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Higher-protein intake and physical activity are associated with healthier body composition and cardiometabolic health in Hispanic adults

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ABSTRACT

Background: Higher protein (HP) intake and physical activity (PA) have been associated with improved lean soft tissue (LST) and reduced fat mass (FM). Puerto Ricans have among the highest age-adjusted prevalence (42.5%) of obesity, which may be associated with inadequate protein consumption and PA. We examined the relationship between protein intake and PA with body composition and biomarkers of cardiometabolic health in Puerto Rican adults.

Methods: Participants included 959 Puerto Rican adults (71.4% women, 28.6% men) from the Boston Puerto Rican Health Study (BPRHS), aged 46-79 y (Women: age, 60.4 ± 7.6 y, BMI, 32.9 ± 6.8; Men: age, 59.8 ± 7.9 y, BMI, 30.1 ± 5.2). Protein intake was assessed using a food frequency questionnaire and expressed as g/kg body weight/day in energy intake-adjusted equal cut point tertile categories (lower, moderate, higher: LP < 0.91 g/kg/d, MP $\geq 0.91 \leq 1.11$ g/kg/d, and HP > 1.11 g/kg/d). PA was assessed by questionnaire and expressed in tertile categories (low, moderate and high; PA1: < 0.8 kilometers (km)/d, PA2: ≥ 0.8 km/d ≤ 3.2 km/d, PA3: > 3.2 km/d).

Results: Participants with energy-adjusted HP had lower appendicular LST (ALST: 16.2 ± 3.8 kg), LST (39.7 ± 8.0 kg) and FM (25.6 ± 8.1 kg) when compared to LP (ALST: 20.1 ± 4.5 kg; LST: 49.5 ± 10.0 kg; FM: 40.8 ± 12.3 kg; $P < 0.001$) and MP (ALST: 18.2 ± 4.3 kg; LST: 44.1 ± 8.8 kg; FM: 32.2 ± 9.8 kg; $P < 0.001$). However, when adjusted for total body weight (kg), relative LST was significantly greater in HP (58 ± 9%) when compared to LP (53 ± 9%; $P < 0.001$) and MP (56 ± 9%; $P < 0.001$). Participants in PA3 had greater ALST (19.5 ± 5.4 kg), and LST (58 ± 10%), compared to PA1 (ALST: 17.2 ± 4.3 kg; LST: 53 ± 9%; $P < 0.001$) or PA2 (ALST: 17.7 ± 4.7 kg; LST: 56 ± 9%; $P < 0.05$). Those in HP+PA3 or MP+PA2 had lower c-reactive protein (CRP; HP+PA3: 5.1 ± 6.8 mg/L; MP+PA2: 6.4 ± 10.0 mg/L), when compared to

LP+PA1 (8.7 ± 8.8 mg/L; $P < 0.05$). Insulin concentration was lower for those in both the HP and PA3 (HP+PA3; 11.4 ± 7.9 IU/mL) compared to those in both the LP and PA1 (LP+PA1; 20.7 ± 16.3 UI/mL) ($P < 0.001$).

Conclusions: The highest tertiles of energy-adjusted protein intake (≥ 1.11 g/kg/d) and PA (> 3.2 km/d) were associated with more desirable indicators of overall body composition and cardiometabolic health, when adjusted for body weight, than those in the lower protein intake and PA in Puerto Rican adults.

Keywords: protein, body composition, lean soft tissue, fat mass, physical activity, Boston Puerto Rican Health Study

BACKGROUND

Obesity is recognized as a major public health concern because of its link to potential fatal complications arising from metabolic and cardiovascular diseases. Currently, an estimated 68% of the United States (US) adult population is considered either overweight (body mass index, BMI = 25 to 29.9 kg/m^2) or obese (BMI ≥ 30 kg/m^2), and this has prompted research aimed at developing strategies to combat this epidemic [1]. The health complications from obesity are particularly high in Hispanic populations living in the U.S. [1]. Maintaining a healthy weight in our obesogenic environment is challenging [2]. Despite many pharmacological advances in this field, lifestyle interventions that emphasize proper nutrition and physical activity (PA) continue to be the primary strategy for individuals to countermeasure excess body weight.

Relatively higher protein intake (> 1.6 g/kg/d) has been suggested to aid in the regulation of body fat, appetite, and energy intake [3,4]. In one study, 23 weeks of whey protein supplementation reduced body weight and fat mass (FM) in overweight and obese (n = 90) free-living adults [5]. In a series of studies, we have demonstrated that the addition of whey protein supplements as part of shorter and longer term weight loss interventions resulted in maintenance of lean soft tissue (LST) mass and reductions in FM in overweight and obese participants [6,7]. Previous research also confirms that both moderate (25%) and high (40%) protein diets improved body composition more than standard protein (15%) diets [6,8,9]. Protein has been shown to have higher satiating effects, compared to either carbohydrates or fat [10,11] suggesting that increased consumption of protein may be helpful in reducing hunger and promoting weight loss. However, while protein is satiating, individual total energy needs vary considerably. For this reason it is necessary to adjust for total energy intake to consider protein intake within the context of total energy because if total energy increases, all macronutrients are likely to increase as well [12–15].

Higher PA levels have also been associated with higher LST and lower FM [16,17]. However, research suggests that the beneficial effects of PA on body composition and metabolic health are amplified with sufficient protein in the diet [16,18]. Higher intensity PA combined with a balanced diet higher protein diet has been shown to elicit beneficial changes in body composition and cardiovascular disease risk in obese individuals, relative to low or moderate PA and a traditional heart healthy diet [8,19].

Given the severity of the obesity epidemic in the US, particularly among the Puerto Rican population (42.5%) [20], higher protein intake, along with greater PA may be related to normal body composition phenotype and overall health. The primary objectives were to analyze associations between 1) protein intake and body composition, 2) PA and body composition, and 3) the interaction of protein intake and PA with biomarkers of cardiometablic health in Puerto Rican adults. We hypothesized that higher protein intake and higher levels of PA would be associated with greater LST and lower FM, as well as better cardiometabolic health, in a population-based cohort of adult Puerto Rican men and women.

METHODS

Participants

Participant data were obtained from the Boston Puerto Rican Health Study (BPRHS) [21], a longitudinal cohort study (2004-2017). The BPRHS aims to understand the relationship between stress, nutrition, and chronic disease conditions in Puerto Rican adults living in the United States, Boston, Massachusetts (MA) area [21–23]. Briefly, participants were recruited using Census data from 2000 to identify community areas with high Hispanic density, and door-to-door enumeration. Participants were also identified at community events, through referrals, and through the use of flyers. Eligible participants were of Puerto Rican descent, able to answer interview questions in English or Spanish, aged 45-75 years, and lived in the Boston, MA area. Bilingual interviewers visited the participants' homes to complete questionnaires, and to collect information on socioeconomic status, health, and health behaviors. All interviewers were trained to administer questionnaires and collect measurements following procedures from National Health and Nutrition Examination Survey (NHANES) II and the MacArthur Studies of Successful Aging [24,25]. A certified phlebotomist collected 12 hour fasted biological samples, including blood, saliva, and urine, the day after the interview or as soon as possible thereafter. Participants were excluded from the study if they could not answer questions due to a serious health condition, if they did not plan to live in the area for more than 2 y, or if they scored $\leq$ 10 on the Mini Mental State Examination. Data were collected at baseline and at 2 y follow up. Body composition with dual-energy X-Ray absorptiometry (DXA) was available only at the 2 y time point (See Methods, below). Therefore, all data presented here are cross-sectional and from the 2 y time point. For the purpose of our analysis, participants were included if body composition, dietary and PA data were available. Procedures were followed in accordance with guidelines approved by the Institutional Review Board of Tufts Medical Center. All participants provided written informed consent prior to the start of the study in the language of preference. The data were approved for use by the Florida State University Institutional Review Board.

Design & Methodology

Dietary intake assessment

Dietary data were collected with a semi-quantitative food-frequency questionnaire (FFQ) adapted and validated for use with the BPRHS cohort [26]. The questionnaire food list was adapted from the National Cancer Institute/Block FFQ, with data from the Hispanic Health and Nutrition Examination Survey (HHANES) dietary recalls for Puerto Rican adults [21,26]. Specific foods and recipes were added to the FFQ to accommodate the typical Puerto Rican diet, which can differ greatly from the average US diet [26]. Participants reported both frequencies of consumption as well as portion size. Dietary analysis was performed using the Nutrition Data System for Research (NDS-R) software (version 2007, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). Outliers for energy intake were excluded when values were < 600 or > 4400 kcal/d [27]. Protein intake (g/kg) was statistically divided into equal tertiles of intake, adjusted for energy intake (kcal) (see statistical analysis), with the following cut point values to create three equal groups (LP: lower protein, < 0.91 g/kg/d, n=317; MP: moderate protein, ≥ 0.91 ≤ 1.11 g/kg/d, n=317; HP: higher protein, > 1.11 g/kg/d, n=316).

Assessment of covariates

Standing height, weight, waist circumference (WC), and hip circumference (HP) were measured in duplicate. Detailed methodology for anthropometric measures is included in Additional file 1. BMI was calculated using weight (kg) divided by height (m^2). Systolic and diastolic blood pressures were measured with an electronic sphygmomanometer (Additional file

1) at three time points during the interview; an average of the second and third readings was used for both measures. Body composition was measured by DXA to obtain LST and index (LSTI, height in m^2), FM and index (FMI, height in m^2), appendicular LST mass commonly termed appendicular skeletal mass as LST from arms and legs is skeletal muscle (ALST, the sum of LST from limbs), and appendicular LST index (ALSTI, ALST/height in m^2). DXA measurements were performed using a Lunar model Prodigy scanner (General Electric) using standard procedures by the manufacturer for a whole body scan. The DXA was calibrated weekly using an external standard aluminum spine phantom (Lunar Radiation Corp) to ensure stability of measurements. The manufacturer lists a coefficient of variation (CV) of less than 0.5% for the quality control phantom scan and similar models have reported CV values of 1.5 and 1.9% for LST and FM respectively [28]. LST and FM measurements were also adjusted for height (m^2) to minimize confounding as standard in body composition research [29]. Additionally, LST was adjusted for total body weight (kg) to determine relative LST associated with energy-adjusted tertiles in order to account for various sizes of individuals [6,9].

PA was assessed through self-report using a modified Paffenbarger questionnaire of the Harvard Alumni Activity Survey, which was validated in an elderly Puerto Rican population [14,30,31]. PA, as measured by distance covered per day (km/day) was collected and was divided into equal tertile categories of PA (PA1 < 0.8 km/d, n=367; PA2 ≥ 0.8 ≤ 3.2 km/d, n=214; PA3 > 3.2 km/d, n=353). Additionally, participants that consumed < 0.91 g protein/kg/d and walked < 0.8 km/d were placed into the LP+PA1 category (n=129), those that consumed ≥ 0.91 ≤ 1.11 g protein/kg/d and walked ≥ 0.8 ≤ 3.2 km/d were placed into the MP+PA2 category (n=141), and those that consumed > 1.11 g protein/kg/d and walked > 3.2 km/d were placed into the HP+PA3 category (n=62).

Blood samples were analyzed for C-reactive protein (CRP), testosterone, insulin-like growth factor 1(IGF-1), tumor necrosis factor (TNF-α), interleukin 6 (IL-6), total cholesterol, HDL, LDL, triglycerides, glucose, and insulin, as described previously [21]. (Additional File 1).

Statistical analysis

All statistical analyses were performed using SPSS version 15.0 (IBM, SPSS Statistics). Mean ± SD were used for continuous variables and frequency and percentages for categorical variables. One way ANOVA was used to compare the mean values of body composition, body weight, dietary intake and biochemical variables in relation to tertiles of protein (energy-adjusted) intake and PA (km/d) levels; separately by sex and age (≤ 65 years and > 65 years). Energy-adjusted protein intake removed the potential confounding factor of total energy intake and is a better representative of protein intake and its relationship to all independent variables. Therefore, the remainder of the results and discussion will focus on energy-adjusted protein intake.

Energy-adjusted protein intake was calculated by regressing reported protein intake as dependent variable on total energy intake as an independent variable [15]. Residual was calculated as: $residual = observed\ protein\ intake - predicted\ protein\ intake$

The residual obtained from the regression model provides a measure of protein intake uncorrelated with total energy intake [15]. The energy-adjusted protein intake was finally calculated as: adjusted protein intake = a + b. Where a = residual obtained from the regression model; b is the expected protein intake with mean energy intake [32].

$$Energy\ adjusted\ protein\ intake = mean\ protein\ intake + residual$$

Linear regression analysis was used to study the association of adjusted protein intake with sex, age, total FM and LST. Interactions of sex and age with total FM and LST were tested with generalized linear models (GLM). GLM models were also used to study the relationship of total FM and LST with PA and energy-adjusted protein intake, and their respective interaction terms. A P-value < 0.05 was used for statistical significance.

RESULTS

When stratified by sex (W: n=690, M: n=269), no significant interactions were observed between energy-adjusted protein tertiles and therefore results are presented as a total population. Outcome variables that were different when stratified by age (< 65 and > 65y) are provided in the Supplemental File. The mean age of the study population was 60 ± 8 y, ranging from 46 to 79 y; ~71% were women and 83% of the women had already experienced menopause. Although, total body weight was greater in Puerto Rican men than women, 62.8% of women and 46.1% of men were categorized as obese.

Body composition and dietary characteristics of the total population, separated by tertiles of energy-adjusted protein intake, are shown in Table 2. Those with HP intake had lower ALST, LST and FM when compared to LP and MP ($P < 0.001$; Table 2). When adjusted for height (m^2), ALSTI (kg/m^2), LSTI (kg/m^2) and FMI (kg/m^2) remained significantly lower in those with HP when compared to LP or to MP ($P < 0.001$). However, when adjusted for total body weight (kg), relative LST was significantly greater in the HP group, when compared to LP or MP ($P < 0.001$). Total energy intake was greater in the LP group, compared to MP or HP ($P < 0.001$). Although macronutrient intake (kcal/d) differed across groups ($P < 0.001$), the percent of total energy from fat was similar (LP: 32%, MP: 32%, HP: 33%). Carbohydrate intake was lower in the HP group (48% total energy) compared to LP (53% total energy) or MP (51% total energy). Mean protein intake in the HP group was 1.4 g/kg/d, relative to 0.91 g/kg/d in LP and 0.81 g/kg/d in MP (Figure 1).

No differences were found between PA tertiles and body weight, WC, LST, or LST (kg/m^2; Table 3). PA3 had greater ALST, ALSTI and relative LST compared to PA1 ($P < 0.001$) or PA2 ($P < 0.05$). PA3 had lower FM compared to PA1 ($P < 0.001$; Figure 2). BMI was significantly lower in PA3 compared to PA1 (Figure 2; $P < 0.001$).

Cardiometabolic biomarkers of health were examined against energy-adjusted protein and PA tertiles (Table 4). HP+PA3 and MP+PA2 had lower CRP, compared to LP+PA1 ($P < 0.05$). IGF-1 was higher in HP+PA3 compared to LP+PA1 ($P < 0.05$) or MP+PA2 ($P < 0.001$, respectively). HP+PA3 was higher in total cholesterol ($P < 0.001$), HDL ($P < 0.05$) and LDL ($P < 0.05$, $P < 0.001$, respectively) when compared to LP+PA1 and MP+PA2. Insulin concentration was lower in HP+PA3 compared to LP+PA1 ($P < 0.001$). There were no significant differences between tertile groups for TNF- α, IL-6, triglycerides or glucose concentrations.

DISCUSSION

In this cross-sectional analysis of the BPRHS, the highest tertile of energy-adjusted dietary protein intake (≥1.11 g/kg/d) and greater PA (> 3.2 km/d) were associated with healthier body composition phenotype and cardiometabolic risk factors. Similarly to the energy-adjusted protein tertiles, PA tertiles resulted comparable body composition differences when adjusted for total body weight. Thus, it appears that higher PA combined with a higher protein diet (HP) is the most advantageous to relative LST. However, PA tertiles showed higher LST (kg) in HP compared to LP and MP without adjusting for total body weight. Indeed, the combination of HP (> 1.11 g/kg/d) and PA (> 3.2 km/d) was associated with healthier body composition as well as reduced CRP and insulin concentrations, indicating reduced inflammatory response and better insulin-sensitivity compared to the combination of LP (< 0.91 g/kg/d) and PA (<0.8 km/d).

In this analysis, higher energy-adjusted protein intake was positively associated with healthier body composition phenotypes. Previous research has shown findings similar to ours in both observational and intervention studies [8,33–36]. In the Health ABC (Aging & Body Composition) Study cohort, those in the highest protein quintile (> 1.1 g/kg/d) retained greater lean mass over a 3-y follow up compared to those in the lowest protein quintile (< 0.7 g/kg/d) [33]. Additionally, moderate protein diets (25% total energy intake) can elicit similar body composition phenotypes to those consuming higher protein diets (40% of total intake) when combined with exercise in an overweight population [8,9,19]. In the current study, there was a significant positive relationship between LST and protein intake (≥1.11g/kg/d; ~19% of total energy intake), which may be a more ideal protein intake for an adult population. Although ≥1.11g/kg/d of may be sufficient protein intake to promote maintenance of LST, it may not be sufficient enough to stimulate FM and body weight loss or to increase LST [37]. Baer et al. showed that whey protein supplementation (56g/d) for 23 weeks stimulated FM loss (-2.3 kg) while maintaining FFM without energy restriction in free-living overweight and obese adults [5,6,19]. Further research is warranted to determine the amount of protein needed to stimulate loss of FM and body weight.

Multiple mechanisms could explain why those who consumed higher protein had significantly lower body weight and higher relative LST and ALST when adjusting for energy intake. In higher protein diets, satiety may be increased [10,11] leading to lower total energy intake which may explain why body weight was lower in HP compared to LP and MP. Higher relative LST is likely influenced by higher metabolic activity of skeletal muscle which is maintained by a diet higher in protein (HP) compared to LP and MP [3,36]. In addition to higher protein intake (HP) being associated with lower body weight and higher relative LST, absolute

FM (kg) was lower in HP compared to LP and MP which largely explains the differences in total body weight between energy-adjusted protein tertiles.

It is well established that higher PA is associated with a healthier body composition and lower cardiometabolic risk factors [18,22,38]. However, in this particular cohort, previous findings suggest that Puerto Rican adults living in the Boston area are likely to present with psychological stress [39], which has been previously associated with being physically inactive [22]. Although PA levels in the current study were low, findings indicate that minor increases in PA were associated with greater relative LST and ALST and lower FM (kg) and BMI (kg/m^2). Higher ALST is particularly important due to its association with functional status and mobility which may influence an individual’s ability to be more physically active The American College of Sports Medicine (ACSM) recommends an accumulation of 10,000 steps/d or approximately 7.6 km [40]. Thus, even the highest PA tertile in this cohort did not perform PA to meet the ACSM recommendations. However, even though < 4.0 km/d (5,250 steps/d) has been associated with many chronic health conditions, there were significant differences in body composition when comparing those that walked < 0.8km/d and those that walked > 3.2 km/d. Although all PA groups were considered to be very low levels of activity, minor increases i.e. PA1 vs. PA3, were associated with healthier body composition.

Previous findings are mixed for CRP and its response to diet where elevated CRP has been found to be a stronger predictor of cardiovascular disease than LDL, and has been associated with body fatness [41,42]. Due et al [41] provided either a higher or lower protein diet to middle aged overweight adults. After six months, no significant effects on inflammatory markers were observed and although the present study did not have a dietary intervention, it is worth noting that energy-adjusted protein intake tertiles alone were not associated with CRP.

However, it is interesting to note that in the preset study, HP+PA3 was associated with significantly lower CRP compared to LP+PA1, suggesting that higher protein intake and PA may be more influential in attenuating the inflammatory response than higher protein intake alone, as supported by previous research [43]. Other proinflammatory cytokines, including IL-6 and TNF-α, did not differ significantly by protein intake or PA level in the present study.

Fasting insulin concentration was significantly lower in men and women that fit both the HP and PA3 (HP+PA3) categories (> 1.11 g/kg/d and >3.2 km/d) compared to LP+PA1 (≤ 0.91 g/kg/d and ≤ 0.8 km/d), which supports previous research suggesting the importance of increased PA for improving insulin regulation [43]. However, in the present study, higher PA was also associated with significantly higher LDL-cholesterol, which contradicts previous findings [44]. The associations between protein intake and PA level with cardiometabolic health observed in this study raise interesting questions in regard to whether diet or PA is the primary regulator cardiometabolic health. Despite our data indicating that differences between LP+PA1, MP+PA2, and HP+PA3 were minimal and that all spent inadequate time being physically active, differences among cardiometabolic biomarkers were still observed. These findings warrant further investigation into the level of PA needed for Puerto Rican adults to maintain a healthy cardiometabolic profile.

Strengths of the current study include the large sample size of Puerto Rican men and women in this cohort; the use of DXA to assess body composition; and the use of an adapted FFQ for the BPRHS population. However, the results should also be interpreted in context of a few limitations. First, the assessment of dietary intake at one time point may not represent long term nutrient intake, and FFQ have the potential to present with reporting inaccuracies [13,33]. Secondly, the observational nature of the current study does not allow us to make causal inferences between protein intake and PA levels with body composition phenotype and cardiometabolic factors.

LIMITATIONS

It should be noted that PA was objectively measured through self-report and not by accelerometry and therefore the PA results may include self-report bias. Additionally, this study did not account for protein source (plant vs. animal) or quality and therefore outcome variables may be influenced by other factors besides total dietary protein. The authors recognize that HP+PA3 had higher LDL when compared to both LP+PA1 and MP+PA2 which may be attributed to protein quality which was not accounted for in this study. Dietary patterns were also not reported and therefore cannot be alluded to in this study.

CONCLUSIONS

In conclusion, the highest tertile of energy-adjusted dietary protein intake (≥1.11 g/kg/d) and greater PA (> 3.2 km/d) were associated with healthier body composition phenotype and cardiometabolic risk factors in the BPRHS cohort. It is important to note that significant associations of PA with healthier body composition and cardiometabolic profiles were observed, despite all PA levels performing inadequate time being physically active. These results also suggest that protein intake ≥ 1.11 g/kg/d may be beneficial for maintaining greater LST (including ALST) when body weight is accounted for in Puerto Rican adults. Given the prevalence of obesity, inflammation and other cardiometabolic risk factors in the population, along with low PA, and lower protein intake, these findings support the need for the development of interventions that focus on the importance of increasing daily PA and protein intake.

Abbreviations

ALST, appendicular lean soft tissue; ALSTI, appendicular lean soft tissue index; CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FMI, fat mass index; LST, lean soft tissue; LSTI; lean soft tissue index; PA, physical activity.

Declarations

Ethics approval and consent to participate

All participants provided written informed consent prior to the start of the study in the language of preference. The data were approved for use by the Florida State University Institutional Review Board.

Availability of data and materials

All data analyzed during this study are included in this published article and its supplementary information files.

Competing interests

MJO serves on the Scientific Advisory Board for Dymatize Nutrition, the International Protein Board, and Clif Bar. PJA serves for Dymatize Nutrition, International Protein Board, and Isagenix. AFB, CMP, SG, SML, and KLT have no conflicts of interest.

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Authors' contributions

The authors' responsibilities were as follows – KLT: original study design for BPRHS; SG, AFB, SML, CMP, and MJO: data analysis; AFB, MJO, CMP, SML, PJA: data interpretation and manuscript writing; AFB, MJO and CMP: study oversight; AFB, MJO, CMP, SG, SML, PJA, KLT: data interpretation and critical revision of the manuscript. The authors had no conflicts of interest.

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Figure Legends

FIGURE 1. Mean macronutrient (kcal/d) consumption across tertiles of protein intake.
Data were adjusted for energy intake (kcal/d) by using linear regression analysis. LP (lower protein tertile, ≤ 0.91 g/kg/d); MP (moderate protein tertile, ≥ 0.91 g/kg/d ≤ 1.11 g/kg/d); HP (higher protein tertile, >1.11 g/kg/d). LP, *n* = 317; MP, *n* = 316; HP, *n* = 316; ** *P* ≤ 0.001between LP and MP; ## *P* ≤ 0.001 between MP and HP; ◆◆ *P* ≤ 0.001 between LP and HP.

FIGURE 2. Fat mass and lean soft tissue across tertiles of protein intake and physical activity.
Data were adjusted for body weight (kg) and energy intake (kcal/d) by using linear regression analysis. LP (lower protein tertile, ≤ 0.91 g/kg/d); MP (moderate protein tertile, ≥ 0.91 g/kg/d ≤ 1.11 g/kg/d); HP (higher protein tertile, >1.11 g/kg/d); PA1 (low physical activity tertile, < 0.8 km/d); PA2 (moderate physical activity tertile, ≥ 0.8 km/d ≤ 3.2 km/d); PA3 (high physical activity tertile >3.2 km/d). LP, *n* = 317; MP, *n* = 316; HP, *n* = 316; PA1, *n* = 364; PA2, *n* = 235; PA3, *n* = 351. ** *P* ≤ 0.001 between LP and MP; ## *P* ≤ 0.001 between MP and HP; ◆◆ *P* ≤ 0.001 between LP and HP; * *P* < 0.05 between PA1 and PA2; ❖❖ *P* < 0.001 between PA1 and PA2.