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Metabolites related to renal function, immune activation, and carbamylation are associated with muscle composition in older adults

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ABSTRACT

Reduced skeletal muscle density in older adults is associated with insulin resistance, decreased physical function, and an increased all-cause mortality risk. To elucidate mechanisms that may underlie the maintenance of skeletal muscle density, we conducted a secondary analysis of previously published muscle composition and serum metabolomic data in 73 older adults (average age, 78 y). Multivariable-adjusted linear regression was used to examine associations between 321 metabolites with muscle composition, defined as the ratio between normal density (NDM) with low density (LDM) thigh muscle cross sectional area (NDM/LDM). Sixty metabolites were significantly ($p \leq 0.05$ and $q < 0.30$) associated with NDM/LDM. Decreased renal function and the immune response have been previously linked with reduced muscle density, but the mechanisms underlying these connections are less clear. Metabolites that were significantly associated with muscle composition were then tested for their association with circulating markers of renal function (blood urea nitrogen, creatinine, uric acid), and with the immune response (neutrophils/lymphocytes) and activation (kynurenine/tryptophan). 43 significant NDM/LDM metabolites (including urea) were co-associated with at least 1 marker of renal function; 23 of these metabolites have been previously identified as uremic solutes. The neutrophil/lymphocyte ratio was significantly associated with NDM/LDM ($\beta \pm SE: -0.3 \pm 0.1$, $p = 0.01$, $q = 0.04$). 35 significant NDM/LDM metabolites were co-associated with immune activation. Carbamylation (defined as homocitrulline/lysine) was identified as a pathway that may link renal function and immune activation with muscle composition, as 29 significant NDM/LDM metabolites were co-associated with homocitrulline/lysine, with at least 2 markers of renal function, and with kynurenine/tryptophan. When considering that elevated urea and uremic metabolites have been linked with an increased systemic microbial burden, that antimicrobial defense can be reduced in the presence of carbamylation, and that adipocytes can promote host defense, we propose the novel hypothesis that the age-related increase in adipogenesis within muscle may be a compensatory antimicrobial response to protect against an elevated microbial burden.

1. Introduction

Aged muscle is characterized by an increase in fat content and a decrease in skeletal muscle density (Goodpaster et al., 2001), a phenotype that is known as myosteatosis (Borkan et al., 1983). Decreased skeletal muscle density is associated with insulin resistance (Goodpaster et al., 1997), reduced mobility and physical function (Goodpaster et al., 2001; Visser et al., 2005), and with an elevated risk for all-cause mortality in older adults (Miljkovic et al., 2015). Because older adults (70 + years) are the fastest growing subpopulation in the world (Affairs and Division, 2009), the development of an improved understanding about mechanisms related to muscle composition will be important for addressing the public health priority of healthy aging.

Decreased renal function and the immune response have been previously linked with reduced skeletal muscle density. First, adult hemodialysis patients have an increased non-contractile cross-sectional area of the ankle dorsiflexors, when compared with age-matched controls (Johansen et al., 2003). More specifically, proliferation and differentiation of satellite cells are impaired, but fibro/adipogenic progenitor (FAP) cells proliferate in mice that have chronic renal disease (CKD), an effect that increases fibrotic tissue and adipocyte gene expression in muscle (Zhang et al., 2010; Dong et al., 2016). In addition, intramuscular fat is derived from FAPs (Joe et al., 2010; Uezumi et al., 2010).

Second, an elevated neutrophil/lymphocyte ratio, as a marker of the immune response (Zahorec, 2001), has been associated with

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myosteatosis in patients with colon cancer (Malietzis et al., 2016). It is important to note that an elevated neutrophil/lymphocyte ratio may also be reflective of an increased circulating and/or systemic microbial burden. For example, neutrophils increase in conjunction with decreased lymphocytes in response to lipopolysaccharide (LPS) (Passler et al., 2013), a component of the outer wall of gram-negative bacteria (Rietschel et al., 1994), and in response to infection with gram-positive bacteria (Dolma et al., 2014) or virus (Holub et al., 2012). Similarly, serum levels of LPS-binding protein (LBP) are elevated in association with an increased neutrophil/lymphocyte ratio (Lemesch et al., 2016).

While decreased renal function and the immune response have been previously linked with muscle composition, the mechanisms that connect these pathways in older adults are less clear. One approach that can be used to elucidate mechanisms between muscle composition with renal function or the immune response is mass spectrometry-based metabolomics. An untargeted metabolomic approach aims to characterize and quantify all of the metabolites in a biological sample, thereby providing an analytical description of complex metabolic processes (Fiehn, 2002). With use of this approach, we have identified potential pathways that may underlie the maintenance of body composition, physical function, and inflammation in older adults (Lustgarten et al., 2013, 2014a, 2014b; Lustgarten and Fielding, 2016).

Accordingly, the goal of the present study was to develop an improved understanding about mechanisms that may underlie muscle composition in older adults. To achieve this objective, we conducted a secondary analysis on the muscle composition and serum metabolomic data reported by Chale et al. (2013) and Lustgarten et al. (2013), respectively. Initially, we examined associations between serum metabolites with the ratio between normal density (NDM) with low density (LDM) thigh muscle cross sectional area (NDM/LDM). To investigate potential links between muscle composition with renal function, with the immune response and activation, and with microbial burden, we then examined associations between significant NDM/LDM metabolites with circulating markers of renal function (blood urea nitrogen, creatinine, uric acid, α-klotho), with the immune response (neutrophils/lymphocytes) and activation (kynurenine/tryptophan), and with microbial burden (LPS, LBP).

2. Materials and methods

2.1. Study design and participants

To identify serum metabolites significantly associated with muscle composition, a secondary analysis on the baseline muscle composition data of Chale et al. (2013) in conjunction with the metabolomic data obtained from the baseline serum samples of Chale et al. (2013), as reported by Lustgarten et al. (2013), was performed. Data for 73 community dwelling, overweight, older adults (average BMI, age: 27.0 kg/m^2, 77.7 y), including 43 women and 30 men, was used. The study was approved by the Tufts University Health Sciences Campus Institutional Review Board.

Inclusion and exclusion criteria were previously reported by Chale et al. (2013). Briefly, all participants were required to be sedentary, defined as the absence of structured exercise during the previous 6 months. Moreover, relevant exclusion criteria were the presence of type I or II diabetes mellitus, and an eGFR $<$ 30 mL/min/1.73 m^2. The median eGFR, calculated with use of the MDRD equation, was 73.1 mL/min/1.73 m^2 (interquartile range: 60.8, 92), a value that is within the range reported for the 2965 older adults ($>$ 70 y) of NHANES III (Coresh et al., 2003).

2.2. Measurement of LDM, NDM, and whole body fat mass

Values for LDM and NDM, as reported by Chale et al. (2013), were obtained with use of computed tomography (CT) imaging (Siemens Somotom Scanner, Erlangen, Germany) of the non-dominant thigh at the midpoint of the femur. CT scans were analyzed by a technician in a blinded manner with use of SliceOmatic v4.2 software (Montreal, Canada). The mean value of all pixels within the range of 0–34 and 35–100 Hounsfield units (HU) was used to quantify the amount of LDM and NDM, respectively. To account for the quantity of normal density muscle relative to the amount of low density muscle, NDM was divided by LDM (NDM/LDM), as an index of muscle composition. A high NDM/LDM is indicative of good muscle composition, whereas a low NDM/LDM is indicative of poor muscle composition. NDM/LDM was strongly correlated with the mean attenuation value (43.3 ± 4.8) for all pixels within the 0–100 HU range (r = 0.9, p = 2.4E − 25).

Values for whole body fat mass, as reported by Chale et al. (2013), were obtained with use of dual-energy X-ray absorptiometry (DXA; Hologic Inc., Bedford, MA). DXA scan acquisition and analysis was performed according to manufacturer guidelines, with three passes over the subject to acquire the full DXA image. Scans were analyzed using Hologic QDR software version 12.3 in array mode.

2.3. Metabolomic analysis

Baseline serum samples obtained from the fasted subjects of Chale et al. (2013) were sent to Metabolon Inc. (Research Triangle Park, NC) for metabolomic data acquisition, as reported by Lustgarten et al. (2013). Briefly, small molecule metabolites were extracted from serum and the reconstituted extracts were resolved using mass spectrometry platforms, including ultrahigh performance liquid chromatography/tandem mass spectrometry and gas chromatography/mass spectrometry, with details of this platform described by Evans et al. (2009).

2.4. Measurement of blood urea nitrogen (BUN), creatinine, uric acid, LPS, LBP, α-klotho, neutrophils and lymphocytes

Baseline serum samples obtained from the fasted subjects of Chale et al. (2013) were used for measurement of BUN, creatinine, uric acid, LPS, LBP, and α-klotho. BUN, creatinine, and uric acid were measured with use of a clinical chemistry automated analyzer (Olympus AU400, Olympus America Inc., Melville, NY), using reagents, calibrators, and standard operating procedures as specified by the manufacturer. LPS was measured using the endpoint chromogenic LAL assay (Lonza, Switzerland). LBP and α-klotho were measured using ELISA kits (human LBP multispecies reactive ELISA kit, Cell Sciences, MA, USA; human soluble α-klotho assay kit, IBL International, Germany).

Blood levels of neutrophils and lymphocytes were quantified in baseline samples obtained from the fasted subjects of Chale et al. (2013) with use of impedance with hydrofocus cytometry (ABX Pentra 60 C +, HORIBA Medical, Irvine, CA).

2.5. Statistics

Box-Cox normality plots (Wessa, 2015) were used to determine the lambda value that results in the optimal fit against the normal distribution for NDM/LDM, BUN, creatinine, uric acid, α-klotho, homocitrulline/lysine, neutrophils/lymphocytes, LPS, LBP, kynurenine/tryptophan, and phenylalanine/tyrosine. These data were then transformed with use of the following lambda values: NDM/LDM (− 0.25), BUN (0.61), creatinine (0.1), uric acid (− 0.05), α-klotho (− 0.78), homocitrulline/lysine (0), neutrophils/lymphocytes (− 0.28), LPS (0.45), LBP (0.6), kynurenine/tryptophan (− 0.56), phenylalanine/tyrosine (− 0.24). To maintain the directionality of associations following transformations with negative lambda values, the data was multiplied by − 1.

Sex, age, and whole body fat mass were each significantly associated with the transformed value for NDM/LDM (β ± SE for sex, age, and whole body fat mass, respectively: 0.1 ± 0.0, p = 0.04; − 0.0 ± 0.0, p = 0.04; − 0.0 ± 0.0, p = 1.5E − 05). Accordingly, sex, age, and whole body fat mass-adjusted linear regression (SAS Enterprise Guide