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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 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 (β ± SE: −0.3 ± 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.

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Conflict of interest

The researchers declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2017.10.003.

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Keywords

Muscle composition; Metabolomics; Renal function; Immune activation; Carbamylation

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 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).

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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 ($r = 0.9$, $p = 2.4E-25$).

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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 4.3) was used to examine

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the association between NDM/LDM with circulating metabolites. Each model included sex, age, whole body fat mass, and individual metabolites.

To explore potential overlapping mechanisms between muscle composition with renal function, the immune response and activation, and microbial burden, metabolites that were significantly associated with NDM/LDM were then investigated for their sex, age, and whole body fat mass-adjusted association with BUN, creatinine, uric acid, α-klotho, homocitrulline/lysine, neutrophils/lymphocytes, LPS, LBP, and kynurenine/tryptophan.

False discovery rates (Benjamini and Hochberg, 1995) were computed with use of the q-value method (Storey and Tibshirani, 2003) to account for multiple comparisons. Q-values were computed based on 870 p-values, including 9 associations between NDM/LDM with BUN, creatinine, uric acid, α-klotho, homocitrulline/lysine, neutrophils/lymphocytes, LPS, LBP, and kynurenine/tryptophan, 321 associations between serum metabolites with NDM/LDM, 60 associations each for the significant NDM/LDM metabolites with BUN (with the exception of the association between serum urea with BUN), creatinine, uric acid, α-klotho, homocitrulline/lysine, neutrophils/lymphocytes, LPS, LBP, and kynurenine/tryptophan (539 total comparisons), and the association between phenylalanine/tyrosine with kynurenine/tryptophan. Statistical significance for all multivariable-adjusted associations was set at p 0.05 and $q < 0.30$, as reported by (Meyers et al., 2010). A q-value of 0.30 indicates that the result is likely to be valid 7 out of 10 times, which we suggest is reasonable in the setting of exploratory discovery.

Stepwise linear regression was used to develop a NDM/LDM predictive model. Sex, age, and whole body fat mass were forced into the stepwise model because of their significant univariate associations with NDM/LDM. The 60 significant NDM/LDM metabolites were then considered as candidate variables, and statistical significance for metabolites to enter and be retained in the model was set at $p < 0.05$.

3. Results

Subject characteristics (pre-transformation) for NDM, LDM, NDM/LDM, whole body fat mass, BUN, creatinine, uric acid, homocitrulline, lysine, homocitrulline/lysine, neutrophils, lymphocytes, neutrophils/lymphocytes, LPS, LBP, kynurenine, tryptophan, and kynurenine/tryptophan are shown in Table 1. Subject demographics, including gender, age, BMI, number of medical diagnoses, number of medications, and ethnicity are shown in Supplementary Table 1.

3.1. Metabolites significantly associated with NDM/LDM

Significant (p 0.05 and $q < 0.30$) and non-significant associations between serum metabolites with NDM/LDM are shown in Table 2 and Supplementary Table 2, respectively. Fifteen metabolites, including amino acids and their degradation products (alanine, asparagine, ergothionine, glutamine, glycine, serine, *trans*-urocanate), bile acid metabolites (7-α-hydroxy-3-oxo-4-cholestenoate, glycoursodeoxycholate), purines (7-methylguanine, xanthine), the carnitine precursor deoxycarnitine, the heme degradation product biliverdin,

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the unsaturated fatty acid 10-undecanoate, and the glucocorticoid cortisone were positively associated with NDM/LDM.

45 metabolites were negatively associated with NDM/LDM, including acylcarnitines (acetylcarnitine, butyrylcarnitine, 2-methylbutroylcarnitine, isobutyrylcarnitine, tiglyl carnitine glutaroylcarnitine, methylglutarylcarnitine, hexanoylcarnitine, octanoylcarnitine, decanoylcarnitine, *cis*-4-decenoyl carnitine, laurylcarnitine), sugar (1,5 anhydroglucitol), sugar alcohols (arabitol, erythritol, mannitol, myo-inositol, threitol) and acids (arabonate, erythronate, glucuronate), *N*-acetylated amino acids (4-acetamidobutanoate, *N*-acetylalanine, N_6-acetyllysine, *N*-acetylmethionine, *N*-acetylserine, *N*-acetylthreonine), steroids (5α-androstan-3β, 17β-diol disulfate, androsterone sulfate, epiandrosterone sulfate), methylated amino acids and purines (dimethylglycine, symmetric dimethylarginine, N_1-methyladenosine, 1-methylurate), glycosylated pyrimidine and amino acids (pseudouridine, *C*-glycosyltrytophan), nitrogen excretion (urea, phenylacetylgluta-mine), hydroxylated and dicarboxylic fatty acids (2-hydroxyisobutyrate, tetradecanedioate), gut bacterial metabolism (phenol sulfate), the tryptophan degradation metabolite indolelactate, the NAD degradation product N_1-methyl-2-pyridone-5-carboxamide, the collagen-related metabolite *trans*-4-hydroxyproline, and a cleavage product of chemotactic peptide, *N*-formylmethionine.

Stepwise linear regression was used to determine a NDM/LDM predictive model. While sex, age, and whole body fat mass explained35.3% of the variability inherent in NDM/LDM, the combination of 4 metabolites (2-hydroxyisobutyrate, mannitol, 7-methylguanine, and 1, 5-anhydroglucitol) explained an additional 31.3%, for a total adjusted R^2 equal to 66.6% (Supplementary Table 3).

3.2. Metabolites significantly associated with NDM/LDM and with circulating markers of renal function

BUN, creatinine, and uric acid are circulating markers of renal function (Gowda et al., 2010) whose values are elevated when renal function is reduced. BUN was significantly negatively associated with NDM/LDM (β ± SE: −0.0 ± 0.0, p = 0.004, q = 0.02), whereas creatinine and uric acid were borderline significant (β ± SE: −0.7 ± 0.4, p = 0.06, q = 0.13; −1.7 ± 0.9, p = 0.06, q = 0.13, respectively). To explore potential overlapping pathways between muscle composition with renal function, metabolites that were significantly associated with NDM/LDM were then investigated for their association with BUN, creatinine, and uric acid. 43, 34, and 23 of the 60 significant NDM/LDM metabolites were significantly associated with 1, 2, and 3 circulating markers of renal function, respectively (Table 3). Data for the 17 significant NDM/LDM metabolites that were not significantly associated with any markers of renal function are shown in Supplementary Table 4.

Moreover, circulating levels of the secreted protein, α-klotho, are decreased in serum in association with reduced renal function (Shimamura et al., 2012). A link between α-klotho with muscle composition is suggested by the findings that muscle mass is reduced in klotho hypomorphic mice (Kuro-o et al., 1997; Phelps et al., 2016), and that α-klotho overexpression increases satellite cell numbers and their proliferation, and increases myotube growth (Wehling-Henricks et al., 2016). We then explored the association between serum α-klotho with NDM/LDM, and between significant NDM/LDM metabolites with α-

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klotho. α-Klotho was not significantly associated with NDM/LDM (β ± SE: 1.5 ± 5.7, $p = 0.79$, $q = 0.60$), nor was there an overlap between any significant NDM/LDM metabolites with α-klotho (Supplementary Table 5).

3.3. Metabolites significantly associated with NDM/LDM and with a circulating marker of carbamylation

When renal function is reduced, circulating levels of urea are elevated. Urea decomposes to form cyanate, which can combine with the amino acid lysine to form the carbamylated metabolite, homocitrulline (Kraus and Kraus, 2001). The homocitrulline/lysine ratio, as an index of carbamylation, is elevated in CKD patients, when compared with subjects that have normal renal function (Koeth et al., 2013). The importance of carbamylation is further illustrated by the finding that it increases during aging, and the accumulation rate of protein carbamylation is negatively correlated with species life expectancy (Gorisse et al., 2016).

To explore the potential link between carbamylation with muscle composition, we investigated the association between the serum homocitrulline/lysine ratio with NDM/LDM, and between the 60 significant NDM/LDM metabolites with homocitrulline/lysine. Although NDM/LDM was not significantly associated with homocitrulline/lysine (β ± SE: −0.2 ± 0.2, $p = 0.25$, $q = 0.34$), 35 significant NDM/LDM metabolites were co-associated with homocitrulline/lysine (Table 4). Significant NDM/LDM metabolites that were not associated with homocitrulline/lysine are shown in Supplementary Table 6.

3.4. Metabolites significantly associated with NDM/LDM and with the neutrophil/ lymphocyte ratio

An elevated neutrophil/lymphocyte ratio has been reported to be negatively associated with muscle composition in colorectal cancer patients (Malietzis et al., 2016). In the older adults of the present study, the neutrophil/lymphocyte ratio was significantly negatively associated with NDM/LDM (β ± SE: −0.3 ± 0.1, $p = 0.01$, $q = 0.04$). Of the 60 significant NDM/LDM metabolites, phenol sulfate (β ± SE:0.0 ± 0.0, $p = 0.03$, $q = 0.08$) and methylglutarylcarnitine (β ± SE:0.0 ± 0.0, $p = 0.04$, $q = 0.10$) were positively associated, whereas xanthine (β ± SE: −0.1 ± 0.0, $p = 0.003$, $q = 0.01$) and 7-α-hydroxy-3-oxo-4-cholestenoate (β ± SE: 0.1 ± 0.0, $p = 0.03$, $q = 0.08$) were negatively associated with the neutrophil/lymphocyte ratio. Metabolites that were significantly associated with NDM/LDM but not with the neutrophil/lymphocyte ratio are shown in Supplementary Table 7.

3.5. Metabolites significantly associated with NDM/LDM and with circulating markers of microbial burden

An elevated neutrophil/lymphocyte ratio may be reflective of an increased systemic microbial burden. For example, neutrophils increase in conjunction with decreased lymphocytes in response to LPS (Passler et al., 2013), and the neutrophil/lymphocyte ratio is elevated in association with increased levels of LPS-binding protein (LBP) (Lemesch et al., 2016). LPS decreases muscle protein synthesis and increases protein degradation (Orellana et al., 2006; Liu et al., 2013), an effect that would be expected to reduce the quantity of normal density muscle. Similarly, elevated serum LBP is associated with reduced skeletal muscle density in 60-year old adults (Tilves et al., 2016). We then tested the association

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between LPS and LBP with NDM/LDM, and with the 60 significant NDM/LDM metabolites. LPS and LBP were not significantly associated with NDM/LDM (β ± SE: –0.0 ± 0.0, p = 0.64, q = 0.55; 0.0 ± 0.0, p = 0.24, q = 0.34, respectively). Of the 60 significant NDM/LDM metabolites, four metabolites were significantly associated with LPS, including butyrylcarnitine (β ± SE:0.2 ± 0.1, p = 0.05, q = 0.12), tiglylcarnitine (β ± SE: 0.2 ± 0.1, p = 0.04, q = 0.10), methylglutarylcarnitine (β ± SE: 0.1 ± 0.0, p = 0.04, q = 0.10), and N_6-acetyllysine (β ± SE: 0.3 ± 0.1, p = 0.04, q = 0.10), whereas only glycine was significantly associated with LBP (β ± SE: 1.2 ± 0.5, p = 0.02, q = 0.06). Associations between metabolites that were significantly associated with NDM/LDM but not with LPS or LBP are shown in Supplementary Tables 8 and 9.

3.6. Metabolites significantly associated with NDM/LDM and with a circulating marker of immune activation

To further explore the link between the immune response with muscle composition, the 60 significant NDM/LDM metabolites were then tested for their association with the serum kynurenine/tryptophan ratio. The degradation of tryptophan to form kynurenine is catalyzed by either tryptophan 2,3-dioxygenase (TDO) or indoleamine-pyrrole 2,3-dioxygenase (IDO) (Yasui et al., 1986; Saito et al., 2000). Whereas TDO is produced primarily by the liver, IDO is induced during immune-related conditions (Badawy, 2017). IDO expression is increased in response to interferon-gamma (IFN-γ) (O'Connor et al., 2009), a cytokine that is produced by immune cells, including monocyte-derived macrophages and dendritic cells (Munn et al., 1999; Hwu et al., 2000), in response to bacterial, viral, and parasitic infection (Yoshida et al., 1979, 1981; Pfefferkorn, 1984; Taylor and Feng, 1991). Kynurenine/tryptophan has been previously reported as a marker of IFN-γ (Apalset et al., 2014). To examine if the kynurenine/tryptophan ratio was related to immune activation, we investigated the association between the serum phenylalanine/tyrosine ratio with kynurenine/tryptophan. The phenylalanine/tyrosine ratio is positively correlated with kynurenine/tryptophan when the immune system is activated (Mangge et al., 2013). Phenylanine/tyrosine was significantly positively associated with kynurenine/tryptophan (β ± SE: 0.1 ± 0.0, p = 0.01, q = 0.04).

Although the association between the kynurenine/tryptophan ratio with NDM/LDM was borderline significant (β ± SE: –0.1 ± 0.1, p = 0.06, q = 0.13), 35 significant NDM/LDM metabolites were co-associated with kynurenine/tryptophan (Table 5). Metabolites that were significantly associated with NDM/LDM but not with kynurenine/tryptophan are shown in Supplementary Table 10.

3.7. Co-overlapping associations between significant NDM/LDM metabolites with circulating markers of renal function, immune activation, and carbamylation

Of the 60 significant NDM/LDM metabolites, 29 were co-associated with at least 2 circulating markers of renal function, with kynurenine/tryptophan, and with homocitrulline/lysine, including sugar alcohols and acids (arabitol, arabonate, erythritol, erythronate, glucuronate, myo-inositol, threitol), acylcarnitines (2-methylbutyrylcarnitine, butyrylcarnitine, *cis*-4-decenoylcarnitine, glutarylcarnitine, isobutyrylcarnitine, tiglylcarnitine), *N*-acetylated amino acids (4-acetamidobutanoate, *N*-acetylalanine, N_6-

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acetyllysine, *N*-acetylserine, *N*-acetylthreonine), glycosylated pyrimidine or amino acids (pseudouridine, *C*-glycosyltryptophan), methylated amino acids or purines (dimethylglycine, N_1-methyladenosine), amino acids (glutamine, serine), urea metabolism (urea, phenylacetylglutamine) and others (*N*-for-mylmethionine, 2-hydroxyisobutyrate, indolelactate).

4. Discussion

The goal of the present study was to develop an improved understanding about mechanisms that may underlie the maintenance of muscle composition in older adults. Initially, we identified significant associations for 60 serum metabolites with the ratio between normal density with low density thigh muscle cross sectional area. Further investigation identified significant, co-overlapping associations for 29 of these metabolites with circulating markers of renal function, with immune activation, and with carbamylation.

In terms of the link between renal function with muscle composition, 31 and 23 of the significant NDM/LDM metabolites (including urea) have been previously identified as early stage markers of reduced renal function (Sekula et al., 2016) and as uremic solutes, respectively (Niewczas et al., 2014; Tanaka et al., 2015). In patients with renal disease, elevated circulating levels of urea are associated with an increase in urease-producing intestinal bacteria (Wong et al., 2014), a finding that suggests a role for an altered gut microbiome on reduced muscle composition in older adults. Within the intestine, key protein components of the colonic tight junction are reduced in the presence of urea and uremic metabolites (Vaziri et al., 2012, 2013), but are greatly depleted in the combined presence of urea and urease (Vaziri et al., 2013). It is important to note that although the subjects of present study did not have renal disease, almost half (33/73) had BUN values 20 mg/dL, which is an amount of urea that significantly reduced tight junction protein levels in isolated human enterocytes (Vaziri et al., 2013). The net effect of elevated urea and uremic metabolites is an increase in gut permeability (Vaziri et al., 2012, 2013). In support of this, 10 significant NDM/LDM metabolites, including deoxycarnitine and seven acylcarnitines, phenylacetylglutamine, and 4-acetamidobutanoate have been reported to be elevated in serum in conjunction with increased gut permeability (Semba et al., 2017). Similarly, the kynurenine/tryptophan ratio, which was borderline significant in its negative association with muscle composition, is elevated in the presence of increased gut permeability (Semba et al., 2016). Collectively, these data suggest that the associations identified between urea and uremic metabolites with muscle composition may be related to an altered gut microbiome, to reduced intestinal tight junction protein levels, and to an increase in intestinal permeability. Future studies aimed at testing this hypothesis are of interest.

Beyond the intestine, bacterial translocation occurs during uremia (de Almeida Duarte et al., 2004), and uremic patients frequently exhibit endotoxemia (Goncalves et al., 2006; Szeto et al., 2008). Furthermore, patients with renal disease have an increased systemic microbial burden, including bloodstream infections with gram-negative and gram-positive bacteria (Berman et al., 2004; Shi et al., 2014), with fungi (Serefhanoglu et al., 2012; Gauna et al., 2013), and viruses (Fabrizi et al., 2000; Gallian et al., 2002). Moreover, levels of circulating microbial products increase during aging. In aged mice, gut permeability was increased in

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conjunction with an elevated plasma level of muramyl dipeptide, a component of the outer bacterial wall (Thevaranjan et al., 2017). In older adult humans, plasma levels of LPS are elevated, when compared with young subjects (Ghosh et al., 2015). Accordingly, to test the hypothesis that microbial burden was elevated in conjunction with poor muscle composition, we examined the association between LPS and LBP with NDM/LDM. LPS and LBP were not significantly associated with muscle composition, and only five significant NDM/LDM metabolites were co-associated with LPS and LBP. While this suggests that gram-negative bacterial burden and its related metabolic products or pathways may not be involved in mechanisms related to muscle composition in older adults, in contrast, a role for gram-positive bacteria, fungi, or viruses is suggested via significant, co-overlapping associations for five short (C4, C5) and five medium chain (C6–C10) acylcarnitines, pseudouridine, and *C*-glycosyltryptophan. In support of this, plasma levels of short and medium chain acylcarnitines are elevated in patients infected with gram-positive bacteria, or with fungi (Schmerler et al., 2012). Expression of the proteins responsible for the degradation of the cognate fatty acids for these acylcarnitines, short and medium-chain acyl-coenzyme A dehydrogenase, is decreased in the presence of viral infection (Bose et al., 2014). Moreover, pseudouridine is elevated in virally infected patients (Colonna et al., 1996), and *C*-glycosyltryptophan is found in viral glycoproteins (Falzarano et al., 2007). When considering that the incidence of bloodstream infections with gram-positive bacteria, with fungi, or viruses are increased by > 5 to15-fold in older adults, when compared with young subjects (Laupland et al., 2008; Zilberberg et al., 2008; Parry et al., 2016), these data may collectively suggest a role for circulating microbial burden on muscle composition in older adults.

If systemic microbial burden is elevated in older adults with poor muscle composition, an immune response and/or activation would be an expected result. In support of this, we identified a significant negative association between the neutrophil/lymphocyte ratio, as a marker of the immune response (Zahorec, 2001), with muscle composition. The neutrophil/lymphocyte ratio has been reported to be elevated in response to infection with gram-positive bacteria (Dolma et al., 2014) or with virus (Holub et al., 2012), and in the presence of bloodstream infections (Yang et al., 2015). Moreover, 35 significant NDM/LDM metabolites were co-associated with kynurenine/tryptophan, as a circulating marker of immune activation (Mangge et al., 2013) and of IFN-γ (Apalset et al., 2014). IFN-γ is produced as an antimicrobial response to bacterial, viral, or parasitic infection (Yoshida et al., 1979, 1981; Pfefferkorn, 1984; Taylor and Feng, 1991), evidence that suggests additional support for the hypothesis that systemic microbial burden may be involved in mechanisms related to muscle composition in older adults. Interestingly, peripheral blood mononuclear cells produce 7-fold more IFN-γ following exposure to gram-positive, when compared against gram-negative bacteria (Skovbjerg et al., 2010), a finding that may drive the search for such pathogens.

Additional support for the hypothesis that antimicrobial defense and immune activation may be related to muscle composition is suggested by co-overlapping associations between significant NDM/LDM metabolites with kynurenine/tryptophan. In terms of antimicrobial defense, eight acylcarnitines were significantly associated with both NDM/LDM and kynurenine/tryptophan-the antimicrobial activity of alkyl containing, quaternary amines (as

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found in acylcarnitines) is well established, with the proposed bactericidal mechanism involving adsorption onto the negatively charged bacterial cell surface, diffusion through the cell wall, binding to and disruption of the cytoplasmic membrane, and eventual cell death (Gilbert and Moore, 2005). Moreover, urea and erythritol have antimicrobial activity against gram-negative and gram-positive bacteria (Weinstein and Mc, 1946), dimethylglycine induces membrane damage and is bactericidal against *Escherichia coli* (Vanhauteghem et al., 2012), and arabitol exhibits antiviral activity (Xu et al., 2016). In terms of immunity, carnitines support the production of CD4+ and CD8+ T cells during infection, and increase lymphocyte proliferation and polymorphonuclear chemotaxis (De Simone et al., 1982; Jirillo et al., 1993). Glutamine is involved in T cell growth and proliferation (Wang et al., 2011). Serine directly modulates adaptive immunity by controlling T cell proliferative capacity (Ma et al., 2017). Myo-inositol improves the ability of macrophages to eliminate antibiotic-resistant *E. coli* (Chen et al., 2015). *N*-formylmethionine is a potent chemoattractant for neutrophils, which are involved in the host's resistance to infection (Schiffmann et al., 1975).

Investigating further, a role for carbamylation on muscle composition is suggested by the finding that 35 significant NDM/LDM metabolites were co-associated with homocitrulline/lysine. Carbamylation is the process by which urea decomposes to form cyanate, and cyanate combines with the amino acid lysine, thereby forming homocitrulline. The homocitrulline/lysine ratio, as an index of carbamylation (Koeth et al., 2013), is strongly correlated with urea (Pietrement et al., 2013), is elevated in CKD patients, (Koeth et al., 2013), and increases during aging (Gorisse et al., 2016). In the presence of carbamylated lysine, the production of IL-10, IFN-γ, and TGFβ are increased (Mydel et al., 2010; Wang et al., 2016). IL-10 and TGFβ are involved in mechanisms that allow FAPs to differentiate and proliferate (Lemos et al., 2015), thereby increasing muscle fibrosis and/or adipogenesis (Vidal et al., 2008; Lemos et al., 2015). Similarly, IFN-γ polarizes M1 macrophages, which are involved in mechanisms that allow FAPs to differentiate, or to undergo apoptosis (Wehling-Henricks et al., 2016). Moreover, in light of the hypothesis that systemic microbial burden may be elevated in older adults with poor muscle composition, in contrast, carbamylation decreases antimicrobial efficacy: immunoglobin carbamylation abrogates complement activation (Koro et al., 2014), and carbamylation of the antimicrobial peptide, LL-37 (cathelicidin), decreases its ability to kill gram-negative and gram-positive bacteria (Koro et al., 2016). Interestingly, an increase in adipogenesis within muscle may be one means for improving antimicrobial defense. In support of this, when exposed to the gram-positive bacterium, *Staphylococcus auerus,* preadipocytes differentiate into adipocytes in conjunction with an 80-fold increased production of cathelicidin antimicrobial peptide (Zhang et al., 2015). When considering that the incidence of bloodstream infections with *S. auerus* is ~15-fold elevated in adults older than 70, when compared with younger adults (Laupland et al., 2008), we propose these data may suggest an antimicrobial role for FAP-derived adipocytes.

In sum, we posit that these data suggest roles for an altered gut microbiome, increased gut permeability and circulating microbial burden, the immune response and activation, and carbamylation on mechanisms related to muscle composition in older adults. Moreover, we propose the novel hypothesis that increased adipogenesis within aged muscle may be a

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compensatory antimicrobial response to protect against an increased systemic microbial burden. Because this was an association-based study, causality between these pathways on muscle composition cannot be determined, but future studies aimed at testing these hypotheses are of interest.

5. Limitations

Our study has several limitations. First, to account for the relatively small sample size (n = 73), validation in a larger cohort is important. Second, when considering the link between muscle composition with BMI (Goodpaster et al., 2000), our findings in the overweight subjects of the present study may not be generalizable to older adults that have a normal weight BMI. Third, all measurements were performed at one time point-studies aimed at examining if these metabolites are markers of longitudinal changes in muscle composition are of interest. Fourth, whether these metabolites influence muscle composition, or muscle composition influences the serum levels of these metabolites, is currently unknown. Alternatively, our findings may be related to variables (e.g. diet) that can influence muscle composition, analytes (e.g. BUN) and metabolites. For example, dietary protein intake is significantly associated with the quantity of lean mass (Houston et al., 2008), and is strongly correlated ($r = 0.98$) with urea production (Young et al., 2000). However, in disagreement with this hypothesis, although dietary protein intake was significantly associated with NDM/LDM ($\beta \pm$ SE: 0.1 ± 0.0, $p = 0.02$; $n = 66$), it was not associated with BUN ($\beta \pm$ SE: 0.8 ± 0.5, $p = 0.13$), and only 2 of the 35 metabolites that were significantly associated with both NDM/LDM and BUN were associated with protein intake (*data not shown*).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Subject characteristics.

	Median (interquartile range)
NDM (cm^2)	68.5 (53.7, 84.3)
LDM (cm^2)	24.8 (19.5, 30.8)
NDM/LDM	2.7 (1.9, 4.1)
Whole body fat mass (kg)	26.4 (19.9, 30.8)
BUN (mg/dL)	18 (16, 24)
Creatinine (mg/dL)	0.9 (0.8, 1.1)
Uric Acid (mg/dL)	5.9 (4.9, 6.5)
Homocitrulline (kilounits)	43 (29, 64)
Lysine (kilounits)	427 (364, 515)
Homocitrulline/lysine	0.10 (0.08, 0.12)
Neutrophils (cells/µL)	2968 (2496, 3758)
Lymphocytes (cells/µL)	1610 (1297, 1991)
Neutrophils/lymphocytes	1.93 (1.87, 1.99)
LPS (EU/mL)	1.5 (0.9, 2.4)
LBP µg/mL)	22.2 (19.3, 28.4)
Kynurenine (kilounits)	528 (490, 591)
Tryptophan (megaunits)	13 (12, 15)
Kynurenine/tryptophan	0.039 (0.038, 0.041)

Data for outcome variables are shown with their pre-transformed median and interquartile range values. Mass spectrometry-obtained values for homocitrulline, lysine, kynurenine, and tryptophan are shown with arbitrary units.

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Table 2

Metabolites significantly associated with muscle composition (NDM/LDM).

	β ± SE	p-Value	q-Value
Mannitol	− 0.0 ± 0.0	1.0E − 05	0.0001
2-Hydroxyisobutyrate	− 0.1 ± 0.0	5.6E − 05	0.0006
Urea	− 0.1 ± 0.0	0.0008	0.005
Butyrylcarnitine	− 0.1 ± 0.0	0.0009	0.006
Pseudouridine	− 0.1 ± 0.0	0.001	0.008
N-acetylthreonine	− 0.1 ± 0.0	0.001	0.008
Tiglyl carnitine	− 0.1 ± 0.0	0.002	0.01
Erythronate	− 0.1 ± 0.1	0.002	0.01
4-Acetamidobutanoate	− 0.1 ± 0.0	0.002	0.01
Glutamine	0.2 ± 0.1	0.003	0.01
C-glycosyltryptophan	− 0.1 ± 0.0	0.003	0.02
Serine	0.1 ± 0.0	0.004	0.02
Erythritol	− 0.1 ± 0.0	0.004	0.02
Glutaroyl carnitine	− 0.1 ± 0.0	0.007	0.03
Glycoursodeoxycholate	0.0 ± 0.0	0.009	0.04
Phenylacetylglutamine	− 0.0 ± 0.0	0.01	0.04
Dimethylglycine	− 0.1 ± 0.0	0.01	0.04
5α-Androstan-3β,17beta-diol disulfate	− 0.0 ± 0.0	0.01	0.04
2-Methylbutyroylcarnitine	− 0.1 ± 0.0	0.01	0.04
Indolelactate	− 0.1 ± 0.0	0.01	0.04
Alanine	0.1 ± 0.0	0.01	0.05
N-acetylalanine	− 0.1 ± 0.1	0.01	0.05
Methylglutarylcarnitine	− 0.0 ± 0.0	0.01	0.05
Octanoylcarnitine	− 0.0 ± 0.0	0.01	0.05
Myo-inositol	− 0.1 ± 0.0	0.01	0.05
Arabitol	− 0.1 ± 0.0	0.01	0.05
Biliverdin	0.0 ± 0.0	0.01	0.05
Arabonate	− 0.0 ± 0.0	0.02	0.05
Isobutrylcarnitine	− 0.0 ± 0.0	0.02	0.05
Decanoylcarnitine	− 0.0 ± 0.0	0.02	0.06
N-formylmethionine	− 0.1 ± 0.0	0.02	0.06
Glycine	0.1 ± 0.0	0.02	0.07
Acetylcarnitine	− 0.1 ± 0.0	0.02	0.07
Phenol sulfate	− 0.0 ± 0.0	0.02	0.07
N_1-methyl-2-pyridone-5-carboxamide	− 0.1 ± 0.0	0.02	0.07
Glucuronate	− 0.0 ± 0.0	0.02	0.07
Trans-4-hydroxyproline	− 0.1 ± 0.0	0.02	0.07
Laurylcarnitine	− 0.0 ± 0.0	0.03	0.07
N-acetylmethionine	− 0.1 ± 0.0	0.03	0.07

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	β ± SE	p-Value	q-Value
Xanthine	0.0 ± 0.0	0.03	0.07
Deoxycarnitine	0.1 ± 0.1	0.03	0.07
Cis-4-decenoyl carnitine	− 0.0 ± 0.0	0.03	0.08
Hexanoylcarnitine	− 0.0 ± 0.0	0.03	0.08
Ergothioneine	0.0 ± 0.0	0.03	0.08
Symmetric dimethylarginine	− 0.1 ± 0.0	0.03	0.08
Cortisone	0.1 ± 0.0	0.03	0.08
Asparagine	0.1 ± 0.0	0.03	0.09
N_6-acetyllysine	− 0.1 ± 0.0	0.03	0.09
Tetradecanedioate	− 0.0 ± 0.0	0.03	0.09
7-Methylguanine	0.1 ± 0.0	0.04	0.09
Trans-urocanate	0.1 ± 0.0	0.04	0.09
10-Undecanoate	0.1 ± 0.0	0.04	0.09
1,5-Anhydroglucitol	− 0.0 ± 0.0	0.04	0.09
Epiandrosterone sulfate	− 0.0 ± 0.0	0.04	0.09
7-α-Hydroxy-3-oxo-4-cholestenoate	0.1 ± 0.0	0.04	0.10
Threitol	− 0.0 ± 0.0	0.05	0.10
1-Methylurate	− 0.0 ± 0.0	0.05	0.10
Androsterone sulfate	− 0.0 ± 0.0	0.05	0.10
N-acetylserine	− 0.1 ± 0.0	0.05	0.10
N_1-methyladenosine	− 0.1 ± 0.1	0.05	0.10

Sex, age and whole body fat mass-adjusted associations between serum metabolites with NDM/LDM are shown with parameter estimates and standard errors (β ± SE), in order of significance (p-value), and with q-values.

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Table 3

Metabolites significantly associated with NDM/LDM and with circulating markers of renal function.

	Vs. BUN			Vs. creatinine			Vs. uric acid		
	β ± SE	**p-Value**	**q-Value**	**p ± SE**	**p-Value**	**q-Value**	**β ± SE**	**p-Value**	**q-Value**
2-Hydroxyisobutyrate	1.8 ± 0.3	8.5E − 09	4.4E − 07	0.0 ± 0.0	1.9E − 05	0.0002	0.0 ± 0.0	0.005	0.02
Butyrylcarnitine	0.9 ± 0.3	0.002	0.01	0.0 ± 0.0	0.24	0.33	0.0 ± 0.0	0.006	0.03
Urea	–	–	–	0.0 ± 0.0	1.6E − 06	3.5E − 05	0.0 ± 0.0	0.005	0.02
Pseudouridine	2.6 ± 0.5	2.8E − 06	5.1E − 05	0.1 ± 0.0	5.9E − 12	1.7E − 09	0.0 ± 0.0	2.3E − 06	4.5E − 05
N-acetylthreonine	2.1 ± 0.4	5.4E − 07	1.7E − 05	0.1 ± 0.0	9.3E − 10	6.5E − 08	0.0 ± 0.0	0.003	0.01
Tiglyl carnitine	1.4 ± 0.3	9.7E − 06	0.0001	0.0 ± 0.0	0.02	0.07	0.0 ± 0.0	0.25	0.34
Erythronate	1.5 ± 0.4	0.0001	0.0009	0.1 ± 0.0	4.5E − 12	1.6E − 09	0.0 ± 0.0	0.002	0.01
4-Acetamidobutanoate	2.0 ± 0.4	1.6E − 05	0.0002	0.1 ± 0.0	6.7E − 08	2.7E − 06	0.0 ± 0.0	0.09	0.20
Glutamine	− 3.8 ± 0.8	1.4E − 05	0.0002	− 0.1 ± 0.0	0.0009	0.006	− 0.0 ± 0.0	0.0001	0.0009
C-glycosyltryptophan	2.7 ± 0.5	6.4E − 07	1.8E − 05	0.1 ± 0.0	2.5E − 10	2.4E − 08	0.0 ± 0.0	0.01	0.04
Serine	− 2.1 ± 0.6	0.001	0.006	− 0.0 ± 0.0	0.01	0.05	− 0.0 ± 0.0	0.37	0.43
Erythritol	1.8 ± 0.4	8.7E − 06	0.0001	0.1 ± 0.0	7.9E − 10	6.4E − 08	0.0 ± 0.0	0.002	0.01
Glutaroyl carnitine	1.3 ± 0.3	8.5E − 05	0.0008	0.0 ± 0.0	0.0002	0.002	0.0 ± 0.0	0.007	0.03
Glycoursodeoxycholate	− 0.1 ± 0.1	0.41	0.44	− 0.0 ± 0.0	0.61	0.55	− 0.0 ± 0.0	0.02	0.07
Phenylacetylglutamine	0.7 ± 0.2	0.0002	0.002	0.0 ± 0.0	0.0001	0.0009	0.0 ± 0.0	0.03	0.09
Dimethylglycine	0.9 ± 0.4	0.02	0.06	0.0 ± 0.0	0.0002	0.002	0.0 ± 0.0	0.0002	0.002
2-Methylbutyroylcarnitine	1.7 ± 0.4	2.5E − 05	0.0003	0.0 ± 0.0	0.0005	0.003	0.0 ± 0.0	0.01	0.05
Indolelactate	1.2 ± 0.3	0.0007	0.005	0.0 ± 0.0	0.0001	0.0009	0.0 ± 0.0	0.28	0.36
N-acetylalanine	3.4 ± 0.6	8.8E − 07	2.2E − 05	0.1 ± 0.0	4.3E − 11	6.1E − 09	0.0 ± 0.0	0.0002	0.002
Methylglutarylcarnitine	0.3 ± 0.2	0.08	0.16	0.0 ± 0.0	0.04	0.09	0.0 ± 0.0	0.49	0.49
Octanoylcarnitine	0.4 ± 0.2	0.12	0.21	0.0 ± 0.0	0.06	0.13	0.0 ± 0.0	0.01	0.04
Myo-inositol	2.0 ± 0.3	4.1E − 08	1.9E − 06	0.1 ± 0.0	1.9E − 10	2.1E − 08	0.0 ± 0.0	0.0008	0.00
Arabitol	3.3 ± 0.6	9.5E − 08	3.6E − 06	0.1 ± 0.0	6.4E − 07	1.8E − 05	0.0 ± 0.0	0.0009	0.006
Arabonate	1.1 ± 0.2	2.1E − 05	0.000	0.0 ± 0.0	2.0E − 06	4.2E − 05	0.0 ± 0.0	0.009	0.036
Isobutrylcarnitine	1.2 ± 0.2	4.0E − 06	6.8E − 05	0.0 ± 0.0	4.2E − 06	7.0E − 05	0.0 ± 0.0	0.22	0.32
Decanoylcarnitine	0.2 ± 0.3	0.34	0.41	0.0 ± 0.0	0.28	0.36	0.0 ± 0.0	0.02	0.07
N-formylmethionine	2.0 ± 0.5	2.5E − 05	0.0003	0.1 ± 0.0	1.1E − 09	6.9E − 08	0.0 ± 0.0	0.008	0.03

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	Vs. BUN			Vs. creatinine			Vs. uric acid		
	β ± SE	p-Value	q-Value	p ± SE	p-Value	q-Value	β ± SE	p-Value	q-Value
Glycine	− 1.2 ± 0.5	0.02	0.06	− 0.0 ± 0.0	0.62	0.55	− 0.0 ± 0.0	0.0008	0.005
Phenol sulfate	0.5 ± 0.2	0.008	0.03	0.0 ± 0.0	0.04	0.11	0.0 ± 0.0	0.05	0.12
N_1-methyl-2-pyridone-5-carboxamide	1.3 ± 0.3	4.4E − 06	7.08E − 05	0.0 ± 0.0	5.6E − 05	0.0006	0.0 ± 0.0	0.006	0.03
Glucuronate	1.3 ± 0.2	4.1E − 07	1.36E − 05	0.0 ± 0.0	0.0003	0.002	0.0 ± 0.0	7.9E − 05	0.0008
N-acetylmethionine	1.0 ± 0.5	0.04	0.10	0.0 ± 0.0	0.004	0.02	0.0 ± 0.0	0.21	0.31
Deoxycarnitine	− 0.3 ± 0.8	0.73	0.58	0.0 ± 0.0	0.008	0.03	− 0.0 ± 0.0	0.46	0.47
Cis-4-decenoyl carnitine	0.5 ± 0.3	0.10	0.19	0.0 ± 0.0	0.03	0.07	0.0 ± 0.0	0.01	0.05
Hexanoylcarnitine	0.6 ± 0.3	0.07	0.15	0.0 ± 0.0	0.11	0.21	0.0 ± 0.0	0.02	0.07
Symmetric dimethylarginine	1.7 ± 0.6	0.007	0.03	0.0 ± 0.0	0.003	0.02	0.0 ± 0.0	0.24	0.34
N_6-acetyllysine	1.4 ± 0.5	0.02	0.05	0.1 ± 0.0	2.2E − 05	0.0002	0.0 ± 0.0	0.02	0.07
Trans-urocanate	− 0.8 ± 0.3	0.01	0.04	− 0.0 ± 0.0	0.12	0.22	− 0.0 ± 0.0	0.17	0.27
10-Undecanoate	− 1.0 ± 0.4	0.02	0.06	0.0 ± 0.0	0.69	0.57	0.0 ± 0.0	0.82	0.61
Threitol	1.2 ± 0.2	4.7E − 06	7.3E − 05	0.0 ± 0.0	1.3E − 11	2.4E − 09	0.0 ± 0.0	0.007	0.03
1-Methylurate	0.3 ± 0.3	0.22	0.32	0.0 ± 0.0	0.03	0.08	0.0 ± 0.0	0.12	0.22
N-acetylserine	1.1 ± 0.4	0.01	0.04	0.0 ± 0.0	0.0003	0.002	0.0 ± 0.0	0.03	0.08
N_1-methyladenosine	2.4 ± 0.8	0.004	0.02	0.1 ± 0.0	2.2E − 06	4.4E − 05	0.0 ± 0.0	0.04	0.11

Metabolites significantly associated with NDM/LDM and with at least one circulating marker of renal function (BUN, creatinine, uric acid) are shown with parameter estimates and standard errors (β ± SE), and with p- and q-values.

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Table 4

Metabolites significantly associated with NDM/LDM and with a circulating marker of carbamylation.

	β ± SE	p-Value	q-Value
C-glycosyltryptophan	0.2 ± 0.0	8.8E − 07	2.2E − 05
Erythronate	0.1 ± 0.0	1.6E − 06	3.5E − 05
N_1-methyladenosine	0.2 ± 0.0	1.1E − 05	0.0001
N-acetylthreonine	0.1 ± 0.0	1.1E − 05	0.0001
Pseudouridine	0.1 ± 0.0	1.8E − 05	0.0002
2-Hydroxyisobutyrate	0.1 ± 0.0	2.3E − 05	0.0003
N-acetylalanine	0.2 ± 0.0	2.6E − 05	0.0003
2-Methylbutyroylcarnitine	0.1 ± 0.0	0.0002	0.002
4-Acetamidobutanoate	0.1 ± 0.0	0.0002	0.002
Threitol	0.1 ± 0.0	0.0002	0.002
N-formylmethionine	0.i ± 0.0	0.0002	0.002
Glutamine	− 0.2 ± 0.0	0.0004	0.003
Myo-inositol	0.1 ± 0.0	0.0004	0.003
Urea	0.1 ± 0.0	0.001	0.007
Phenylacetylglutamine	0.0 ± 0.0	0.001	0.007
Tiglyl carnitine	0.1 ± 0.0	0.001	0.008
Erythritol	0.1 ± 0.0	0.001	0.008
Arabonate	0.0 ± 0.0	0.003	0.01
Glutaroyl carnitine	0.1 ± 0.0	0.003	0.01
Isobutrylcarnitine	0.0 ± 0.0	0.003	0.01
Dimethylglycine	0.1 ± 0.0	0.003	0.01
Serine	− 0.1 ± 0.0	0.01	0.05
N-acetylserine	0.1 ± 0.0	0.01	0.05
Methylglutarylcarnitine	0.0 ± 0.0	0.02	0.05
Asparagine	− 0.1 ± 0.0	0.02	0.06
Glucuronate	0.0 ± 0.0	0.02	0.07
Indolelactate	0.0 ± 0.0	0.02	0.07
N_6-acetyllysine	0.1 ± 0.0	0.02	0.07
Arabitol	0.1 ± 0.0	0.02	0.07
Phenol sulfate	0.0 ± 0.0	0.03	0.07
Cis-4-decenoyl carnitine	0.0 ± 0.0	0.03	0.08
Butyrylcarnitine	0.0 ± 0.0	0.03	0.09
Hexanoylcarnitine	0.0 ± 0.0	0.04	0.09
Glycine	− 0.1 ± 0.0	0.04	0.09
Mannitol	0.0 ± 0.0	0.05	0.12

Metabolites significantly associated with NDM/LDM and with a circulating marker of carbamylation (homocitrulline/lysine) are shown with parameter estimates and standard errors (β ± SE), and with p- and q-values.

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Table 5

Metabolites significantly associated with NDM/LDM and with a circulating marker of immune activation.

	β ± SE	p-Value	q-Value
C-glycosyltryptophan	0.4 ± 0.1	4.4E – 09	2.5E – 07
Erythritol	0.3 ± 0.1	6.3E – 08	2.7E – 06
Pseudouridine	0.4 ± 0.1	1.2E – 07	4.2E – 06
1-Methylurate	0.2 ± 0.0	8.0E – 07	2.1E – 05
4-Acetamidobutanoate	0.3 ± 0.1	9.2E – 07	2.2E – 05
N_1-methyladenosine	0.4 ± 0.1	2.5E – 06	4.7E – 05
N-acetylalanine	0.4 ± 0.1	3.2E – 06	5.6E – 05
N-formylmethionine	0.3 ± 0.1	4.8E – 06	7.3E – 05
Arabonate	0.1 ± 0.0	1.3E – 05	0.0002
Urea	0.2 ± 0.0	2.1E – 05	0.0002
Phenylacetylglutamine	0.1 ± 0.0	2.9E – 05	0.0003
Erythronate	0.2 ± 0.0	5.9E – 05	0.0006
Myo-inositol	0.2 ± 0.0	0.0001	0.0009
Glutaroyl carnitine	0.1 ± 0.0	0.0002	0.002
2-Hydroxyisobutyrate	0.2 ± 0.0	0.0004	0.003
Threitol	0.1 ± 0.0	0.0004	0.003
Glucuronate	0.1 ± 0.0	0.0005	0.003
N_6-acetyllysine	−0.2 ± 0.1	0.001	0.007
N-acetylserine	0.2 ± 0.0	0.001	0.008
Dimethylglycine	0.2 ± 0.0	0.002	0.009
Isobutrylcarnitine	0.1 ± 0.0	0.003	0.01
N-acetylthreonine	0.2 ± 0.1	0.003	0.01
Methylglutarylcarnitine	0.1 ± 0.0	0.003	0.01
Arabitol	0.2 ± 0.1	0.003	0.01
Serine	− 0.2 ± 0.1	0.003	0.02
N_1-methyl-2-pyridone-5-carboxamide	0.1 ± 0.0	0.005	0.02
Tiglyl carnitine	0.1 ± 0.0	0.006	0.03
Cis-4-decenoyl carnitine	0.1 ± 0.0	0.007	0.03
Ergothioneine	− 0.1 ± 0.0	0.007	0.03
2-Methylbutyroylcarnitine	0.1 ± 0.1	0.009	0.04
Cortisone	− 0.2 ± 0.1	0.01	0.05
Butyrylcarnitine	0.1 ± 0.0	0.02	0.06
Hexanoylcarnitine	0.1 ± 0.0	0.03	0.07
Indolelactate	0.1 ± 0.0	0.04	0.10
Glutamine	− 0.2 ± 0.1	0.05	0.12

Metabolites significantly associated with NDM/LDM and with a circulating marker of immune activation (kynurenine/tryptophan) are shown with parameter estimates and standard errors (β ± SE), and with p- and q-values.