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The Influence of Circulating Polya mines and Amino Acids on Lean Body Mass

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Abstract

Polyamines have been implicated in a number of age-related phenotypes. The relationship between polyamines and lean body mass has been less explored. Moreover, the relative influence of amino acids and polyamines on skeletal muscle mass is unknown. Therefore, the purpose of the present study was to investigate circulating amino acid and polyamine patterns and determine their relative influence on skeletal muscle mass. Subjects consisted of 10 females and 53 males. Targeted metabolomics analysis was performed using the AbsoluteIDQ™ p180 kit combined with liquid chromatography tandem mass spectrometry. Analysis of lean body mass index (LBI), lean body mass divided by height squared, found that males had a higher LBI than females (P<0.01) and LBI decreased with advancing age (P<0.05). Using forward stepwise linear regression, only spermidine was related to LBI independent of age and gender. For circulating amino acids, only tyrosine (Tyr) was associated with LBI independently of age and gender. When circulating Tyr was combined with spermidine in the regression model, both spermidine and Tyr were related to LBI independently of age and gender. We conclude that circulating spermindine and tyrosine independently influence LBIt.

Keywords: Polyamines • Amino acids • Spermidine • Lean body mass

Introduction

Age-related decline in lean body mass is a common problem in the aging population, leading to impairment in mobility and independence [1]. The mechanism underlying an age-related reduction in muscle mass may involve reduced growth hormone [2] and male sex hormone levels, particularly testosterone [3]. In addition, polyamines, such as spermidine, have been implicated in a number of age-related phenotypes. Spermidine has been identified as a calorie restriction mimetic [4], which may be beneficial in preventing the age-related decline in body function. The relationship between polyamines, including spermidine, spermine and putrescine, and lean body mass and bone mass has yet to be fully explored.

Anabolic resistance, defined as a poor muscle protein synthesis, has also been implicated as a mechanism underlying age-related sarcopenia [5]. Amino acids, particularly the essential amino acid leucine, stimulate muscle protein synthesis [6]. One study showed older adults with sarcopenia and frailty were characterized by an amino acid profile distinct from those without the condition [7]. As the amino acid profile is related to dietary intake, which can be influenced by distinct dietary patterns related to culture and ethnicity, it is currently unclear if the circulating amino acid profile characteristic of subjects with sarcopenia in previous studies is generalizable to other populations. Moreover, the relative influence of amino acids and polyamines on skeletal muscle mass remains unknown. Therefore, the purpose of the present study was to investigate circulating amino acid and polyamines patterns and their relative influence on skeletal muscle mass in Thais.

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Materials and Methods

Subjects

Participants were recruited from the population studied in the Electricity Generating Authority of Thailand (EGAT) study in 2009, cohort 3 (EGAT 3). Details of the study cohort have been published previously [8]. Briefly, subjects in the cohort were employees of EGAT who volunteered to participate in a health survey. All participants completed a medical evaluation and had routine laboratory investigations including urinalysis. Blood was drawn after a 12 h fast. There were three EGAT cohorts. In 1985, 3499 workers of EGAT (half of the total employees) were randomly enrolled as EGAT 1 cohort. In 1998, 2999 employees were randomly enrolled as EGAT 2 cohort. In 2009, 2584 participants were recruited to the EGAT 3 cohort. EGAT 3 was resurveyed in 2014. Each time, the same individuals were contacted by telephone and invitation letter to attend the follow-up examination. Information about cause of death was sought for those known to have died during the interim period. At each follow-up visit, subjects underwent similar medical evaluations and had routine laboratory investigations as the baseline visit.

A total of 63 participants (10 females, 53 males) were enrolled in this retrospective study. The participants were randomly selected from those who did not have a previous history of diabetes and had a fasting plasma glucose (FPG) ≥ the 85th percentile (5.4 mmol/L), but <7 mmol/L. Body composition was determined after at least 3 h of fasting using multi-frequency bioelectrical impedance analysis with eight-point tactile electrodes (InBody 720; Biospace, Seoul, Korea). Body mass index (BMI) was calculated using body mass (kg)/height (m)2. Likewise, LBI was calculated using skeletal muscle mass from body composition measurement (kg)/height (m)2. Serum samples +were obtained and kept at −80°C until analysis.

Ethical approval

The Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine, Ramathibodi Hospital, Mahidol University approved the study, and it conformed with the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). All participants gave their written informed consent before participating in the study.

Polyamine and amino acid assessment

Serum levels of polyamines and amino acids were measured using an AbsoluteIDQ™ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria), involving separation by liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS) measurements. Sample preparation was performed in accordance with the manufacturer's instructions and samples were analyzed on a QTRAP 5500 mass spectrometer (ABsciex, Framingham, MA, USA), using electrospray ionization and operating in the multiple reaction monitoring mode, coupled to an Agilent HPLC 1260 Series (Agilent Technologies, Santa Clara, CA, USA) equipped with an Agilent Zobax Eclipse XDB C18, 3.0 mm × 100 mm, 3.5 µm column and a Phenomenex C18, 4.0 mm × 3.0 mm SecurityGuard column. Amino acids and biogenic amines were analyzed by LC-MS/MS in positive mode. Metabolites were quantified according to the manufacturer's protocol, using the MetIDQ™ Carbon software for targeted metabolomic data processing and management. This assay quantifies targeted metabolites that encompass 21 amino acids and 21 biogenic amines.

Statistical analysis

Statistical analysis was performed using Rstudio version 1.0.136 and R version 3.3.2 (RStudio Inc., Boston, USA.). Comparisons between groups were performed by the Wilcoxon rank-sum test. Linear regression analyses were used to assess statistical association. P<0.05 was considered statistically significant.

Results

The clinical characteristics of the participants are shown in Table 1. Their mean age was 41.9 ± 6.8 years, and most (84.1%) were males, because of the demographic structure of the workforce in the organization where the participants were recruited. The participants were generally obese, having a mean (± SD) BMI of 27.0 ± 4.4 kg/m2. Their mean baseline FPG was 5.93 ± 0.48 mmol/L.

Our analysis found that males had a higher lean body mass index (LBI) than females (P < 0.01) and LBI decreased with advancing age (P<0.05) Table 2. Spermine, spermidine and putrescine were then analyzed for their relationship with LBI. As shown in Table 3, spermine and spermidine were significantly associated with LBI, while no association with LBI and putrescine was found. Using forward stepwise linear regression, it was found that only spermidine was related to LBI independent of age and gender. Spermine was no longer correlated to LBI when spermidine was included in the model.

Using hierarchical cluster analysis, spermine, spermidine and putrescine were clustered into two distinct groups Figure 1, with the levels of the polyamines in group one (Gr. 1) significantly higher than group 2 (Gr. 2), as shown in Table 4. Subjects in Gr. 1 had a significantly higher LBI compared with subjects in Gr. 2 (10.7 ± 0.2 vs. 10.3 ± 0.1 kg/m2, respectively, P<0.05). Using multivariate analysis, it was found that the polyamine clusters were also associated with LBI independently of age and gender Table 5. However, with stepwise analysis, the effect of the polyamine clusters could not be demonstrated when spermidine was present in the model.

For circulating amino acids, it was found that only tyrosine (Tyr) was associated with LBI independently of age and gender. No effect of branch-chained amino acids (BCAAs) or essential amino acids was found. When circulating Tyr was included in the regression model in addition spermidine,

Table 1. Clinical characteristics of the study population (n = 63).

Variables	Mean ± SD or number (%)
Age (year)	41.9 ± 6.8
Male (%)	53 (84.1%)
Body mass index (kg/m^2)	27.0 ± 4.4
Lean body mass index (kg/m^2)	10.5 ± 1.1
Fasting plasma glucose (mmol/L)	5.93 ± 0.48

Table 2. Negative association of age and positive association of male gender with lean body mass index.

Variables	Beta coefficient	P value
Age (year)	–0.26	<0.05
Male gender	0.35	<0.01

Table 3. Association of polyamines with lean body mass index after adjusting for age and gender. A. spermine, B. spermidine, C. Putrescine.

A

Variables	Beta coefficient	*P* value
Age (year)	–0.24	<0.05
Male gender	0.31	<0.01
Spermine (nM)	0.23	<0.05

B

Variables	Beta coefficient	*P* value
Age (year)	–0.31	<0.01
Male gender	0.31	<0.01
Spermidine (nM)	0.30	<0.01

C

Variables	Beta coefficient	*P* value
Age (year)	–0.26	<0.05
Male gender	0.35	<0.01
Putrescine (nM)	–0.02	0.88

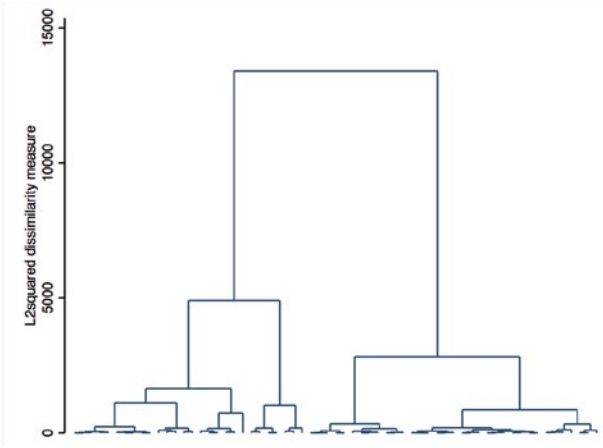

Figure 1. Hierarchical clustering of spermine, spermidine and putrescine.

Table 4. Difference in circulating spermidine, spermine and putrescine between subjects in groups one (Gr. 1) and two (Gr. 2).

Variables	Gr. 1	Gr. 2	*P* value
Spermidine (nM)	352.3 ± 13.8	214.7 ± 8.3	<0.001 (rank sum)
Spermine (nM)	180.9 ± 6.9	161.1 ± 4.4	<0.05
Putrescine (nM)	109.9 ± 12.5	64.6 ± 4.5	<0.01

Table 5. The association of polyamine clusters with lean body mass index independently of age and gender.

Variables	Beta coefficient	*P* value
Age (year)	-0.33	<0.01
Male gender	0.33	<0.01
Polyamine clusters	0.28	<0.05

Table 6. Associations and standardized regression coefficients of the regression of circulating amino acids (nM) on lean body mass index.

Variables	Beta coefficient	*p* value
Age (year)	–0.26	0.06
Male gender	–0.40	<0.01
Alanine	0.26	0.12
Arginine	–0.14	0.37
Asparagine	–0.16	0.32
Aspartate	0.12	0.45
Citrulline	–0.19	0.16
Glutamine	0.15	0.37
Glutamate	0.08	0.70
Glycine	0.25	0.12
Histidine	–0.11	0.44
Isoleucine	–0.36	0.19
Leucine	0.24	0.38
Lysine	–0.14	0.46
Methionine	–0.02	0.93
Ornithine	–0.14	0.46
Phenylalanine	0.22	0.21
Proline	–0.16	0.22
Serine	–0.25	0.14
Threonine	0.20	0.27
Tryptophan	0.09	0.63
Tyrosine	0.41	< 0.05
Valine	0.08	0.62

Table 7. The effect of circulating spermidine and tyrosine on lean body mass index independently of age and gender.

Variables	Beta coefficient	*p* value
Age (year)	–0.22	<0.05
Male gender	0.25	<0.05
Spermidine (nM)	0.21	<0.05
Tyrosine (nM)	0.44	<0.01

it was found that both spermidine and Tyr were related to LBI independently of age and gender.

Discussion

Polyamines are involved in the growth, differentiation and function of many tissues. Studies of the influence of polyamines in skeletal muscle have identified altered polyamine levels in various skeletal muscle diseases, such as Duchenne muscular dystrophy [9]. Moreover, spermidine oxidase, a multitasking enzyme involved in polyamine metabolism, is highly expressed in muscle tissue [10], further supporting the important functional role of polyamines in skeletal muscle. (Mouse spermine oxidase gene splice variants. Nuclear subcellular localization of a novel active isoform) In the present study, we demonstrated that polyamine clusters derived from the hierarchical clustering of circulating levels of putrescence, spermidine and spermine, particularly spermidine, were associated with LBI independent of circulating amino acid profiles and other clinical variables. When examining the differences between clusters, it was evident that the cluster associated with a lower skeletal muscle mass was that with higher putrescine levels, but with relatively lower both spermidine and spermine levels. This may suggest a decrease in the activity of spermidine synthase, the enzyme catalyzing the conversion of putrescine to spermidine.

Aging is associated with an alteration in the protein metabolism of muscle tissues, and BCAAs have been shown to stimulate muscle protein synthesis in the elderly, possibly involving the direct effect of leucine on the initiation of mRNA translation [11]. However, in the present study, an association between BCAAs or essential amino acids with LBI was not found. Instead, we found that circulating Tyr was highly independently associated with LBI. Tyrosine is a non-essential amino acid that is the precursor for many biological mediators. In humans, supplementation with L-tyrosine can improve cognitive function [12]. Considering skeletal muscle function, L-tyrosine can improve aerobic power in animal models [13], as well as deficits in locomotion after cold-swim stress [14]. Therefore, it is likely that the association between L-tyrosine and LBI found in the present study may have a causal nature. Further investigations into the effects of L-tyrosine supplementation on skeletal muscle and function in humans are therefore warranted.

As the assessment of muscle and sarcopenia is not readily available, potential serum biomarkers to reflect low muscle mass have been investigated. For example, in subjects without diabetes, BCAAs and Tyr have been shown to be associated with muscle volume and glucose metabolism [15]. Consistent with this previous finding, we also found that Tyr was associated with LBI in subjects with prediabetes. However, no influence of BCAAs was found in our study. Moreover, the effect of essential amino acids was not evident in our study, despite a previous study showing the association of essential amino acids with skeletal muscle mass in cachectic patients [16]. Using a number of relevant predefined serum biomarkers, such as interleukin-6, secreted protein acidic and rich in cysteine, macrophage migration inhibitory factor and insulin-like growth factor-1, previous research found that serum biomarkers in combination can achieve a higher diagnostic accuracy than individual biomarkers, and may be utilized for early diagnosis and prognosis of sarcopenia [17]. It remains to be investigated if the diagnostic performance of the biomarker panel would improve if amino acids and polyamines were included in the predictive model.

A number of studies in animal models and humans have investigated the effect of spermidine supplementation. With regard to safety, it has been demonstrated that spermidine supplements using a spermidine-rich plant extract was safe and well-tolerated in mice and older adults [18]. In a pilot trial, spermidine supplementation was associated with a positive impact on memory performance in older adults with subjective cognitive decline [19]. To our knowledge, there has been no human study looking into the effect of spermidine or polyamine supplementation on muscle or body composition. However, in a study in piglets, polyamines supplementation before weaning increased piglet growth at weaning [20].

Conclusion

Circulating spermindine and tyrosine possess independent influences on the LBI. It is therefore warranted to invesigate the effect of spermidine and tyrosine supplements on the prevention of sarcopenia in the elderly.

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