Individuals Diagnosed with Phenylketonuria are Not at Greater Risk for Cardiovascular Disease

Nadine Assaf, MS, RDN, LDN[1*], Ruth Williams-Hooker, MS, RD, EdD, FAND[2], Dale Bowman, PhD[3]

[*1]Nutrition Therapy Department, Methodist Le Bonheur Healthcare, 7691 Poplar Avenue, Germantown, TN 38138, USA.

[2]College of Health Sciences, University of Memphis, 495 Zach Curlin Drive, Memphis, TN 38152, USA.

[3]Department of Mathematical Sciences, Institute for Intelligent Systems, University of Memphis, 495 Zach Curlin Drive, Memphis, TN 38152, USA.

RESEARCH

Please cite this paper as: Assaf N, Williams-Hooker R, Bowman D. Individuals diagnosed with phenylketonuria are not at greater risk for cardiovascular disease. Journal of Food & Nutritional Sciences [2020] 2(1): 48-58.

***Corresponding Author:**

Nadine Assaf, MS, RDN, LDN

Nutrition Therapy Department, Methodist Le Bonheur Healthcare, 7691 Poplar Avenue, Germantown, TN 38138, USA; Tel: (901) 340-2859; E-mail: nadineassaf11@gmail.com

ABSTRACT

Phenylketonuria (PKU) is an inborn error of metabolism in which the phenylalanine hydroxylase (PAH) enzyme, that converts phenylalanine to tyrosine, is defected. This defect results in a high serum and brain phenylalanine level, which can lead to serious health complications. The primary treatment of PKU is maintaining the level of serum phenylalanine by following a protein restricted diet. Although the PKU diet is mainly plant-based, many items that are phenylalanine-free are also higher in calories, carbohydrates and fat, predisposing this population to higher fat intakes and obesity risk. High fat diets and obesity are two indicators of increased cardiovascular disease (CVD) risk. Therefore, the aim of this retrospective study was to look at fat intakes and obesity to investigate whether individuals, between the ages of 1-50, with PKU were at an increased risk of CVD. In this study, body mass index (BMI), body mass index percentage (BMI%) and weight per length (WPL) statistics were used to analyze obesity, while percentage of calories coming from fat (PCF) was used to analyze fat intake. This study did not identify a significant increased risk for CVD in its participants as all studied average variables were found to be within or below the standard. Although the standard for obesity was not met, this study did identify mean values in the overweight category and upper confidence limits in both the overweight and obese categories for individuals younger than the age of two and individuals older than the age of eighteen. Moreover, higher BMI and PCF values were found in females than males. With these findings, this study did recognize that females and certain age groups are at greater risk for the development of CVD. This study also identified lower PCF values in younger-aged participants, indicating a risk of fat, fatty acid, and fat-soluble vitamin deficiencies.

Key Words: phenylketonuria, phenylalanine hydroxylase enzyme, cardiovascular disease, body mass index, weight per length, percentage of calories coming from fat.

LIST OF ABBREVIATIONS

Word	Abbreviation
Phenylketonuria	PKU
Cardiovascular Disease	CVD

Body Mass Index	BMI
Body Mass Index Percentile	BMI%
Weight Per Length	WPL
Percentage of Calories Coming from Fat	PCF
Phenylalanine Hydroxylase	PAH
University of Tennessee Health Science Center	UTHSC
Boling Center for Developmental Disabilities	BCDD
Percent	%
Meters	m
Kilograms	kg
Less Than	<
Less Than or Equal to	≤
Greater Than	>
Greater Than or Equal to	≥

INTRODUCTION

Phenylketonuria (PKU) is a rare, autosomal recessive, metabolic disorder in which the essential amino acid phenylalanine builds up in the body's blood and tissues. This is the result of a defect in the phenylalanine hydroxylase (PAH) enzyme, that is responsible for converting phenylalanine to tyrosine [1-3]. Phenylalanine is often found naturally in proteins, such as beef, poultry, fish, milk, yogurt, eggs, cheese, soy products, nuts, and seeds [4]. Aspartame, one of the most common artificial sweeteners, is formed from phenylalanine, aspartic acid, and methanol [5,6]. Two brands of artificial sweeteners containing aspartame are NutraSweet and Equal [5]. A buildup of phenylalanine can lead to serious, irreversible complications, such as memory loss, epilepsy, and developmental delay. Therefore, a life-long natural protein restricted diet is recommended to prevent these complications [1-3]. This can be achieved by being on a phenylalanine-free formula, a low-protein diet, and a low-aspartame diet [2,7,8]. The level of diet restriction depends on the severity of the PAH enzymatic defect [9]. However, the allowed amount of fat and calories in the PKU diet is not restricted, and is based on an individual's recommended dietary intake, like that of healthy individuals [10,11]. The intake of fat is a minor concern for those following the PKU diet; however, higher intakes of fat are strongly associated with obesity and cardiovascular disease (CVD) [12].

The aim of this study was to investigate the potential risk of CVD in the PKU population by analysing their dietary fat intake and, depending on age, body mass index (BMI), body mass index percentage (BMI%), or weight per length (WPL), as described in Table 1. By using these variables, this study determined whether or not this population was at a higher risk for developing CVD. For the purpose of this study, obesity was analysed through BMI, BMI% and WPL statistics and fat intake was analysed through percentage of calories coming from fat (PCF). Lower BMI, BMI%, WPL, and PCF values indicated a lower risk for CVD. While opposite, a higher BMI, BMI%, WPL, and PCF indicated a higher risk for CVD [13,14].

METHODS

This retrospective study was done in Memphis, Tennessee at The University of Tennessee Health Science Center's Boling Center for Developmental Disabilities (UTHSC BCDD) and the University of Memphis from January to November of 2017. Medical records for participants diagnosed with PKU were obtained from the office of nutrition at the BCDD for analysis. Approvals from the institutional review boards at both the UTHSC BCDD and the University of Memphis were obtained.

Study Population and Recruitment

Participants included in this study met the following criteria:

a. Between the ages 1-50 years
b. Diagnosed with PKU (Phenylketonuria)
c. On a low-phenylalanine diet since birth
d. Treated at the University of Tennessee Boling Center for Developmental Disabilities

e. Had 10 food record and/or anthropometric measurements available for use in medical chart

Food Records and Analysis

At the BCDD, patients or their guardians are asked to bring a three-day food record to every appointment they have with the dietitian. All foods and drinks, including formula, consumed the three days directly before the appointment are noted on the three-day record. These three-day food records are then analysed by the metabolic dietitian using an online program called MetabolicPro (Genetic Metabolic Dietitians International). The dietary analyses are then stored in medical charts, which were then accessed at the time of this study to collect the PCF data. All food records used in this study were analysed using MetabolicPro between 2010-2017. The dietary analyses created by MetabolicPro from food records provide calories, protein, fat, carbohydrates, percentage of energy coming from each macronutrient, and micronutrients.

Anthropometric Measurements and Analysis

At each participant's appointment with the dietitian at the BCDD, the nursing staff at UTHSC utilizes various anthropometric scales to obtain weight, height, BMI, BMI%, and WPL measurements. The anthropometrics are recorded into each patient's chart, which were then collected at the time of this study. The data collected was noted on a Microsoft Excel Data Collection and Analysis spread sheet by the parameter.

To obtain height, the nurse assisted each participant, older than two years, to stand in front of a stadiometer, which provides height measurements in both centimeters and inches. The nurse then converted the height measurements to feet and inches. The height measurement was then inserted into a computer system, which was connected to a floor scale. As the nurse assisted the individual onto the large floor scale, the connected computer indicated the participant's weight and BMI, which was later converted into a percentile by the nurse. For purposes of this study, BMI% data was collected for analysis on participants between the ages three-eighteen years, while BMI data was collected on participants nineteen years and older. Based on BMI, participants were categorized by normal weight, underweight and overweight, indicated in Figure 1 [13].

For participants two years old and younger, a length measurement was taken by the nursing staff through an infantometer. This length measurement was then compared to the child's weight through growth charts, obtaining the participant's weight per length percentile. In this study, the WHO (0-24 months) growth chart was used for participants younger than twenty-four months [15]. The weight and height of any individual between twenty-four and thirty-six months were plotted on the CDC (0-36 months) growth chart [16].

Both BMI% and WPL in the medical charts were indicated as percentile ranges; therefore, the online website, PediTools.org, was used to obtain exact BMI and WPL percentages by inserting participant age, gender, height, and length information. PediTools is an online website that allows anthropometrics to be inserted and compared to different growth charts. Microsoft Excel Statistics (Microsoft, 2015) was used for the data collection process, patient stratification, and statistical analysis. Then, exact BMI% and WPL measurements were categorized to weight status categories using Figure 2 [14].

Patient Stratification

Participants were divided into different age groups (Groups 1, 2, and 3) based on their recommended PCF ranges (Table 2). Each group was further divided into males and females, forming six new groups for the gender specific analyses. The groups used in the PCF analysis were also used in the BMI and BMI% analysis. However, length measurements are taken on individuals younger than the age of two, as they are too young for height measurements; therefore, WPL data was collected. Due to this, participants in Group 1 were further divided into two separate groups: 1-2 years old (Group 1a) and 2-3 years old (Group 1b). A

WPL analysis, instead of BMI and BMI% analyses, was completed on those in Group 1a. Participants were likely included into more than one age group, as the aim of this study was to collect a data set of ten PCF, BMI, BMI%, WPL values, which therefore, would have been reflective of appointments at different times of a participant's life.

Statistical Analysis

The statistical analysis included the use of confidence intervals, which were constructed for the mean values of the PCF, BMI, BMI%, and WPL variables. If needed, a multivariate t-test, or Hoteling T^2, was used in order to test whether the variable's means were equal to the recommended values within each age group. Individual t-tests were used in this study, testing PCF values to the lower and upper limits of the recommended ranges for each age group, BMI to ≥30 kg/m2, BMI% to ≥95%, and WPL to ≥95%.

A statistical analysis was also performed on the six gender-specific formed groups. This study was to perform analyses on both genders in Group 1, 2, and 3; however, Group 1 was not analysed for gender differences because there was only 1 female so no estimate of variance could be obtained. Confidence intervals were performed to compare the recommended PCF, BMI, WPL, and BMI% values to the participant's values. The individual t-tests were performed to test PCF values to the lower and upper limits of the recommended range, BMI% to ≥95%, WPL to ≥95%, and BMI values to ≥ 30. Also, individual t-tests were performed to compare tested variables between male and female groups.

RESULTS

Although the aim of this study was to include thirty-seven participants, there were a total of thirty-eight participants, ranging from 1 to 50 years old. With realization that collecting ten records for every participant was not feasible, it was required that every participant had at least one record that summarized their appointment with the metabolic dietitian at the UTHSC BCDD. Each record consisted of three-day food records and/or anthropometric measurements. Medical records utilized were from different times throughout participants' lives; therefore, some participants were included into different age groups. The participant related information for the PCF analysis is shown in Table 3. Although all participants were stratified according to PCF recommended intake ranges, the variance in age affected the BMI, BMI%, and WPL data collected; therefore, the groups had to be further divided, as shown in Table 4. Furthermore, although BMI% values were collected on participants three years-old and older, two two-year old participants were found during data collection to have both WPL and BMI% data noted in their charts. These two participants were therefore included in both Group 1a and Group 1b.

To compare the fat intake and BMI of these patients with recommended values, 95% confidence intervals were computed as summarized in Table 5.

Individual t-tests were conducted to test whether the fat intake was greater than the upper limit of the recommended range for each age and whether the BMI is 30 or greater (indicating obesity). Then analysis was done considering gender and there was no significant difference in male vs female in any of the groups. The results are summarized in Table 6.

CONCLUSIONS AND DISCUSSION

An earlier study analysed the glycemic index, glycemic load, and metabolic profile of twenty-one PKU diagnosed individuals and established that these individuals consumed a high intake of carbohydrate and fiber and a low intake of fat and protein [17]. Consistent to these findings, a low to normal intake of fat was observed in this study. While mean values for every group fell within the recommended ranges, the lower confidence levels for groups 2M, 2F, and 3M fell below the recommended ranges. Not only did the lower confidence value for males between the ages of 4-18 (Group 2M) fall below the recommended range, but also the group's mean value; therefore, indicating that this age and gender group consumes less fat and are therefore, at risk for fat, fatty acid and fat-soluble vitamin deficiencies. Despite this, previous research has suggested that along with table food, these individuals are also consuming high fat, high caloric formulas, providing more means for individuals to meet their nutrient needs [18]. This study showed that individuals with PKU between the ages of one-three years, who are most likely to consume both table food and formula, are at high risk for fat, fatty acid, and fat-soluble vitamin deficiencies, as the mean PCF, lower confidence limit, and upper confidence limit fell below the recommended range. Of note, this study also found that the older individuals, especially those within the 19-50 age group, were likely to consume higher amounts of fat in their diet than those of younger ages. Although, all PCF values were within recommended ranges, except group 1 whose intake was below the recommended ranges.

Additionally, authors Blundell JE, Finlayson G, Gibbons C, Caudwell P, and Hopkins M looked at the connection between fat-free mass, resting metabolic rate, and energy intake and determined that men, having a higher amount of fat-free mass, consume more food than women [19]. The current study's findings were inconsistent with those of the Blundell study as higher fat intakes were identified in females in both Group 2 and Group 3. Although higher than the male groups, both values fell within the normal range for total fat intake. Showing strong correlation, not only did the male-specific groups (Group 2M and 3M) have the lower PCF values, but also the lower BMI values when compared to the female groups (Group 2F and 3F). This finding is consistent with the findings of two studies done by authors, Gokmen Ozel H, Ahring K, Bélanger-Quintana A, et al and Burrage LC, McConnell J, Haesler R, et al which is, females with PKU have a higher prevalence of obesity than males [20,21].

Since, individuals with PKU are introduced to an atypical lifestyle at a young age, [22] such as being on phenylalanine-free formulas and protein restricted diets, substituting low-protein foods and adult formulas for high-caloric foods and high-fat infant formulas are believed to be reasons to which PKU individuals are predisposed to obesity [23]. A new interest in the dietary habits of PKU diagnosed individuals has arisen, specifically in regards to obesity prevalence and CVD [24,25]. The mean BMI values for participants 18-50 years (Group 3, Group 3M, and Group 3F) all categorized as overweight, falling between the BMI range of 25.0-29.9 kg/m^2. Furthermore, the upper confidence limit for these participants were all within the obesity category, which identified that obesity is a risk commonly seen in PKU diagnosed individuals between the ages of 19-50 years. Mean BMI% values for participants younger than the age of 18 (Group 1a, Group 1b, Group 2, Group 2M, and Group 2F) were all found to be within normal limits; therefore, the risk for obesity within younger aged PKU diagnosed individuals was not indicated. However, this study did find that although the mean WPL and BMI% values fell within the recommended range, participants in this study younger than the age of 3 years were at the higher end of the recommended range, with an upper limit that fell within the obesity category.

Fat intakes and obesity are strongly associated and are two risk factors for CVD [25,26]. The type of fat consumed has a greater effect on increasing the risk of CVD than does the amount of fat [26]. However, both the presence of adipose tissue and obesity have been found to increase CVD risk through certain mechanisms, such as inflammation, oxidative stress, and endothelial dysfunction [27]. Although the participants is this study neither consumed excessive amounts of fat nor were obese, some groups did have BMI mean values that categorized in the overweight category and upper confidence levels that categorized in the obesity category. Individuals with higher BMI values, specifically those categorized as overweight and obese, are at higher risk for CVD. Obesity has been found to be the leading cause of CVD, with an impact on coronary artery disease, congestive heart failure, arrhythmias, and sudden cardiac death [27,28]. An excessive amount of body fat can raise blood cholesterol levels, blood triglyceride levels, and blood pressures. It has also been shown to cause diabetes, lower HDL levels, and affect blood circulation and fluid levels, which all contribute to a higher risk of CVD [29].

In conclusion, this study analysed the relationship among dietary fat intake, BMI, BMI%, WPL, obesity, and CVD within the PKU population. This study included participants between the ages of 1-50 years and took into consideration gender differences. Based on previous research, it was believed that PKU diagnosed individuals were going to have a higher fat intake and higher BMI values because of their food restriction. However, as none of the participant groups had a mean PCF value higher than the recommended ranges, it was determined that individuals with PKU do not consume an excessive fat intake. More specifically, this study further recognized that individuals of younger ages are likely to be deficient in fat. Although this study did not look at the intake of fatty acids or fat-soluble vitamins specifically, this study suspects deficiencies in both due to the deficiency in fat and recommends this as a further topic of research. In regard to obesity, the mean values for participants in this study were within the normal recommended range; therefore, did not observe obesity in any of the groups. However, this study did recognize mean values that fell into the overweight category, and upper confidence levels that fell in either overweight or obese categories. Furthermore, this study found higher average values in females than males, indicating a higher risk of both fat intake and obesity. With this, this study determined that based on the studied population's average values, individuals with PKU are not at a greater risk for CVD but encourages individuals with PKU to sustain a healthy intake of all macronutrients, not only limited to protein.

STUDY LIMITATIONS

This study has several limitations. One, the study had a small sample size and was only conducted at one facility. Also, three-day food records were used and can contain estimations, inaccuracies, and human error. However, since three-day food records are easy and straight-forward, it was the best method to obtain the information needed for this study. Lastly, this study wants to acknowledge that the plan was to collect ten BMI, BMI%, or WPL and ten PCF values on all participants; however, as data collection began, it was determined not possible. This data reflects the study population well; however, some individuals may have had less data than others.

Acknowledgements

A sincere thank you to my preceptor, Dr. Lee Wallace for all your advice, time, and mentorship through this process. Without you, this would not have been possible.

REFERENCES

1. Vockley, J., Anderson, HC., Antshel, KM., et al. Phenylalanine hydroxylase deficiency: Diagnosis and management guideline. Genetics in Medicine: Official Journal of the American College of Medical Genetics. 2014; 16(2): 188-200.
2. National PKU Alliance. My PKU Binder. https://npkua.org/portals/0/pdfs/pkubinder/PKU%20Binder%202011.pdf
3. About PKU. National PKU Alliance. 2017.
4. Ehrlich S, NMD. Phenylalanine. University of Maryland Medical Center. 2016.
5. The American Cancer Society Medical and Editorial Content Team. Aspartame. American Cancer Society. 2014.
6. Aspartame. What is Aspartame. Calorie Control Council. 2013.
7. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. The Lancet. 2010; 376(9750): 1417-1427.
8. Castro G, Hamilton V, Cornejo V. Chilean nutrition management protocol for patients with phenylketonuria. Journal of Inborn Errors of Metabolism and Screening. 2017, volume 5: 1-6.
9. Linda Casey. Caring for children with phenylketonuria. Canadian Family Physician. 2013; 59(8): 837.
10. Feillet F, Agostoni C. Nutritional issues in treating phenylketonuria. J Inherit Metab Dis. 2010; 33(6): 659-664.

11. Johnston CS, Tjonn SL, Swan PD. High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults. The Journal of Nutrition. 2004; 134(3): 586.
12. The American Heart Association's Diet and Lifestyle Recommendations. The American Heart Association, 2017.
13. "About Adult BMI." Centers for Disease Control and Prevention. 15 May 2015.
14. "About Child & Teen BMI." Centers for Disease Control and Prevention. 15 May 2015.
15. Chou JH. Growth WHO (0-24 months). PediTools. 2020; 22(1): e16204.
16. Chou JH. Growth CDC (0-36 months). PediTools. 2020; 22(1): e16204.
17. Moretti F, Pellegrini N, Salvatici E, et al. Dietary glycemic index, glycemic load and metabolic profile in children with phenylketonuria. Nutrition, Metabolism and Cardiovascular Diseases. 2017; 27(2): 176-182.
18. Belanger-Quintana A, Martínez-Pardo M. Physical development in patients with phenylketonuria on dietary treatment: A retrospective study. Molecular Genetics and Metabolism. 2011; 104(4): 480-484.
19. Blundell JE, Finlayson G, Gibbons C, Caudwell P, Hopkins M. The biology of appetite control: Do resting metabolic rate and fat-free mass drive energy intake? Physiology & Behavior. 2015; 152(Pt B): 473-478.
20. Gokmen Ozel H, Ahring K, Bélanger-Quintana A, et al. Overweight and obesity in PKU: The results from 8 centres in europe and turkey. Molecular Genetics and Metabolism Reports. 2014; 1: 483-486.
21. Burrage LC, McConnell J, Haesler R, et al. High prevalence of overweight and obesity in females with phenylketonuria. Molecular Genetics and Metabolism. 2012; 107(1-2): 43.
22. MacDonald A, Rocha JC, van Rijn M, Feillet F. Nutrition in phenylketonuria. Molecular Genetics and Metabolism. 2011; 104: S18.
23. Belanger-Quintana A, Martínez-Pardo M. Physical development in patients with phenylketonuria on dietary treatment: A retrospective study. Molecular Genetics and Metabolism. 2011; 104(4): 480-484.
24. Rocha JC, MacDonald A, Trefz F. Is overweight an issue in phenylketonuria? Molecular Genetics and Metabolism. 2013; 110 Suppl: S24.
25. DiNicolantonio JJ, O'Keefe JH. Preventing cardiovascular disease. Progress in Cardiovascular Diseases. 2016; 58(5): 463.
26. Dietary fat and risk of cardiovascular disease: Recent controversies and advances. Annual Review of Nutrition. 2017; 37: 423.
27. Chrostowska M, Szyndler A, Hoffmann M, Narkiewicz K. Impact of obesity on cardiovascular health. Best Practice & Research. Clinical Endocrinology & Metabolism. 2013; 27(2): 147.
28. American Heart Association, Gidding SS, Dennison BA, et al. Dietary recommendations for children and adolescents: A guide for practitioners. Pediatrics. 2006; 117(2): 544-559.
29. Obesity Information. American Heart Association. 2016.

PEER REVIEW

Not commissioned. Externally peer reviewed.

TABLES

Table 1: Definitions of Variables Used.

- Percentage of calories coming from fat (PCF): indicates how much fat is contributing to the total calorie intake
- Body mass index (BMI): the ratio of body weight, in kilograms, and height, in meters. Ratio is compared to a standard (<18.5 (underweight), 18.5-24.9 (healthy), 25-29.9 (overweight), and ≥30 (obese), as shown below in Figure 1 [13,14] was used for participants nineteen years and older.
- Body mass index percentage (BMI%): obtained by plotting BMI and age on growth charts; represents the comparison of an individual's BMI% value to that of other individuals who share the same gender and age [14] was used for participants three to eighteen years of age.
- Weight per length (WPL): similar to BMI% but uses length measurements instead; indicated in two years and younger population.

Table 2: Patient Stratification Based on Age and Fat Recommendations [12].

Group Number and Age Range	Recommended Percentage of Calories Coming from Fat Ranges
Group 1: 1-3 years old	30-40%
Group 2: 4-18 years old	25-35%
Group 3: 19-50 years old	20-35%

Table 3: Percentage of Calories Coming from Fat (PCF) Analyses Participants.

Measure	**Group**	**n (Participants)***	**Gender Ratio**	**Total Number of Records**
PCF	Group 1	8	7 Males : 1 Female	62
PCF	Group 2	19	11 Males : 8 Females	124
PCF	Group 3	19	6 Males : 13 Females	108

Table 4: Weight Per Length (WPL), Body Mass Index Percentage (BMI%), and Body Mass Index (BMI) Analyses Participants.

Measure	Group	n (Participants)*	Gender Ratio	Total Number of Records
WPL	Group 1a	6	5 Males : 1 Female	43
BMI%	Group 1b	6	6 Males : 0 Females	34
BMI%	Group 2	19	11 Males : 8 Females	138
BMI	Group 3	19	6 Males : 12 Females	95

*Some participants were included into more than one group

Table 5: Results for the 95% Confidence Interval Analyses for Groups 1, 2, and 3.

Age Group	Measure	Mean	Variance	Lower Confidence limit	Upper Confidence limit	Recommended	Mean Group Value in Comparison to Recommended
Group 1	PCF (%)	26.22	11.58	23.37	29.06	30-40	Low
Group 1a	WPL (%)	80.16	303.5	61.88	98.44	5th to <85th	Normal
Group 1b	BMI% (%)	66.91	689.48	39.36	94.47	5th to <85th	Normal
Group 2	PCF (%)	25.66	34.75	22.82	28.50	25-35	Normal
	BMI% (%)	64.16	696.84	51.43	76.88	5th to <85th	Normal
Group 2M	PCF (%)	23	6.24	21.72	25.08	25-35	Low
	BMI% (%)	56.97	786.71	38.12	75.81	5th to <85th	Normal
Group 2F	PCF (%)	28.87	67.14	22.02	35.72	25-35	Normal
	BMI% (%)	74.04	475.09	55.82	92.26	5th to <85th	Normal
Group 3	PCF (%)	27.88	24.39	25.50	30.26	20-35	Normal
	BMI (kg/m^2)	27.68	82.75	23.30	32.07	18.5-24.9	Overweight
Group 3M	PCF (%)	26.92	56.50	19.03	34.81	20-35	Normal
	BMI (kg/m^2)	25.42	27.19	19.95	30.90	18.5-24.9	Overweight
Group 3F	PCF 9%)	28.80	19.85	26.10	31.49	20-35	Normal
	BMI (kg/m^2)	29.51	109.92	22.85	36.17	18.5-24.9	Overweight

Table 6: Results for the Individual T-Test Analyses for Groups 1, 2, 3, and Gender Comparison.

Age Group	**Null Hypothesis**	**Alternative Hypothesis**	**p-value**
		PCF	
Group 1	H_0: mean % fat intake ∈[30,40]	H_1: mean % fat intake > 40	1.0
	H_0: mean % fat intake ∈[30,40]	H_1: mean % fat intake < 30	0.0008
Group 2	H_0: mean % fat intake ∈[25,35]	H_1: mean % fat intake > 35	1.0
	H_0: mean fat intake ∈[25,35]	H_1: mean % fat intake <25	0.302
Group 2M	H_0: mean % fat intake ∈[25,35]	H_1: mean % fat intake > 35	1.0
	H_0: mean fat intake ∈[25,35]	H_1: mean % fat intake <25	0.03
Group 2F	H_0: mean PCF intake ∈[20,35]	H_1-a: mean PCF intake > 35	0.96
	H_0: mean PCF intake ∈[20,35]	H_1-b: mean PCF intake < 20	0.89
Group 3	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake > 35	1.0
	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake < 20	1.0
Group 3M	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake > 35	1.0
	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake < 20	1.0
Group 3F	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake > 35	1.0
	H_0: mean % fat intake ∈[20,35]	H_1: mean % fat intake < 20	1.0
Group 2: M vs F	H_0: mean PCF of M = mean PCF of F	H_1: the mean PCF of M ≠ mean PCF of F	0.1053
Group 3: M vs F	H_0: mean PCF of M = mean PCF of F	H_1: the mean PCF of M ≠ mean PCF of F	0.589
		WPL, BMI%, BMI	
Group 1a	H_0: mean WPL** ≤ 95	H_1: mean WPL > 95	0.954
Group 1b	H_0: mean BMI%*** ≤ 95	H_1: mean BMI% > 95	0.976
Group 2	H_0: mean BMI% ≤ 95	H_1:mean BMI% > 95	1.0
Group 2M	H_0: mean BMI% ≤ 95	H_1:mean BMI% > 95	0.999
Group 2F	H_0: mean BMI% ≤ 95	H_1:mean BMI% > 95	0.999
Group 3	H_0: mean BMI ≤ 30 kg/m	H_1:mean BMI > 30 kg/m	0.730
Group 3M	H_0: mean BMI ≤ 30 kg/m	H_1:mean BMI > 30 kg/m	0.730
Group 3F	H_0: mean BMI ≤ 30 kg/m	H_1:mean BMI > 30 kg/m	0.730
Group 2: M vs F	H_0: mean BMI% of M = mean BMI% of F	H_1: mean BMI% of M ≠ mean BMI% of F	0.169
Group 3: M vs F	H_0: mean BMI of M = mean BMI of F	H_1: mean BMI of M ≠ mean BMI of F	0.286

FIGURES

Figure 1: Weight Categories Based on Body Mass Index for Participants Nineteen Years Old and Older [13].

BMI	Weight Status
Below 18.5	Underweight
18.5 – 24.9	Normal or Healthy Weight
25.0 – 29.9	Overweight
30.0 and Above	Obese

Figure 2: Weight Categories Based on Weight per Length and Body Mass Percentile for Participants One to Eighteen Years of Age [14,15].

Weight Status Category	Percentile Range
Underweight	Less than the 5th percentile
Normal or Healthy Weight	5th percentile to less than the 85th percentile
Overweight	85th to less than the 95th percentile
Obese	Equal to or greater than the 95th percentile