0.96, 2.75), p=0.07	17%	0.26 (0.11, 0.60), p=0.002	7%
Organoheterocyclic compounds:					
Imidazole propionate	0.40 (0.23, 0.71), p=0.002	0.44 (0.25, 0.80), p=0.007	11%	0.27 (0.11, 0.65), p=0.004	9%
Indole-3-propionate	1.56 (1.03, 2.34), p=0.03	1.38 (0.91, 2.11), p=0.13	26%	0.26 (0.11, 0.59), p=0.002	7%
1-methylnicotinamide	1.80 (1.18, 2.73), p=0.006	1.55 (0.98, 2.45), p=0.06	25%	0.28 (0.12, 0.66), p=0.004	13%
Bilirubin	1.66 (1.10, 2.52), p=0.02	1.47 (0.92, 2.34), p=0.10	24%	0.26 (0.11, 0.61), p=0.002	8%
Phenylpropanoids and polyketides:					
Hydrocinnamate	1.94 (1.21, 3.13), p=0.006	2.02 (1.15, 3.56), p=0.01	-6%	0.23 (0.10, 0.56), p=0.001	-1%

*Modeling the probability of high walking ability versus low walking ability

Attenuation=100*(beta coefficient from unadjusted model – beta coefficient from adjusted model) / beta coefficient from unadjusted model

Table 11. Medication-adjusted associations between walking ability extremes and 9 metabolites that were associated with total number of prescription medications using conditional logistic regression adjusting for matched design among 120 CHS All Stars

HMDB Taxonomy super class Log-transformed/standardized metabolites	**Model 1: Unadjusted**	**Model 2: Metabolite + Total number of prescription medications**			
		Metabolite*		Total number of prescription medications*	
	Odds ratio* (95% CI), p-value	Odds ratio** (95% CI), p-value	Attenuation	Odds ratio** (95% CI), p-value	Attenuation
Total number of medications*	0.63 (0.42, 0.93), p=0.02	-----			
Lipids and lipid-like molecules: C34:3 PC plasmalogen	1.78 (1.11, 2.84), p=0.02	1.61 (1.00, 2.60), p=0.05	17%	0.68 (0.45, 1.02), p=0.06	17%
C36:5 PC plasmalogen-B	1.49 (0.99, 2.24), p=0.05	1.33 (0.87, 2.03), p=0.19	29%	0.66 (0.44, 1.01), p=0.05	13%
C38:3 PE plasmalogen	1.55 (1.03, 2.32), p=0.03	1.36 (0.89, 2.08), p=0.15	29%	0.68 (0.45, 1.03), p=0.07	18%
C34:5 PC plasmalogen	1.85 (1.17, 2.91), p=0.008	1.70 (1.07, 2.70), p=0.02	13%	0.67 (0.44, 1.03), p=0.07	15%
Organic acids and derivatives: Cinnamoylglycine	1.85 (1.14, 3.00), p=0.01	1.79 (1.05, 3.03), p=0.03	6%	0.65 (0.43, 1.0), p=0.05	10%
Organoheterocyclic compounds: 3-methylxanthine	0.57 (0.36, 0.90), p=0.02	0.64 (0.40, 1.02), p=0.06	19%	0.69 (0.46, 1.04), p=0.08	21%
7-methylxanthine	0.67 (0.44, 1.03), p=0.07	0.74 (0.48, 1.16), p=0.19	26%	0.67 (0.44, 1.00), p=0.048	13%
Bilirubin	1.63 (1.07, 2.46), p=0.02	1.43 (0.94, 2.18), p=0.10	26%	0.69 (0.46, 1.05), p=0.08	21%
Organic oxygen compounds: Glycerate	1.63 (1.08, 2.44), p=0.02	2.34 (1.36, 4.04), p=0.002	-75%	0.46 (0.28, 0.75), p=0.002	-66%

*All continuous variables were standardized to a mean of zero and standard deviation of one

**Modeling the probability of high walking ability versus low walking ability

Attenuation=100*(beta coefficient from unadjusted model – beta coefficient from adjusted model) / beta coefficient from unadjusted model

Table 12. Interleukin-6-adjusted associations between walking ability extremes and 32 metabolites that were significantly associated with interleukin-6 using conditional logistic regression adjusting for matched design among 120 CHS All Stars

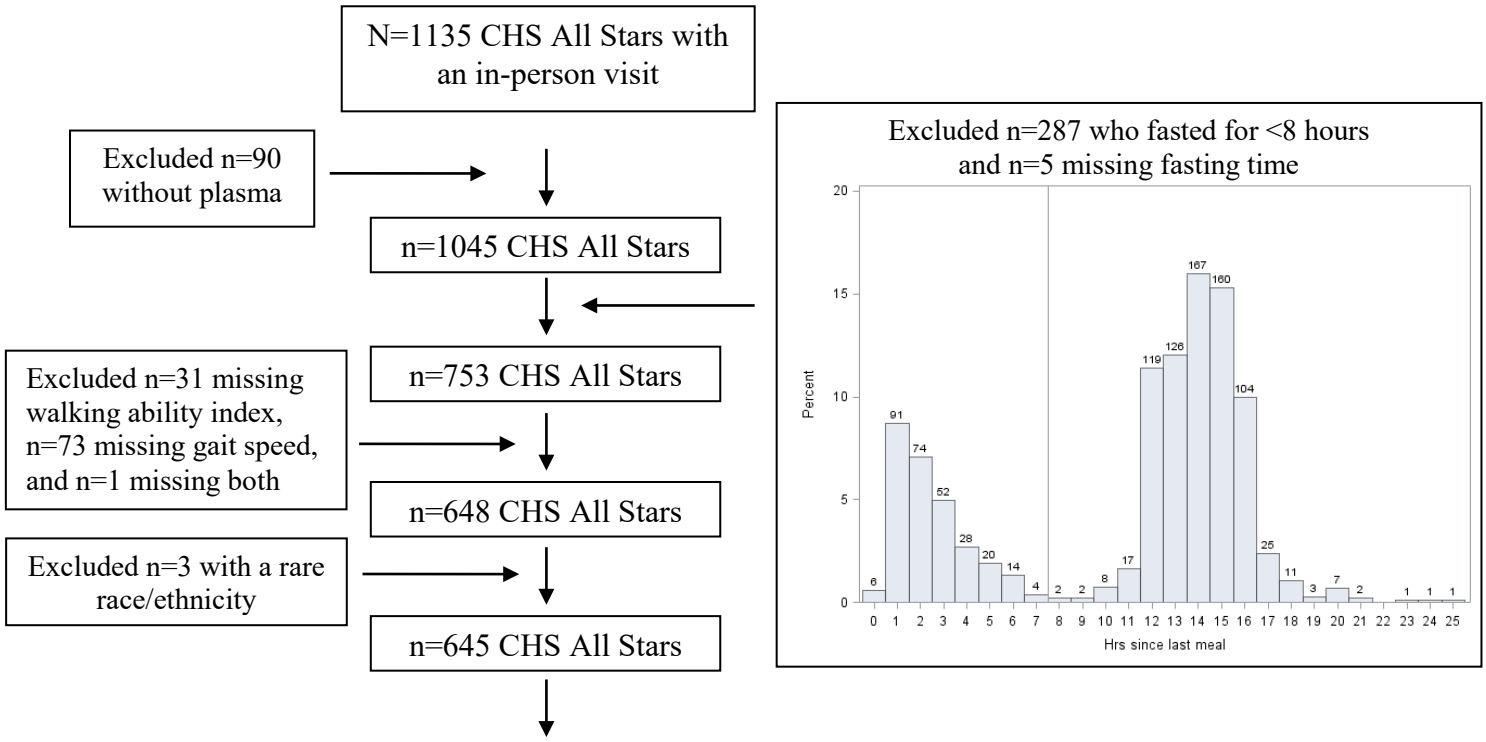
HMDB Taxonomy super class Log-transformed/standardized metabolites	**Model 1: Unadjusted**	**Model 2: Metabolite + Interleukin-6**			
		Metabolite*		Interleukin-6*	
	Odds ratio* (95% CI), p-value	Odds ratio** (95% CI), p-value	Attenuation	Odds ratio** (95% CI), p-value	Attenuation
Interleukin-6*	0.43 (0.24, 0.75), p=0.003	-----			
Lipids and lipid-like molecules:					
C58:10 TAG	2.69 (1.44, 5.02), p=0.002	2.69 (1.28, 5.63), p=0.009	0%	0.46 (0.25, 0.85), p=0.01	10%
C56:9 TAG	2.58 (1.41, 4.74), p=0.002	2.36 (1.21, 4.60), p=0.01	10%	0.49 (0.27, 0.89), p=0.02	17%
C58:11 TAG	2.75 (1.47, 5.13), p=0.002	2.48 (1.25, 4.92), p=0.009	10%	0.50 (0.28, 0.92), p=0.03	20%
C56:10 TAG	2.75 (1.42, 5.34), p=0.003	2.40 (1.18, 4.92), p=0.02	13%	0.51 (0.28, 0.93), p=0.03	22%
C54:9 TAG	2.59 (1.32, 5.08), p=0.006	2.18 (1.07, 4.44), p=0.03	18%	0.51 (0.28, 0.92), p=0.02	21%
C52:1 TAG	0.62 (0.42, 0.94), p=0.02	0.64 (0.41, 1.01), p=0.05	6%	0.44 (0.25, 0.78), p=0.005	5%
C52:2 TAG	0.63 (0.42, 0.95), p=0.03	0.67 (0.42, 1.06), p=0.09	13%	0.45 (0.26, 0.80), p=0.006	7%
C24:0 LPC	1.90 (1.12, 3.22), p=0.02	1.76 (0.99, 3.10), p=0.05	12%	0.45 (0.25, 0.81), p=0.007	6%
C34:3 PC plasmalogen	1.90 (1.14, 3.19), p=0.01	1.87 (1.04, 3.39), p=0.04	2%	0.44 (0.25, 0.79), p=0.006	5%
C38:3 PE plasmalogen	1.66 (1.07, 2.57), p=0.02	1.54 (0.96, 2.49), p=0.08	14%	0.45 (0.25, 0.80), p=0.006	6%
C22:0 LPE	2.22 (1.26, 3.93), p=0.006	1.72 (0.95, 3.11), p=0.07	32%	0.50 (0.28, 0.92), p=0.02	20%
C36:3 PS plasmalogen	0.50 (0.30, 0.85), p=0.01	0.60 (0.34, 1.04), p=0.07	24%	0.48 (0.27, 0.87), p=0.01	15%
Organic acids and derivatives:					
Proline	0.28 (0.13, 0.62), p=0.001	0.32 (0.14, 0.73), p=0.007	11%	0.51 (0.28, 0.94), p=0.03	21%
Homocitrulline	0.59 (0.35, 0.98), p=0.04	0.73 (0.41, 1.30), p=0.29	42%	0.46 (0.26, 0.82), p=0.009	10%
N6-acetyllysine	0.50 (0.26, 0.99), p=0.046	0.66 (0.33, 1.34), p=0.25	40%	0.47 (0.27, 0.83), p=0.01	12%
4-acetamidobutanoate	0.63 (0.37, 1.06), p=0.08	0.80 (0.45, 1.41), p=0.44	52%	0.45 (0.25, 0.80), p=0.007	7%
Cystine	0.55 (0.32, 0.93), p=0.03	0.70 (0.40, 1.24), p=0.22	41%	0.47 (0.26, 0.84), p=0.01	11%
Diacetylspermine	0.48 (0.27, 0.84), p=0.01	0.57 (0.32, 1.02), p=0.06	25%	0.49 (0.27, 0.87), p=0.01	16%
Malonate	1.59 (1.01, 2.49), p=0.04	1.29 (0.79, 2.08), p=0.31	46%	0.47 (0.26, 0.83), p=0.01	11%
Dimethylguanidino valeric acid	0.60 (0.36, 0.98), p=0.04	0.76 (0.43, 1.34), p=0.34	47%	0.47 (0.26, 0.83), p=0.01	11%
Organoheterocyclic compounds:					
Imidazole propionate	0.44 (0.24, 0.80), p=0.008	0.45 (0.23, 0.85), p=0.01	2%	0.43 (0.23, 0.78), p=0.006	0%
1-methylguanine	0.37 (0.20, 0.69), p=0.002	0.46 (0.24, 0.89), p=0.02	22%	0.53 (0.30, 0.96), p=0.04	26%
7-methylguanine	0.55 (0.31, 0.97), p=0.04	0.64 (0.34, 1.22), p=0.17	26%	0.46 (0.26, 0.81), p=0.007	9%
Dimethylurate	0.61 (0.38, 0.98), p=0.04	0.72 (0.42, 1.24), p=0.24	34%	0.46 (0.26, 0.82), p=0.008	9%
Nucleosides, nucleotides, and analogues:					
N2,N2-dimethylguanosine	0.40 (0.21, 0.76), p=0.005	0.51 (0.26, 0.99), p=0.048	25%	0.51 (0.29, 0.90), p=0.02	21%
1-methyladenosine	0.57 (0.34, 0.96), p=0.03	0.73 (0.41, 1.30), p=0.29	44%	0.47 (0.26, 0.84), p=0.01	11%
Pseudouridine	0.57 (0.32, 1.02), p=0.06	0.75 (0.39, 1.43), p=0.38	48%	0.46 (0.26, 0.82), p=0.008	9%
Organic oxygen compounds:					
Glycerate	1.53 (1.00, 2.35), p=0.05	1.32 (0.83, 2.11), p=0.25	35%	0.45 (0.25, 0.81), p=0.007	7%
Benzenoids:					
p-hydroxyphenylacetate	0.63 (0.40, 1.01), p=0.05	0.63 (0.38, 1.07), p=0.09	1%	0.42 (0.24, 0.76), p=0.004	-1%
Mandelate	0.55 (0.33, 0.91), p=0.02	0.65 (0.38, 1.12), p=0.12	27%	0.47 (0.27, 0.83), p=0.01	12%
Alkaloids and derivatives:					
Trigonelline	0.77 (0.49, 1.19), p=0.24	0.95 (0.57, 1.58), p=0.83	79%	0.43 (0.24, 0.77), p=0.004	2%
Taxonomy class not available:					
C-glycosyltryptophan	0.55 (0.33, 0.94), p=0.03	0.74 (0.42, 1.29), p=0.28	48%	0.47 (0.26, 0.85), p=0.01	12%

*All continuous variables were standardized to a mean of zero and standard deviation of one; interleukin-6 was log-transformed prior to standardization

**Modeling the probability of high walking ability versus low walking ability

Attenuation=100*(beta coefficient from unadjusted model – beta coefficient from adjusted model) / beta coefficient from unadjusted model

2.7 FIGURES



Tertiles of the walking ability index:	Tertiles of gait speed (15 ft)		
	<0.7 m/s (worst)	≥0.7 to < 0.9 m/s	≥0.9 m/s (best)
0-1 (worst)	n=81 Row: 59% Column: 37%	n=37 Row: 27% Column: 22%	n=19 Row: 14% Column: 7%
2-6	n=89 Row: 37% Column: 41%	n=71 Row: 29% Column: 41%	n=83 Row: 34% Column: 32%
7-9 (best)	n=47 Row: 18% Column: 22%	n=64 Row: 24% Column: 37%	n=154 Row: 58% Column: 60%

Figure 3. Flow chart of CHS All Stars eligible for our nested case-control study examining metabolites associated with walking ability extremes

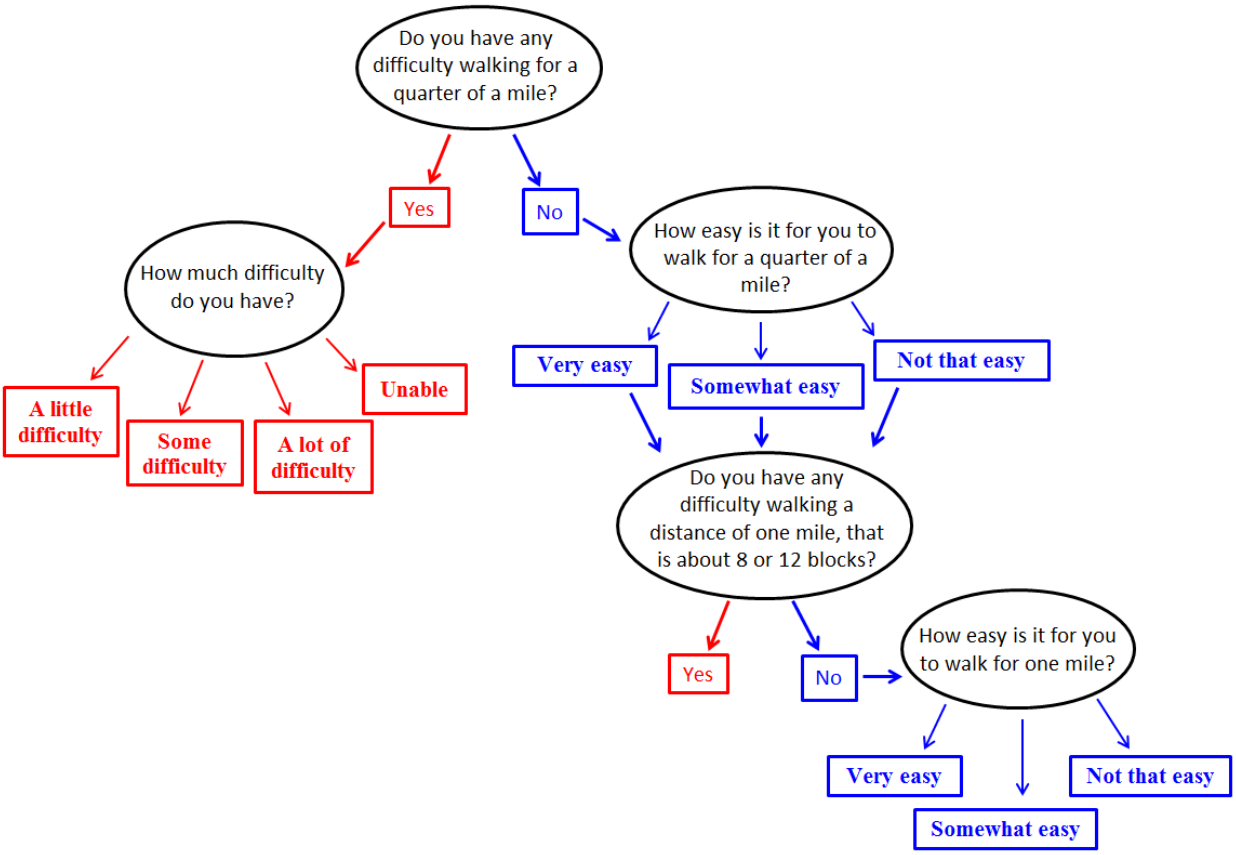


Figure 4. Flow chart of potential responses and follow-up questions used to calculate the original Walking Ability Index in the Health, Aging, and Body Composition study

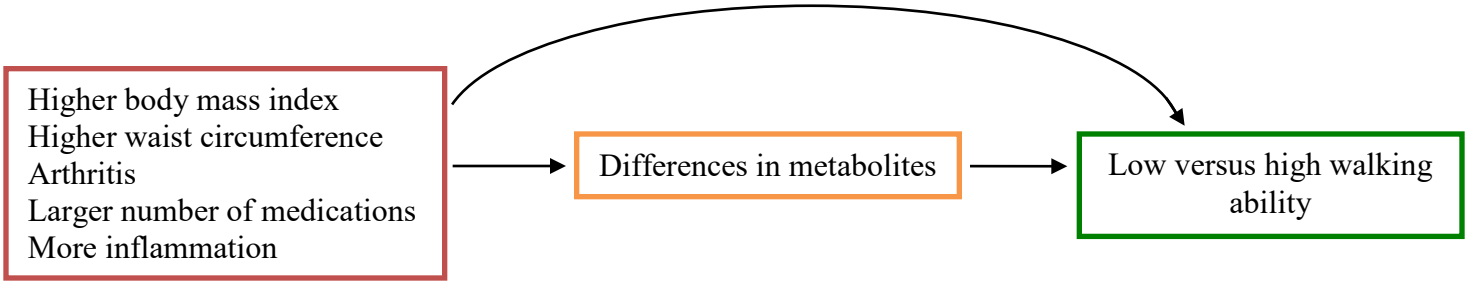
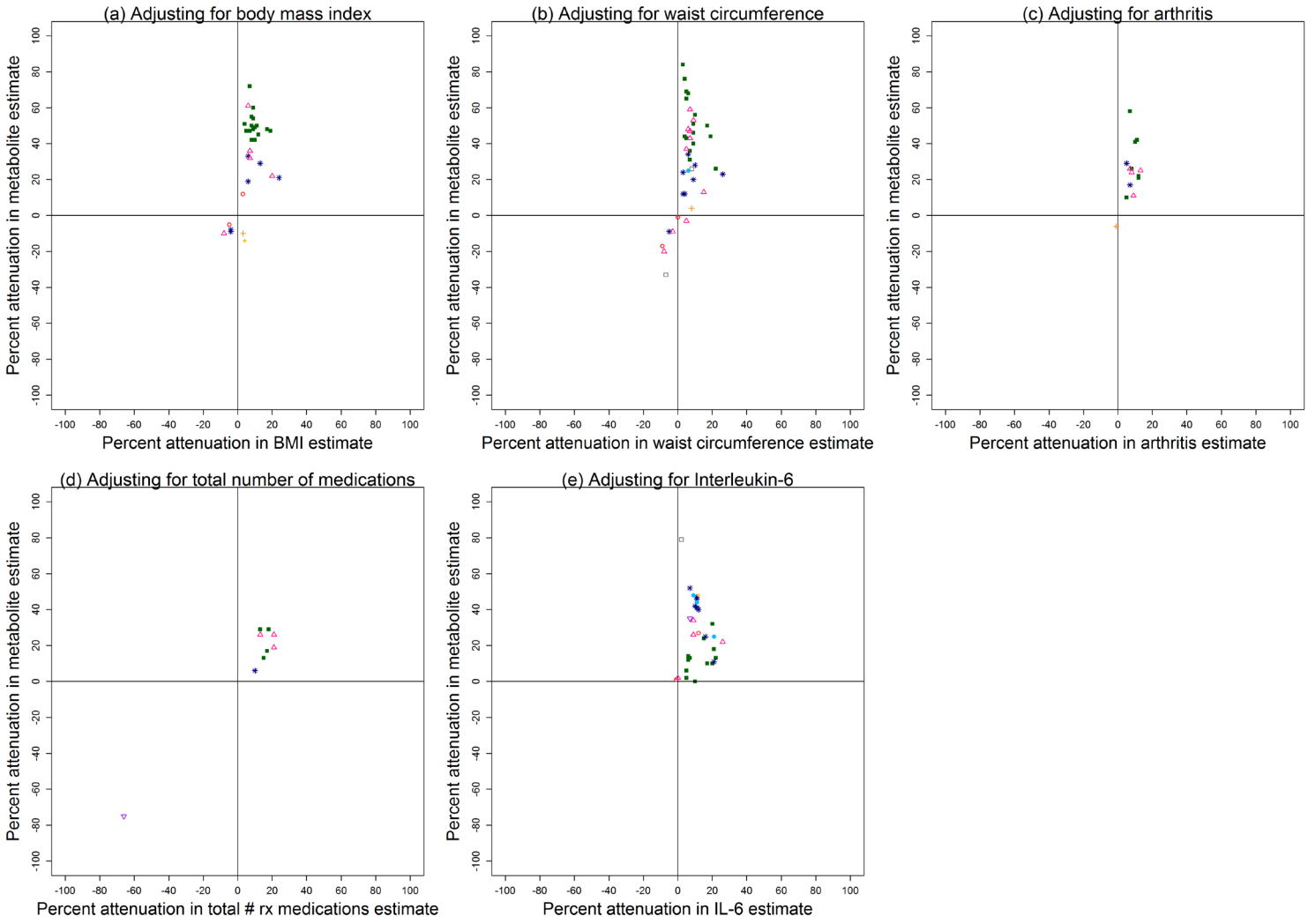


Figure 5. Simplified hypothetical causal diagram of more commonly measured risk factors, metabolites, and walking ability



Each point is a single metabolite organized by its taxonomy super class, according to the Human Metabolome Database: green squares are lipids and lipid-like molecules; navy blue asterisks are organic acids and derivatives; pink triangles are organoheterocyclic compounds; light blue circles are nucleosides, nucleotides, and analogues; purple upside-down triangles are organic oxygen compounds; red circles are benzenoids; orange plus sign is phenylpropanoids and polyketides; grey square is alkaloids and derivatives; and gold diamonds are unclassified metabolites.

Figure 6. Percent attenuations in the associations between select metabolites and walking ability after adjusting for a more commonly measured variable (y-axis) *versus* percent attenuations in the association between a more commonly measured variable and walking ability after adjusting for a metabolite (x-axis), organized by taxonomy class among 120 CHS All Stars

3.0 METABOLITES ASSOCIATED WITH VIGOR TO FRAILTY AMONG COMMUNITY-DWELLING OLDER BLACK MEN

3.1 ABSTRACT

Black versus white older Americans are more likely to experience frailty, a condition associated with multiple major health outcomes. To reduce racial disparities in health, a complete understanding of the pathophysiology of frailty is needed. Metabolomics may further our understanding by characterizing differences in the body during a vigorous versus frail state. Here, we sought to identify metabolites and biological pathways associated with vigor to frailty using a cohort of 287 black men ages 70-81 from the Health, Aging, and Body Composition study. Using liquid chromatography-mass spectrometry, 350 metabolites were measured in overnight-fasting plasma. Vigor to frailty was measured using the scale of aging vigor in epidemiology (SAVE), based on weight change, strength, energy, gait speed, and physical activity. Thirty-seven metabolites correlated with SAVE scores ($p<0.05$) adjusting for age and site, 14 remained significant after multiple comparisons adjustment (false discovery rate<0.30). *Lower* values of amino acids (tryptophan, methionine, tyrosine, and asparagine), C14:0 sphingomyelin, and 1-methylnicotinamide and *higher* values of glucoronate, N-carbamoyl-beta-alanine, isocitrate, creatinine, C4-OH carnitine, cystathionine, hydroxyphenylacetate, and putrescine were associated with frailer SAVE scores. Pathway analyses identified nitrogen

metabolism, aminoacyl-tRNA biosynthesis, and the citric acid cycle associated with SAVE scores. Future studies need to confirm whether these metabolites and pathways characterize vigor versus frailty in late-life, which will indicate novel mechanisms potentially involved in the frailty syndrome that can then be intervened on to promote healthy, vigorous aging.

3.2 INTRODUCTION

In the United States, black compared to white older adults are more likely to be frail (141). In fact at every age group, older community-dwelling black men and women had a higher prevalence of frailty than white men and women, respectively (54). To reduce racial disparities in health and further the understanding of the biology and pathophysiology of frailty, a deeper characterization of frailty is needed. One way to provide a deeper characterization is by identifying metabolites associated with the full spectrum of healthy aging from vigor to frailty. Using metabolites to identify differences in the body during a frail state may reveal new insights into altered biological processes that adapt to maintain homeostasis in the presence of evolving frailty.

A pilot study measured metabolites in a subset of randomly selected black men from the Health, Aging, and Body Composition (Health ABC) study (113). Such a well-characterized cohort allows for identifying metabolites associated with vigor to frailty, while controlling for important confounders. Thus, the aims of this report were first, to identify novel metabolites and biologic pathways associated with vigor to frailty and second, to determine whether these associations are attenuated after adjusting for more commonly measured variables.

3.3 METHODS

3.3.1 Health, Aging, and Body Composition (Health ABC) study

The Health ABC study was a prospective cohort of 3075 black and white men and women recruited from Pittsburgh, Pennsylvania and Memphis, Tennessee during March 1997 to July 1998. The study was originally designed to address the role of weight-related health conditions and body composition in the onset of disability (142). Eligible participants were ages 70-79 during recruitment and self-reported no difficulty walking ¼ mile, climbing ten steps, or with basic activities of daily living. Ineligibility included history of active cancer treatment in the past three years or planning on moving from the study area within the next three years. The study was approved by each site's institutional review board. Participants provided written informed consent.

An ancillary pilot study measured 350 known and numerous unknown metabolites in a randomly selected subset of 319 black men from the second visit of the Health ABC study to provide insight on the influence of lean mass and adiposity in human metabolism (123). The study was limited in size, so it was restricted to black men since there is a higher prevalence of obesity and obesity-related health conditions, but more muscle mass among black versus white Americans, and to limit heterogeneity due to differences in body composition by sex. The randomly selected black men were healthier than the whole sample of Health ABC black men since the second visit was used and attrition had occurred during the first year (123).

3.3.2 Metabolites

Metabolites were measured in plasma extracts collected at visit 2 in the morning after an overnight fast of at least eight hours (mean=14 hours). We used plasma samples that had never been thawed and were stored at -80ºC from the time of collection (1998-1999) until 2016 when metabolites were measured. Using liquid chromatography-mass spectrometry (LC-MS), metabolite profiling platforms measured: 1) amines and polar metabolites (e.g., amino acids, dipeptides), 2) central metabolites and polar metabolites (e.g., sugars, organic acids, purine and pyrimidines), and 3) lipids (e.g., triglycerides). Metabolite values used for this report are LC-MS peak areas, analyzed using TraceFinder (ThermoFisher Scientific, US) and Progenesis QI (Nonlinear Dynamics, UK). Peaks were confirmed manually using known standards. Metabolites below the limit of quantitation (signal/noise<10) were classified as unquantifiable (120). The median intraclass correlation coefficient of known metabolites from 16 blinded duplicates was 0.92 (interquartile range: 0.81-0.97), indicating high reliability (123).

Positive ion mode detection used a 4000 QTRAP triple quadrupole mass spectrometer (SCIEX) coupled to an 1100 Series pump (Agilent) and an HTS PAL autosampler (Leap Technologies) with a 4.5kV ion spray voltage and at 450ºC source temperature. Using protein precipitation, plasma samples (10µL) were prepared with the addition of nine volumes of 74.9:24.9:0.2 (v/v/v) acetonitrile/methanol/formic acid containing stable isotope-labeled internal standards (0.2ng/µL valine-d8, Isotec; and 0.2ng/µL phenylalanine-d8; Cambridge Isotope Laboratories). Samples were centrifuged for 10 minutes (9,000g, 4°C). Resulting supernatants were injected onto a 150×2mm Atlantis HILIC column that was eluted at a 250µL/min flow rate. Initial conditions were set at 5% mobile phase A (10mM ammonium formate and 0.1% formic

acid in water) for one minute and then altered linearly over ten minutes to 40% mobile phase B (acetonitrile with 0.1% formic acid) (120, 121).

Negative ion mode detection used a 5500 QTRAP triple quadrupole mass spectrometer (SCIEX) coupled to an ACQUITY UPLC (Waters) with a modified hydrophilic interaction chromatography method and −4.5kV ion spray voltage and at 500ºC source temperature. Using protein precipitation, plasma samples (30µL) were prepared with the addition of 120µL of 80% methanol containing 0.05 ng/µL inosine-15N4, 0.05 ng/µL thymine-d4, and 0.1ng/µL glycocholate-d4 as internal standards. Samples were centrifuged (10 min, 9,000×g, 4°C) and 10µL of supernatants were injected onto a 150×2.0mm Luna NH2 column (Phenomenex) that underwent elution at a 400µL/min flow rate. Initial conditions were set at 10% mobile phase A (20 mM ammonium acetate and 20 mM ammonium hydroxide; Sigma-Aldrich) in water (VMR) along with 90% mobile phase B (10 mM ammonium hydroxide in 75:25 v/v acetonitrile/methanol (VWR)) and then altered linearly over ten minutes to 100% mobile phase A (120, 121).

Lipids were detected using an Exactive Plus orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) coupled to a Nexera X2 UHPLC (Shimadzu, Marlborough, MA) with electrospray ionization and positive ion mode Q1 scans. The ion spray voltage was 5.0 kV with 400ºC source temperature. Plasma samples (10 µL) were extracted using 190µL of isopropanol containing 0.25 ng/µL 1-dodecanoyl-2-tridecanoyl-sn-glycero-3-phosphocholine (Avanti Polar Lipids). Samples were centrifuged and 10 µL of supernatants were injected onto a 150×3.0 mm Prosphere HP C4 column (Grace). The column was eluted with initial conditions set at 80% mobile phase A (95:5:0.1 vol/vol/vol 10mM ammonium acetate/methanol/acetic acid), then after two minutes, changed linearly over one minute to 80% mobile phase B (99.9:0.1 vol/vol

methanol/acetic acid), followed by a linear change over 12 minutes to 100% mobile phase B. Conditions remained at 100% mobile phase B for 10 minutes (120, 121).

3.3.3 Scale of Aging Vigor in Epidemiology (SAVE)

The Scale of Aging Vigor in Epidemiology (SAVE) was developed by modifying the Fried frailty phenotype (54) to allow for measuring both the healthy (i.e., vigorous), in addition to the unhealthy (i.e. frail), extremes (59, 60). The SAVE was calculated using information from five items assessed at year 2: weight change, physical activity, grip strength, gait speed, and energy level (11). Weight change was the difference between measurements at year one and two. Self-reported time spent doing major chores, walking, climbing stairs, working, volunteering, and caregiving in the past week was converted to kilocalories/kilogram/week and summed to get an estimate of weekly physical activity. Grip strength was the maximum of two trials on the right hand using a hand-held dynamometer. Gait speed was the average over 20 meters. Participants self-reported usual energy level in the past month on a scale of 0 (no energy) to 10 (most energy ever had). Scores on each of the five items were ranked into tertiles using information from all Health ABC men (Table 13). Individuals who scored in the best, middle, or worst tertile for a component received a score of 0, 1, or 2, respectively. SAVE scores were the sum of tertile scores for the five items, ranging from 0 (most vigorous) to 10 (most frail), and examined continuously and as tertiles. SAVE tertiles were determined using information from all Health ABC participants and ranged from 0-3 (most vigorous), 4-5, and 6-10 (most frail).

3.3.3.1 Health ABC black men with information on metabolites and the SAVE

Among the 319 black men with metabolites measured, 287 (90%) had complete information to calculate the SAVE. In this report, we focused on the known metabolites. Among the 350 known metabolites, 301 were measured in all 287 participants and 33 were measured in at least 80% of participants, of which missing values were assumed to be due to the true values being below the detectable limit and were replaced with half the minimum recorded value for that respective metabolite (123). Sixteen (5%) metabolites were excluded from the current analysis because they were measured in less than 80% of participants (124). Thus, we examined 334 metabolites among 287 black men.

3.3.4 Potential confounders of metabolites and SAVE scores

Participants self-reported age, race, highest level of education, and smoking habits at baseline. Height and weight were recorded at year 2. Baseline history or presence of cardiovascular disease, hypertension, diabetes, cancer, peripheral arterial disease, osteoarthritis, depression, pulmonary disease, and kidney disease were based on self-report of a physician diagnosis. Participants were also classified as having cardiovascular disease, hypertension, diabetes, cancer, depression, or pulmonary disease if taking medication for those diseases and peripheral arterial disease if self-reported intermittent claudication, leg pain, or leg artery bypass or angioplasty. Participants brought all prescription medications used in the last two weeks to the visit at year 2 for a medication inventory.

Daily calories, protein, and fat intake at year 2 were determined using a 108-item interviewer-administered food frequency questionnaire estimating usual nutrient intake over the past year and was developed for the Health ABC study by Block Dietary Data Systems

(Berkeley, CA) using food lists obtained from a 24-hour recall among participants who were ages 65 or older, black or white race, and living in the Northeastern or Southern U.S. from the Third National Health and Nutrition Examination Survey (143). Protein per kilogram of body weight and percent of kilocalories from protein and from fat were also examined.

Body composition at year 2 was estimated using total body dual-energy x-ray absorptiometry (Hologic QDR 4500A; Hologic, Bedford, MA). Appendicular lean mass was the bone-free lean mass in the arms and legs standardized to height^2. Percent fat was examined relative to total body mass. A core laboratory at Wake Forest University measured interleukin-6 and C-reactive protein in serum and EDTA plasma, respectively, collected at year 2 in the morning after an overnight fast. Cystatin C and creatinine were measured in serum at year 1 by the Laboratory for Clinical Biochemistry Research at the University of Vermont. Glomerular filtration rate was estimated as 133*(cystatin C/0.8)-y*0.996age, where y=0.499 when cystatin C ≤0.8 mg/L and y=1.328 when cystatin C >0.8 mg/L (144).

3.3.5 Statistical analysis

Mean (standard deviation) or frequency (percent) described differences in potential confounders by SAVE tertiles and were tested using Analysis of Variance or Kruskal-Wallis for continuous measures and chi-square tests or Fisher's exact test for categorical measures. Metabolites were log-transformed and standardized. Partial Pearson correlation coefficients identified metabolites associated with SAVE scores, while minimally adjusting for age and study site. A Benjamini-Hochberg correction was used for multiple comparisons (99). Since this was a hypothesis-generating report, we used a liberal 30% false discovery rate (124). We examined the extent to which more commonly measured variables explained the age- and study site-adjusted

associations between metabolites and SAVE scores using percent attenuation calculated as $100*(r_1-r_2)/r_1$, where r_1 is the age- and study site-adjusted correlation coefficients between SAVE scores and metabolites and r_2 is the correlation coefficients after further adjusting for a more commonly measured variable.

Metabolites associated with SAVE scores at a $p<0.05$ were examined in a pathway analysis using MetaboAnalyst (129), which compared the set of associated metabolites against established sets of metabolites involved in metabolic pathways. A Fisher's exact test determined whether the number of SAVE-associated metabolites involved in a pathway was more than expected by chance. Impact scores indicated how centrally located SAVE-associated metabolites were in particular pathways, i.e., the amount of impact on the pathway if the values for those metabolites were altered. Impact scores range from zero to one, indicating matched metabolites account for none to all of the pathway importance, respectively (129).

3.4 RESULTS

3.4.1 Characteristics by SAVE score tertiles among Health ABC black men

Participants were 75 years old, on average. There was no difference in daily calories, protein intake, or body composition by SAVE tertiles (Table 14). Median levels of markers of inflammation and kidney disease were slightly higher among frailer participants. The frailest individuals had the highest prevalence of cardiovascular disease, diabetes, and pulmonary disease, as well as were taking a larger number of medications, specifically medications for hypertension, diabetes, and pulmonary diseases.

3.4.2 Metabolites correlated with SAVE scores

Among 334 metabolites, 37 correlated with SAVE scores ($p<0.05$) adjusting for age and site (Table 15), most of the associations did not appear to be driven by a single item used to calculate SAVE scores (Table 16). However, only 2 of the 37 metabolites correlated with weight change ($p<0.05$), whereas more than a third correlated with gait speed and physical activity (Table 16). Among the 37 metabolites, 14 remained significant after multiple comparisons adjustment (false discovery rate<0.30). Eight metabolites (glucoronate, N-carbamoyl-beta-alanine, isocitrate, creatinine, C4-OH carnitine, cystathionine, hydroxyphenylacetate, and putrescine) were positively correlated with SAVE scores, indicating lower metabolite values associated with vigor and higher values associated with frailty. The remaining six metabolites (tryptophan, methionine, tyrosine, C14:0 SM, 1-methylnicotinamide, asparagine) were negatively correlated with SAVE scores, indicating higher metabolite values associated with vigor and lower values associated with frailty.

3.4.3 Attenuating associations between metabolites and SAVE scores

Figure 7 illustrates the percent attenuations of the correlations between SAVE scores and the 37 top metabolites after further adjusting for more commonly measured variables, in addition to age and study site, where each data point on the plot is a different metabolite organized by its taxonomy super class according to the Human Metabolome Database (84). Adjusting for current smoking status or body mass index minimally attenuated the associations between SAVE scores and metabolites (Figure 7A-B; attenuations≤0.5%; Table 17). Adjusting for percent fat and appendicular lean mass, daily protein intake, or inflammation markers attenuated more of the

associations between SAVE scores and metabolites (Figure 7C-E), though attenuations were still <12% (Table 17). Among the more commonly measured variables considered, adjusting for creatinine, prevalent diseases, or medications resulted in the most attenuation between SAVE scores and metabolites (Figure 7F-H; attenuations <84%, <59%, and <69%, respectively). Adjusting for multiple more commonly measured variables (Figure 7I) attenuated the associations between SAVE scores and 11 metabolites by at least 40%: salicylurate, 5-aminolevulinic acid, hydroxyphenylacetate, creatinine, symmetric dimethylarginine, trimethylamine-N-oxide, 2-hydroxyglutarate, glucoronate, homogentisate, C36:4 PE, and isocitrate. Conversely, adjusting for multiple more commonly measured variables did not explain the association between SAVE scores and 13 metabolites (attenuations<10%). Specifically, the associations between SAVE scores and leucine, 1-methylnicotinamide, C54:10 triacylglycerol, C34:3 PE plasmalogen, glycodeoxycholate, and C22:0 sphingomyelin were actually strengthened by ≥10%, after further adjusting for multiple more commonly measured variables (Table 17).

3.4.4 Pathway analysis

Among the 37 metabolites correlated with SAVE scores at a $p<0.05$, 35 were in the Human Metabolome Database Version 4.0 (84) and were included in the pathway analysis. Table 18 includes the top ten pathways among 36 that involved at least one SAVE-associated metabolite. The most significant pathways were nitrogen metabolism, aminoacyl-transfer RNA biosynthesis, and the citric acid cycle. The match status for nitrogen metabolism was 5/39, meaning 39 known metabolites are involved in nitrogen metabolism and five of them were associated with SAVE scores (tyrosine, tryptophan, asparagine, histidine, and cystathionine). The match status was 6/75

for aminoacyl-transfer RNA biosynthesis (tyrosine, tryptophan, asparagine, histidine, methionine, and leucine) and 3/20 for the citric acid cycle (isocitrate, malate, and fumarate). However, low impact scores were observed for these pathways (Table 18).

3.5 DISCUSSION

We identified unique patterns of plasma metabolites differing across the range of health from vigorous to frail older black men. Thirty-seven metabolites correlated with SAVE scores, of which 14 remained significant after multiple comparisons adjustment. Nitrogen metabolism, aminoacyl-transfer RNA biosynthesis, and the citric acid cycle were top metabolic pathways associated with SAVE scores, suggesting differences in functioning of these pathways may be present during a frail versus vigorous state. Since many other factors influence metabolism, it was notable that several metabolites were associated with SAVE scores independent of more commonly measured variables, such as body composition, smoking status, daily protein intake, inflammation markers, several chronic conditions, and medication use.

Several amino acids were associated with SAVE scores, where lower values were correlated with frailer scores. Lower values of tryptophan, methionine, tyrosine, and leucine were also correlated with less appendicular lean mass among the Health ABC black men (123) and lower values of leucine and other branched-chain amino acid-related metabolites were correlated with lower thigh muscle cross-sectional area and fat-free mass index among functionally-limited older adults (145). To date, few studies examined metabolites associated with frailty. A targeted set of metabolites in muscle biopsies similarly found tryptophan, methionine, tyrosine, asparagine, and histidine lower among frail older adults (103). In addition,

blood-based tryptophan and tyrosine measured among a Spanish older adult cohort were lower among frail participants (146). Conversely, higher levels of amino acids have been associated with obesity, diabetes, and cardiovascular disease (114-116). Notably, frailer Health ABC black men were more likely to have diabetes, though there was no difference in body mass index by level of frailty. It may be that the difference in direction of associations between amino acids and adverse health outcomes may be explained by a U-shaped relationship, where higher values of certain amino acids are associated with metabolic disorders, such as obesity, diabetes, and cardiovascular disease, but lower values are associated with wasting disorders that are further along in pathogenesis, such as frailty.

It was previously reported that seven metabolites (glucoronate, tryptophan, asparagine, C24:1 Ceramide (d18:1), 2-hydroxyglutarate, salicylurate, and C54:10 TAG) correlated with gait speed among the Health ABC black men (98), of which all were similarly associated with SAVE scores, as expected since gait speed is an item of the SAVE. In addition, eight metabolites (N-carbamoyl-beta-alanine, creatinine, C4-OH carnitine, 5-aminolevulinic acid, inosine, symmetric dimethylarginine, C36:4 PE, and C18:2 CE) that predicted incident disability (98) and nine metabolites (glucoronate, N-carbamoyl-beta-alanine, isocitrate, creatinine, hydroxyphenylacetate, 5-aminolevulinic acid, symmetric dimethylarginine, urate, and trimethylamine-N-oxide) that were associated with extremes of a healthy aging index (107) among the Health ABC black men were also associated with SAVE scores. N-carbamoyl-beta-alanine, creatinine, inosine, and symmetric dimethylarginine are indicators of kidney functioning (98) and may be important markers of healthy aging.

SAVE-associated metabolites involved in nitrogen metabolism and aminoacyl-transfer RNA biosynthesis were mostly amino acids. In a healthy individual, plasma levels of amino acid

are tightly regulated within a fixed range (147, 148). The rate of appearance of amino acids in plasma is a result of dietary protein intake and release of amino acids by muscles and other tissues, whereas, the rate of disappearance from plasma is due to amino acid oxidation, metabolism, and incorporation into proteins, and, to a lesser degree, loss through excretion. Hypoaminoacidemia may occur from insufficient protein intake/storage and/or stress (147). Protein intake in Health ABC did not vary by level of frailty and appeared to be sufficient with an overall average of 0.97 g/kg/day. However, it is possible that this level of intake is insufficient in the frailer men to overcome aging-related anabolic resistance, where the body's ability to utilize amino acids to synthesize muscle proteins appears to be altered (149-151). Additionally or alternatively, the frailer participants may have lower levels of tryptophan, methionine, tyrosine, asparagine, and histidine due to an increased stress response causing conversion of plasma amino acids to glucose (147). There may be specific mechanisms that adapt to aging-related disease states, but by doing so have adverse effects that potentially lead to altered energy pathways and then eventually frailty.

A limitation of this report was that measurements for metabolites were unit-less LC-MS peak areas. If we instead had concentration of metabolites, we could determine whether the metabolites that were either lower or higher among the frailer participants were more extreme than what is considered within a healthy range. In addition, we measured frailty severity using the SAVE, which only describes how frail an individual is relative to the rest of their cohort, whereas that same individual may appear much less frail if they were instead compared to the United States population of older adults. Other limitations include studying only black men, limiting the generalizability and comparability of results; using self-reported dietary information from the Food Frequency Questionnaire, which may not be accurate to what the participants

were actually consuming, as well as it only provides information on usual diet; and the potential for false positives given the liberal false discovery rate. Strengths were the well-characterized cohort of ambulatory older adults, allowing us to examine whether several potential factors attenuated the associations between metabolites and SAVE scores, as well as information on a large number of metabolites from blood plasma carefully collected and stored after an overnight fast.

Several metabolites, particularly amino acids, were associated with vigor to frailty scores among older black men from the Health ABC study, which may help us better understand the mechanisms underlying progression of frailty. Multisystem decline with frailty makes it impossible to pinpoint any one organ system responsible; instead the aggregate of multisystem dysfunction may actually be responsible for these metabolic characteristics. The generalizability of these findings needs to be confirmed. Once confirmed, more research will be needed to identify biologic mechanisms causing these differences in metabolites that are associated with frailty, either through animal models that can directly alter specific pathways or through interventions in humans that attempt to enhance a pathway, for example, by providing a certain supplement coupled with physical activity.

3.6 TABLES

Table 13. Tertile cut-offs of the five items used to calculate SAVE scores for Health ABC men

SAVE items:	Best tertile=0	Mid tertile=1	Worst tertile=2
Weight change (kg)	> 0.68	> -1.36 to ≤ 0.68	≤ -1.36
Physical activity* (kcal/kg/week)	≥ 43	> 11 to < 43	≤ 11
20 meter walk time (sec)**	≤ 16	> 16 to ≤ 18	> 18
Grip strength (kg):			
BMI <24	> 38	>32 to ≤ 38	≤ 32
BMI ≥24	> 41	>35 to ≤ 41	≤ 35
Usual energy level	8 to 10	6 to 7	0 to 5

*Total physical activity is only based off of kcal/kg/week doing major chores, walking and climbing stairs, working, volunteering and caregiving (did not collect data for kcal/kg/week of exercise/recreation).
**Tertiles for walk time did not differ for men by mean height

Table 14. Characteristics of 287 Health ABC black men by tertiles of SAVE scores

Mean (standard deviation) or Frequency (percent)	SAVE tertiles			Overall p-value, Pairwise comparisons
	Vigorous (T1) n=73	Average (T2) n=105	Frail (T3) n=109	
SAVE scores	2.4 (0.7) Range: 0-3	4.5 (0.5) Range: 4-5	7.0 (1.1) Range: 6-10	-----
Age	74 (3)	75 (3)	75 (3)	.006, T1<T2,T3
Pittsburgh site	34 (47%)	56 (53%)	63 (58%)	.33
More than high school education	28 (38%)	24 (23%)	28 (26%)	.06
Current smoker at baseline	9 (12%)	22 (21%)	21 (19%)	.31
Body mass index (kg/m^2)	27 (4)	27 (4)	27 (5)	.82
Dietary intake:				
Total calories (Kcal/day)	2329 (1111)	2199 (1022)	2095 (1038)	.35
Protein intake (g/day)	81 (44)	75 (37)	73 (39)	.41
Percent of daily kilocalories from protein	14 (3)	14 (3)	14 (3)	.82
Daily protein intake per body weight (g/kg)	1.0 (0.6)	0.97 (0.5)	0.94 (0.5)	.71
Fat intake (g/day)	92 (51)	87 (49)	81 (48)	.30
Percent of daily kilocalories from fat	35 (6)	35 (8)	34 (8)	.57
Body composition:				
Appendicular lean mass (kg/m^2)	8.4 (1)	8.3 (1)	8.3 (1)	.68
Percent fat	28 (5)	28 (5)	28 (6)	.92
Inflammation markers:				
Interleukin-6 (pg/mL)	4.2 (5.9) Median=2.5 IQR: 1.4, 4.1	3.2 (2.2) Median=2.4 IQR: 1.6, 3.8	4.2 (3.4) Median=3.0 IQR: 1.8, 5.4	.05
C-reactive protein (ug/mL)	5.4 (8.9) Median=2.8 IQR: 1.4, 6.2	5.3 (9.7) Median=2.1 IQR: 1.1, 6.2	8.4 (16) Median=3.9 IQR: 1.6, 8.8	.05
Markers of kidney disease at baseline:				
Creatinine (mg/dL)	1.2 (0.2) Median=1.2 IQR: 1.0, 1.3	1.2 (0.3) Median=1.2 IQR: 1.0, 1.3	1.3 (0.4) Median=1.2 IQR: 1.1, 1.5	.04
Cystatin C (mg/L) at baseline	1.0 (0.2) Median=0.96 IQR: 0.87, 1.1	1.0 (0.3) Median=1.0 IQR: 0.87, 1.1	1.1 (0.3) Median=1.1 IQR: 0.94, 1.2	.05
Estimated glomerular filtration rate	77 (17)	75 (19)	70 (19)	.03, T1>T3
Prevalent disease at baseline:				
Cardiovascular disease	11 (15%)	36 (34%)	39 (36%)	.006, T1<T2,T3
Hypertension	34 (47%)	65 (62%)	67 (61%)	.08
Diabetes	8 (11%)	18 (17%)	37 (34%)	.0004, T1,T2<T3
Cancer	10 (14%)	11 (10%)	11 (10%)	.72
Peripheral artery disease	2 (3%)	7 (7%)	9 (8%)	.32
Osteoarthritis	2 (3%)	9 (9%)	11 (10%)	.17
Depression	4 (5%)	5 (5%)	8 (7%)	.71
Pulmonary disease	7 (10%)	8 (8%)	21 (19%)	.02, T2<T3
Kidney disease	1 (1%)	2 (2%)	0	.36
Medication use:				
Total number of prescription medications	2.2 (2)	3.0 (3)	4.0 (4)	.0003, T1,T2<T3
Antihypertensive medications	35 (48%)	64 (61%)	74 (68%)	.03, T1<T3
Antilipemic medications	14 (19%)	11 (10%)	17 (16%)	.25
Medications for diabetes:	5 (7%)	17 (16%)	36 (33%)	<.0001, T1,T2<T3
Insulin	0	2 (2%)	10 (9%)	.004, T1,T2<T3
Oral hypoglycemic	5 (7%)	15 (14%)	28 (26%)	.003, T1,T2<T3
Medications for prostate disease	10 (14%)	14 (13%)	19 (17%)	.83, P=0.66
Medications for pulmonary diseases	5 (7%)	2 (2%)	13 (12%)	.02, T2<T3
Spasmolytics (theophylline and others)	0	1 (1%)	5 (5%)	.09
Anti-inflammatory	24 (33%)	43 (41%)	52 (49%)	.09

IQR=interquartile range

Table 15. Correlation of 37 metabolites with SAVE scores ($p<0.05$) among 287 Health ABC black men adjusted for age and study site

Log-transformed and standardized Metabolites	HMDB ID number	Taxonomy sub class	Continuous SAVE scores		
			Partial Pearson Correlation	p-value	False discovery rate
Glucuronate	00127	Carbohydrates and carbohydrate conjugates	0.21	0.0003	0.08
Tryptophan	00929	Indolyl carboxylic acids and derivatives	-0.21	0.0005	0.08
Methionine	00696	Amino acids, peptides, and analogues	-0.19	0.001	0.15
N-carbamoyl-beta-alanine	00026	Ureas	0.17	0.004	0.22
Tyrosine	00158	Amino acids, peptides, and analogues	-0.17	0.004	0.22
Isocitrate	00193	Tricarboxylic acids and derivatives	0.17	0.004	0.22
Creatinine	00562	Amino acids, peptides, and analogues	0.16	0.008	0.27
C4-OH carnitine	13127	Beta hydroxy acids and derivatives	0.16	0.009	0.27
C14:0 SM	12097	Phosphosphingolipids	-0.15	0.009	0.27
Cystathionine	00099	Amino acids, peptides, and analogues	0.15	0.009	0.27
Hydroxyphenylacetate	00020	1-hydroxy-2-unsubstituted benzenoids	0.15	0.01	0.27
Putrescine	01414	Amines	0.15	0.01	0.27
1-methylnicotinamide	00699	Pyridinecarboxylic acids and derivatives	-0.15	0.01	0.27
Asparagine	00168	Amino acids, peptides, and analogues	-0.15	0.01	0.27
Leucine	00687	Amino acids, peptides, and analogues	-0.14	0.02	0.35
5-aminolevulinic acid	01149	Amino acids, peptides, and analogues	0.14	0.02	0.36
Inosine	00195	Not available	0.14	0.02	0.39
Histidine	00177	Amino acids, peptides, and analogues	-0.13	0.03	0.39
C34:3 PE plasmalogen	11343	Glycerophosphoethanolamines	-0.13	0.03	0.39
Symmetric dimethylarginine (SDMA)	03334	Amino acids, peptides, and analogues	0.13	0.03	0.39
C24:1 Ceramide (d18:1)	04953	Ceramides	0.13	0.03	0.39
C36:4 PE	08937	Glycerophosphoethanolamines	0.13	0.03	0.39
Urate	00289	Purines and purine derivatives	0.13	0.03	0.39
C18:2 CE	00610	Steroid esters	-0.13	0.03	0.39
Trimethylamine-N-oxide	00925	Aminoxides	0.13	0.03	0.39
2-hydroxyglutarate	00694	Fatty acids and conjugates	0.13	0.03	0.39
C24:0 SM	11697	Phosphosphingolipids	-0.13	0.03	0.39
Fumarate	00134	Dicarboxylic acids and derivatives	0.13	0.03	0.39
C22:0 SM	12103	Phosphosphingolipids	-0.13	0.03	0.39
C20:5 LPC	10397	Glycerophosphocholines	-0.12	0.04	0.39
Salicylurate	00840	Benzoic acids and derivatives	0.12	0.04	0.41
Homogentisate	00130	Phenylacetic acids	0.12	0.04	0.41
Glycodeoxycholate	00631	Bile acids, alcohols and derivatives	-0.12	0.04	0.42
Malate	00156	Beta hydroxy acids and derivatives	0.12	0.04	0.42
5-hydroxytryptophan	00472	Tryptamines and derivatives	-0.12	0.04	0.42
C54:10 TAG	----	Triradylcglycerols	-0.12	0.046	0.43
C44:13 PE plasmalogen	----	Glycerophosphoethanolamines	-0.12	0.049	0.44

HMDB= Human Metabolome Database (84)

Table 16. Correlations of components of the SAVE and 37 top metabolites among N=287 Health ABC black men, adjusting for age and study site

Log-transformed and standardized metabolites:	SAVE scores	Individual components of SAVE scores (coded by tertiles: 0=best, 1=mid, 2=worst; see Table S2)				
		Weight change	Physical activity	Gait speed	Grip strength	Energy level
Glucuronate	0.21 (p=0.0003)	-0.04 (p=0.48)	0.14 (p=0.01)	0.19 (p=0.001)	0.24 (p<0.0001)	-0.001 (p=0.98)
Tryptophan	-0.21 (p=0.0005)	0.01 (p=0.80)	-0.19 (p=0.001)	-0.13 (p=0.02)	-0.14 (p=0.02)	-0.06 (p=0.34)
Methionine	-0.19 (p=0.001)	-0.03 (p=0.60)	-0.08 (p=0.16)	-0.11 (p=0.07)	-0.06 (p=0.32)	-0.18 (p=0.003)
N-carbamoyl-beta-alanine	0.17 (p=0.004)	-0.0009 (p=0.99)	0.11 (p=0.07)	0.12 (p=0.05)	0.10 (p=0.08)	0.10 (p=0.11)
Tyrosine	-0.17 (p=0.004)	-0.05 (p=0.40)	-0.13 (p=0.02)	-0.06 (p=0.35)	0.01 (p=0.89)	-0.18 (p=0.003)
Isocitrate	0.17 (p=0.004)	-0.05 (p=0.36)	0.14 (p=0.02)	0.14 (p=0.02)	0.13 (p=0.03)	0.07 (p=0.26)
Creatinine	0.16 (p=0.008)	0.06 (p=0.28)	0.13 (p=0.03)	0.10 (p=0.10)	0.07 (p=0.23)	0.02 (p=0.76)
C4-OH carnitine	0.16 (p=0.009)	0.03 (p=0.60)	0.17 (p=0.004)	0.10 (p=0.08)	-0.01 (p=0.84)	0.08 (p=0.18)
C14:0 SM	-0.15 (p=0.009)	-0.15 (p=0.01)	0.01 (p=0.91)	-0.13 (p=0.03)	-0.07 (p=0.25)	-0.04 (p=0.49)
Cystathionine	0.15 (p=0.009)	0.09 (p=0.12)	0.08 (p=0.17)	0.07 (p=0.26)	0.16 (p=0.007)	-0.02 (p=0.76)
Hydroxyphenylacetate	0.15 (p=0.01)	0.04 (p=0.51)	0.11 (p=0.06)	0.12 (p=0.04)	0.08 (p=0.19)	0.02 (p=0.73)
Putrescine	0.15 (p=0.01)	0.01 (p=0.86)	0.12 (p=0.04)	0.09 (p=0.14)	0.10 (p=0.09)	0.04 (p=0.46)
1-methylnicotinamide	-0.15 (p=0.01)	-0.05 (p=0.43)	-0.05 (p=0.36)	-0.11 (p=0.07)	-0.02 (p=0.71)	-0.13 (p=0.02)
Asparagine	-0.15 (p=0.01)	0.04 (p=0.49)	-0.05 (p=0.44)	-0.17 (p=0.003)	-0.07 (p=0.21)	-0.12 (p=0.05)
Leucine	-0.14 (p=0.02)	0.02 (p=0.78)	-0.07 (p=0.22)	-0.11 (p=0.06)	-0.09 (p=0.15)	-0.10 (p=0.09)
5-aminolevulinic acid	0.14 (p=0.02)	-0.01 (p=0.86)	0.10 (p=0.09)	0.11 (p=0.08)	0.17 (p=0.004)	-0.01 (p=0.81)
Inosine	0.14 (p=0.02)	-0.07 (p=0.27)	0.13 (p=0.03)	0.14 (p=0.02)	0.11 (p=0.06)	0.03 (p=0.61)
Histidine	-0.13 (p=0.03)	0.08 (p=0.17)	-0.05 (p=0.40)	-0.12 (p=0.05)	-0.09 (p=0.14)	-0.16 (p=0.008)
C34:3 PE plasmalogen	-0.13 (p=0.03)	-0.07 (p=0.23)	-0.09 (p=0.13)	-0.11 (p=0.05)	-0.04 (p=0.49)	0 (p=0.97)
Symmetric dimethylarginine (SDMA)	0.13 (p=0.03)	0.07 (p=0.27)	0.09 (p=0.15)	0.09 (p=0.14)	0.12 (p=0.04)	-0.04 (p=0.54)
C24:1 Ceramide (d18:1)	0.13 (p=0.03)	0.05 (p=0.36)	0.17 (p=0.005)	0.05 (p=0.39)	-0.02 (p=0.74)	0.06 (p=0.34)
C36:4 PE	0.13 (p=0.03)	-0.01 (p=0.87)	0.12 (p=0.04)	0.09 (p=0.15)	0.06 (p=0.28)	0.06 (p=0.35)
Urate	0.13 (p=0.03)	0.04 (p=0.53)	0.15 (p=0.009)	0.10 (p=0.08)	0.01 (p=0.92)	0.01 (p=0.88)
C18:2 CE	-0.13 (p=0.03)	-0.04 (p=0.51)	-0.12 (p=0.05)	-0.06 (p=0.32)	-0.11 (p=0.05)	0.01 (p=0.86)
Trimethylamine-N-oxide	0.13 (p=0.03)	0.01 (p=0.87)	0.14 (p=0.01)	0.06 (p=0.31)	0.10 (p=0.10)	-0.0003 (p=0.996)
2-hydroxyglutarate	0.13 (p=0.03)	-0.05 (p=0.36)	0.04 (p=0.50)	0.19 (p=0.001)	0.13 (p=0.03)	0.01 (p=0.80)
C24:0 SM	-0.13 (p=0.03)	-0.13 (p=0.02)	-0.06 (p=0.32)	-0.04 (p=0.56)	-0.07 (p=0.22)	-0.01 (p=0.91)
Fumarate	0.13 (p=0.03)	-0.10 (p=0.08)	0.10 (p=0.09)	0.12 (p=0.04)	0.04 (p=0.53)	0.15 (p=0.01)
C22:0 SM	-0.13 (p=0.03)	-0.11 (p=0.06)	-0.04 (p=0.49)	-0.05 (p=0.37)	-0.11 (p=0.06)	0.01 (p=0.92)
C20:5 LPC	-0.12 (p=0.04)	0.03 (p=0.57)	-0.12 (p=0.04)	-0.05 (p=0.38)	-0.10 (p=0.10)	-0.07 (p=0.26)
Salicylurate	0.12 (p=0.04)	0.01 (p=0.89)	0.03 (p=0.56)	0.17 (p=0.005)	0.01 (p=0.82)	0.08 (p=0.18)
Homogentisate	0.12 (p=0.04)	-0.02 (p=0.74)	0.08 (p=0.17)	0.13 (p=0.03)	0.11 (p=0.05)	-0.004 (p=0.94)
Glycodeoxycholate/Glycochenodeoxycholate	-0.12 (p=0.04)	-0.02 (p=0.70)	-0.07 (p=0.22)	-0.09 (p=0.12)	-0.04 (p=0.48)	-0.07 (p=0.27)
Malate	0.12 (p=0.04)	-0.05 (p=0.43)	0.12 (p=0.05)	0.10 (p=0.10)	0.02 (p=0.71)	0.10 (p=0.10)
5-hydroxytryptophan	-0.12 (p=0.04)	0.04 (p=0.49)	-0.16 (p=0.006)	-0.11 (p=0.07)	-0.04 (p=0.47)	-0.02 (p=0.79)
C54:10 TAG	-0.12 (p=0.046)	-0.03 (p=0.58)	-0.11 (p=0.07)	-0.13 (p=0.03)	0.01 (p=0.93)	-0.02 (p=0.73)
C44:13 PE plasmalogen	-0.12 (p=0.049)	-0.06 (p=0.32)	-0.12 (p=0.05)	-0.03 (p=0.63)	-0.01 (p=0.90)	-0.07 (p=0.26)

Note: blue shaded boxes indicate associations with p≤0.10

Table 17. Attenuation of correlation coefficients of SAVE scores and top metabolites after further adjustments in addition to age and study site

Log-transformed and standardized Metabolites	Age- and site-adjusted correlation with SAVE	Percent attenuation[a] of the association between metabolite and SAVE scores after further adjusting for more commonly measured variables, in addition to age and study site								
		A) Adjusting for smoking status	B) Adjusting for body mass index	C) Adjusting for % fat and appendicular lean mass	D) Adjusting for daily protein intake	E) Adjusting for IL-6 and CRP	F) Adjusting for creatinine	G) Adjusting for prevalent diseases[b]	H) Adjusting for medications[c]	I) Adjusting for multiple more commonly measured variables[d]
Organic acids and derivatives:										
Methionine	-0.1889	0.17%	-2.1%	5.6%	4.5%	4.1%	-0.61%	11%	13%	16%
Tyrosine	-0.1716	0.18%	-6.9%	-8.2%	3.6%	-1.2%	9.0%	-4.5%	-14%	-5.1%
Isocitrate	0.1703	0.06%	0.21%	6.1%	7.4%	-3.4%	30%	8.5%	11%	40%
Creatinine	0.1564	-2.1%	0.29%	-6.8%	-1.4%	5.0%	74%	16%	30%	89%
Cystathionine	0.1544	0.26%	0.26%	0.36%	5.2%	1.4%	25%	-15%	1.8%	32%
Asparagine	-0.1495	0.30%	0.25%	5.9%	0.57%	4.5%	-2.5%	11%	21%	13%
Leucine	-0.1433	0.33%	-8.6%	0.95%	0.71%	-1.6%	3.7%	-29%	-41%	-34%
5-aminolevulinic acid	0.1409	-1.9%	0.36%	7.7%	0.82%	4.9%	69%	20%	32%	113%
Histidine	-0.1325	0.27%	-1.1%	7.5%	4.4%	7.5%	-3.9%	12%	-2.3%	4.3%
Symmetric dimethylarginine	0.1312	-1.6%	-1.5%	3.9%	-1.3%	3.1%	83%	5.2%	18%	86%
Fumarate	0.1268	0.33%	-0.84%	2.9%	5.3%	-8.7%	12%	-3.1%	-11%	-6.6%
C 4-OH carnitine	0.1550	0.13%	0.02%	2.1%	2.8%	-5.1%	15%	23%	21%	35%
Malate	0.1194	0.36%	-2.4%	2.3%	3.2%	-7.0%	14%	0.09%	-35%	-8.8%
N-carbamoyl-beta-alanine	0.1722	0.05%	-0.16%	2.7%	2.4%	1.5%	26%	20%	26%	39%
Lipids and lipid-like molecules:										
C34:3 PE plasmalogen	-0.1318	-0.05%	-0.69%	-4.0%	-2.4%	4.2%	-6.3%	0.86%	-35%	-15%
C36:4 PE	0.1301	0.22%	-0.26%	-10%	-1.9%	-2.4%	17%	24%	16%	43%
C44:13 PE plasmalogen	-0.1168	0.50%	0.39%	4.8%	-2.2%	8.6%	-4.4%	16%	-5.3%	23%
C20:5 LPC	-0.1249	0.23%	-0.19%	5.8%	-2.1%	11%	0.69%	23%	13%	40%
C14:0 SM	-0.1546	0.23%	-3.3%	-7.3%	-2.9%	-1.5%	-0.32%	-0.73%	-0.62%	-6.2%
C24:0 SM	-0.1269	0.42%	-1.4%	-4.9%	0.03%	4.2%	-7.3%	2.4%	0.86%	-2.5%
C22:0 SM	-0.1254	0.44%	-2.5%	-8.9%	-2.7%	-0.15%	-6.4%	2.4%	0.37%	-10%
C24:1 Ceramide (d18:1)	0.1306	0.009%	-0.74%	0.07%	-0.29%	1.2%	4.1%	1.3%	-5.4%	-6.9%
C18:2 CE	-0.1296	-0.14%	-0.91%	-0.33%	-2.2%	4.8%	-4.6%	17%	6.5%	22%
Glycodeoxycholate	-0.1209	-0.49%	0.50%	-4.9%	-6.9%	-2.3%	0.72%	7.3%	8.2%	-13%
2-hydroxyglutarate	0.1270	-0.59%	-0.03%	11%	0.15%	-0.19%	47%	15%	4.2%	49%
C54:10 TAG	-0.1183	0.47%	-3.2%	-5.1%	-0.60%	2.3%	-5.7%	-14%	-13%	-23%
Organoheterocyclic compounds:										
Tryptophan	-0.2052	0.14%	-2.3%	5.1%	-2.2%	0.66%	16%	1.8%	6.8%	15%
1-methylnicotinamide	-0.1501	0.29%	0.18%	-3.8%	-5.3%	4.7%	-12%	-3.4%	-13%	-25%
Urate	0.1299	0.04%	-0.75%	-2.8%	3.2%	-1.1%	32%	-1.4%	10%	21%
5-hydroxytryptophan	-0.1193	-2.0%	-0.27%	11%	2.2%	-0.59%	41%	0.52%	21%	31%

Table 17 Continued

Log-transformed and standardized Metabolites	Age- and site-adjusted correlation with SAVE	Percent attenuation[a] of the association between metabolite and SAVE scores after further adjusting for more commonly measured variables, in addition to age and study site								
		A) Adjusting for smoking status	B) Adjusting for body mass index	C) Adjusting for % fat and appendicular lean mass	D) Adjusting for daily protein intake	E) Adjusting for IL-6 and CRP	F) Adjusting for creatinine	G) Adjusting for prevalent diseases[b]	H) Adjusting for medications[c]	I) Adjusting for multiple more commonly measured variables[d]
Benzenoids:										
Hydroxyphenylacetate	0.1526	0.29%	0.32%	7.1%	-4.5%	3.0%	41%	32%	45%	103%
Salicylurate	0.1230	0.25%	0.10%	11%	4.9%	4.3%	36%	59%	68%	118%
Homogentisate	0.1222	-0.04%	0.32%	-8.6%	9.2%	3.2%	22%	32%	24%	45%
Organic nitrogen compounds:										
Putrescine	0.1506	-4.7%	0.03%	-1.7%	7.9%	-0.10%	19%	-2.6%	6.5%	16%
Trimethylamine-N-oxide	0.1278	0.28%	0.35%	9.5%	-11%	0.03%	47%	24%	49%	80%
Nucleosides/nucleotides/ analogues:										
Inosine	0.1368	0.24%	0.03%	1.8%	7.2%	3.6%	11%	3.3%	-11%	16%
Organic oxygen compounds:										
Glucuronate	0.2115	-0.08%	0.01%	5.2%	3.9%	0.61%	25%	15%	30%	49%

Shading: blue indicates negative attenuation (stronger association after adjustment) and red indicates positive attenuation (weaker association after adjustment)

[a]Percent attenuation=100*(r1-r2)/r1; where r1=correlation coefficient of SAVE scores and a metabolite adjusting for age and study site, r2=same correlation coefficient after further adjustments

[b]Prevalent diseases: cardiovascular diseases, diabetes, and pulmonary diseases

[c]Medications: total number of prescription medications, anti-hypertensives, and medications for diabetes

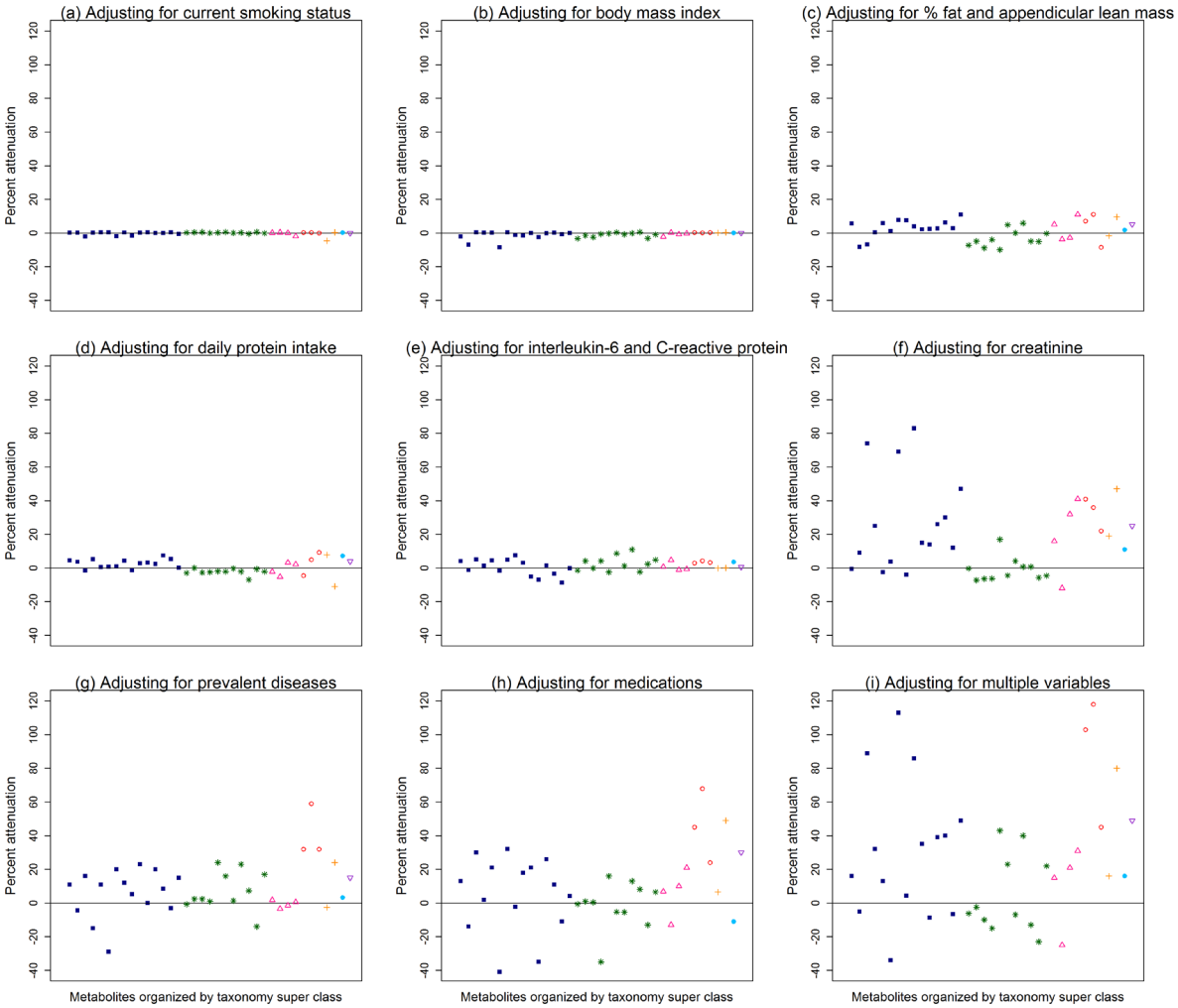
[d]More commonly measured variables: current smoking status, body mass index, appendicular lean mass, percent fat, daily protein intake, interleukin-6, C-reactive protein, creatinine, cardiovascular disease, diabetes, pulmonary diseases, and total number of prescription medications

Table 18. Top results from pathway analysis of metabolites* correlated with SAVE scores ($p<0.05$) among 287 Health ABC black men

Pathway name	Match status	Fisher's exact test p-value	False discovery rate	Impact score
Nitrogen metabolism	5/39	0.00009	0.007	0.008
Aminoacyl-tRNA biosynthesis	6/75	0.0002	0.01	0
Citric acid cycle (TCA cycle)	3/20	0.002	0.05	0.12
Tyrosine metabolism	4/76	0.01	0.27	0.15
Phenylalanine metabolism	3/45	0.02	0.28	0
Glycine, serine and threonine metabolism	3/48	0.02	0.28	0
Alanine, aspartate and glutamate metabolism	2/24	0.03	0.37	0.05
Sphingolipid metabolism	2/25	0.04	0.37	0.30
Phenylalanine, tyrosine and tryptophan biosynthesis	2/27	0.04	0.37	0.007
beta-Alanine metabolism	2/28	0.05	0.37	0.04

*35 metabolites with Human Metabolome Database identification number: glucuronate, tryptophan, methionine, N-carbamoyl-beta-alanine, tyrosine, isocitrate, creatinine, C4-OH carnitine, C14:0 SM, cystathionine, hydroxyphenylacetate, putrescine, 1-methylnicotinamide, asparagine, leucine, 5-aminolevulinic acid, inosine, histidine, C34:3 PE plasmalogen, SMDA, C24:1 ceramide (d18:1), C36:4 PE, urate, C18:2 CE, trimethylamine-N-oxide, 2-hydroxyglutarate, C24:0 SM, fumarate, C22:0 SM, C20:5 LPC, salicylurate, homogentisate, glycodeoxycholate/glycochenodeoxycholate, malate, 5-hydroxytryptophan

3.7 FIGURES



Each point is a single metabolite, organized by its taxonomy super class, according to the Human Metabolome Database: **Navy blue asterisks are organic acids and derivatives; green squares are lipids and lipid-like molecules; pink triangles are organoheterocyclic compounds; red circles are benzenoids; orange plus sign is organic nitrogen compounds; light blue circles are nucleosides, nucleotides, and analogues; and purple upside-down triangles are organic oxygen compounds.**

Percent attenuation=100*(r1-r2)/r1, where r1 is the age- and site-adjusted correlation coefficient between SAVE scores and a metabolite and r2 is the correlation coefficient after further adjusting for more commonly measured variable(s).

*Prevalent diseases: cardiovascular diseases, diabetes, and pulmonary diseases

**Medications: total number of prescription medications, anti-hypertensives, and medications for diabetes

***More commonly measured variables: current smoking status, body mass index, appendicular lean mass, percent fat, daily protein intake, interleukin-6, C-reactive protein, creatinine, cardiovascular disease, diabetes, pulmonary diseases, and total number of prescription medications

Figure 7. Percent attenuations of the correlation between metabolites and 37 SAVE scores after further adjusting for more commonly measured variables in addition to age and study site, organized by metabolite taxonomy super class

4.0 EXPLAINING THE FRAILTY-ASSOCIATED MORTALITY RISK WITH A NOVEL METABOLITE COMPOSITE SCORE

4.1 ABSTRACT

Frailty is more common with advanced age and is a risk factor for mortality. In the previous section, I identified 37 metabolites, mostly organic acids and derivatives and lipids and lipid-like molecules that potentially better characterize the body during a frail versus vigorous state. Here, I sought to develop a novel metabolite score composed of the 37 previously identified metabolites to determine whether it could explain the heightened mortality risk associated with frailty. Metabolites were measured in overnight-fasting plasma using liquid chromatography-mass spectrometry among 287 community-dwelling black men ages 70-81. Our metabolite score was a tertile-ranked sum of the 37 previously identified metabolites. Frailty to vigor was measured using the scale of aging vigor in epidemiology (SAVE). One standard deviation frailer SAVE score was associated with 30% higher mortality (p=0.0002), adjusting for age and study site. Additionally adjusting for our metabolite composite score explained 56% of the higher mortality risk associated with frailer SAVE scores and 100% of its statistical significance. The majority of the attenuation was driven by organic acids and derivatives (mostly amino acids) and lipids and lipid-like molecules (mostly glycerophospholipids and sphingolipids). In the same model, one standard deviation higher metabolite composite score was associated with 46%

higher mortality ($p<0.0001$). The metabolite composite score also significantly predicted mortality risk among an independent validation cohort; one standard deviation higher metabolite composite score was associated with 30% higher mortality among 120 older adults from the CHS All Stars study ($p=0.045$), while adjusting for age and gender. Our set of plasma metabolites may provide a deeper characterization of the body at the time of frailty assessment that explains the increased vulnerability to death. Future work is needed to assess temporality between metabolites and frailty and further investigate the underlying mechanisms leading to mortality through frailty progression, which can then be intervened on.

4.2 INTRODUCTION

As discussed in Section 1.3, the prevalence of frailty is higher with older age (54). With a growing U.S. older adult population that is projected to more than double by year 2060 (1), we will see more individuals affected by frailty than ever before. In addition to its increased likelihood with advanced age, frailty is also a risk factor for multiple major health outcomes (54), including mortality, making it a major public health concern. To guide future research and inform effective interventions targeted at reducing frailty severity and its associated mortality risk, we need to better understand the altered molecular processes occurring during a frail state that contribute to its higher mortality risk.

In Section 3.0, I examined the association between metabolites and vigor to frailty scores in a community-dwelling cohort of older black men, with the aim of better characterizing the body during a frail versus vigorous state and potentially furthering our understanding of the pathophysiology of frailty. We identified 37 metabolites associated with vigor to frailty scores.

The majority were lipids and lipid-like molecules (e.g., glycerophospholipids) and organic acids and derivatives (e.g., amino acids, peptides, and analogues; Table 19). These metabolites potentially indicate altered metabolic processes that occur as a consequence of frailty and/or altered metabolic processes that contribute to the progression of frailty severity, where in both cases the metabolites may contribute to mortality risk either directly or indirectly through frailty.

In this current report, I sought to take this information a step further by examining whether select metabolites can characterize the higher mortality risk associated with frailty. To do so, I first developed a metabolite composite score using information on the 37 metabolites associated with vigor to frailty scores from Section 3.0. My specific aims were then to: 1) confirm that frailer black men in our study had a higher mortality risk than more vigorous participants, 2) determine the extent to which the metabolite composite score predicts mortality risk and explains the frailty-associated higher mortality risk, and 3) validate the metabolite composite score against all-cause mortality in an independent cohort of 120 community-dwelling older adults from the Cardiovascular Health Study (CHS) All Stars study. I hypothesized that the metabolite composite score would explain a greater proportion of the higher mortality risk associated with frailty than any single metabolite alone.

4.3 METHODS

4.3.1 Health, Aging, and Body Composition (Health ABC) study

As described in Section 3.3.1, the Health ABC study was a prospective cohort of 3075 black and white men and women recruited from Pittsburgh, Pennsylvania and Memphis, Tennessee from

March 1997 to July 1998. The study was aimed to examine the role of weight-related health conditions and body composition in the onset of disability (142). Individuals were eligible if they were ages 70-79 during recruitment and self-reported no difficulty walking ¼ mile, climbing ten steps, or with basic activities of daily living. Exclusion criteria included a history of active cancer treatment in the past three years or planning on moving from the study area within the next three years. The institutional review boards from each site approved the study and all participants provided written informed consent.

In 2016, an ancillary study measured 350 known and numerous unknown metabolites in a randomly selected subset of 319 black men from year 2 of the Health ABC study, with the goal of determining metabolites associated with body composition (123). The ancillary study was limited in size, so it was restricted to black participants since there is a higher prevalence of obesity and obesity-related health conditions, but more muscle mass among black versus white Americans, and the study was restricted to men to limit heterogeneity in body composition differences by sex.

4.3.2 CHS All Stars study as an independent validation cohort

As described in Section 2.3.2, the CHS All Stars study was an ancillary study of 1862 men and women alive at year 18 (2005 to 2006) of the Cardiovascular Health Study (CHS) (118, 119). CHS was a prospective population-based cohort of 5888 older men and women recruited from Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania. Eligible participants were ≥65 years old during recruitment. Ineligibility included wheelchair bound, unable to participate in a clinic examination, undergoing active cancer treatment, or planning to move out of the study area

during the next three years. All CHS participants still alive and willing to participate were recruited for the CHS All Stars study. The study was designed to examine healthy aging and longevity. The CHS and the CHS All Stars study were both approved by the Human Research Protection Office at each participating university and all participants provided informed consent.

4.3.3 Metabolites

Metabolites in the Health ABC study were measured in plasma extracts collected at year 2 after an overnight fast of at least eight hours, average fasting time was 14 hours. Plasma samples had never been thawed and were stored at -80ºC from collection until metabolite measurement. Using liquid chromatography-mass spectrometry (LC-MS), metabolite profiling platforms measured: 1) amines and polar metabolites (e.g., amino acids, dipeptides), 2) central metabolites and polar metabolites (e.g., sugars, organic acids, purine and pyrimidines), and 3) lipids (e.g., triglycerides). The LC-MS peak areas were used as the metabolite values, which were analyzed using using TraceFinder (ThermoFisher Scientific, US) and Progenesis QI (Nonlinear Dynamics, UK). See Section 3.3.2 for more detail on the methods of measuring metabolites.

In this report, we only focused on the following known metabolites associated with vigor to frailty scores in the subset of 319 older black men from the Health ABC study (see Section 3.0; Table 15): glucoronate, tryptophan, methionine, N-carbamoyl-beta-alanine, tyrosine, isocitrate, creatinine, C4-OH carnitine, C14:0 SM, cystathionine, hydroxyphenylacetate, putrescine, 1-methylnicotinamide, asparagine, leucine, 5-aminolevulinic acid, inosine, histidine, C34:3 PE plasmalogen, symmetric dimethylarginine, C24:1 ceramide (d18:1), C36:4 PE, urate, C18:2 CE, trimethylamine-N-oxide, 2-hydroxyglutarate, C24:0 SM, fumarate, C22:0 SM, C20:5 LPC, salicylurate, homogentisate, glycodeoxycholate, malate, 5-hydroxytryptophan, C54:10

TAG, and C44:13 PE plasmalogen. Four of these metabolites (5-hydroxytryptophan, cystathionine, C4-OH carnitine, C54:10 TAG) were not measured in all 287 participants, but were measured in more than 80% of the cohort, so missing values were assumed to be due to the true values being below the detectable limit and half the minimum recorded value for that respective metabolite was imputed to replace missing values (123). Metabolites were log-transformed and standardized to a mean of zero and standard deviation of one.

Metabolites were similarly measured among a subset of 120 participants from the CHS All Stars study by the same company as in the Health ABC study. Information on 32 of the 37 metabolites examined in this report was available in the 120 CHS All Stars. The five metabolites that were not detected in the plasma samples of the CHS All Stars were: glucoronate, cystathionine, homogentisate, C54:10 TAG, and C44:13 PE plasmalogen. Two metabolites were not measured in all 120 participants. Inosine and glycodeoxycholate were measured in 113 (94%) and 119 (99%) participants, respectively. We assumed the missing values for these two metabolites were due to the true values being below the detectable limit and were replaced with half the minimum recorded value for the respective metabolite. The 32 metabolites were log-transformed and standardized using to the respective means and standard deviations among the 120 CHS All Stars.

4.3.4 Calculating a metabolite composite score

We constructed the metabolite composite score similar to how the Physiologic Index of Comorbidity (48), the Healthy Aging Index (108), and the Scale of Aging Vigor in Epidemiology (59, 60) were constructed. We first ranked the top 37 SAVE-associated metabolites into tertiles (Table 20). Then we gave a score to the three tertiles for each metabolite.

Individuals who scored in the best, middle, or worst tertile for a metabolite received a score of 0, 1, or 2, respectively. The "best" tertile for a metabolite was determined to be the tertile that was associated with more vigorous SAVE scores. Similarly, the "worst" tertile for a metabolite was the tertile that was associated with frailer SAVE scores (Table 20). Each participant's metabolite composite score was then calculated as the sum of the tertile scores for the 37 metabolites. The metabolite composite score ranged from zero (best) to 74 (worst). If a participant had a score of zero then that meant their values for all 37 metabolites fell into the "best" tertile, or in other words fell into the tertile that was associated with more vigorous SAVE scores. Similarly, the maximum score of 74 indicated a participant had values for all 37 metabolites that fell into the "worst" tertile, or in other words fell into the tertile that was associated with frailer SAVE scores.

For validation, the metabolite composite score was also calculated in 120 participants from the CHS All Stars study. The composite score was calculated in the same way as it was for the Health ABC black men, using the Health ABC-specific tertile cut offs and scoring for each metabolite (Table 20). The metabolite composite score was validated using a subset of 32 out of the 37 metabolites, since five of the metabolites were not measured in the CHS All Stars.

4.3.4.1 Metabolite composite score by taxonomy super class

To determine if results from our metabolite composite were based on a single subset of metabolites, we also calculated separate metabolite composite scores by their taxonomy super class according to the Human Metabolome Database (84). The taxonomy super classes of the 37 metabolites are listed in Table 19 and Table 20. Five separate metabolite composite scores were calculated for the 14 organic acids and derivatives; 12 lipids and lipid-like molecules; 4 organoheterocyclic compounds; 3 benzenoids; and the remaining five metabolites of taxonomy super classes: organic nitrogen compounds, organic oxygen compounds, and nucleosides,

nucleotides, and analogues. These five metabolite composite scores were calculated identically to how the total metabolite composite score was calculated, but with a subset of the 37 metabolites.

4.3.5 Scale of Aging Vigor in Epidemiology (SAVE)

Weight change, physical activity, grip strength, gait speed, and energy level assessed at year 2 of the Health ABC study were used to calculate the SAVE (11). Weight change was the difference in weight from year 1 to year 2. An estimate of weekly physical activity was the sum of kilocalories/kilogram/week of self-reported time spent doing major chores, walking, climbing stairs, working, volunteering, and caregiving in the past week. Grip strength was assessed twice on the right hand using a hand-held dynamometer, of which the maximum was used for analysis. Gait speed was the average over 20 meters. Participants self-reported usual energy level in the past month, ranging from 0 (no energy) to 10 (most energy ever had). Scores on each of the five items of the SAVE were ranked into tertiles using information from all Health ABC men (Table 13). Individuals who scored in the best, middle, or worst tertile for a component received a score of 0, 1, or 2, respectively. SAVE scores were the sum of tertile scores for the five items, ranging from 0 (most vigorous) to 10 (most frail). To illustrate the association between SAVE scores and mortality, we plotted the Kaplan-Meier all-cause mortality curve by SAVE tertiles. Tertiles were determined using information from all Health ABC participants and ranged from 0-3 (most vigorous), 4-5, and 6-10 (most frail). Among the 319 Health ABC black men with metabolites measured, 287 (90%) had complete information to calculate the SAVE.

4.3.5.1 Mortality

Participants in the Health ABC study were contacted every six months after the baseline visit (March 1997 to July 1998), with a 17.4-year maximum follow-up. During that time, deaths were identified by obituaries and proxy interviews. At the time of analysis, those alive were censored at their last interview date. Median follow-up after year 2 was 10.3 years for the 287 black men. Similar to all Health ABC black men, 76% (n=218) died during follow-up.

Participants in the CHS All Stars study were contacted during follow-up by telephone interviews. Deaths were identified by obituaries and proxy interviews. Among the 120 CHS All Stars, 69 (58%) died during follow-up. At the time of analysis, those alive were censored at their last interview date. Median follow-up after the All Stars visit (year 18 of CHS) was 7.4 years among the 120 participants.

4.3.6 Participant characteristics

Health ABC participants self-reported their age, race, highest level of education, and smoking habits at baseline. Body mass index was calculated from height and weight recorded at year 2. History or presence of cardiovascular disease, hypertension, diabetes, cancer, peripheral arterial disease, osteoarthritis, depression, and pulmonary disease were based on a self-report of a physician diagnosis. Participants were also classified as having cardiovascular disease, hypertension, diabetes, cancer, depression, or pulmonary disease if taking medication for those diseases and peripheral arterial disease if self-reported intermittent claudication, leg pain, or leg artery bypass or angioplasty. Participants brought all prescription medications used in the last two weeks to the second visit (year 2) for a medication inventory.

4.3.7 Statistical analysis

Mean and standard deviation or frequency and percent were used to characterize participants according to tertiles of SAVE scores. Differences were tested using Analysis of Variance or Kruskal-Wallis when normality could not be approximated for continuous measures and chi-square tests or Fisher's exact test for categorical measures. Pairwise comparisons were made when an overall difference was observed at a 0.05 significance level. Mortality rates per 100 person-years were estimated according to SAVE tertiles and Kaplan-Meier survival curves were plotted for each tertile of SAVE scores and for each tertile of the metabolite composite score. The associations between log-transformed and standardized metabolites and SAVE scores were examined using partial Pearson correlation coefficients adjusting for age and study site.

The association between SAVE scores and mortality risk was estimated using Cox proportional hazards regression models adjusting for age and study site. We determined the extent to which additionally adjusting for top SAVE-associated metabolite(s) or the metabolite composite score attenuated the association between SAVE scores and mortality risk using percent attenuation. Percent attenuation was calculated as $100*(b_1-b_2)/b_1$, where b_1 was the age- and study site-adjusted beta coefficient of the association between SAVE scores and mortality risk from a Cox proportional hazards regression model and b_2 was the same beta coefficient after further adjusting for either a single SAVE-associated metabolite, multiple SAVE-associated metabolites, or the metabolite composite score. As a sensitivity analysis, we re-ran mortality analyses using Aalen additive hazards models and found similar attenuations.

The mortality predictive power for age, the SAVE, and the metabolite composite score was examined using area under the receiver operating characteristic curve (more commonly known as AUC). The AUC estimates the ability to discriminate between participants who died

during follow-up based on information included in a logistic regression model with an indicator for morality as the outcome. Pearson correlation coefficients were used to examine the associations between age, SAVE scores, and metabolite composite scores.

4.4 RESULTS

4.4.1 Cohort characteristics

The 287 Health ABC black men were 75 (2.8) years old, on average, where those with the frailest SAVE scores were older (Table 21). SAVE scores were not associated with body mass index or baseline current smoking status. Frailer participants were more likely to self-report a physician diagnosis of cardiovascular disease, diabetes, and pulmonary disease and were taking more prescription medications, on average, than remaining participants. The 120 CHS All Stars used for validation were 85 (2.9) years old on average, 48 (40%) were men, and 12 (12%) were black.

4.4.2 SAVE scores and mortality

A Kaplan-Meier all-cause mortality curve by tertiles of SAVE scores (Figure 8) among the 287 Health ABC black men illustrated that more vigorous participants had a greater survival probability than frailer participants. Participants in the most vigorous, average, and frailest tertiles of SAVE scores had a median survival (95% confidence interval) of 13.8 (11.3, 15.3), 10.1 (7.4, 12.5), and 8.8 (7.1, 10.3) years, respectively, after year 2.

4.4.3 Metabolite composite score and mortality

A Kaplan-Meier all-cause mortality curve was also illustrated by tertiles of the metabolite composite score among the Health ABC black men (Figure 9). Participants in the best, middle, and worst tertile of the metabolite composite score had a median survival (95% confidence interval) of 12.6 (10.7, 15.0), 11.5 (9.5, 13.3), and 6.8 (5.0, 8.6), years, respectively, after year 2. One standard deviation higher metabolite composite score was associated with a 52% higher mortality risk among the Health ABC black men, while adjusting for age and study site (95% confidence interval: 1.33, 1.75; $p<0.0001$).

The metabolite composite score was also significantly associated with mortality risk among the 120 CHS All Stars; one standard deviation higher metabolite composite score was associated with a 30% higher mortality risk, adjusting for age and gender (95% confidence interval: 1.01, 1.67; $p=0.045$). Since the metabolite composite score was developed using information from only men in the Health ABC study, I examined the association between the metabolite composite score and mortality restricted to men from the CHS All Stars study and found a stronger association. One standard higher metabolite composite score was associated with a 52% higher mortality risk among the 48 men from the CHS All Stars study, while adjusting for age (95% confidence interval: 1.04, 2.22; $p=0.03$). Whereas, the metabolite composite score was not significantly associated with mortality risk among the 72 women from the CHS All Stars study (age-adjusted hazard ratio: 1.11; 95% confidence interval: 0.78, 1.58; $p=0.57$).

4.4.4 Attenuation of the higher mortality risk associated with frailer SAVE scores

One standard deviation frailer SAVE score was associated with a 30% higher mortality risk among the 287 black men, adjusting for age and study site (p=0.0002). Table 22 includes information on how the association between frailer SAVE scores and mortality risk among the Health ABC black men was attenuated after further adjusting for metabolite(s), the metabolite composite score, or more commonly measured risk factors, in addition to age and study site. Adjusting for a single SAVE-associated metabolite resulted in small attenuations in the association between frailer SAVE scores and mortality risk (all attenuations <17%). Forcing all 37 SAVE-associated metabolites into the same model attenuated the age and study site-adjusted association between frailer SAVE scores and mortality risk by only 33%. Applying a backwards stepwise selection approach (p<0.10 criterion) to the model containing all 37 SAVE-associated metabolites while forcing SAVE scores, age, and study site, left us with 10 metabolites (Model 3 in Table 22). Though the model restricted to these ten metabolites only attenuated the age and study site-adjusted association between frailer SAVE scores and mortality by 26%. When adjusting for our metabolite composite score, in addition to age and study site, the association between SAVE scores and mortality risk was attenuated by 56% and the statistical significance of the association was completely explained away (Model 4 in Table 22).

We also examined whether more commonly measured risk factors resulted in the same amount of attenuation in the association between the SAVE and mortality risk when compared to adjusting for our metabolite composite score among the Health ABC black men (Models 5 versus 4; Table 22). When additionally adjusting for education, smoking status, body mass index, and chronic conditions, in addition to age and study site, the association between SAVE scores and mortality risk was only attenuated by 12%. Adjusting for our metabolite composite

score and the more commonly measured risk factors resulted in the greatest amount of attenuation (63%) in the age- and study site-adjusted association between frailer SAVE scores and mortality risk, of which the attenuation was mostly driven by the metabolite composite score. SAVE scores and more commonly measured risk factors minimally attenuated the association between the metabolite composite score and mortality risk.

4.4.5 Mortality predictive power for age, the SAVE, and the metabolite composite score

The mortality predictive power for age, the SAVE, and the metabolite composite score were compared in Table 23 using AUC among the Health ABC black men. Age alone had an AUC of 0.65 (Model a), which was somewhat higher than the predictive power of the SAVE alone (AUC=0.61; Model b) and somewhat lower than the predictive power of the metabolite composite score alone (AUC=0.69; Model c). Interestingly, adding the metabolite composite score to a model that included age and study site resulted in a significantly higher predictive power for mortality (AUC=0.74, p=0.01; Model f versus d), whereas adding the SAVE to a model that included age and study site did not (AUC=0.69, p=0.25; Model e versus d).

4.4.6 Stratifying the metabolite composite score by taxonomy super class

I examined whether a subset of metabolites, based on taxonomy super class according to the Human Metabolome Database (84), within the total metabolite composite score appeared to be driving the attenuation between frailer SAVE scores and mortality risk among the Health ABC black men (Table 24). When adjusting for the metabolite composite score that was *only* based on the 14 organic acids and derivatives or that was only based on the 12 lipids and lipid-like

molecules, the age- and study site-adjusted association between frailer SAVE scores and mortality risk was attenuated by 35% and 24%, respectively. Other metabolite composite scores that were *only* based on a subset of metabolites according to other taxonomy super classes resulted in minimal attenuations between the SAVE scores and mortality risk. Our total metabolite composite score was highly correlated with both the subset metabolite composite score containing organic acids and derivatives (r=0.82) and the subset metabolite composite score containing lipids and lipid-like molecules (r=0.67; Table 25). When adjusting for both the subset metabolite composite score containing organic acids and derivatives and the subset metabolite composite score containing lipids and lipid-like molecules, the age- and study site-adjusted association between frailer SAVE scores and mortality risk was attenuated by 50% (Model viii, Table 24), almost accounting for the full amount of attenuation that we saw when adjusting for the total metabolite composite score (Model 4, Table 22).

4.4.7 Other methods of combining metabolites into a composite score

Last, I developed several other metabolite composite scores (listed in Table 26) among the 287 Health ABC black men, in addition to our final tertile-ranked sum score, to determine which method explained the association between SAVE scores and mortality risk the most. In Section 3.4.4, I found nitrogen metabolism, aminoacyl-transfer RNA biosynthesis, and the citric acid cycle to be the top three most significant pathways associated with SAVE scores. There were 39 known metabolites involved in the nitrogen metabolism pathway according to MetaboAnalyst (129), of which I had information on 11 in the Health ABC cohort. Using those 11 metabolites involved in nitrogen metabolism, I ran a factor analysis and got three significant factors and calculated factor scores. Next, I examined whether adjusting for the three factor scores consisting

of metabolites involved in the nitrogen metabolism pathway explained the association between frailer SAVE score and mortality risk, but found minimal attenuation (6%). I repeated this same process for the citric acid cycle and aminoacyl-transfer RNA biosynthesis pathways and similarly found that adjusting for their respective factor scores minimally attenuated the association between SAVE scores and mortality (attenuation ≤10%; Method #1 in Table 26). Next, I used the 37 top metabolites associated with SAVE scores ($p<0.05$) to calculate four different metabolite composite scores. The first composite score involved applying a factor analysis to the 37 SAVE-associated metabolites, the second involved the tertile-ranked sum of the 37 metabolites, the third involved summing the z-scores of the 37 metabolites, and the fourth involved applying LASSO regression to the 37 metabolites with SAVE scores as the outcome to get a predicted SAVE score. Adjusting for one of these 4 metabolite composite scores explained a similar amount of the association between frailer SAVE scores and mortality risk (attenuations ranging from 41% to 56%; Methods #2-5 in Table 26). Since all four methods resulted in similar amounts of attenuation, the final metabolite composite score used for this report was the tertile-ranked sum since it was the simplest and a commonly used method of combining measurements among older adults (e.g., the physiologic index of comorbidity (48), the healthy aging index (108), and the Scale of Aging Vigor in Epidemiology (59, 60) are all calculated using a tertile-ranked sum of their respective markers).

4.5 DISCUSSION

We developed a novel composite score using information from 37 metabolites associated with vigor to frailty scores among older black men. Frailer SAVE scores were significantly associated

with a higher risk of all-cause mortality, of which my metabolite composite score explained 56% of the association and all of its statistical significance. My metabolite composite score was also significantly associated with mortality independent of frailer SAVE scores, age, study site, and more commonly measured risk factors, such as education, smoking status, body mass index, and chronic conditions. The metabolite composite score also significantly predicted mortality among an independent validation cohort of community-dwelling older adults, where the association was stronger when restricted to men. This select set of plasma metabolites, especially those classified as organic acids and derivatives or lipids and lipid-like molecules, may provide a deeper characterization of the human body at the time of frailty, particularly among older men, that explains the increased vulnerability to major health outcomes, such as death.

Among the 37 metabolites included in our composite score, 70% were classified as organic acids and derivatives (e.g., amino acids) or lipids and lipid-like molecules (e.g., glycerophospholipids). Composite scores based on the 26 metabolites classified into these two taxonomy super classes explained the higher mortality risk experienced by frailer black men in our cohort most prominently. Most of the lipids and lipid-like molecules (75%, m=9) included in our composite score were inversely associated with SAVE scores, indicating lower levels associated with being more frail. A U- or J-shaped association, or in some cases even an inverse association has been observed for lipids with respect to mortality among older adults (152). For example, levels of total cholesterol and LDL cholesterol in the lowest quartile (≤175 mg/dL and ≤97.8 mg/dL, respectively) were significantly associated with a 70% and 90% higher mortality risk, respectively, when compared to levels in the highest quartile (>226 mg/dL and >144.0 mg/dL, respectively) among community-dwelling older adults, even after adjusting for several potential confounders (152). Total cholesterol and LDL cholesterol appear to decline over time

among older adults (153-155). For example, total cholesterol and LDL-cholesterol decreased by an average of 13.7 mg/dL and 18.9 mg/dL, respectively, over 15 years in a prospective longitudinal cohort of community-dwelling Finnish older adults ages ≥70 who were not taking lipid-lowering medications (153). Predictors of a decline in lipids during follow-up in the Cardiovascular Health Study were male gender, older age, and a higher white blood cell count at baseline, as well as weight loss during follow-up (156). Acquired low levels of cholesterol have been suggested as a surrogate marker for frailty (152) and are thought to be a result of chronic conditions and immune dysregulation (157). Specifically, an increase in cytokines are thought to inhibit lipoprotein production in the liver and increase lipoprotein catabolism (157).

In addition to observing an inverse association between most of the lipids and lipid-like molecules involved in our composite score and the SAVE, we also observed an inverse association between all amino acids involved in our composite score and the SAVE. Metabolic pathways involving the metabolism of lipids and amino acids are located in the mitochondrial matrix (158). Mitochondria are responsible for energy production and are especially vulnerable to oxidative stress (158). The lipid membrane of cells is vulnerable to oxidative stress, or reactive oxygen species, because of vulnerable double bonds between carbon atoms (158). In addition, one factor influencing the rate of disappearance of amino acids from plasma is amino acid oxidation. Is was previously shown that frail versus non-frail community-dwelling older adults had higher levels of oxidative stress estimated using plasma lipid peroxidation determined by malondialdehyde and protein oxidation (68). Lipid replacement therapy has been suggested as a way to improve cellular function, specifically improve mitochondrial functioning by providing membrane phospholipids and antioxidants to a body that has accumulated age-related damage to phospholipids by oxidation (159).

As discussed in Section 3.5, hypoaminoacidemia may be a result of insufficient protein intake or storage, and/or stress (147). The recommended amount of dietary protein for adults is 0.8 grams/kilogram/day. However, recently this recommendation has been thought to be too low for older adults, especially those with acute or chronic disease (149-151), to overcome aging-related anabolic resistance, where the body's ability to utilize amino acids to synthesize muscle proteins is not as efficient in older adults when compared to younger adults (150). Average (standard deviation) dietary protein intake was 0.97 (0.54) grams/kilogram/day among the Health ABC black men, with no difference by SAVE tertiles. Though, the Health ABC black men with frailer SAVE scores and lower plasma levels of certain amino acids may have needed more dietary protein to maintain nitrogen balance and homeostasis during their frailer state. More research is needed to identify processes that adapt to aging-related disease states, but by doing so have adverse effects that can lead to further dysregulation.

I validated the metabolite composite score against all-cause mortality among 120 participants from the CHS All Stars study. Those participants were about 12 years older, on average, when compared to the 287 Health ABC black men. As well as, 62 (52%) were white women, 46 (38%) were white men, 10 (8%) were black women, and 2 (2%) were black men, whereas the 287 Health ABC participants with metabolites measured were all black men. Even with these demographic differences between the two cohorts, we still found a significant association between the metabolite composite score and all-cause mortality among the 120 CHS All Stars, where the association was stronger when restricted to men. It may be that the molecular characterization of the body in the presence of frailty that causes an increased vulnerability to death differs between older men versus women.

Frailty is defined as a dynamic state at which physiologic reserves are so reduced that an older adult cannot tolerate additional challenges, placing them at an increased vulnerability to disease, disability, and death (160). Metabolomics has the potential to identify new biomarkers that can be used as therapeutic targets to improve physiologic reserve and thus, reverse the vulnerability that comes during a frail state. It is thought that dysregulated immune, endocrine, stress, and energy responses are involved in the pathophysiology of frailty (55). More research is needed to replicate the findings from this report, as well as determine the underlying mechanisms causing differences in metabolite profiles among those who are frail versus vigorous, which can then be used to inform secondary and tertiary intervention efforts to alleviate symptoms of frailty, improve quality of life, and reduce the frailty-associated mortality risk.

Similar to Section 3.0, a limitation was that metabolite values were unit-less LC-MS peak areas, which do not provide information on whether values were outside a healthy range. Another limitation was that we do not have a measure of clinically manifested frailty. Instead, we only have a measure of vigor to frailty relative to the participants in the Health ABC cohort, which was a relatively healthy cohort of older adults recruited to be non-disabled at year 1. It may be that a participant with a frail SAVE score in Health ABC may not actually appear frail when compared to a more general older adult population. There were several strengths of this report, including the well-characterized cohort with detailed mortality information available on every participant, plasma samples that were carefully collected after an overnight fast of at least eight hours, information on known metabolites among a unique sample of older black men, and validating the metabolite composite score against all-cause mortality in an independent cohort of community-dwelling older adults.

In this study, I sought to better characterize the increased vulnerability to death that occurs during a frail state at a molecular level. I developed a composite score of 37 metabolites that appeared to be a meaningful marker of the higher mortality risk associated with frailty versus vigor among older black men from the Health ABC study, and validated this metabolite composite score among older adults from the CHS All Stars study. Future work will need to determine temporality between the metabolite composite score and vigor to frailty to better inform points of intervention that can have the largest and most sustainable impact among older adults. For example, if the metabolite composite score is causing frailty progression, then interventions targeted at improving those specific metabolite levels may successfully halt frailty progression, whereas if frailty severity is instead causing the metabolite composite score then an intervention targeted at improving those specific metabolite levels may only soften the negative impact that frailty has on an individual, rather than fixing the underlying issue.

4.6 TABLES

Table 19. Taxonomy classification of 37 SAVE-associated metabolites among 287 Health ABC black men

Taxonomy super class	Taxonomy class	Taxonomy sub class
Organic acids and derivatives (m=14)	Carboxylic acids and derivatives (m=11)	Amino acids, peptides, and analogues (m=9)
		Tricarboxylic acids and derivatives (m=1)
		Dicarboxylic acids and derivatives (m=1)
	Hydroxy acids and derivatives (m=2)	Beta hydroxy acids and derivatives (m=2)
	Organic carbonic acids and derivatives (m=1)	Ureas (m=1)
Lipids and lipid-like molecules (m=12)	Glycerophospholipids (m=4)	Glycerophosphoethanolamines (m=3)
		Glycerophosphocholines (m=1)
	Sphingolipids (m=4)	Phosphosphingolipids (m=3)
		Ceramides (m=1)
	Steroids and steroid derivatives (m=2)	Bile acids, alcohols and derivatives (m=1)
		Steroid esters (m=1)
	Glycerolipids (m=1)	Triradylcglycerols (m=1)
	Fatty Acyls (m=1)	Fatty acids and conjugates (m=1)
Organoheterocyclic compounds (m=4)	Indoles and derivatives (m=2)	Indolyl carboxylic acids and derivatives (m=1)
		Tryptamines and derivatives (m=1)
	Imidazopyrimidines (m=1)	Purines and purine derivatives (m=1)
	Pyridines and derivatives (m=1)	Pyridinecarboxylic acids and derivatives (m=1)
Benzenoids (m=3)	Benzene and substituted derivatives (m=2)	Benzoic acids and derivatives (m=1)
		Phenylacetic acids (m=1)
	Phenols (m=1)	1-hydroxy-2-unsubstituted benzenoids (m=1)
Organic nitrogen compounds (m=2)	Organonitrogen compounds (m=2)	Amines (m=1)
		Aminoxides (m=1)
Organic oxygen compounds (m=1)	Organooxygen compounds (m=1)	Carbohydrates and carbohydrate conjugates (m=1)
Nucleosides, nucleotides, and analogues (m=1)	Purine nucleosides (m=1)	Not available (m=1)

Table 20. Tertile cut-offs based on standardized values from 37 metabolites associated with SAVE scores in Health ABC black men, organized by taxonomy super class according to the Human Metabolome Database

Taxonomy super class Log-transformed and standardized metabolite	SAVE Pearson correlation, adjusted for age and site	Best tertile=0	Mid tertile=1	Worst tertile=2
Organic acids and derivatives:				
Methionine	-0.19	≥ 0.37	≥ -0.37 to < 0.37	< -0.37
Tyrosine	-0.17	≥ 0.49	≥ -0.43 to < 0.49	< -0.43
Isocitrate	0.17	< -0.43	≥ -0.43 to < 0.37	≥ 0.37
N-carbamoyl-beta-alanine	0.17	< -0.452	≥ -0.452 to < 0.228	≥ 0.228
Creatinine	0.16	< -0.439	≥ -0.439 to < 0.30	≥ 0.30
C4-OH carnitine	0.16	< -0.25	≥ -0.25 to < 0.499	≥ 0.499
Cystathionine	0.15	< -0.30	≥ -0.30 to < 0.30	≥ 0.30
Asparagine	-0.15	≥ 0.37	≥ -0.45 to < 0.37	< -0.45
Leucine	-0.14	≥ 0.5339	≥ -0.348 to < 0.5339	< -0.348
5-aminolevulinic acid	0.14	< -0.442	≥ -0.442 to < 0.264	≥ 0.264
Histidine	-0.13	≥ 0.40	≥ -0.386 to < 0.40	< -0.386
Symmetric dimethylarginine (SDMA)	0.13	< -0.445	≥ -0.445 to < 0.30	≥ 0.30
Fumarate	0.13	< -0.39	≥ -0.39 to < 0.24	≥ 0.24
Malate	0.12	< -0.45	≥ -0.45 to < 0.32	≥ 0.32
Lipids and lipid-like molecules:				
C14:0 SM	-0.15	≥ 0.41	≥ -0.42 to < 0.41	< -0.42
C34:3 PE plasmalogen	-0.13	≥ 0.42	≥ -0.33 to < 0.42	< -0.33
C36:4 PE	0.13	< -0.341	≥ -0.341 to < 0.518	≥ 0.518
C24:0 SM	-0.13	≥ 0.40	≥ -0.352 to < 0.40	< -0.352
C22:0 SM	-0.13	≥ 0.47	≥ -0.366 to < 0.47	< -0.366
C24:1 Ceramide (d18:1)	0.13	< -0.354	≥ -0.354 to < 0.53	≥ 0.53
C18:2 CE	-0.13	≥ 0.48	≥ -0.323 to < 0.48	< -0.323
2-hydroxyglutarate	0.13	< -0.43	≥ -0.43 to < 0.20	≥ 0.20
C44:13 PE plasmalogen	-0.12	≥ 0.47	≥ -0.406 to < 0.47	< -0.406
C20:5 LPC	-0.12	≥ 0.457	≥ -0.394 to < 0.457	< -0.394
Glycodeoxycholate	-0.12	≥ 0.375	≥ -0.515 to < 0.375	< -0.515
C54:10 TAG	-0.12	≥ 0.305	≥ -0.27 to < 0.305	< -0.27
Organoheterocyclic compounds:				
Tryptophan	-0.21	≥ 0.52	≥ -0.29 to < 0.52	< -0.29
1-methylnicotinamide	-0.15	≥ 0.26	≥ -0.395 to < 0.26	< -0.395
Urate	0.13	< -0.36	≥ -0.36 to < 0.456	≥ 0.456
5-hydroxytryptophan	-0.12	≥ 0.54	≥ -0.18 to < 0.54	< -0.18
Benzenoids:				
Hydroxyphenylacetate	0.15	< -0.51	≥ -0.51 to < 0.37	≥ 0.37
Salicylurate	0.12	< -0.57	≥ -0.57 to < 0.25	≥ 0.25
Homogentisate	0.12	< -0.45	≥ -0.45 to < 0.388	≥ 0.388
Organic nitrogen compounds:				
Putrescine	0.15	< -0.39	≥ -0.39 to < 0.39	≥ 0.39
Trimethylamine-N-oxide	0.13	< -0.475	≥ -0.475 to < 0.224	≥ 0.224
Organic oxygen compounds:				
Glucuronate	0.21	< -0.461	≥ -0.461 to < 0.10	≥ 0.10
Nucleosides, nucleotides, and analogues:				
Inosine	0.14	< -0.24	≥ -0.24 to < 0.32	≥ 0.32

Table 21. Characteristics of 287 Health ABC black men by tertiles of SAVE scores

Mean (standard deviation) or Frequency (percent)	SAVE tertiles			Overall p-value, Pairwise comparisons
	Vigorous (T1) n=73	Average (T2) n=105	Frail (T3) n=109	
SAVE scores	2.4 (0.7) Range: 0-3	4.5 (0.5) Range: 4-5	7.0 (1.1) Range: 6-10	-----
Age	74 (3)	75 (3)	75 (3)	.006, T1<T2,T3
Pittsburgh site	34 (47%)	56 (53%)	63 (58%)	.33
More than high school education	28 (38%)	24 (23%)	28 (26%)	.06
Current smoker at baseline	9 (12%)	22 (21%)	21 (19%)	.31
Body mass index (kg/m^2)	27 (4)	27 (4)	27 (5)	.82
Prevalent disease at baseline: Cardiovascular disease	11 (15%)	36 (34%)	39 (36%)	.006, T1<T2,T3
Hypertension	34 (47%)	65 (62%)	67 (61%)	.08
Diabetes	8 (11%)	18 (17%)	37 (34%)	.0004, T1,T2<T3
Cancer	10 (14%)	11 (10%)	11 (10%)	.72
Peripheral artery disease	2 (3%)	7 (7%)	9 (8%)	.32
Osteoarthritis	2 (3%)	9 (9%)	11 (10%)	.17
Depression	4 (5%)	5 (5%)	8 (7%)	.71
Pulmonary disease	7 (10%)	8 (8%)	21 (19%)	.02, T2<T3
Total number of prescription medications	2.2 (2)	3.0 (3)	4.0 (4)	.0003, T1,T2<T3
All-cause mortality: Number (%) of deaths	46 (63%)	82 (78%)	90 (83%)	.008
Mortality rate per 100 person-years	5.3 (4.0, 7.0)	8.5 (6.8, 10.5)	9.5 (7.7, 11.7)	------

Table 22. Attenuation of mortality hazard ratio per standard deviation higher SAVE score and one standard deviation older age after further adjusting for SAVE-associated metabolites among N=287 black men

Model	Covariates*	Mortality hazard ratio (95% CI), p-value	Beta coefficient (s.e.)	Percent attenuation**
Model 0	SAVE	1.30 (1.14, 1.50), p=0.0002	0.2654 (0.07)	Reference
	Age	1.25 (1.08, 1.44), p=0.002	0.2199 (0.07)	
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	
Model 1a	SAVE	1.25 (1.08, 1.44), p=0.002	0.2230 (0.07)	16%
	Age	1.24 (1.07, 1.42), p=0.004	0.2108 (0.07)	4%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	Glucuronate	1.18 (1.05, 1.34), p=0.007		
Model 1b	SAVE	1.27 (1.10, 1.46), p=0.001	0.2383 (0.07)	10%
	Age	1.24 (1.08, 1.43), p=0.003	0.2166 (0.07)	2%
	Pittsburgh site	0.72 (0.54, 0.94), p=0.02	------	------
	Tryptophan	0.89 (0.78, 1.02), p=0.08		
Model 1c	SAVE	1.28 (1.11, 1.47), p=0.0007	0.2445 (0.07)	8%
	Age	1.25 (1.09, 1.44), p=0.002	0.2248 (0.07)	-2%
	Pittsburgh site	0.68 (0.52, 0.90), p=0.006	------	------
	Methionine	0.91 (0.80, 1.04), p=0.17		
Model 1d	SAVE	1.27 (1.10, 1.46), p=0.0008	0.2395 (0.07)	10%
	Age	1.24 (1.08, 1.43), p=0.003	0.2144 (0.07)	3%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	N-carbamoyl-beta-alanine	1.19 (1.04, 1.37), p=0.01		
Model 1e:	SAVE	1.32 (1.15, 1.53), p=0.0001	0.2806 (0.07)	-6%
	Age	1.25 (1.09, 1.44), p=0.002	0.2230 (0.07)	-1%
	Pittsburgh site	0.69 (0.52, 0.91), p=0.007	------	------
	Tyrosine	1.07 (0.94, 1.23), p=0.30		
Model 1f:	SAVE	1.25 (1.08, 1.43), p=0.002	0.2203 (0.07)	17%
	Age	1.25 (1.08, 1.44), p=0.002	0.2225 (0.07)	-1%
	Pittsburgh site	0.72 (0.55, 0.95), p=0.02	------	------
	Isocitrate	1.43 (1.24, 1.66), p<0.0001		
Model 1g:	SAVE	1.3 (1.12, 1.49), p=0.0004	0.2586 (0.07)	3%
	Age	1.24 (1.08, 1.43), p=0.002	0.2185 (0.07)	1%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.009	------	------
	Creatinine	1.04 (0.89, 1.20), p=0.65		
Model 1h:	SAVE	1.27 (1.11, 1.47), p=0.0007	0.2424 (0.07)	9%
	Age	1.25 (1.08, 1.44), p=0.002	0.2224 (0.07)	-1%
	Pittsburgh site	0.68 (0.52, 0.90), p=0.006	------	------
	C4-OH carnitine	1.20 (1.04, 1.39), p=0.02		
Model 1i:	SAVE	1.29 (1.12, 1.48), p=0.0004	0.2535 (0.07)	4%
	Age	1.24 (1.07, 1.42), p=0.003	0.2119 (0.07)	4%
	Pittsburgh site	0.72 (0.55, 0.96), p=0.02	------	------
	C14:0 SM	0.91 (0.79, 1.05), p=0.18		
Model 1j:	SAVE	1.28 (1.12, 1.48), p=0.0005	0.2486 (0.07)	6%
	Age	1.23 (1.07, 1.41), p=0.004	0.2048 (0.07)	7%
	Pittsburgh site	0.70 (0.54, 0.93), p=0.01	------	------
	Cystathionine	1.19 (1.03, 1.38), p=0.02		
Model 1k	SAVE	1.27 (1.10, 1.46), p=0.001	0.2367 (0.07)	11%
	Age	1.24 (1.08, 1.44), p=0.003	0.2186 (0.07)	1%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	Hydroxyphenylacetate	1.16 (1.01, 1.34), p=0.04		
Model 1l	SAVE	1.31 (1.14, 1.51), p=0.0001	0.2730 (0.07)	-3%
	Age	1.25 (1.08, 1.44), p=0.002	0.2202 (0.07)	0%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	Putrescine	0.94 (0.82, 1.08), p=0.37		
Model 1m	SAVE	1.30 (1.13, 1.50), p=0.0002	0.2632 (0.07)	1%
	Age	1.25 (1.08, 1.44), p=0.002	0.2206 (0.07)	0%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.009	------	
	1-methylnicotinamide	0.98 (0.85, 1.12), p=0.74		
Model 1n	SAVE	1.29 (1.12, 1.49), p=0.0004	0.2559 (0.07)	4%
	Age	1.25 (1.09, 1.44), p=0.002	0.2254 (0.07)	-2%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	------
	Asparagine	0.95 (0.82, 1.09), p=0.43		

Table 22 Continued

Model	Covariates*	Mortality hazard ratio (95% CI), p-value	Beta coefficient (s.e.)	Percent attenuation**
Model 1o	SAVE	1.28 (1.12, 1.48), p=0.0005	0.2490 (0.07)	6%
	Age	1.23 (1.07, 1.42), p=0.004	0.2074 (0.07)	6%
	Pittsburgh site	0.70 (0.53, 0.91), p=0.009	------	------
	Leucine	0.86 (0.75, 0.99), p=0.04		
Model 1p	SAVE	1.27 (1.11, 1.46), p=0.0008	0.2402 (0.07)	9%
	Age	1.22 (1.06, 1.4), p=0.007	0.1968 (0.07)	11%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.009	------	------
	5-aminolevulinic acid	1.17 (1.03, 1.33), p=0.02		
Model 1q	SAVE	1.30 (1.13, 1.49), p=0.0003	0.2599 (0.07)	2%
	Age	1.25 (1.08, 1.44), p=0.002	0.2209 (0.07)	0%
	Pittsburgh site	0.70 (0.53, 0.92), p=0.009	------	------
	Inosine	1.04 (0.91, 1.19), p=0.53		
Model 1r	SAVE	1.27 (1.11, 1.46), p=0.0007	0.2412 (0.07)	9%
	Age	1.26 (1.09, 1.45), p=0.002	0.2280 (0.07)	-4%
	Pittsburgh site	0.65 (0.50, 0.86), p=0.003	------	------
	Histidine	0.82 (0.72, 0.94), p=0.005		
Model 1s	SAVE	1.30 (1.13, 1.49), p=0.0002	0.2617 (0.07)	1%
	Age	1.25 (1.08, 1.44), p=0.002	0.2201 (0.07)	0%
	Pittsburgh site	0.68 (0.51, 0.89), p=0.005	------	------
	C34:3 PE plasmalogen	0.90 (0.78, 1.05), p=0.20		
Model 1t	SAVE	1.28 (1.11, 1.47), p=0.0007	0.2431 (0.07)	8%
	Age	1.19 (1.03, 1.38), p=0.02	0.1776 (0.07)	19%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	------
	Symmetric dimethylarginine	1.18 (1.02, 1.38), p=0.03		
Model 1u	SAVE	1.30 (1.13, 1.49), p=0.0003	0.2598 (0.07)	2%
	Age	1.25 (1.08, 1.44), p=0.002	0.2202 (0.07)	0%
	Pittsburgh site	0.69 (0.52, 0.91), p=0.008	------	------
	C24:1 Ceramide (d18:1)	1.05 (0.91, 1.20), p=0.53		
Model 1v	SAVE	1.30 (1.13, 1.49), p=0.0003	0.2599 (0.07)	2%
	Age	1.24 (1.08, 1.43), p=0.003	0.2170 (0.07)	1%
	Pittsburgh site	0.69 (0.52, 0.90), p=0.007	------	------
	C36:4 PE	1.06 (0.92, 1.24), p=0.42		
Model 1w	SAVE	1.29 (1.12, 1.49), p=0.0004	0.2569 (0.07)	3%
	Age	1.25 (1.09, 1.45), p=0.002	0.2260 (0.07)	-3%
	Pittsburgh site	0.70 (0.53, 0.92), p=0.009	------	------
	Urate	1.05 (0.92, 1.19), p=0.48		
Model 1y	SAVE	1.28 (1.11, 1.47), p=0.0006	0.2465 (0.07)	7%
	Age	1.25 (1.08, 1.44), p=0.002	0.2194 (0.07)	0%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	------
	C18:2 CE	0.88 (0.77, 1.00), p=0.06		
Model 1x	SAVE	1.29 (1.13, 1.49), p=0.0003	0.2578 (0.07)	3%
	Age	1.25 (1.08, 1.44), p=0.002	0.2199 (0.07)	0%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.009	------	------
	Trimethylamine-N-oxide	1.10 (0.96, 1.25), p=0.17		
Model 1z	SAVE	1.29 (1.12, 1.48), p=0.0005	0.2519 (0.07)	5%
	Age	1.25 (1.09, 1.44), p=0.002	0.2223 (0.07)	-1%
	Pittsburgh site	0.68 (0.51, 0.89), p=0.005	------	------
	2-hydroxyglutarate	1.27 (1.11, 1.46), p=0.0007		
Model 1aa	SAVE	1.29 (1.12, 1.48), p=0.0004	0.2547 (0.07)	4%
	Age	1.24 (1.07, 1.42), p=0.003	0.2115 (0.07)	4%
	Pittsburgh site	0.66 (0.50, 0.87), p=0.003	------	------
	C24:0 SM	0.86 (0.75, 0.99), p=0.03		
Model 1ab	SAVE	1.30 (1.13, 1.50), p=0.0002	0.2638 (0.07)	1%
	Age	1.23 (1.07, 1.41), p=0.005	0.2032 (0.07)	8%
	Pittsburgh site	0.65 (0.49, 0.86), p=0.003	------	------
	Fumarate	1.13 (1.02, 1.27), p=0.03		
Model 1ac	SAVE	1.28 (1.12, 1.48), p=0.0005	0.2491 (0.07)	6%
	Age	1.24 (1.07, 1.42), p=0.004	0.2111 (0.07)	4%
	Pittsburgh site	0.67 (0.51, 0.88), p=0.004	------	------
	C22:0 SM	0.84 (0.73, 0.97), p=0.01		
Model 1ad	SAVE	1.29 (1.12, 1.48), p=0.0004	0.2525 (0.07)	5%
	Age	1.25 (1.09, 1.44), p=0.002	0.2245 (0.07)	-2%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	------
	C20:5 LPC	0.92 (0.80, 1.06), p=0.24		

Table 22 Continued

Model	Covariates*	Mortality hazard ratio (95% CI), p-value	Beta coefficient (s.e.)	Percent attenuation**
Model 1ae	SAVE	1.29 (1.12, 1.48), p=0.0004	0.2546 (0.07)	4%
	Age	1.23 (1.07, 1.42), p=0.004	0.2099 (0.07)	5%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	Salicylurate	1.14 (0.99, 1.32), p=0.07		
Model 1af	SAVE	1.30 (1.13, 1.49), p=0.0003	0.2588 (0.07)	2%
	Age	1.25 (1.09, 1.44), p=0.002	0.2232 (0.07)	-1%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	------	------
	Homogentisate	1.05 (0.92, 1.19), p=0.47		
Model 1ag	SAVE	1.32 (1.15, 1.52), p=0.0001	0.2793 (0.07)	-5%
	Age	1.25 (1.08, 1.44), p=0.002	0.2202 (0.07)	0%
	Pittsburgh site	0.68 (0.52, 0.90), p=0.006	------	------
	Glycodeoxycholate	1.07 (0.94, 1.23), p=0.32		
Model 1ah	SAVE	1.30 (1.13, 1.49), p=0.0003	0.2589 (0.07)	2%
	Age	1.21 (1.05, 1.39), p=0.009	0.1879 (0.07)	15%
	Pittsburgh site	0.66 (0.50, 0.87), p=0.003	------	------
	Malate	1.25 (1.09, 1.44), p=0.002		
Model 1ai	SAVE	1.29 (1.12, 1.48), p=0.0005	0.2511 (0.07)	5%
	Age	1.24 (1.08, 1.43), p=0.003	0.2142 (0.07)	3%
	Pittsburgh site	0.70 (0.54, 0.92), p=0.011	------	------
	5-hydroxytryptophan	0.90 (0.79, 1.02), p=0.11		
Model 1aj	SAVE	1.30 (1.13, 1.49), p=0.0002	0.2607 (0.07)	2%
	Age	1.23 (1.07, 1.42), p=0.004	0.2099 (0.07)	5%
	Pittsburgh site	0.69 (0.52, 0.90), p=0.007	------	------
	C54:10 TAG	0.93 (0.83, 1.05), p=0.23		
Model 1ak	SAVE	1.29 (1.12, 1.49), p=0.0004	0.2555 (0.07)	4%
	Age	1.25 (1.08, 1.44), p=0.002	0.2196 (0.07)	0%
	Pittsburgh site	0.69 (0.53, 0.91), p=0.009	------	------
	C44:13 PE plasmalogen	0.95 (0.83, 1.08), p=0.45		
Model 2	SAVE	1.20 (1.01, 1.41), p=0.04	0.1782 (0.08)	33%
	Age	1.09 (0.92, 1.29), p=0.31	0.0849 (0.08)	61%
	Pittsburgh site	0.60 (0.42, 0.84), p=0.004	------	------
	Glucuronate	1.08 (0.89, 1.32), p=0.44		
	Tryptophan	1.05 (0.83, 1.33), p=0.68		
	Methionine	0.78 (0.58, 1.03), p=0.08		
	N-carbamoyl-beta-alanine	0.87 (0.72, 1.04), p=0.12		
	Tyrosine	1.31 (1.05, 1.64), p=0.02		
	Isocitrate	1.27 (1.01, 1.59), p=0.04		
	Creatinine	0.71 (0.55, 0.92), p=0.01		
	C4-OH carnitine	1.10 (0.89, 1.37), p=0.36		
	C14:0 SM	0.87 (0.71, 1.05), p=0.15		
	Cystathionine	1.18 (0.97, 1.44), p=0.10		
	Hydroxyphenylacetate	1.17 (0.93, 1.48), p=0.18		
	Putrescine	0.97 (0.83, 1.13), p=0.69		
	1-methylnicotinamide	0.98 (0.85, 1.13), p=0.77		
	Asparagine	1.29 (1.05, 1.58), p=0.02		
	Leucine	0.94 (0.78, 1.14), p=0.52		
	5-aminolevulinic acid	1.07 (0.85, 1.34), p=0.58		
	Inosine	0.98 (0.84, 1.15), p=0.83		
	Histidine	0.74 (0.59, 0.93), p=0.009		
	C34:3 PE plasmalogen	0.92 (0.75, 1.14), p=0.45		
	Symmetric dimethylarginine	1.44 (1.10, 1.87), p=0.007		
	C24:1 Ceramide (d18:1)	0.99 (0.84, 1.18), p=0.93		
	C36:4 PE	1.02 (0.85, 1.24), p=0.82		
	Urate	0.86 (0.73, 1.02), p=0.09		
	C18:2 CE	1.20 (0.94, 1.53), p=0.14		
	Trimethylamine-N-oxide	0.95 (0.80, 1.13), p=0.58		
	2-hydroxyglutarate	1.17 (0.96, 1.42), p=0.12		
	C24:0 SM	1.00 (0.70, 1.42), p=0.98		
	Fumarate	1.02 (0.83, 1.26), p=0.84		
	C22:0 SM	0.73 (0.51, 1.06), p=0.10		
	C20:5 LPC	0.87 (0.68, 1.11), p=0.25		
	Salicylurate	1.11 (0.92, 1.34), p=0.28		
	Homogentisate	1.05 (0.89, 1.24), p=0.59		
	Glycodeoxycholate	1.06 (0.91, 1.25), p=0.45		
	Malate	1.02 (0.80, 1.31), p=0.87		

Table 22 Continued

Model	Covariates*	Mortality hazard ratio (95% CI), p-value	Beta coefficient (s.e.)	Percent attenuation**
Model 2 Continued	**5-hydroxytryptophan**	0.95 (0.80, 1.14), p=0.61		
	C54:10 TAG	1.05 (0.88, 1.24), p=0.60		
	C44:13 PE plasmalogen	0.95 (0.74, 1.23), p=0.71		
Model 3	SAVE	1.22 (1.05, 1.41), p=0.01	0.1968 (0.08)	26%
	Age	1.16 (0.99, 1.35), p=0.06	0.1460 (0.08)	34%
	Pittsburgh site	0.65 (0.48, 0.88), p=0.005	------	------
	Methionine	0.80 (0.63, 1.01), p=0.06		
	Tyrosine	1.32 (1.10, 1.59), p=0.003		
	Isocitrate	1.38 (1.17, 1.62), p=0.0001		
	Creatinine	0.81 (0.66, 1.00), p=0.05		
	C14:0 SM	0.86 (0.72, 1.02), p=0.08		
	Hydroxyphenylacetate	1.21 (1.02, 1.42), p=0.03		
	Asparagine	1.20 (1.01, 1.44), p=0.04		
	Histidine	0.72 (0.59, 0.88), p=0.002		
	Symmetric dimethylarginine	1.31 (1.06, 1.62), p=0.01		
	C22:0 SM	0.85 (0.72, 1.01), p=0.06		
Model 4	SAVE	1.12 (0.96, 1.31), p=0.14	0.1164 (0.08)	56%
	Age	1.22 (1.06, 1.41), p=0.007	0.1968 (0.07)	11%
	Pittsburgh site	0.68 (0.52, 0.90), p=0.0059	------	------
	Metabolite composite score	1.46 (1.25, 1.69), p<0.0001		
Model 5	SAVE	1.26 (1.09, 1.47), p=0.002	0.2346	12%
	Age	1.19 (1.02, 1.38), p=0.02	0.1717	22%
	Pittsburgh site	0.71 (0.54, 0.94), p=0.01	------	------
	More than high school education	0.73 (0.53, 1.01), p=0.05		
	Current smoker at baseline	1.43 (1.00, 2.04), p=0.05		
	Body mass index	0.89 (0.77, 1.03), p=0.11		
	Cardiovascular disease	1.34 (0.99, 1.80), p=0.06		
	Hypertension	1.19 (0.90, 1.58), p=0.23		
	Diabetes	1.46 (1.04, 2.05), p=0.03		
Model 6	SAVE	1.10 (0.94, 1.30), p=0.23	0.0990	63%
	Age	1.17 (1.00, 1.36), p=0.05	0.1524	31%
	Pittsburgh site	0.70 (0.53, 0.92), p=0.01	------	------
	More than high school education	0.83 (0.60, 1.15), p=0.26		
	Current smoker at baseline	1.37 (0.96, 1.96), p=0.08		
	Body mass index	0.89 (0.77, 1.03), p=0.11		
	Cardiovascular disease	1.35 (1.00, 1.81), p=0.05		
	Hypertension	1.14 (0.86, 1.51), p=0.38		
	Diabetes	1.47 (1.05, 2.06), p=0.02		
	Metabolite composite score	1.41 (1.21, 1.65), p<0.0001		

CI = confidence interval

s.e. = standard error

Model 0 is the reference model, examining how SAVE associated with mortality, while adjusting for age and study site.

Models 1a – 1ak is Model 0 plus additionally adjusting for one of the 37 top SAVE-associated metabolites.

Model 2 is Model 0 plus additionally adjusting for all 37 SAVE associated metabolites in the same model.

Model 3 is Model 2 after performing backwards stepwise selection on the 37 SAVE-associated metabolites.

Model 4 is Model 0 plus additionally adjusting for the metabolite composite score, rather than individual metabolite values.

*All continuous covariates in the models were standardized prior to analysis.

**Percent attenuation was calculated as:

100*[(beta coefficient from reduced model - beta coefficient from more complex model) / beta coefficient from reduced model]

Table 23. Mortality predictive power for age, SAVE scores, and the metabolite composite index alone and together with study site using area under the receiver operating characteristic curve (AUC) among N=287 black men

Model	**Covariates***	**Model AUC (95% CI)****
Model a	Age	0.65 (0.58, 0.73)
Model b	SAVE	0.61 (0.54, 0.69)
Model c	Metabolite composite score	0.69 (0.62, 0.76)
Model d	Age	**0.67 (0.60, 0.74), reference**
	Pittsburgh site	
Model e	SAVE	0.69 (0.62, 0.76), p=0.25
	Age	
	Pittsburgh site	
Model f	Age	0.74 (0.67, 0.80), p=0.01
	Pittsburgh site	
	Metabolite composite score	
Model g	SAVE	0.74 (0.67, 0.80), p=0.02
	Age	
	Pittsburgh site	
	Metabolite composite score	

CI = confidence interval

*All continuous covariates in the models were standardized prior to analysis.

**AUC is the area under the receiver operating characteristic curve, outcome=mortality indicator.

Table 24. Attenuation of mortality hazard ratio per one standard deviation higher SAVE score and one standard deviation older age after further adjusting for subsets of our metabolite composite score based on taxonomy super class among N=287 black men

Model	Covariates*	Mortality hazard ratio (95% CI), p-value	Beta coefficient (standard error)	Percent attenuation**
Model i	SAVE	1.30 (1.14, 1.50), p=0.0002	0.2654 (0.07)	Reference
	Age	1.25 (1.08, 1.44), p=0.002	0.2199 (0.07)	
	Pittsburgh site	0.69 (0.53, 0.91), p=0.008	------	
Model ii	SAVE	1.19 (1.03, 1.38), p=0.02	0.1726 (0.07)	35%
	Age	1.21 (1.05, 1.40), p=0.009	0.191 (0.07)	13%
	Pittsburgh site	0.67 (0.51, 0.88), p=0.004	-0.3971 (0.14)	------
	Composite score of 14 organic acids and derivatives	1.35 (1.17, 1.56), p<0.0001	0.2988 (0.07)	
Model iii	SAVE	1.23 (1.06, 1.42), p=0.007	0.2026 (0.07)	24%
	Age	1.24 (1.07, 1.43), p=0.004	0.212 (0.07)	4%
	Pittsburgh site	0.68 (0.52, 0.89), p=0.005	-0.3896 (0.14)	------
	Composite score of 12 lipids and lipid-like molecules	1.25 (1.09, 1.44), p=0.002	0.2249 (0.07)	
Model iv	SAVE	1.26 (1.09, 1.46), p=0.001	0.2331 (0.07)	12%
	Age	1.26 (1.09, 1.45), p=0.002	0.2294 (0.07)	-4%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	-0.3433 (0.14)	------
	Composite score of 4 Organoheterocyclic compounds	1.16 (1.01, 1.33), p=0.03	0.1487 (0.07)	
Model v	SAVE	1.28 (1.11, 1.48), p=0.0005	0.249 (0.07)	6%
	Age	1.24 (1.08, 1.43), p=0.003	0.2166 (0.07)	2%
	Pittsburgh site	0.71 (0.54, 0.93), p=0.01	-0.3428 (0.14)	------
	Composite score of 3 Benzenoids	1.10 (0.96, 1.26), p=0.19	0.0909 (0.07)	
Model vi	SAVE	1.29 (1.12, 1.48), p=0.0005	0.2516 (0.07)	5%
	Age	1.25 (1.08, 1.43), p=0.002	0.2188 (0.07)	0.5%
	Pittsburgh site	0.69 (0.52, 0.90), p=0.007	-0.3763 (0.14)	------
	Composite score of 5 remaining metabolites* **	1.07 (0.93, 1.23), p=0.33	0.068 (0.07)	
Model vii	SAVE	1.12 (0.96, 1.31), p=0.14	0.117 (0.08)	56%
	Age	1.21 (1.05, 1.40), p=0.01	0.1898 (0.07)	14%
	Pittsburgh site	0.68 (0.52, 0.90), p=0.008	-0.3808 (0.14)	------
	Composite score of 14 organic acids and derivatives	1.28 (1.09, 1.51), p=0.003	0.2481 (0.08)	
	Composite score of 12 lipids and lipid-like molecules	1.20 (1.05, 1.38), p=0.009	0.1836 (0.07)	
	Composite score of 4 Organoheterocyclic compounds	1.04 (0.90, 1.21), p=0.61	0.0386 (0.08)	
	Composite score of 3 Benzenoids	1.08 (0.93, 1.25), p=0.32	0.0734 (0.07)	
	Composite score of 5 remaining metabolites* **	1.01 (0.87, 1.16), p=0.94	0.0056 (0.07)	
Model viii	SAVE	1.14 (0.98, 1.33), p=0.08	0.1329 (0.08)	50%
	Age	1.20 (1.04, 1.39), p=0.01	0.1852 (0.07)	16%
	Pittsburgh site	0.67 (0.51, 0.87), p=0.003	-0.4077 (0.14)	------
	Composite score of 14 organic acids and derivatives	1.31 (1.13, 1.52), p=0.0003	0.2712 (0.07)	
	Composite score of 12 lipids and lipid-like molecules	1.20 (1.05, 1.38), p=0.009	0.1828 (0.07)	

CI = confidence interval

Model i is the reference model, examining how SAVE associated with mortality, while adjusting for age and study site.

Models ii – vi is Model i plus additionally adjusting for a portion of the metabolite composite score based on taxonomy super class.

Model vii is Model i plus additionally adjusting for all portions of the metabolite composite score by taxonomy super class.

Model viii is Model vii after performing backwards stepwise selection, forcing SAVE, age, and site.

*All continuous covariates in the models were standardized prior to analysis.

**Percent attenuation was calculated as:

100*[(beta coefficient from reduced model - beta coefficient from more complex model) / beta coefficient from reduced model]

***Two organic nitrogen compounds, one organic oxygen compounds, and one nucleosides, nucleotides, and analogues.

Table 25. Pearson correlation coefficients of SAVE scores, age, and total metabolite composite score and by taxonomy super class among N=287 black men

	SAVE	Age	Metabolite composite score	Metabolite composite score by taxonomy super class:			
				Organic acids and derivatives (m=14)	Lipids and lipid-like molecules (m=12)	Organoheterocyclic compounds (m=4)	Benzenoids (m=3)
Age	0.17 (p=0.004)						
Metabolite composite score	0.42 (p<0.0001)	0.13 (p=0.03)					
Metabolite composite score by taxonomy super class: Organic acids and derivatives (m=14)	0.31 (p<0.0001)	0.15 (p=0.01)	0.82 (p<0.0001)				
Lipids and lipid-like molecules (m=12)	0.29 (p<0.0001)	0.08 (p=0.17)	0.67 (p<0.0001)	0.29 (p<0.0001)			
Organoheterocyclic compounds (m=4)	0.22 (p=0.0002)	-0.04 (p=0.48)	0.53 (p<0.0001)	0.42 (p<0.0001)	0.13 (p=0.03)		
Benzenoids (m=3)	0.18 (p=0.002)	0.07 (p=0.24)	0.36 (p<0.0001)	0.16 (p=0.008)	0.03 (p=0.63)	0.11 (p=0.07)	
Remaining metabolites* (m=5)	0.27 (p<0.0001)	0.06 (p=0.33)	0.47 (p<0.0001)	0.26 (p<0.0001)	0.10 (p=0.10)	0.15 (p=0.009)	0.37 (p<0.0001)

*Organic nitrogen compounds (m=2), organic oxygen compounds (m=1), and nucleosides, nucleotides, and analogues (m=1)

Table 26. Comparing how different methods of calculating metabolite composite scores attenuate the association between frailer SAVE scores and mortality risk among 287 Health ABC black men

Method of combining metabolites:	Scale of Aging Vigor in Epidemiology (SAVE)	
	Mortality Hazard Ratio (95% confidence interval), adjusting for metabolite scores, age, site	Percent attenuation[a]
1. Pathway scores: Nitrogen metabolism[b]	beta=0.25038 1.29 (1.11, 1.48), p=0.0006	6%
Citric acid cycle[c]	beta=0.25287 1.29 (1.12, 1.48), p=0.0004	5%
Aminoacyl-tRNA biosynthesis[d]	beta=0.23934 1.27 (1.10, 1.46), p=0.0009	10%
2. Factor analysis: 37 SAVE-associated metabolites[e]	beta=0.14738 1.16 (0.99, 1.35), p=0.06	44%
3. Tertile-ranked sum: 37 SAVE-associated metabolites[f]	beta=0.11643 1.12 (0.96, 1.31), p=0.14	**56%**
4. Sum of z-scores: 37 SAVE-associated metabolites[g]	beta=0.11703 1.12 (0.96, 1.31), p=0.14	56%
5. LASSO regression: 37 SAVE-associated metabolites[h]	beta=0.15695 1.17 (1.01, 1.36), p=0.04	41%

[a]Percent attenuation = 100*(b1-b2)/b1, where b1 is the age-and site-adjusted beta coefficient of the association between SAVE scores and mortality risk (=0.2654) and b2 is the same beta coefficient after further adjusting for a metabolite composite score.

Note: For each factor analysis, factors with eigenvalues >1 were retained. Metabolites with absolute loadings ≥0.20 were considered to load onto a factor. Factor scores were calculated as a weighted sum of the metabolites that loaded onto the factor, where weights were the standardized scoring coefficients from the rotated factor pattern.

[b]Among the 39 metabolites involved in nitrogen metabolism, 11 were measured in the Health ABC study. Applying a factor analysis to those 11 metabolites resulted in three factors. Factor 1 consisted of tryptophan, phenylalanine, and tyrosine; factor 2 consisted of tyrosine, taurine, histidine, glutamine, and asparagine; and factor 3 consisted of glutamine, tyrosine, AMP, taurine.
[c]Among the 20 metabolites involved in the citric acid cycle, 9 were measured in the Health ABC study. Applying a principal factor analysis to those 9 metabolites resulted in one factor (aconitate and malate).
[d]Among the 75 metabolites involved in the aminoacyl-transfer RNA biosynthesis pathway, 18 were measured in the Health ABC study. Applying a principal factor analysis to those 18 metabolites resulted in two factors. Factor 1 consisted of leucine, valine, methionine, and tyrosine; and factor 2 consisted of leucine, valine, methionine, glutamine, asparagine, serine, histidine, arginine, lysine, and threonine.

[e]ApplyingApplying a factor analysis to the 37 metabolites that were associated with SAVE scores ($p<0.05$) resulted in six factors. Factor 1 consisted of symmetric dimethylarginine, creatinine, glucoronate, 5-aminolevulinic acid, hydroxyphenylacetate, and isocitrate; factor 2 consisted of C22:0 SM, C24:0 SM, and C18:2 CE; factor 3 consisted of methionine, tryptophan, and tyrosine; factor 4 consisted of hydroxyphenylacetate, isocitrate, malate, and fumarate; factor 5 consisted of hydroxyphenylacetate, C22:0 SM, methionine, tyrosine, C44:13 PE plasmalogen, C20:5 LPC, and C34:3 PE plasmalogen; and factor 6 consisted of symmetric dimethylarginine, creatinine, glucoronate, hydroxyphenylacetate, C18:2 CE, tyrosine, C44:13 PE plasmalogen, histidine, C36:4 PE, C14:0 SM, salicylurate, homogentisate, and inosine.

[f]Details on how the tertile-ranked sum was calculated is in Section 4.3.4.1.

[g]The z-scores for the 17 metabolites that were negatively associated with SAVE scores were multiplied by -1, then the z-scores for the 37 SAVE-associated metabolites were summed.

[g]Lasso regression kept 11 of the 37 metabolites: glucoronate, tryptophan, methionine, isocitrate, creatinine, C4:OH carnitine, C14:0 SM, hydroxyphenylacetate, putrescine, 1-methylnicotinamide, histidine.

4.7 FIGURES

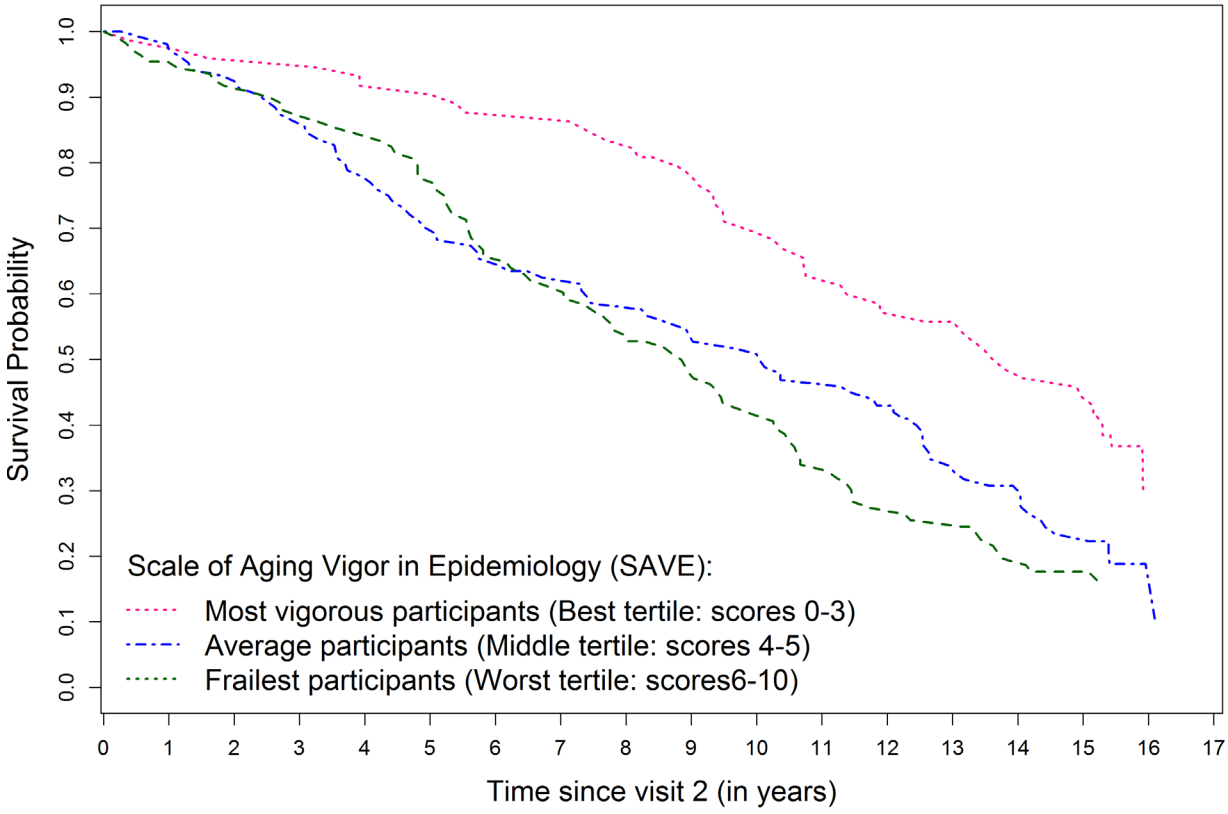


Figure 8. Kaplan-Meier all-cause mortality curve by tertiles of the Scale of Aging Vigor in Epidemiology (SAVE) among 287 Health ABC black men

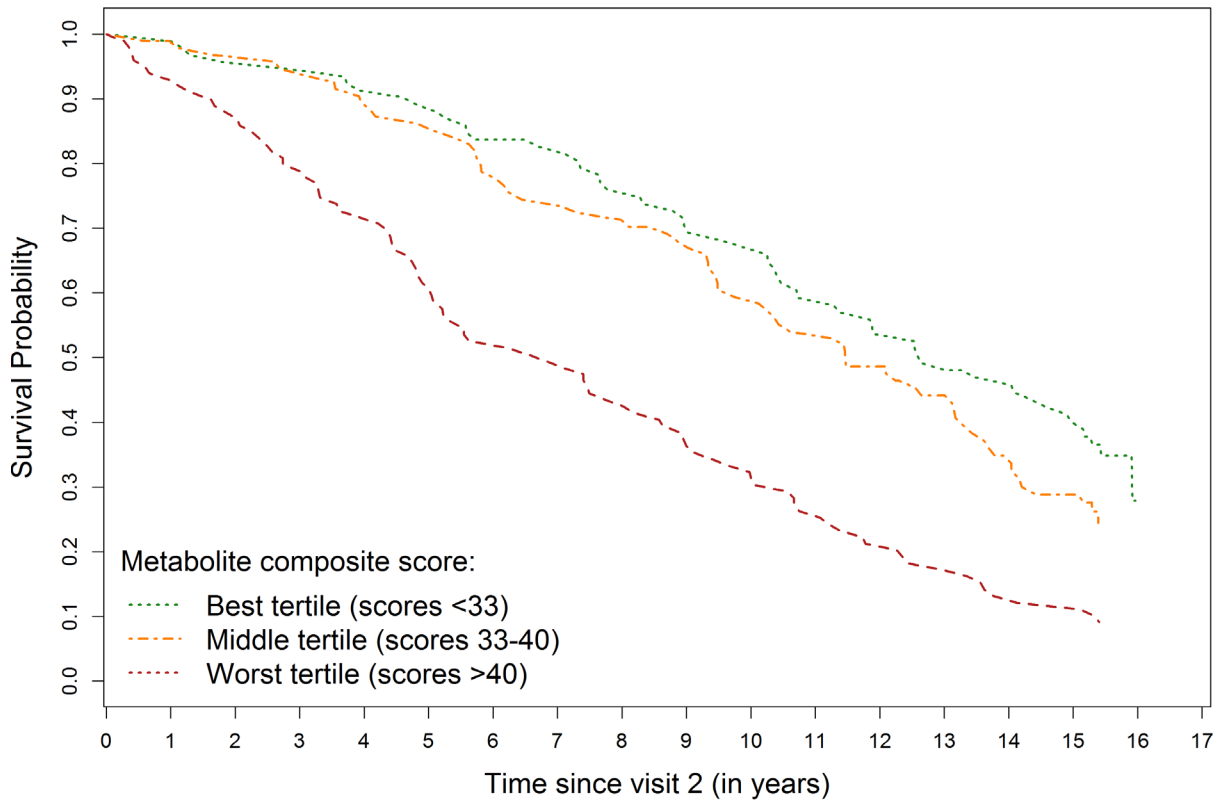


Figure 9. Kaplan-Meier all-cause mortality curve by tertiles of the metabolite composite score among 287 Health ABC black men

5.0 OVERALL DISCUSSION

Using information from community-dwelling older adult cohorts, I identified several metabolites associated with vigor to frailty scores and walking ability extremes. A pattern of differences in metabolites including amino acids, glycerophospholipids, and spingolipids was associated with vigor to frailty in older black men from the Health ABC study. Top metabolites associated with walking ability extremes included proline, triacylglycerols, and glycerophospholipids in our nested case-control study of older adults from the CHS All Stars study. Lipids and lipid-like molecules and organic acids and derivatives were two classes of metabolites most commonly associated with these aging-related phenotypes.

Differences in multiple triacylglycerols were observed by walking ability extremes among the CHS All Stars subset. The triacylglycerols that were higher among those with better walking ability consisted mostly of polyunsaturated fatty acids, whereas the triacylglycerols that were higher among those with worse walking ability consisted mostly of saturated or monounsaturated fatty acids. Behavioral differences in diet and physical activity is an underlying modifiable risk factor that has the potential to explain these differences in lipids by walking ability. Older adults with better walking ability likely have a more optimal diet consisting of more omega-3 fatty acids and less saturated fats. Caloric restriction and physical activity has long been suggested as methods to promote healthy aging (161, 162). Thus, older adults with low

walking ability may benefit most by multi-factorial interventions consisting of nutritional education with a reduced daily calorie goal and an increased daily physical activity goal.

Frailer black men from the Health ABC study had lower levels of multiple amino acids and lipids than more vigorous participants, potentially indicating frailer individuals may benefit most from a nutritional intervention focused on improving the amount of amino acids and healthy lipids in their bodies. A specialized physical activity intervention for frail individuals would also likely have a benefit, since physical activity has shown to improve the body's ability to utilize amino acids and other nutrients (163, 164). Lipid-replacement therapy has also been suggested as a way to improve mitochondrial functioning, and thus, increase energy levels, by providing membrane phospholipids and antioxidants to overcome age-related damage caused by oxidation (159). Another potential intervention for frail older adults is to evaluate their current medications and determine whether all medications still have more benefit than risk given their health status.

Frailty may be viewed as further along in the pathogenesis of aging-related morbidity when compared to a slowed walking ability. However, it should be noted that the Health ABC black men were younger and had a faster gait speed, on average, than the CHS All Stars. Using the walking ability extreme definitions that were applied to the CHS All Stars report, only 4 (1%) Health ABC black men would be classified as having low walking ability (gait speed <0.7 meters/second and 0-1 score on the walking ability index), whereas 171 (60%) would be classified as having high walking ability (gait speed ≥0.9 meters/second and 7-9 score on the walking ability index). There were only three metabolites that were significantly associated with vigor to frailty in the Health ABC black men *and* associated with walking ability extremes in the CHS All Stars: hydroxyphenylacetate, 1-methylnicotinamide, and symmetric dimethylarginine.

Hydroxyphenylacetate and symmetric dimethylarginine were also associated with the healthy aging index (107), and thus, may be important markers of healthy aging. The differences in demographics and health status between the Health ABC black men and the CHS All Stars likely explain why there was not more overlap in individual metabolites associated with the two aging-related phenotypes.

There is a need to validate the associations observed in this dissertation between metabolites and vigor to frailty and metabolites and walking ability to determine the reproducibility and generalizability of these findings. The underlying mechanisms contributing to these differences in profiles of metabolites that potentially cause unhealthy versus healthy aging need to be determined to inform interventions to promote healthy aging and increase independence in the older adult population. Examining additional –omics methods may be one way to further investigate underlying mechanisms by identifying patterns in dysregulation at different molecular levels that may contribute to these alterations in metabolites. Unfortunately, with a cross-sectional study design, we cannot infer causal relationships between metabolites and vigor to frailty or walking ability. However, results obtained from this dissertation can inform longitudinal studies aimed at determining temporality.

To offset the financial and societal challenges of a growing older adult population, we need to positively shift their distribution of health, so that a greater proportion of older adults are living free of morbidity with preserved independence and high quality of life. Lipids and lipid-like molecules and organic acids and derivatives (e.g., amino acids) were two classes of metabolites commonly associated with the aging-related phenotypes, frailty and walking ability. Differences in these classes of metabolites may indicate metabolic processes that become altered as a result of aging. The public health significance of this dissertation is that knowledge on

differences in these metabolites and metabolic pathways associated with vigor to frailty and walking ability has the potential to better characterize these complex aging-related phenotypes and can inform points in their pathophysiology that can be intervened on to reduce their progression and ultimately promote healthy aging with preserved independence in the population.

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