Original Article

The Effect of Pyridoxine Hydrochloride Supplementation on Leptin, Adiponectin, Glycemic Indices, and Anthropometric Indices in Obese and Overweight Women

Fatemeh Haidari [ID],[1] Majid Mohammadshahi [ID],[1] Mehdi Zarei [ID],[2] Mohammad Hosein Haghighizadeh [ID],[3] Fatemeh Mirzaee [ID] [4]

[1]Department of Nutrition, Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Iran
[2]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz 61357-43337, Iran
[3]Department of Statistics and Epidemiology, Faculty of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Iran
[4]Department of Nutrition, Diabetes Research Center, Health Research Institute, Faculty of Paramedical Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Iran

ORCID iDs
Fatemeh Haidari [ID]
https://orcid.org/0000-0001-9104-4639
Majid Mohammadshahi [ID]
https://orcid.org/0000-0002-4987-8194
Mehdi Zarei [ID]
https://orcid.org/0000-0002-2965-7773
Mohammad Hosein Haghighizadeh [ID]
https://orcid.org/0000-0002-4197-0199
Fatemeh Mirzaee [ID]
https://orcid.org/0000-0003-0131-1583

ABSTRACT

Obesity has reached epidemic proportions globally. Among several methods for treating obesity, the use of dietary supplements is common recently. One supplement that can help in this regard might be vitamin B6 in high doses. The objective of this study was to evaluate the effect of pyridoxine hydrochloride supplementation on anthropometric indices, body composition, visceral adiposity index (VAI), and metabolic status in obese and overweight women. In this randomized controlled clinical trial, 44 obese and overweight women aged 18–50 years were selected and divided randomly into 2 groups: an intervention group (receiving 80 mg pyridoxine hydrochloride supplement for 8 weeks) and a control group (receiving placebo for 8 weeks). In the pyridoxine hydrochloride group, weight ($p = 0.03$), body mass index ($p = 0.023$), fat mass ($p = 0.003$), waist circumference ($p = 0.005$), VAI ($p = 0.001$), fasting insulin, insulin resistance (homeostasis model assessment of insulin resistance; HOMA-IR), total cholesterol, low-density lipoprotein, triglycerides (TG) and leptin ($p < 0.001$) decreased whereas adiponectin ($p < 0.001$) increased in comparison to the baseline values. There was a significant difference in fat mass, VAI, fasting insulin, HOMA-IR, and TG between pyridoxine hydrochloride and control groups following intervention in adjusted models ($p < 0.05$). The findings suggest that vitamin B6 supplementation may be effective in reducing BMI and improving body composition and biochemical factors associated with obesity.

Trial Registration: Iranian Registry of Clinical Trials Identifier: IRCT20181002041206N1

Keywords: Vitamin B6; Pyridoxine; Obesity; Glycemic indices; Adipokines

INTRODUCTION

Abnormal or excessive fat accumulation in the body is defined as overweight and obesity, which may affect health status. In 2016, more than 1.9 billion adults aged 18 years and older were overweight worldwide. Of these over 650 million adults were obese [1]. Genetic factors, sedentary lifestyle, mild inflammation in the body, the consumption of certain medications, some diseases, the imbalance between calorie intake and calories expenditure, sleep deprivation, or stress are all known factors associated with obesity [2]. Obesity is related to chronic diseases, such as cardiovascular disease, diabetes, hypertension, stroke, musculoskeletal disorders, and some cancers [1,3]. There are several strategies for weight loss including adequate physical activity, weight loss medications, and various types of obesity surgery [4]. Since drug therapy and surgeries are accompanied by various side effects, using natural or synthetic dietary supplements has attracted the attention of researchers.

Vitamin B6 is one of the water-soluble vitamins. There are three forms of vitamin B6: pyridoxamine (PM), pyridoxal, and pyridoxine [5]. The deficiency of this vitamin is rare because it is abundant in food sources, such as meat, whole grains, vegetables, and nuts. Besides dietary sources, the gut microbiota is an important source of vitamin B6 production in the colon [6]. Vitamin B6 acts as an important coenzyme in various reactions in the body, including the metabolism of amino acids, glucose release from glycogen, modulation and regulation of steroid hormone receptors, tryptophan conversion to niacin, and biosynthesis of sphingolipids [6]. It also plays a role in the biosynthesis of dopamine, serotonin, and gamma-aminobutyric acid neurotransmitters that regulate various functions like blood pressure, mental state, depression, and appetite [7-9]. In some studies, the effects of pyridoxine on calcium ion signaling have been observed that increases lipolysis and decreases fatty acid synthesis [10-12]. In some studies, it has also been shown to decrease insulin resistance [13-16] and to improve lipid profile [13,17-19]. The antioxidant [20] and anti-inflammatory [15] effects of this vitamin are also seen. The effect of different forms of vitamin B6 administration in mice has been shown to significantly decrease body and adipose tissue weight [21], fasting blood glucose [13,22], liver triglycerides (TG) and cholesterol levels and hepatic lipid accumulation [13], fasting insulin levels and increase insulin sensitivity [3,14] in previous studies.

Since the effect of oral pyridoxine hydrochloride (vitamin B6) supplementation on weight loss in obese and overweight subjects has not been studied, the current study was aimed to evaluate the effect of oral pyridoxine hydrochloride supplement on anthropometric measurements, body composition, visceral adiposity index (VAI), glycemic and lipidemic risk factors and serum levels of leptin and adiponectin in obese and overweight women.

MATERIALS AND METHODS

Participants

This randomized, double-blind, placebo-controlled clinical trial was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (reference number: ir.ajums.rec.1397.523) and registered in the registration center for clinical trials in Iran (code: IRCT20181002041206N1). Patients were recruited from Jundishapur University of Medical Science, in Ahvaz, Iran, in 2019. Initial anthropometric measurements, including weight, height, waist circumference (WC), and waist-to-hip circumference ratio (WHR), were recorded. Inclusion criteria were age range of 18–50 years, body mass index (BMI) equal

to or higher than 25 kg/m², and patient satisfaction. Exclusion criteria included pregnancy; lactation; menopause; smoking; alcohol and drug abuse; participation in exercise or weight reduction programs; medications known to affect weight in the past 6 months; history or presence of other diseases such as diabetes, cardiovascular diseases, hypertension, and infection; other inflammatory disorders; liver and kidney disorders; steroids or hormonal drugs; and the consumption of vitamins and minerals in the past 6 months. According to the study conducted by Zemel and Bruckbauer [23] on insulin levels and considering the confidence interval of 95% and power of 90%, the sample size was estimated to be 18 participants in each group. However, considering a 20% probable withdrawal, 22 participants were enrolled into each group. After explaining the projects, we requested each patient to complete the consent form.

Study design

The participants were randomly allocated into 2 groups to take either 80 mg/day pyridoxine hydrochloride supplement (2 × 40 mg tablets) or placebo (2 × starch tablets) for 8 weeks. Enrolled patients were divided into 2 groups by randomized block allocation using a random number table. Randomization and allocated number to each group were performed by an independent consultant statistician. Pyridoxine hydrochloride supplements were purchased from the pharmacy and placebo tablets were manufactured by Pharmaceutical Incubator, Jundishapur University of Medical Science (Ahvaz, Iran). Based on randomized block allocation, pyridoxine hydrochloride and placebo tablets were distributed to the persons by research assistants who were not aware of group allocation. Data collectors, interviewers, and principal investigators were not aware of the patient allocation during the intervention period. The patient recruitment flowchart for this clinical trial is presented in **Figure 1**.

At the first visit, all tablets were given to the participants, and they were asked to take 2 tablets per day. To enhance compliance with the study, short messages were sent to all participants' cell phones every day to remind them about taking the tablets and phone calls
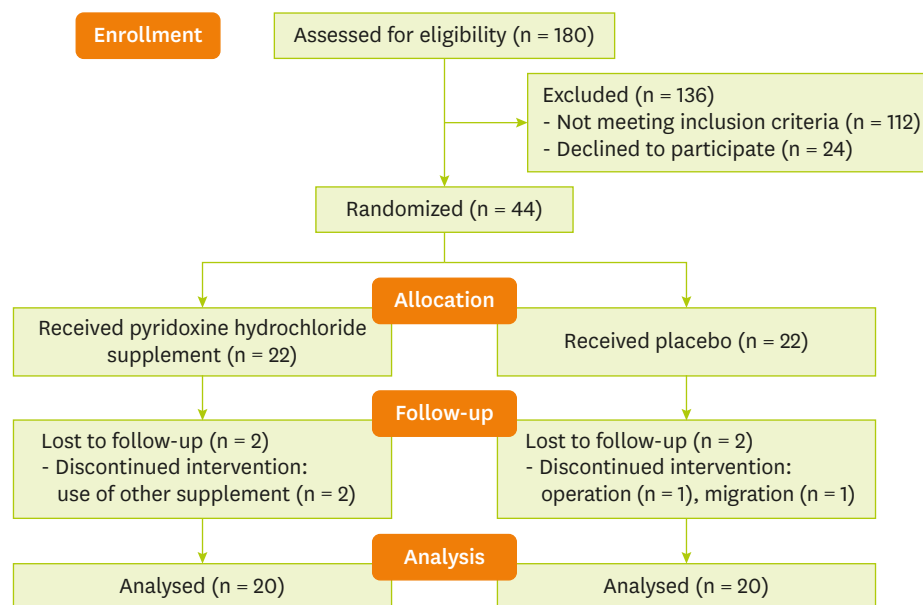

Figure 1. Flowchart of participant recruitment for the clinical trial of pyridoxine hydrochloride supplementation in obese and overweight women.

were made to people every 2 weeks. Biochemical, anthropometric, and nutritional factors were evaluated only at the beginning and end of the study. The degree of compliance for each patient was determined according to the amount of the returned pills. In the case that the patients consumed less than 90% of the tablets, they were excluded from the study.

Dietary assessment and anthropometric measurement

The diet was recorded before and at the end of the study by using a 3-day food recall, including one weekend day and two weekdays, to assess their total energy and macronutrient intake. We asked participants not to change their level of physical activity during the study. An International Physical Activity Questionnaire (IPAQ) was used to assess physical activity. Data from the IPAQ were converted to metabolic equivalent (MET)-minutes/week by using the existing guidelines [24]. Demographic information and medical history were recorded for each participant, and anthropometric measurements including weight and height were recorded, and the BMI (kg/m^2) was calculated. The height was measured using a non-stretchable tape to the nearest 0.5 cm while standing barefoot with heels sticking to the wall, head straightened, and eyes looking forward. The weight and body composition were measured with minimum clothing by using a BF511-Omron body composition monitor (OMRON Healthcare Co., Ltd., Kyoto, Japan). Subsequently, the BMI was calculated using the formulae BMI = weight in kilograms divided by the square of the height in meters.

Biochemical analysis

At the beginning and the end of the study, blood samples were collected from all participants after 12–14 hours of overnight fasting. Serum samples were prepared and stored at −70°C until used for biochemical testing. The parameters were fasting blood sugar (FBS), total cholesterol, low-density lipoprotein (LDL) - and high-density lipoprotein (HDL) - cholesterol, TG, serum insulin, leptin, and adiponectin. Serum glucose, TG, and total and HDL cholesterol were measured using the enzymatic method. LDL was calculated using the Friedewald formula = Total cholesterol − (HDL + TG/5) [26]. VAI, suggested as a valid indicator of visceral fat performance, was determined by $\left(\frac{WC}{36.58 + (1.89 \times BMI)}\right) \times \left(\frac{TG}{0.81}\right) \times \left(\frac{1.52}{HDL - C}\right)$ [25]. Serum insulin was measured using an enzyme-linked immune sorbent assay (ELISA) test kit (DiaPlus, North York, ON, Canada), and the HOMA-IR was calculated using the following formula: Fasting plasma glucose (mmol/L) × Fasting insulin (µU/mL)/22.5 [27]. Serum leptin was measured using a leptin ELISA test kit (Diagnostics Biochem Canada Inc, London, ON, Canada). For quantitatively detecting human adiponectin in samples, we used a human adiponectin ELISA test kit (Boster Biological Technology, Pleasanton, CA, USA). To prevent possible hormonal changes, none of the samples were in the menstrual cycle on the day of blood sampling.

Statistical analysis

The normality of all variables was checked using the Kolmogorov-Smirnov test. For normal distribution variables, independent sample t-test and paired sample t-test were used to compare parameters at the beginning and the end of the study between and within groups, respectively. Mann-Whitney U test and Wilcoxon signed-rank test as nonparametric alternatives were applied to compare sample parameters between and within groups, respectively. Patients' medications were compared between the 2 groups and were evaluated by the χ^2 test. To control confounding variables, including baseline values, age, baseline BMI, physical activity, and baseline calorie intake, analysis of covariance was used. The data were expressed as mean ± standard deviation. All statistical analyses were performed using the SPSS software (version 22; SPSS Inc., Chicago, IL, USA), and p < 0.05 was considered to be statistically significant.

RESULTS

Demographic and anthropometric characteristics of the participants at the baseline and the end of the study have been presented in **Table 1**. The results of this information show no significant differences between the two groups at the beginning of the study (p > 0.05). There were no significant changes in BMI, weight, muscle mass, WC, WHR, VAI, and physical

Table 1. Demographic and anthropometric characteristics of the study population

Variable	Pyridoxine hydrochloride group (n = 20)	Placebo group (n = 20)	p*
Age (year)	33.86 ± 9.86	30.90 ± 12.32	0.34
Height (cm)	159.04 ± 5.97	160.02 ± 5.09	0.72
Weight (kg)			
Week 0	75.95 ± 11.64	77.22 ± 8.00	0.37
Week 8	74.88 ± 11.41	77.55 ± 8.13	0.33
Mean difference	1.07 ± 1.88	−0.33 ± 1.50	
p†	0.03	0.34	
BMI (kg/m²)			
Week 0	30.04 ± 4.52	30.20 ± 3.20	0.42
Week 8	29.61 ± 4.43	34.63 ± 13.57	0.09
Mean difference	0.43 ± 0.75	−4.42 ± 13.97	
p†	0.023	0.081	
Fat mass (kg)			
Week 0	33.96 ± 9.11	35.51 ± 5.99	0.13
Week 8	32.75 ± 8.32	36.22 ± 5.87	0.02
Mean difference	1.22 ± 1.90	−0.7 ± 2.01	
p†	0.003	0.223	
Muscle mass (kg)			
Week 0	18.09 ± 2.16	17.76 ± 2.35	0.39
Week 8	18.26 ± 2.28	17.61 ± 2.14	0.15
Mean difference	−0.16 ± 0.67	0.14 ± 1.12	
p†	0.291	0.733	
Fat mass/muscle mass			
Week 0	1.87 ± 0.42	2.02 ± 0.36	0.133
Week 8	1.79 ± 0.37	2.06 ± 0.32	0.005
Mean difference	0.084 ± 0.13	−0.045 ± 0.16	
p†	0.005	0.709	
WC (cm)			
Week 0	98.36 ± 21.88	94.59 ± 5.89	1.000
Week 8	96.91 ± 21.86	95.09 ± 6.09	0.46
Mean difference	1.44 ± 2.06	−0.50 ± 1.13	
p†	0.005	0.065	
WHR (cm)			
Week 0	0.90 ± 0.16	0.86 ± 0.06	0.69
Week 8	0.89 ± 0.16	0.86 ± 0.06	0.92
Mean difference	0.005 ± 0.01	−0.003 ± 0.008	
p†	0.073	0.145	
VAI			
Week 0	3.87 ± 2.19	4.12 ± 1.86	0.25
Week 8	3.25 ± 1.72	3.89 ± 1.79	0.11
Mean difference	0.62 ± 0.78	0.225 ± 0.776	
p†	0.001	0.223	
Physical activity (MET of task-min/wk)			
Week 0	2,096 ± 12.44	2,096 ± 14.34	0.90
Week 8	2,102 ± 23.24	2,094 ± 15.22	0.24
Mean difference	−5.72 ± 25.95	2.72 ± 11.95	
p†	0.34	0.39	

Values are presented as mean ± standard deviation.
BMI, body mass index; WC, waist circumference; WHR, waist-to-hip circumference ratio; VAI, visceral adiposity index; MET, metabolic equivalent.
*p values are between-group comparison of the variables; independent sample t-test (for age, height, weight, and BMI) and Mann-Whitney U test (for other variables);
†p values are within-group comparison of the variables; paired sample t-test (for age, height, weight, and BMI) and Wilcoxon signed-rank test (for other variables).

activity in the subjects after consuming pyridoxine hydrochloride and placebo. At the end of the study, we just observed a significant difference in fat mass changes (p = 0.02). In the intervention group, weight (p = 0.03), BMI (p = 0.023), fat mass (p = 0.003), WC (p = 0.005) and VAI (p = 0.001) decreased significantly after 8 weeks. The placebo group showed no significant change after eight weeks (p > 0.05).

Table 2 shows dietary macronutrient and vitamin B6 intake in 2 groups. No significant change was observed in these variables between two groups at baseline and the end of the study (p > 0.05).

Table 3 shows the biochemical indices at the beginning and the end of the study in the two groups. There was no significant difference in FBS, fasting insulin, HOMA-IR, and lipid profile (TG, total cholesterol, LDL-, HDL- cholesterol) between the 2 groups at baseline (p > 0.05). At the end of the study, we observed a significant difference in fasting insulin (p < 0.001), HOMA-IR (p < 0.001), total cholesterol (p = 0.006), LDL-cholesterol (p = 0.03), and TG (p = 0.009) between the 2 groups. This result remained significant for fasting insulin (p < 0.001), HOMA-IR (p < 0.001), and TG (p = 0.02) after adjustment for weight, BMI, energy intake, and physical activity levels. In the post-intervention, fasting insulin (p < 0.001), HOMA-IR (p < 0.001), total cholesterol (p < 0.001), LDL-cholesterol (p < 0.001), and TG (p < 0.001). and leptin (p < 0.001) decreased significantly in the intervention group. Also, adiponectin (p < 0.001) increased significantly after 8 weeks of vitamin B6 supplementation. The placebo group showed no significant change after 8 weeks (p > 0.05).

Table 4 shows leptin, and adiponectin levels at the beginning and the end of the study in the 2 groups. There was no significant difference in leptin, and adiponectin between the 2 groups at baseline (p > 0.05). At the end of the study, the serum adiponectin levels in the intervention group were higher than the control group. Of course, this difference has been marginally significant (p = 0.05). However, in the adjusted model, there was no statistically significant difference in serum adiponectin levels at the end of the study (p > 0.05). The results did not show a significant difference in serum leptin levels between the 2 groups at the end of the study (p = 0.18). In the post-intervention, leptin (p < 0.001) decreased significantly and, adiponectin (p < 0.001) increased significantly after 8 weeks of vitamin B6 supplementation. The placebo group showed no significant change after eight weeks (p > 0.05).

Side effects
There was not any report of complication or side effect due to intake of pyridoxine hydrochloride supplement by the participants during the study.

DISCUSSION

To the best of our knowledge, this is the first randomized, double-blinded, controlled clinical trial to evaluate the effects of 80 mg/day pyridoxine hydrochloride supplement on anthropometric measurements, body composition, VAI, glycemic and lipidemic risk factors, and serum levels of leptin and adiponectin in obese and overweight women. The current recommended daily amount (RDA) for B6 is 1.6 mg for Iranian adults over 19 [28]. No adverse effects have been seen from the consumption of B6 food sources. Although some studies have shown severe sensory neuropathy due to treatment with 2–6 g/day pyridoxine

Table 2. Dietary intake at the baseline and end of the study in 2 groups

Variable	Pyridoxine hydrochloride group (n = 20)	Placebo group (n = 20)	p*
Energy (kcal)			
Week 0	1,709 ± 150	1,708 ± 187	0.98
Week 8	1,678 ± 246	1,758 ± 202	0.29
Mean difference	30.72 ± 152.93	−49.72 ± 124.85	
p†	0.21	0.49	
Protein (percent of energy)			
Week 0	18 ± 2.31	17 ± 1.88	0.64
Week 8	18 ± 1.98	17 ± 2.02	0.06
Mean difference	−0.20 ± 2.78	0.59 ± 2.81	
p†	0.72	0.06	
Carbohydrate (percent of energy)			
Week 0	53 ± 2.51	53 ± 2.63	0.69
Week 8	54 ± 2.29	53 ± 2.12	0.18
Mean difference	−0.69 ± 3.08	0.96 ± 3.59	
p†	0.65	0.24	
Fat (percent of energy)			
Week 0	28 ± 2.71	28 ± 2.46	0.43
Week 8	27 ± 3.15	29 ± 3.35	0.16
Mean difference	0.27 ± 5.11	−0.56 ± 4.75	
p†	0.60	0.38	
SFA (g)			
Week 0	13 ± 1.54	12 ± 1.61	0.10
Week 8	12 ± 1.55	13 ± 1.43	0.07
Mean difference	1.11 ± 1.80	−0.80 ± 1.81	
p†	0.38	0.41	
MUFA (g)			
Week 0	13 ± 1.59	13 ± 1.27	0.52
Week 8	14 ± 1.67	12 ± 1.32	0.06
Mean difference	−0.036 ± 1.23	0.42 ± 1.18	
p†	0.17	0.09	
PUFA (g)			
Week 0	15 ± 1.36	14 ± 1.72	0.82
Week 8	18 ± 1.74	16 ± 1.31	0.63
Mean difference	−3.02 ± 1.15	−1.92 ± 1.83	
p†	0.82	0.91	
Cholesterol (g)			
Week 0	133 ± 15.12	125 ± 11.74	0.06
Week 8	122 ± 9.99	121 ± 15.38	0.51
Mean difference	10.95 ± 17.13	3.63 ± 14.42	
p†	0.09	0.17	
Fiber (g)			
Week 0	10.39 ± 2.16	11.01 ± 1.92	0.63
Week 8	10.25 ± 2.05	10.27 ± 2.06	0.81
Mean difference	0.14 ± 1.38	0.42 ± 2.56	
p†	0.49	0.13	
Vitamin B6 (mg)			
Week 0	1.87 ± 0.72	1.90 ± 0.46	0.65
Week 8	1.89 ± 0.48	2.21 ± 0.46	0.06
Mean difference	−0.02 ± 0.65	−0.30 ± 0.60	
p†	0.46	0.14	

Values are presented as mean ± standard deviation.
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
*p values are between-group comparison of the variables; independent sample t-test (for energy and carbohydrate) and Mann-Whitney U test (for other variables);
†p values are within-group comparison of the variables; paired sample t-test (for energy and carbohydrate) and Wilcoxon signed-rank test (for other variables).

hydrochloride [29]. The amount of upper limit for this vitamin is 100 mg/day [30]. Results of the present study showed that 8-week consumption of pyridoxine hydrochloride reduced BMI and improved body composition compared with the placebo.

Table 3. Effect of pyridoxine hydrochloride supplement on FBS, fasting insulin and lipid profile in the study population

Variable	Pyridoxine hydrochloride group (n = 20)	Placebo group (n = 20)	p*	p†
FBS (mg/dL)				
Week 0	82.09 ± 6.76	85.40 ± 8.56	0.23	0.47
Week 8	83.22 ± 4.91	87.54 ± 9.70	0.06	0.43
Mean difference	−1.13 ± 7.38	−2.13 ± 5.33		
p‡	0.78	0.39		
Fasting insulin (u/mL)				
Week 0	11.73 ± 1.30	12.15 ± 1.23	0.31	0.65
Week 8	10.28 ± 1.03	12.20 ± 1.23	0.000	0.000
Mean difference	1.45 ± 1.14	−0.05 ± 1.25		
p‡	0.000	0.728		
HOMA-IR				
Week 0	2.34 ± 0.29	2.53 ± 0.40	0.07	0.31
Week 8	1.92 ± 0.20	2.60 ± 0.40	0.000	0.000
Mean difference	0.42 ± 0.22	−0.07 ± 0.33		
p‡	0.000	0.91		
Total cholesterol (mg/dL)				
Week 0	161.59 ± 21.83	166.63 ± 26.48	0.664	0.939
Week 8	148.90 ± 18.45	167.50 ± 24.16	0.006	0.066
Mean difference	12.68 ± 8.39	−0.86 ± 12.51		
p‡	0.000	0.643		
LDL cholesterol (mg/dL)				
Week 0	97.09 ± 19.41	100.63 ± 22.51	0.54	0.97
Week 8	85.78 ± 17.49	98.73 ± 22.24	0.03	0.28
Mean difference	11.30 ± 8.45	1.90 ± 9.01		
p‡	0.000	0.355		
HDL cholesterol (mg/dL)				
Week 0	47.09 ± 6.05	46.50 ± 7.37	0.35	0.64
Week 8	47.95 ± 6.55	47.86 ± 7.51	0.78	0.82
Mean difference	−0.86 ± 5.30	−1.36 ± 3.57		
p‡	0.63	0.17		
TG (mg/dL)				
Week 0	87.04 ± 36.01	97.50 ± 36.35	0.47	0.69
Week 8	75.86 ± 32.44	97.68 ± 30.25	0.009	0.02
Mean difference	11.18 ± 10.21	−0.18 ± 16.33		
p‡	0.000	0.559		

Values are presented as mean ± standard deviation.
FBS, fasting blood sugar; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; BMI, body mass index.
*p values are between-group comparison of the variables; independent sample t-test (for FBS, HDL cholesterol, and TG) and Mann-Whitney U test (for other variables); †p values are between-group comparison of the variables at baseline and after the intervention; analysis of covariance in the adjusted models (adjusted for weight, BMI, dietary intake of energy, and physical activity); ‡p values are within-group comparison of the variables; paired sample t-test (for FBS, HDL cholesterol, and TG) and Wilcoxon signed-rank test (for other variables).

Table 4. Effect of pyridoxine hydrochloride supplement on leptin and adiponectin pre and post the intervention

Variable	Pyridoxine hydrochloride group (n = 20)	Placebo group (n = 20)	p*	p†
Leptin (ng/mL)				
Week 0	31.25 ± 6.62	28.75 ± 4.35	0.13	0.16
Week 8	25.66 ± 6.12	27.64 ± 4.85	0.18	0.23
Mean difference	5.59 ± 4.47	1.11 ± 2.70		
p‡	0.000	0.094		
Adiponectin (μg/mL)				
Week 0	7.40 ± 0.79	7.64 ± 0.77	0.41	0.09
Week 8	8.32 ± 1.09	7.66 ± 1.17	0.05	0.11
Mean difference	−0.91 ± 0.86	−0.027 ± 1.23		
p‡	0.000	0.970		

Values are presented as mean ± standard deviation.
*p values are between-group comparison of the variables; Mann-Whitney U test; †p values are between-group comparison of the variables at baseline and after the intervention; analysis of covariance in the adjusted models (adjusted for weight, BMI, dietary intake of energy, and physical activity); ‡p values are within-group comparison of the variables; Wilcoxon signed-rank test.

In some studies, the effects of pyridoxine on calcium ion signaling have been observed that increases lipolysis and decreases fatty acid synthesis [10-12]. Zemel and Bruckbauer [23] studied the effects of leucine and pyridoxine-containing nutraceutical on body weight and composition in obese subjects. They recently demonstrated leucine to modulate energy partitioning between adipose tissue and muscle. Further, leucine exhibits synergy with B6, resulting in reduced adipocyte lipid storage coupled with increased muscle fat oxidation and insulin sensitivity, and reduced oxidative and inflammatory stress [15]. They found that this nutraceutical combination improves oxidative capacity and thereby significantly augments weight and fat loss [23]. Pyridoxal phosphate inhibits adipocyte Ca^{2+} influx in vitro, resulting in significant decreases in adipocyte fatty acid synthase expression and activity and corresponding reductions in adipocyte TG content [15] because Ca^{2+} signaling coordinately stimulates fatty acid synthase activity and inhibits lipolysis in adipocytes [31-35].

Our results showed that pyridoxine hydrochloride had beneficial effects on lipid profile. Similar effect of vitamin B6 has been shown in previous studies [17-19]. Pyridoxine supplementation decreased plasma total cholesterol, HDL-cholesterol, and TG; and increased LDL-cholesterol [17]. Also, vitamin B6 supplementation was associated with a significant reduction in total cholesterol and HDL-cholesterol [18]. We observed a significant reduction in fasting insulin and HOMA-IR. In some studies, it has also been shown to decrease fasting blood glucose [13,22] and insulin resistance [13-16]. Unoki-Kubota et al. [14] examined pyridoxamine supplementation, an inhibitor of advanced glycation end products (AGE) formation could ameliorate insulin resistance in obese mice with type 2 diabetes. In this study administration of pyridoxamine decreased fasting insulin levels and improved insulin sensitivity in mice [14]. Spellacy et al. [16] in 1976 investigated the role of vitamin B6 (pyridoxine) treatment in women with gestational diabetes. This study demonstrated that one group treated with vitamin B6 resulted in an improvement in glucose tolerance and reduced plasma insulin [16]. In 2009, Hagiwara et al. conducted a study to investigate the effects of pyridoxamine on glucose intolerance and obesity in mice. It showed that the antioxidative effect of pyridoxamine is associated with improvement of glucose intolerance and obesity in mice fed a high-fat diet (HFD). They said that pyridoxamine may be useful in the treatment of obesity-associated metabolic syndrome [21]. Moreover, they recently demonstrated the effect of pyridoxamine, an AGE inhibitor, on the improvement of glucose intolerance in type 2 diabetes mellitus mice [36]. In our study, serum level of leptin decreased, and adiponectin increased significantly in the pyridoxine hydrochloride group. Maessen et al. [37] reported that PM intervention prevents body weight gain, improves metabolic characteristics (fasting plasma glucose, cholesterol, insulin, and leptin levels), prevents mild vascular dysfunction, and reduces the lipid content of the liver in HFD-induced obese mice. Altogether, these findings highlight the potential of PM to serve as an intervention strategy in obesity [37].

The possible mechanism of action of pyridoxine hydrochloride on anthropometric indices and visceral fat index can be stated that pyridoxine may be useful in regulating adipocytokines levels and can improve obesity by increasing fat oxidation in skeletal muscle [21]. Pyridoxine can also stimulate the production of serotonin and cause weight loss [22]. Regarding the possible mechanism of the effects of pyridoxine hydrochloride on metabolic factors, several effects are suggested:

Studies on the possible effects of pyridoxamine as an inhibitor of the formation of AGEs and oxidative stress on improving glucose tolerance and insulin resistance in diabetic rats have been reported [14,21,38,39]. AGEs accumulate in the adipose tissue of obese people,

causing insulin dysfunction in fat cells and skeletal muscle. There is considerable evidence that hyperglycemia eventually leads to the production of reactive oxygen species (ROS) and oxidative stress in various tissues, which in turn can lead to insulin resistance. Activation of nicotinamide adenine dinucleotide phosphate oxidase (NADPH) by inflammatory cytokines also causes the production of other active molecules including superoxide, and hydrogen peroxide [38,39]. Prolonged activation of stress-sensitive signaling pathways by active molecules induces insulin resistance and impairs insulin secretion [40,41]. Pyridoxamine decreases levels of hydrogen peroxide, serum levels of AGEs and NADPH expression, as well as increases the expression of antioxidant enzymes and improves adipocyte levels of adipose tissue. On the other hand, it improves the activity of protein kinase B (an important cellular molecule in the insulin signaling pathway and is required for glucose transport) and replaces GLUT4 (an insulin-dependent glucose transporter) in skeletal muscle [42]. Previous studies have shown that leucine stimulates Sirtuin 1 pathways, resulting in increased mitochondrial function and increased fat metabolism in muscle and fat cells [43,44]. Studies show that a combination of leucine and vitamin B6 than leucine alone improves the oxidative capacity of cells by increasing the expression of adipocyte 5' adenosine monophosphate-activated protein kinase and peroxisome proliferator-activated receptor gamma coactivator 1 protein and increasing mitochondrial function, which leads to increased fatty acid oxidation in muscle and fat cells [23].

The exact mechanism by which vitamin B6 alters lipid profiles is unclear. Vitamin B6 is known to play a role in the desaturation and elongation of fatty acids, methylation of phospholipids, and mobilization of unsaturated fatty acids from TG to phospholipids. Increased delta-desaturase activity reportedly stimulates prostaglandin E1 synthesis, which in turn inhibits cholesterol biosynthesis and subsequently modifies cholesterol levels [45,46]. Moreover, glycosylated LDL cholesterol has diminished ability to be bound and degraded, and thus the inhibition of LDL cholesterol glycosylation by pyridoxal phosphate would enhance the catabolism of LDL cholesterol [47].

AGEs accumulated in the adipose tissue of obese individuals, in addition to causing insulin resistance, impair the expression of adipokines, including interleukin-6 and adiponectin [48-50]. We believe that inhibition of the formation of these products by pyridoxine hydrochloride can improve serum adipokine levels [37].

The design of the study, inclusion and exclusion criteria, controlling for covariates, and repeated assessment of dietary components are amongst the strengths of this study. The randomization method distributes fairly the unknown confounding factors between the intervention and control groups. We also used the double-blind method to minimize selection and information biases. The limitation of the present study was that only one dosage of pyridoxine hydrochloride supplement was used. Further studies with larger sample sizes, longer duration, and different dosages of pyridoxine hydrochloride are recommended.

CONCLUSIONS

This study demonstrated that 8-week supplementation with pyridoxine hydrochloride can reduce BMI and improve body composition and biochemical factors associated with obesity. Pyridoxine hydrochloride may have protective effects against overweight and obesity.

CLINICAL NUTRITION RESEARCH

ACKNOWLEDGEMENTS

REFERENCES

1. World Health Organization. Fact sheets: obesity and overweight [Internet]. Available from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight [cited 2021]. c2021.

2. Lysen LK, Israel DA. Nutrition in weight management. In: Mahan LK, Raymond JL, editors. Krause's food & the nutrition care process. 13th ed. St. Louis (MO): Elsevier Inc.; 2012. p. 462-88.

3. Upadhyay J, Farr O, Perakakis N, Ghaly W, Mantzoros C. Obesity as a disease. Med Clin North Am 2018;102:13-33.
   PUBMED | CROSSREF

4. Haidari F, Samadi M, Mohammadshahi M, Jalali MT, Engali KA. Energy restriction combined with green coffee bean extract affects serum adipocytokines and the body composition in obese women. Asia Pac J Clin Nutr 2017;26:1048-54.
   PUBMED | CROSSREF

5. Spinneker A, Sola R, Lemmen V, Castillo MJ, Pietrzik K, González-Gross M. Vitamin B6 status, deficiency and its consequences--an overview. Nutr Hosp 2007;22:7-24.
   PUBMED

6. Lee Gallagher M. The nutrients and their metabolism. In: Mahan LK, Raymond JL, editors. Krause's food and the nutrition care process. 13th ed. St. Louis (MO): Elsevier Inc.; 2012. p. 74-88.

7. Kleijnen J, Knipschild P. Niacin and vitamin B6 in mental functioning: a review of controlled trials in humans. Biol Psychiatry 1991;29:931-41.
   PUBMED | CROSSREF

8. Mikkelsen K, Stojanovska L, Prakash M, Apostolopoulos V. The effects of vitamin B on the immune/cytokine network and their involvement in depression. Maturitas 2017;96:58-71.
   PUBMED | CROSSREF

9. Konarzewska B, Stefańska E, Wendołowicz A, Cwalina U, Golonko A, Małus A, Kowzan U, Szulc A, Rudzki L, Ostrowska L. Visceral obesity in normal-weight patients suffering from chronic schizophrenia. BMC Psychiatry 2014;14:35.
   PUBMED | CROSSREF

10. Lal KJ, Sharma SK, Dakshinamurti K. Regulation of calcium influx into vascular smooth muscle by vitamin B6. Clin Exp Hypertens 1993;15:489-500.
    PUBMED | CROSSREF

11. Dakshinamurti K, Lal KJ, Ganguly PK. Hypertension, calcium channel and pyridoxine (vitamin B6). Mol Cell Biochem 1998;188:137-48.
    PUBMED | CROSSREF

12. Vasdev S, Ford CA, Parai S, Longerich L, Gadag V. Dietary vitamin B6 supplementation attenuates hypertension in spontaneously hypertensive rats. Mol Cell Biochem 1999;200:155-62.
    PUBMED | CROSSREF

13. Liu Z, Li P, Zhao ZH, Zhang Y, Ma ZM, Wang SX. Vitamin B6 prevents endothelial dysfunction, insulin resistance, and hepatic lipid accumulation in $Apoe^{-/-}$ mice fed with high-fat diet. J Diabetes Res 2016;2016:1748065.
    PUBMED | CROSSREF

14. Unoki-Kubota H, Yamagishi S, Takeuchi M, Bujo H, Saito Y. Pyridoxamine, an inhibitor of advanced glycation end product (AGE) formation ameliorates insulin resistance in obese, type 2 diabetic mice. Protein Pept Lett 2010;17:1177-81.
    PUBMED | CROSSREF

15. Zemel MB, Bruckbauer A. Effects of a leucine and pyridoxine-containing nutraceutical on fat oxidation, and oxidative and inflammatory stress in overweight and obese subjects. Nutrients 2012;4:529-41.
    PUBMED | CROSSREF

16. Spellacy WN, Buhi WC, Birk SA. Vitamin B6 treatment of gestational diabetes mellitus: studies of blood glucose and plasma insulin. Am J Obstet Gynecol 1977;127:599-602.
PUBMED | CROSSREF

17. de Gómez Dumm NT, Giammona AM, Touceda LA. Variations in the lipid profile of patients with chronic renal failure treated with pyridoxine. Lipids Health Dis 2003;2:7.
PUBMED | CROSSREF

18. Hlais S, Reslan DR, Sarieddine HK, Nasreddine L, Taan G, Azar S, Obeid OA. Effect of lysine, vitamin B6, and carnitine supplementation on the lipid profile of male patients with hypertriglyceridemia: a 12-week, open-label, randomized, placebo-controlled trial. Clin Ther 2012;34:1674-82.
PUBMED | CROSSREF

19. Virk RS, Dunton NJ, Young JC, Leklem JE. Effect of vitamin B-6 supplementation on fuels, catecholamines, and amino acids during exercise in men. Med Sci Sports Exerc 1999;31:400-8.
PUBMED | CROSSREF

20. Tambasco-Studart M, Titiz O, Raschle T, Forster G, Amrhein N, Fitzpatrick TB. Vitamin B6 biosynthesis in higher plants. Proc Natl Acad Sci U S A 2005;102:13687-92.
PUBMED | CROSSREF

21. Hagiwara S, Gohda T, Tanimoto M, Ito T, Murakoshi M, Ohara I, Yamazaki T, Matsumoto M, Horikoshi S, Funabiki K, Tomino Y. Effects of pyridoxamine (K-163) on glucose intolerance and obesity in high-fat diet C57BL/6J mice. Metabolism 2009;58:934-45.
PUBMED | CROSSREF

22. Abraham PM, Kuruvilla KP, Mathew J, Malat A, Joy S, Paulose CS. Alterations in hippocampal serotonergic and INSR function in streptozotocin induced diabetic rats exposed to stress: neuroprotective role of pyridoxine and Aegle marmelose. J Biomed Sci 2010;17:78.
PUBMED | CROSSREF

23. Zemel MB, Bruckbauer A. Effects of a leucine and pyridoxine-containing nutraceutical on body weight and composition in obese subjects. Diabetes Metab Syndr Obes 2013;6:309-15.
PUBMED | CROSSREF

24. Wolin KY, Heil DP, Askew S, Matthews CE, Bennett GG. Validation of the International Physical Activity Questionnaire-short among blacks. J Phys Act Health 2008;5:746-60.
PUBMED | CROSSREF

25. Amato MC, Giordano C. Visceral adiposity index: an indicator of adipose tissue dysfunction. Int J Endocrinol 2014;2014:730827.
PUBMED | CROSSREF

26. Chen Y, Zhang X, Pan B, Jin X, Yao H, Chen B, Zou Y, Ge J, Chen H. A modified formula for calculating low-density lipoprotein cholesterol values. Lipids Health Dis 2010;9:52.
PUBMED | CROSSREF

27. Mahmoud AM, Ashour MB, Abdel-Moneim A, Ahmed OM. Hesperidin and naringin attenuate hyperglycemia-mediated oxidative stress and proinflammatory cytokine production in high fat fed/streptozotocin-induced type 2 diabetic rats. J Diabetes Complications 2012;26:483-90.
PUBMED | CROSSREF

28. D-A-CH Reference values for nutrient intakes. Frankfurt: Umschau Verlag; 2000.

29. Baer RL. Cutaneous skin changes probably due to pyridoxine abuse. J Am Acad Dermatol 1984;10:527-8.
PUBMED | CROSSREF

30. Food and Nutrition Board. Dietary reference intakes for thiamine, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington D.C.: National Academy Press; 1998.

31. Xue B, Zemel MB. Relationship between human adipose tissue agouti and fatty acid synthase (FAS). J Nutr 2000;130:2478-81.
PUBMED | CROSSREF

32. Kim JH, Kiefer LL, Woychik RP, Wilkison WO, Truesdale A, Ittoop O, Willard D, Nichols J, Zemel MB. Agouti regulation of intracellular calcium: role of melanocortin receptors. Am J Physiol 1997;272:E379-84.
PUBMED | CROSSREF

33. Xue B, Greenberg AG, Kraemer FB, Zemel MB. Mechanism of intracellular calcium ($[Ca^{2+}]_i$) inhibition of lipolysis in human adipocytes. FASEB J 2001;15:2527-9.
PUBMED | CROSSREF

34. Xue B, Moustaid-N , Wilkison WO, Zemel MB. The agouti gene product inhibits lipolysis in human adipocytes via a Ca^{2+}-dependent mechanism. FASEB J 1998;12:1391-6.
PUBMED | CROSSREF

35. Shi H, Moustaid-Moussa N, Wilkison WO, Zemel MB. Role of the sulfonylurea receptor in regulating human adipocyte metabolism. FASEB J 1999;13:1833-8.
PUBMED | CROSSREF

36. Tanimoto M, Gohda T, Kaneko S, Hagiwara S, Murakoshi M, Aoki T, Yamada K, Ito T, Matsumoto M, Horikoshi S, Tomino Y. Effect of pyridoxamine (K-163), an inhibitor of advanced glycation end products, on type 2 diabetic nephropathy in KK-A^y/Ta mice. Metabolism 2007;56:160-7.
   PUBMED | **CROSSREF**

37. Maessen DE, Brouwers O, Gaens KH, Wouters K, Cleutjens JP, Janssen BJ, Miyata T, Stehouwer CD, Schalkwijk CG. Delayed intervention with pyridoxamine improves metabolic function and prevents adipose tissue inflammation and insulin resistance in high-fat diet-induced obese mice. Diabetes 2016;65:956-66.
   PUBMED | **CROSSREF**

38. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
   PUBMED | **CROSSREF**

39. Zalba G, San José G, Moreno MU, Fortuño MA, Fortuño A, Beaumont FJ, Díez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. Hypertension 2001;38:1395-9.
   PUBMED | **CROSSREF**

40. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes 2003;52:1-8.
   PUBMED | **CROSSREF**

41. Bruce CR, Carey AL, Hawley JA, Febbraio MA. Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism. Diabetes 2003;52:2338-45.
   PUBMED | **CROSSREF**

42. Voziyan PA, Hudson BG. Pyridoxamine as a multifunctional pharmaceutical: targeting pathogenic glycation and oxidative damage. Cell Mol Life Sci 2005;62:1671-81.
   PUBMED | **CROSSREF**

43. Sun X, Zemel MB. Leucine and calcium regulate fat metabolism and energy partitioning in murine adipocytes and muscle cells. Lipids 2007;42:297-305.
   PUBMED | **CROSSREF**

44. Sun X, Zemel MB. Leucine modulation of mitochondrial mass and oxygen consumption in skeletal muscle cells and adipocytes. Nutr Metab (Lond) 2009;6:26.
   PUBMED | **CROSSREF**

45. Harripersad R, Burger FJ. The effect of a subnormal dose of vitamin B6 on plasma lipid in the rat. Int J Vitam Nutr Res 1997;67:95-101.
   PUBMED

46. Dionyssiou-Asteriou A, Triantafyllou A, Lekakis J, Kalofoutis A. Influence of prostaglandin E1 on high density lipoprotein-fraction lipid levels in rats. Biochem Med Metab Biol 1986;36:114-7.
   PUBMED | **CROSSREF**

47. Gonen B, Baenziger J, Schonfeld G, Jacobson D, Farrar P. Nonenzymatic glycosylation of low density lipoproteins in vitro. Effects on cell-interactive properties. Diabetes 1981;30:875-8.
   PUBMED | **CROSSREF**

48. Gaens KH, Goossens GH, Niessen PM, van Greevenbroek MM, van der Kallen CJ, Niessen HW, Rensen SS, Buurman WA, Greve JW, Blaak EE, van Zandvoort MA, Bierhaus A, Stehouwer CD, Schalkwijk CG. Nε-(carboxymethyl)lysine-receptor for advanced glycation end product axis is a key modulator of obesity-induced dysregulation of adipokine expression and insulin resistance. Arterioscler Thromb Vasc Biol 2014;34:1199-208.
   PUBMED | **CROSSREF**

49. Schalkwijk CG, Brouwers O, Stehouwer CD. Modulation of insulin action by advanced glycation endproducts: a new player in the field. Horm Metab Res 2008;40:614-9.
   PUBMED | **CROSSREF**

50. Sandu O, Song K, Cai W, Zheng F, Uribarri J, Vlassara H. Insulin resistance and type 2 diabetes in high-fat-fed mice are linked to high glycotoxin intake. Diabetes 2005;54:2314-9.
   PUBMED | **CROSSREF**