

# Animal-Based Dietary Protein Intake Is Not A Risk Factor For Metabolic Syndrome Among Young Or Middle-Aged Females

Nutrition and Metabolic Insights  
Volume 15: 1–7  
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DOI: 10.1177/11786388221107800



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## ABSTRACT

**BACKGROUND:** Metabolic syndrome (MetS) increases risk for morbidity and premature mortality. Blood pressure, waist circumference, and fasting triglycerides (TG), blood glucose (BG), and high-density lipoprotein cholesterol (HDL) are factors for determining MetS. The Simple Method for Quantifying Metabolic Syndrome (siMS) score and risk score estimate risk of MetS. The purpose for this study was to examine the relationship of animal-based (ABP) and plant-based protein (PLP) with MetS as estimated by siMS score and risk score. Physical activity is another important consideration in MetS as it can reduce blood pressure, waist circumference and blood glucose, and affect blood lipid and lipoprotein concentrations.

**METHODS:** A cross-sectional study examined whether physical activity (PA) level and dietary protein source (i.e., animal- or plant-based) among young (18-24 years) and middle-aged (45-60 years) females were associated with siMS score and siMS risk score. Average time spent in sedentary, light, and moderate-to-vigorous PA (MVPA; min/wk), steps (steps/day), energy intake (kcal/day), percent dietary protein to total energy intake, ABP and PLP dietary intake, and ABP:PLP ratio (g/day) were included in the analysis. Volunteers were recruited from North Dakota and Minnesota from 2017 to 2019.

**RESULTS:** Eighty-one female participants (mean  $\pm$  SD; young,  $n=38$ ,  $20.4 \pm 1.7$  years, middle-aged,  $52.5 \pm 4.8$  years) were included in the independent *t*-tests used to examine group differences in age, body mass index, HDL, BG, TG, systolic blood pressure, waist circumference, energy intake, energy intake percentage of total carbohydrates, fat, protein, ABP, and PLP, ABP:PLP, siMS score, and siMS risk score. Step-wise linear regressions were used to evaluate whether PA level and dietary protein source were predictors of siMS score and siMS risk score among young and middle-aged adult females. There was an inverse relationship between PLP intake and siMS score. The model explained 6.9% of the variance in siMS risk score ( $F_{1,80}=5.93$ ). Plant-based protein intake was inversely related to siMS risk score while light PA was positively associated with siMS risk score. The model explained 16% of the variance in siMS risk score ( $F_{1,80}=7.53$ ). Animal-based dietary protein intake did not impact siMS score ( $p=0.180$ ) and siMS risk score ( $p=0.283$ ).

**CONCLUSIONS:** Plant-based protein intake was associated with a lower risk of MetS via siMS scores, while ABP was not associated. Given the nature of the cross-sectional design of this study, no causal relationship can be determined, but longitudinal studies or randomized control trials to confirm the results from this study are needed in the future.

**KEYWORDS:** Diabetes risk, blood lipids, plant-based dietary protein, physical activity, siMS score

**RECEIVED:** December 20, 2021. **ACCEPTED:** May 28, 2022.

**TYPE:** Original Research

**FUNDING:** The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We acknowledge research funding support from the North Dakota Beef Commission.

**DECLARATION OF CONFLICTING INTERESTS:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Introduction

Metabolic syndrome (MetS) is related to increased morbidity and mortality rates, and the prevalence of MetS increases with age.<sup>1</sup> In the United States (U.S.), MetS is defined as having a cluster of three of the five following risk factors: a waist circumference  $\geq 35$  inches or 88 cm for females (other countries differ), triglycerides (TG)  $\geq 150$  mg/dL or 1.7 mM, high-density lipoprotein (HDL) of  $< 50$  mg/dL or 1.03 mM, systolic

blood pressure (SBP)  $\geq 130$  mm Hg and/or diastolic  $\geq 85$  mm Hg, and blood glucose (BG)  $> 100$  mg/dL or 5.6 mM.<sup>2,3</sup> When multiple risk factors for MetS occur together, an individual has a higher probability of cardiovascular disease (CVD) and type 2 diabetes (T2D) due to microvascular and macrovascular damage.<sup>2,4</sup> Individuals who have direct family members with CVD are genetically predisposed to this lifestyle disease and may need early medical intervention for prevention.<sup>1</sup>



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Dyslipidemia, characterized by high total cholesterol and low HDL increases risk for CVD, the leading cause of death in the U.S. for both men and women.<sup>3</sup> In the 2015–2016 wave of the National Health and Nutrition Examination, more than 12% of adults aged 20 years and older had total cholesterol higher than 240mg/dL, more than 18% of adults had HDL levels less than 40mg/dL, and 7% of U.S. children and adolescents ages 6 to 19 had high total cholesterol.<sup>3</sup> Elevated blood cholesterol has no symptoms and must be determined by blood analysis.<sup>5</sup> As such, dyslipidemia often goes unnoticed.<sup>3</sup> Healthy adults, 20 years and older, should have blood lipid levels checked every 4 to 5 years, and individuals with a family history of CVD and T2D should have levels checked more often.<sup>3</sup> The disease burden on public health and acute care of both CVD and T2D may be attributed to the MetS risk factors,<sup>1-3,6,7</sup> thus assessing the risk of MetS earlier in life is important for preventing chronic diseases later on.<sup>1-3</sup>

Each of the five MetS risk factors can be calculated into the Simple Method for Quantifying Metabolic Syndrome (siMS) score shown in Figure 1.<sup>8</sup> The score can be utilized in research to provide insight for population group recommendations to decrease MetS risk, track trends of siMS over time, and can be utilized for screening in the primary care arena.<sup>8</sup> At least one study has used siMS score to identify younger study participants who were at risk for MetS even though they did not present with >2 of the defining conditions.<sup>9</sup>

Another method for quantifying metabolic risk is the siMS risk score shown in Figure 2. The siMS risk score is calculated using siMS score and follows the recommended risk factor definition of the International Diabetes Federation and the American Heart Association.<sup>1,6</sup>

Beyond the effects of aging, including family history is relevant, as a history of cardio- or cerebrovascular events that occur at an early age (<55 years for brother or father or <65 years for mother or sister) is related to increased CVD risk.<sup>1,2,6</sup>

Increasing age is associated with increasing prevalence of MetS, but MetS also increases if recommended lifestyle choices such as optimal physical activity (PA) and nutrition are ignored.<sup>13</sup> Physical activity is a determinant of siMS score.<sup>10-12</sup> For example, increased frequency and duration of moderate-to-vigorous physical activity (MVPA) can impact siMS score by lowering blood pressure, BG, waist circumference, TG, and increasing HDL.<sup>10-12</sup> Although emerging adults, defined as those 18–25 years and striving to establish independence, are at lower risk for MetS than middle-aged adults due to their younger age,<sup>13,14</sup> younger individuals can develop CVD or T2D later in life as the result of if poor habits formed or cemented in early adulthood, such as a sedentary lifestyle and unhealthy dietary patterns.<sup>1,10</sup> Thus, health screening is essential to determine baseline biomarkers for previously identified risky behaviors affecting this age group,<sup>15</sup> such as excess energy intake, lack of daily MVPA, and increased substance abuse.<sup>16</sup>

$$\text{siMS score} = \frac{2 \times \text{Waist}}{\text{Height}} + \frac{\text{Gly}}{\text{ref.}} + \frac{\text{Tg}}{\text{ref.}} + \frac{\text{TA}_{\text{systolic}}}{\text{ref.}} - \frac{\text{HDL}}{\text{ref.} \left( \frac{\text{male}}{\text{female}} \right) }$$

**Figure 1.** Depicts the equation to calculate siMS score. \*Gly=Glucose, ref.=5.6; Tg=Triglycerides, ref.=1.7; TA<sub>systolic</sub>=Traditional systolic blood pressure, ref.=130, HDL=High-density lipoprotein, ref. male=1.02, ref. female=1.28.

$$\text{siMS risk score} = \text{siMS score} \times \left( \frac{\text{Age}}{45 \text{ or } 50 \text{ (males or females)}} \right) \times \left( \begin{array}{l} \text{Family history of cardio or} \\ \text{cerebro - vascular event} \\ \text{(event = 1.2, else = 1)} \end{array} \right)$$

**Figure 2.** Depicts the equation to calculate siMS risk score.

Daily PA, especially in the recommended dose of volume and intensity,<sup>10-12</sup> can lower risk for MetS. The physiological effects of MVPA, in the recommended amount of at least 150 minutes per week, can reduce weight, reduce blood pressure, improve blood lipid values (such as raise HDL and lower TG), and decrease insulin resistance.<sup>17</sup>

Middle-aged females, 45–60 years, may be at a greater risk for MetS compared to younger females, independent of obesity, as this age group has unique health circumstances (e.g., menopause) that impact MetS risk.<sup>2,13</sup> In support of this notion, the overall prevalence of dyslipidemia was higher in adults aged 40–59 years (17.1%) compared to those aged 20–39 years (7.9%) and 60 years and beyond (12.5%).<sup>3</sup> Outside of differences in age-based cohorts, if females in this age group have an increased waist circumference, elevated TG levels, and hypertension, they are more likely to be at risk for MetS than age-matched males.<sup>2,4</sup> In addition to the greater prevalence of dyslipidemia,<sup>3</sup> middle-aged individuals often have greater access to family medical histories.<sup>4</sup> Therefore, middle-aged adults are more likely to answer “yes” to questions regarding familial cardio- or cerebro-vascular events, increasing siMS risk score.

Dietary macronutrient percentage intake distribution can influence MetS risk variables and siMS score.<sup>12</sup> An inappropriate energy balance for one’s body weight, height, age, and sex can increase BMI and MetS risk factors.<sup>1</sup> There may also be an association of high carbohydrate consumption (i.e., >65% of total caloric intake) and MetS equation factors, especially TG.<sup>18</sup> Additionally, limited data is available comparing dietary protein percentage intake of total energy consumption and MetS risk values.<sup>19</sup> Plant-based protein (PLP) diets, at the exclusion of any animal-based dietary protein (ABP), have become more popular in recent years. The Adventist Health Study, which used data collected from 2002 to 2007, concluded that a high contribution of “meat” in the diet increases risk of CVD-related mortality.<sup>20</sup> However, the “meat” pattern used for the Adventist

study included animal-based fat such as butter, and other foods high in saturated fat, which are known to increase total blood cholesterol values, specifically LDL cholesterol.<sup>20</sup> Despite the recent popularity of exclusive PLP intake for health, confirmation of the disparate health effects attached to PLP compared to ABP are mixed and based on patterns including “high red meat” which not only included red meat but also potatoes and gravy.<sup>21</sup>

The primary aim of this study was to examine relationships for both siMS score and siMS risk score with PA level and dietary protein source (i.e., ABP or PLP) among young and middle-aged adult females. The secondary aim was to observe differences among ABP and PLP, PA, and other variables between young and middle-aged healthy adult females.

## Methods

### Design

A cross-sectional study was designed to observe whether PA level and dietary protein source (i.e., ABP or PBP) among healthy young and middle-aged adult females were predictors of siMS score and siMS risk score. Average time spent in sedentary, light PA and MVPA (min/wk), average steps (steps/day), energy intake (kcal/day), percentage protein energy to total energy, total grams of ABP, and PLP, and ABP:PLP ratio (g/day) were included in the analysis.

### Participants

Healthy female volunteers aged 18–24 years (young) and 45–60 years (middle-aged) were recruited from the Fargo, ND and Moorhead, MN and surrounding areas between October 2017 and December 2019. The inclusion/exclusion criteria were the following: not currently using any nicotine product, free of any untreated or nonresponsive diseases or conditions including neuromuscular disease or conditions that might undermine muscle health, such as diabetes, ambulatory without any assistance, and had to include both animal-based and plant-based foods in their diets. Recruitment flyers and contact information were posted at local community centers, shopping malls, coffee shops, fitness facilities, and universities. Participants were also recruited using word of mouth, social media, and numerous email listservs. After obtaining IRB approval from North Dakota State University, participants were invited to attend a voluntary instructional session which included study-specific training. After signing informed consents, all participants completed the Physical Activity Readiness Questionnaire (PAR-Q)<sup>22</sup> and a standard health history questionnaire which included a “yes” or “no” check box for the siMS risk score specific question “Have your father or brother(s) had heart disease prior to age 55 OR mother or sister(s) had heart disease prior to age 65?” The answer to this health history question was utilized for the siMS risk score.

### Measures

*Blood spot testing, blood pressure, and anthropometric measurements.* Blood was collected within 1 hour of waking between 6:00 a.m. and 9:00 a.m. Capillary blood drops were collected from the fingertip and dried on filter paper as dried blood spots (DBS).<sup>23,24</sup> Blood spots were dried for at least 4 hours, and DBS filter cards were then stored at  $-80^{\circ}\text{C}$  until shipment in bulk to ZRT Laboratory (Beaverton, OR) for testing. The DBS assays were performed by immunoassays.<sup>23,24</sup> The ZRT Laboratory is a CLIA-approved laboratory. Results of the laboratory analyses were provided to the study investigators. Dried blood spot testing has shown strong correlation with conventional serum tests, making it a reliable and convenient tool for screening cardiometabolic risk factors.<sup>23,24</sup> The blood spot samples were taken after 8–12 hours of fasting by study-trained research assistants. Participants whose blood values were out-of-range (i.e., TG values over  $> 600\text{ mg/dL}$ , HbA1c  $< 3.5\%$ , and HDL  $< 20\text{ mg/dL}$ ) were excluded ( $n = 7$ ) from the data set as outliers. The HbA1c value from the blood spot samples was converted into estimated average BG (mmol/l) for the siMS score equation using a formula from American Diabetes Association.<sup>25</sup>

Resting blood pressure readings were obtained in the seated position after 5 minutes of rest using a manual sphygmomanometer and stethoscope (American Diagnostic Corporation, Hauppauge, NY). After resting measures, height was measured to the nearest 0.1 cm, using a stadiometer (Seca 213, Chino, CA) and body mass, to nearest 0.1 kg, was recorded (Denver Instrument DA-150, Arvada, CO). Anthropometric measurements to collect data regarding the MetS risk factors for weight, waist and height were taken with shoes and outer layer of clothing removed. Waist circumference was measured between the iliac crest and the lowest rib, typically at the level of the umbilicus, using a Gulick (Fitness Mart Division of Country Technology Inc., Gays Mills, WI) spring-loaded measuring tape to the nearest cm and the means of two waist measurements were used for the analysis.

*Physical activity.* Accelerometers were given to and sent home with participants. Physical activity was recorded using Actigraph (Pensacola, FL) GT9X accelerometers worn on the non-dominant wrist for seven consecutive days.<sup>26</sup> Participants were instructed to wear the accelerometer during all waking hours except activities involving water (e.g., bathing or swimming). The raw acceleration data were collected at 80 Hz, and processed in R software (<http://cran.r-project.org>) using the GGIR package (version 1.10-10).<sup>27</sup> A sleep log was provided to help delineate non-wear time from time spent sleeping. Non-wear time was defined as intervals of at least 90 minutes of zero counts with allowance of two-minute interval of non-zero counts within a 30-minute window,<sup>26</sup> thus only valid time during waking hours of each day was included for statistical

analyses. The minimum number of wear days was four, including one weekend or one non-routine day, over the weeklong collection period, with a minimum wear time of 10 hours/day.

*Three-day dietary log.* During the same 7 days as accelerometer tracking, participants were required to fill out a three-day dietary log along with demographic questions. Participants were asked to record all dietary intake for 3 days: two typical, or weekdays, and one atypical, or weekend day. Dietary logs were carefully imported into the Food Processor software (ESHA, Salem, OR), supervised by a registered dietitian nutritionist.<sup>28</sup>

### Statistical analysis

All statistical analyses were performed using SPSS version 24 (IBM, Armonk, NY). Descriptive statistics are reported as mean  $\pm$  SD, and statistical significance was set at  $\alpha=0.05$ . Stepwise linear regression models evaluated our research questions: whether PA level and dietary protein source were associated with siMS score and siMS risk score among young and middle-aged females. Sedentary behavior, light PA, MVPA, steps, energy intake, total protein percent of energy, ABP, and PLP, and ABP:PLP were included in these stepwise analyses. Independent t-tests explored differences between age-based cohorts, comparing BMI, HDL, BG, TG, SBP, waist circumference, energy intake, energy intake percentage of total carbohydrates, fat, protein, ABP, and PLP, ABP:PLP, siMS score, and siMS risk score between young and middle-aged females.

## Results

### Participants

A total of 81 females were included in the analysis (young = 38; middle-aged = 43) (Table 1); it was estimated using G\*Power (version 3.1.9.6, Kiel, Germany) assuming a “medium” effect size that the 81-person sample had 96% power. Only one participant in the young group and three participants in the middle-aged group reported a family history of cardio- or cerebro-vascular disease for the siMS risk score analysis question.

### Physical activity

Middle-aged females had significantly higher levels of light PA compared to young females. No significant differences were observed for sedentary time, MVPA, or steps (Table 2).

### Cardiometabolic profiles

Young females had significantly lower values of BG compared to middle-aged females. No significant differences were observed for TG or HDL (Table 3).

**Table 1.** Descriptive statistics of young (18-24 years) and middle-aged females (45-60 years).

	YOUNG (N=38)	MIDDLE-AGED (N=43)	P (GROUP)
Age (years)	20.4 $\pm$ 1.7	52.5 $\pm$ 4.8	<0.001 <sup>†</sup>
BMI (kg/m <sup>2</sup> )	24.5 $\pm$ 3.2	26.0 $\pm$ 4.9	0.111
Waist (cm)	99.5 $\pm$ 7.6	85.0 $\pm$ 13.0	<0.001 <sup>†</sup>
SBP (mmHg)	114.8 $\pm$ 7.3	120.2 $\pm$ 10.5	0.008 <sup>*</sup>
DBP (mmHg)	72.8 $\pm$ 8.1	81.0 $\pm$ 8.6	<0.001 <sup>†</sup>

Note. All values are represented as mean  $\pm$  SD.

BMI, body mass index, SBP, systolic blood pressure, DBP, diastolic blood pressure.

<sup>\*</sup>Significant difference from young females ( $P < 0.050$ ).

<sup>†</sup>Significant difference from young females ( $P < 0.001$ ).

### Dietary intake

Young females consumed significantly greater PLP and lower total percentage fat intake. No significant differences were observed for energy, total carbohydrate, total protein, ABP, and ABP:PLP between young and middle-aged females (Table 4).

### siMS Score and siMS Risk Score

Middle-aged females showed significantly higher siMS risk score than young females (Table 5).

### Regression Analysis

Of sedentary, light, and MVPA, PLP, ABP, ABP:PLP, total steps, energy (kcal), and percent of kcal from protein, only PLP, not ABP, was predictive for siMS score and only light PA and PLP were predictors for siMS risk score. There was an inverse relationship between PLP intake in both siMS score and siMS risk score (see Table 6).

For every 1% increase in energy from PLP, siMS score was lowered by 0.135 and siMS risk score was lowered by 0.179. The regression model for siMS explained 6.9% of the variance in siMS score ( $F_{1,80} = 5.93$ ,  $R^2 = 0.069$ , adjusted  $R^2 = 0.057$ ,  $p = 0.017$ ). Plant-based protein intake was inversely related to siMS risk score while light PA was positively associated with siMS risk score. The regression model for siMS risk explained 16% of the variance in siMS risk score ( $F_{1,80} = 7.53$ ,  $R^2 = 0.160$ , adjusted  $R^2 = 0.139$ ,  $p = 0.043$ ).

## Discussion

The main findings of this study were the significant inverse relationships observed between PLP and siMS score and siMS risk score. In support of the benefits of PLP, a recent review that dissected dietary intake and CVD risk reported nutritional patterns heavier in PLP had lower risk for CVD when compared to “the typical American diet.”<sup>21</sup>

**Table 2.** Physical activity of young (18-24 years) and middle-aged females (45-60 years).

	YOUNG (N=38)	MIDDLE-AGED (N=43)	P (GROUP)
Sedentary Time (min/wk)	398.3 ± 91.6	378.8 ± 79.8	0.312
Light PA (min/wk)	307.4 ± 73.3	357.7 ± 55.5	<0.001†
MVPA (min/wk)	90.5 ± 27.4	96.4 ± 29.9	0.354
Steps (#/day)	10841.0 ± 2733.1	11897.8 ± 2802.9	0.088

Note. All values are represented as mean ± SD.

PA, Physical activity; MV, Moderate-to-vigorous.

†Significant difference from young females ( $P < 0.001$ ).

**Table 3.** Cardiometabolic profile of young (18-24 years) and middle-aged females (45-60 years).

	YOUNG (N=38)	MIDDLE-AGED (N=43)	P (GROUP)
Glucose mmol/L (mg/dL)	4.4 ± 0.8 (79.3 ± 14.4)	5.0 ± 1.1 (90.1 ± 19.8)	0.007*
Triglycerides mmol/L (mg/dL)	1.9 ± 0.6 (168.1 ± 53.14)	1.9 ± 0.9 (168.1 ± 79.7)	0.905
HDL mmol/L (mg/dL)	1.6 ± 0.4 (61.9 ± 15.6)	1.8 ± 0.7 (69.6 ± 27.1)	0.120

Note. All values are represented as mean ± SD.

HDL, High density lipoprotein.

\*Significant difference from young females ( $P < 0.050$ ).

**Table 4.** Dietary intake of young (18-24 years) and middle-aged females (45-60 years).

	YOUNG (N=38)	MIDDLE-AGED (N=43)	P (GROUP)
Energy Intake (kcal/day)	2118.6 ± 619.3	1998.3 ± 472.0	0.332
Protein (%)	17.4 ± 6.2	16.6 ± 3.8	0.478
ABP (%)	11.7 ± 6.2	11.7 ± 3.9	0.976
PLP (%)	5.2 ± 1.5	4.6 ± 1.3	0.035*
ABP:PLP (g)	2.4 ± 1.3	2.9 ± 1.6	0.135
Carbohydrate (%)	47.5 ± 6.0	45.0 ± 8.2	0.108
Fat (%)	35.1 ± 6.2	38.4 ± 7.1	0.027*

Note. All values are represented as mean ± SD.

ABP, Animal-based protein; PLP, Plant-based protein.

\*Significant difference from young females ( $P < 0.050$ ).

However, this review also concluded that consumption of unprocessed ABP and low saturated fat ABP (e.g., lean beef and pork, fat-free dairy) lowered incidence of cerebral and cardiovascular events.<sup>21</sup> Although our results indicate a beneficial effect of increasing PLP intake, our study does not imply that one should reduce ABP, as ABP was unrelated to siMS score or siMS risk score.

**Table 5.** siMS score and SiMS risk score of young (18-24 years) and middle-aged females (45-60 years).

	YOUNG (N=38)	MIDDLE-AGED (N=43)	P (GROUP)
siMS score	2.4 ± 0.5	2.5 ± 0.9	0.474
siMS risk score	1.0 ± 0.2	2.7 ± 1.0	<0.001†

Note. All values are represented as mean ± SD.

†Significant difference from young females ( $P < 0.001$ ).

**Table 6.** Stepwise regression determinants for siMS score and SiMS risk score.

	β ± SE	P	R <sup>2</sup>	ADJUSTED R <sup>2</sup>
<b>siMS score</b>			0.069	0.057
Constant	3.156 ± 0.283	<0.001		
PLP (%)	-0.135 ± 0.056	0.017		
<b>siMS risk score</b>			0.160	0.139
Constant	1.193 ± 0.831	0.155		
Light PA (min/wk)	0.005 ± 0.002	0.009		
PLP (%)	-0.179 ± 0.087	0.043		

Note: β, Beta; SE, Standard error; PLP, Plant-based protein; PA, Physical activity.

In addition, we found a positive relationship between light PA and siMS risk score. For every additional minute of light PA per week, siMS risk score increased by 0.005. Light PA (minimal active steps, e.g., low intensity walking at home, school, or office setting) has been identified to be more beneficial than sedentary activity for improving SBP and BG. However, more time (minutes per week) spent in light PA may imply less time spent in MVPA. This suggests that females who spend less time in light PA, such as through MVPA, could decrease siMS risk score.

In this investigation, dietary intake of PLP was significantly higher in young females than middle-aged females. Lin et al. (2019) examined dietary intake and PA patterns of U.S. college students (aged  $20.6 \pm 2.07$  years;  $n=237$ ; females=172) and found 33% of the female participants consumed legumes at least one to two times per week.<sup>15</sup> This may suggest that the young female college students have greater access to PLP through campus meal plans or rely on quick and affordable PLP options at home, such as rice and beans.<sup>16</sup> In addition to this, young females may associate ABP with unnecessary weight gain, regardless of dietary recommendations.<sup>16</sup> Young females are more likely to be influenced by social and cultural norms regarding diet, which may lead to restriction of ABP during this life period.<sup>19</sup> These influences could contribute to dietary choices of foods that contain less bioavailable forms of iron and vitamin B12 than ABP foods such as lean meat.<sup>20</sup> Both iron and vitamin B12 are nutrients of concern for young females who are at an increased risk for deficiency related to monthly menstrual cycles.<sup>20</sup>

A significantly higher energy intake percentage of fat was observed in middle-aged females ( $38.4 \pm 7.1$  %) compared to young females ( $35.1 \pm 6.2$  %). Women in our work ate more fat compared to data from the Centers for Disease Control and Prevention, in which females aged 25-44 years consumed an average dietary fat of 34.8%, whereas females aged 45-64 consumed an average of 35.1%.<sup>29</sup> As mentioned previously, diets that include unprocessed and low saturated fat ABP foods (e.g., lean beef and pork, fat-free dairy) have been shown to lower incidence of cerebral and cardiovascular events.<sup>21</sup> This leads us to believe the significantly higher dietary fat intake in middle-aged females was negligible and may be explained by an increased awareness to consume a more heart healthy diet for reducing CVD risk while aging.<sup>11</sup>

In this study, young females consumed a slightly higher percentage of carbohydrate intake ( $47.5\% \pm 6.0$ ) than middle-aged females ( $45.0\% \pm 8.2$ ). While carbohydrate intake and TG values were not significantly different between groups ( $P=0.108$  and  $p = 0.905$ ), BG values were ( $P=0.007$ ). Middle-aged females had significantly higher BG ( $5.0 \text{ mg/dL} \pm 1.1$ ) in comparison to young females ( $4.4 \text{ mg/dL} \pm 0.8$ ). There are metabolic changes that occur with menopause can alter body composition by increasing fat mass and decrease lean mass.<sup>30</sup>

An interesting, yet concerning finding, was the significant difference in the waist circumference between young ( $99.5 \pm 7.6$  cm) and middle-aged ( $85.0 \pm 13.0$  cm) females. Waist circumference is a well-recognized method for the assessment of CVD risk.<sup>1,2</sup> While young females did not have a significantly higher siMS score and siMS risk score than middle-aged females, waist circumference tends to increase with age related to increased abdominal adipose tissue. This may lead to greater increases in siMS score and siMS risk score for these young females later in life.<sup>31</sup>

The strengths of the investigation included the objective measure of PA by accelerometry and a contribution of literature on underrepresented middle-aged females. Another strength was the three-day dietary log training, completed by a registered dietitian nutritionist, that included food portioning handouts and serving size guides. While beneficial for assessing dietary intake, the limitations of predicting energy and macronutrient consumption from dietary logs are well known and are only estimates of self-reported information.<sup>28</sup>

## Conclusion

In sum, dietary consumption of PLP was associated with lower siMS score and siMS risk score, whereas ABP was unrelated to siMS score and risk score. We also found a positive association of light PA with MetS with more light PA. The siMS score and risk score provide simple methods for early screening for MetS. Future studies should investigate the role of dietary protein source plays in MetS, using longitudinal or randomized controlled-trial design.<sup>5</sup>

## Acknowledgements

We wish to recognize Johanna Weber, who painstakingly separated animal- from plant-based protein sources from participant reported dietary intake. We also acknowledge Linh Tran, Lindsey Johnson, and Johanna Weber for entry of the dietary log data.

## Author Contributions


Study concept and design: KH, SS; acquisition of data: BS, KT, CK, NJ, AB, RS, KH, WB, and SS; analysis and interpretation of data: BS, KT, CK, WB; drafting of the manuscript: BS, KT, and SS; critical revision of the manuscript: BS, CK, and SS; statistical analysis: KT, CK, NJ; obtained funding: KH and SS; administrative, technical, or material support: SS; and study supervision: KH and SS.

## Ethics declaration

The host university's Institutional Review Board for the protection of human participants approved all procedures.

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