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Folic Acid Supplementation Improves Vascular Function in Professional Dancers With Endothelial Dysfunction

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Abstract

Objective. To determine if folic acid supplementation improves vascular function (brachial artery flow-mediated dilation [FMD]) in professional dancers with known endothelial dysfunction.

Design. Prospective cross-sectional study.

Setting. Academic institution in the Midwestern United States.

Subjects. Twenty-two professional ballet dancers volunteered for this study.

Main Outcome Measures. Subjects completed a 3-day food record to determine caloric and micronutrient intake. Menstrual status was determined by interview and questionnaire. Endothelial function was determined as flow-induced vasodilation measured by high-frequency ultrasound of the brachial artery. A change in brachial diameter of <5% to hyperemic flow stimulus was defined a priori as endothelial dysfunction. Subjects with abnormal FMD took 10 mg of folic acid daily for 4 weeks, and FMD testing was then repeated. Serum whole blood was measured for folic acid levels before and after supplementation.

Results. Sixty-four percent of dancers (n = 14) had abnormal brachial artery FMD (<5%) (mean ± standard deviation, 2.9% ± 1.5%). After 4 weeks of folic acid supplementation (10 mg/day), FMD improved in all the subjects (7.1% ± 2.3%; *P* < .0001).

Conclusions. This study reveals that vascular endothelial function improves in dancers after supplementation with folic acid (10 mg/day) for at least 4 weeks. This finding may have clinically important implications for future cardiovascular disease risk prevention.

Introduction

Previous studies have shown that amenorrheic athletes have evidence of impaired vascular function manifested by reduced brachial artery flow-mediated dilation (FMD) to hyperemic stimuli [1,2]. Subjects in these studies consisted of college runners and endurance athletes in Sweden; none were dancers. Several studies have shown that ballet dancers have a high prevalence of amenorrhea [3–5] and disordered eating [6–8]. Therefore this group also may be at high risk for endothelial dysfunction.

Previous studies in athletes have shown a relationship between estrogen levels and brachial artery FMD [9]. It is known that coronary and peripheral vessels contain estrogen receptors, which allow estrogen to play a regulatory role in vascular function. Estrogen stimulates the production of nitric oxide (NO) through both genomic and non-genomic effects, leading to increased production of endothelial-derived NO, causing vasodilatation [10,11]. Estrogen exerts long-term genomic effects via alteration of gene and protein

expression, mostly at the level of gene transcription, resulting in increased endothelial NO synthase (eNOS) synthesis and activity [10,11]. In addition to these genomic effects, evidence supports acute increases in eNOS via interaction with estrogen receptors on endothelial cells [12]. Finally, amenorrhea associated with athletes is known to have a steroid hormonal profile similar to that observed in menopause. Therefore low estrogen levels, whether in a post-menopausal woman or a young athlete with athletic-associated amenorrhea, will theoretically cause impaired endothelial cell function and resultant impaired arterial dilation [1,2].

Abnormal endothelial dysfunction manifested as reduced brachial artery dilation has been shown to have a positive predictive value (95%) for coronary endothelial dysfunction [13]. In addition, Schachinger and colleagues [14] have shown that coronary endothelial vasodilator dysfunction predicts long-term atherosclerotic disease progression and cardiovascular event rates. Woo and associates [15] have shown that brachial endothelial dysfunction correlates with the extent of coronary atherosclerosis. Therefore non-invasive assessment of brachial endothelial function by high-frequency ultrasound can provide valuable information about endothelial function in an otherwise healthy athletic population.. Early treatment in this group is important because cardiovascular disease is the number one cause of death in women in the United States.

Rickenlund and colleagues [16] have shown that administration of oral contraceptives (30 μg of ethinyl estradiol and 150 μg of levonorgestrel) significantly improved brachial artery FMD in young amenorrheic runners. However, the Women's Health Initiative study showed an increased risk of breast cancer, heart disease, stroke, and blood clots among postmenopausal women who were using hormone replacement therapy that included both estrogen and progestin [17,18]. More recently, Chlebowski and colleagues [19] determined that synthetic hormone replacement therapy increases breast cancer risk and that these cancers tend to be more aggressive and lethal. Although these findings have not been found in premenopausal women, seeking an alternative treatment approach to reduced FMD in young athletes is reasonable.

Folic acid has potential benefits with regard to cardiovascular parameters such as endothelial function, arterial stiffness, blood pressure, and thrombotic activity [20–28]. Several studies have already shown that folic acid supplementation improves endothelial function in various disease states, including hypercholesterolemia [29,30], hypertension [28], diabetes [31], coronary artery disease [32,33], and hyperhomocystinemia [15]. These studies suggest that higher folic acid intake by specific populations may have vasculoprotective effects.

Several mechanisms explain the effectiveness of folic acid therapy. Folates are believed to participate in the endogenous regeneration of tetrahydrobiopterin [34], an essential cofactor for eNOS production of NO, and therefore may result in increased NO production after supplementation. Folic acid has a known homocysteine-lowering effect that could contribute to improvements in endothelial function. In addition, folic acid may have a direct antioxidant effect in the vasculature, increasing NO bioavailability, which is a major factor influencing the improvement in FMD [13,32]. Therefore the purpose of this study was to determine if folic acid supplementation of 10 mg per day would increase FMD in professional dancers with reduced FMD.

Methods

Subjects and Experimental Design

This study was reviewed and approved by multi-institutional human subjects internal review boards. Inclusion criteria included professional female dancers, ages 18–35 years, from a single dance company in the Midwest. Exclusion criteria included a history of heart disease, vascular disease, hypertension, diabetes, hypercholesteremia, chromosomal disorder, current pregnancy, pituitary tumor, oral contraceptive use, anomalies of the reproductive system, and thyroid disease. Any subject taking a cardiac, cholesterol, hypertension, or thyroid medication was excluded from the study. Subjects were not excluded based on ethnicity. Twenty-two women met the criteria and completed the study. No women were excluded based on our criteria. The study was explained in person, and informed consent was

obtained. Medical history and menstrual status were obtained through questionnaires and interview.

Serum Folic Acid Levels

Fasting whole serum folate levels were measured by the Bayer ADVIA Centaur assay (Siemens Medical Solutions Diagnostics, Deerfield, IL) before and after supplementation with folic acid. This assay is a competitive immunoassay that uses direct chemiluminescent technology. The inter-assay coefficient of variation was 5%–7%, the intra-assay coefficient of variation was 4%–8%, the lower limit of sensitivity was 0.35 ng/mL, and the upper limit of sensitivity was ≥24 ng/mL. Blood was drawn early in the morning after an overnight fast. Subjects with FMD <5% were instructed to take folic acid (10 mg/day) for 4 weeks. For standardization purposes, subjects were instructed to take their supplements in the morning with food. Subjects kept written logs and were randomly called to assess compliance.

Dietary History

Caloric micronutrient intake was determined from a prospective 3-day food diary recorded during 2 weekdays and 1 weekend day during the dance training season. A registered research dietitian met with each dancer for education and training in proportions or size of food servings, proper recording of foods, and the use of the food diary in general. Dietary analysis was performed by the research dietician with use of Nutrition Data System for Research software version 2007 (developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN) to determine total calories; total composition of fat, protein, and carbohydrates; and micronutrient content (ie, folic acid, L-arginine, vitamin C, vitamin E, and vitamin B12). Subjects were encouraged to weigh their food portions for recording.

Menstrual Status

Self-administered questionnaires were used to determine menarche, number of periods per year, number of days between periods, skipped periods, history of thyroid disease or pituitary tumor,

and use and type of birth control. A formal interview regarding menstrual status and medical history was conducted by the principal investigator. For the purpose of this study, eumenorrhea or regular cycling was defined as menstrual bleeding every 28–30 days. Primary amenorrhea was defined as absence of menarche by age 15 years and secondary amenorrhea was defined as cessation of menses for >3 consecutive cycles after onset of menarche, excluding pregnancy. Oligomenorrhea was represented by menstrual cycles with intervals greater than 35 days for at least 12 months. Luteal phase dysfunction was defined as a menstrual cycle less than 22 days. If subjects were taking oral contraceptives for treatment of secondary amenorrhea, they were identified as amenorrhea—oral contraceptive group. If they were taking oral contraceptives for any other reason, they were excluded from the study.

Cardiovascular Status

Brachial artery FMD and velocity were determined by techniques previously used in this laboratory [1,35,36]. Subjects were studied in a supine resting position following an 8-hour fast in a temperature-controlled room. Systemic arterial pressure was periodically monitored in the nondominant arm with an automated sphygmomanometer. Subjects were instrumented for continuous recording of electrocardiogram, heart rate, and intermittent blood pressure. All subjects were tested at approximately the same time of day, with eumenorrheic, oligomenorrheic, and amenorrheic subjects studied in the early follicular phase of the menstrual cycle (day 4–8) [37–39]. Amenorrheic subjects were tested randomly as schedules permitted, but also in the early morning.

Statistics

Descriptive statistics are presented as mean ± standard deviation (SD) for each measurement at baseline and after 4 weeks of folic acid supplementation. The primary outcome of interest for this study was change in brachial artery FMD before and after folic acid supplementation. A paired *t*-test was used to compare FMD before and after supplementation. All comparisons with the untreated group used an unpaired *t*-test, whereas all pre-post comparisons used a paired *t*-

test. Based on Box-Cox analysis, all nutritional intake values were log transformed before the paired *t*-test. Because folic acid levels had an upper quantification threshold at 24 ng/mL, a log-rank test was used when comparing the folic acid levels (ng/mL) of the abnormal FMD (5%) group (n = 14) with the normal FMD (>5%) group (n = 8) before supplementation with folic acid. The mean and SD of the underlying distribution were estimated by fitting a truncated normal distribution. The pre-treatment and post-treatment folic acids levels were compared using McNemar's exact test on the indicator of exceeding the 24 ng/mL threshold. All analyses were performed using R software version 2.10.1 [40].

Results

Subject Characteristics

Twenty-two elite dancers consented to participate and completed this study. Twenty dancers self-reported their ethnicity as Caucasian, one reported Asian ethnicity, and one reported Hispanic ethnicity, with an age of 23.2 ± 4.7 years (mean ± SD). Table 1 provides descriptive demographics of the cohort including height, weight, percent body fat, body mass index, age, menarche, and years dancing divided into 2 groups based on whether FMD was abnormal (<5%) or normal (>5%). No difference in body mass index, percent body fat, or menarche was found between the 2 groups. A difference in age and years of dancing was found; the abnormal FMD group was younger and had danced fewer total years compared with the normal group.

Table 1. *Descriptive demographics of cohort*

| Characteristic	Group With Abnormal FMD (N = 14)	Group With Normal FMD (N = 8)	P Value
Age (y)	21.1 ± 3.2	27.0 ± 4.3	.002
Height (in)	64.8 ± 2.1	64.7 ± 1.8	.94
Weight (lb)	115.6 ± 11.2	113.3 ± 7.5	.62
Body fat (%)	17.6 ± 5.2	15.2 ± 3.4	.28
Body mass index (kg/m^2)	19.3 ± 1.3	19.0 ± 0.7	.58
Menarche (y)	13.2 ± 1.8	14.1 ± 1.5	.27
Dance history (y)	15.5 ± 3.1	19.5 ± 5.0	.04

FMD = flow-mediated dilation.
Values are mean ± standard deviation unless otherwise indicated.
P value based on 2-sample t-test.

Vascular Reactivity

Brachial artery data are summarized in Table 2 and Figure 1. Table 2 includes data on heart rate (beats per minute), systolic arterial pressure (mm Hg), diastolic arterial pressure (mm Hg), temperature (°F), relative humidity (%), baseline brachial artery diameter (mm), FMD (%), and peak change in flow velocity (%). Abnormal FMD was defined by less than 5% dilation in response to an ischemic challenge [35,36]. This definition is based on data from our laboratory (normal = 7.0% ± 1.5%; unpublished data) and others [41–43]. Subjects were subsequently divided into a normal group (>5%, n = 8) and a reduced group (<5%, n = 14). Brachial artery FMD was reduced in 14 of 22 dancers (64%). Baseline brachial diameters, arterial pressures, resting heart rate, temperature, humidity, and peak change in flow velocity were not significantly different (*P* > .05) between subjects with reduced and normal FMD (Table 2). After folic acid supplementation of 10 mg/day for 4 weeks, all subjects had an increase in FMD (from 2.9% ± 1.5% to 7.1% ± 2.3%, *P* < .0001) (Figure 1). Individual data for each subject before and after folic acid supplementation also is provided (Figure 2).

Brachial Artery Flow-Mediated Dilation
Before & After Supplementation
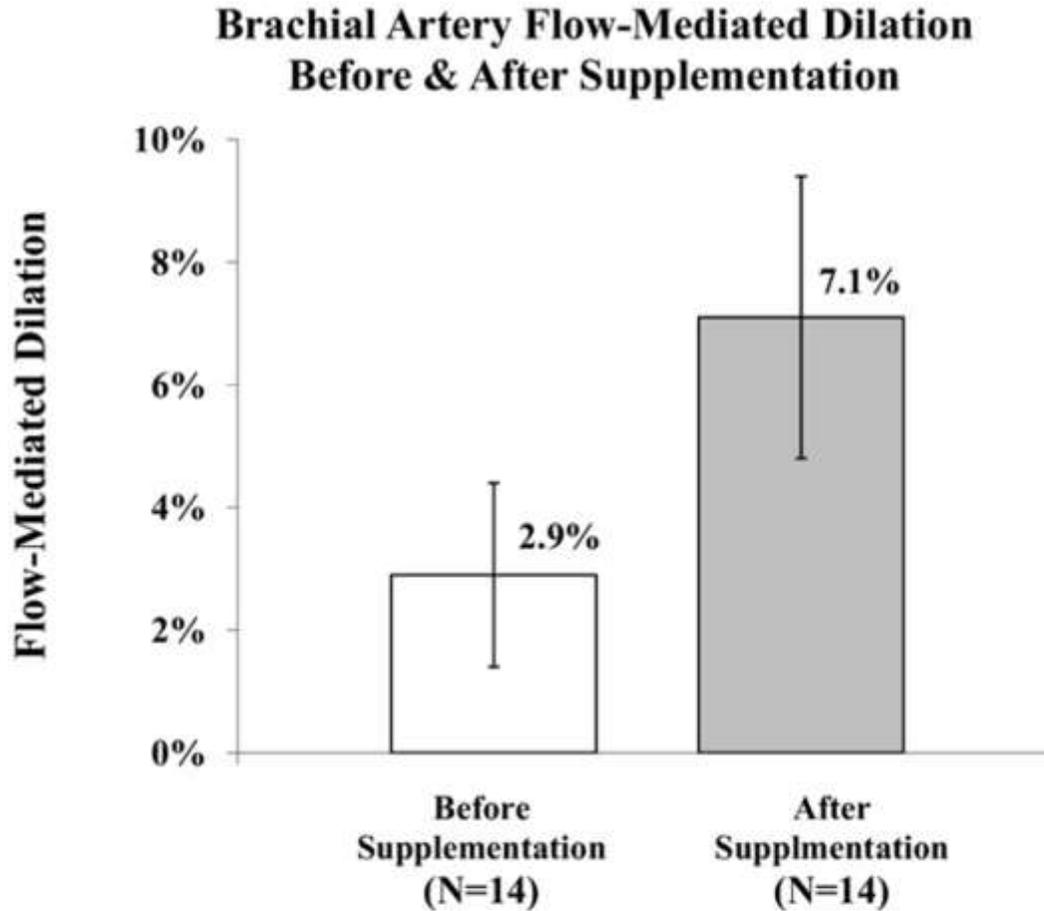
Abnormal defined a priori as <5%
P values based on paired *t* test
Mean ± SD shown

Figure 1. Fourteen dancers had reduced flow-mediated dilation (defined a priori as < 5%). Flow-mediated dilation increased after folic acid supplementation.

Ballet Subjects with Abnormal FMD Values Before & After Folic Acid Supplementation
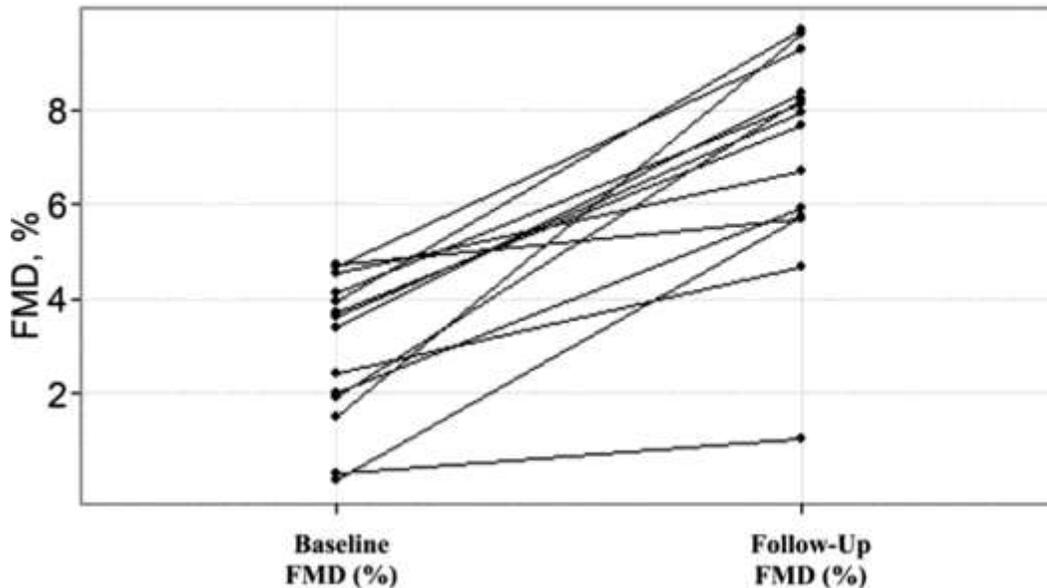

Figure 2. Individual data of the 14 dancers with reduced flow-mediated dilation before and after folic acid supplementation. FMD = flow-mediated dilation.

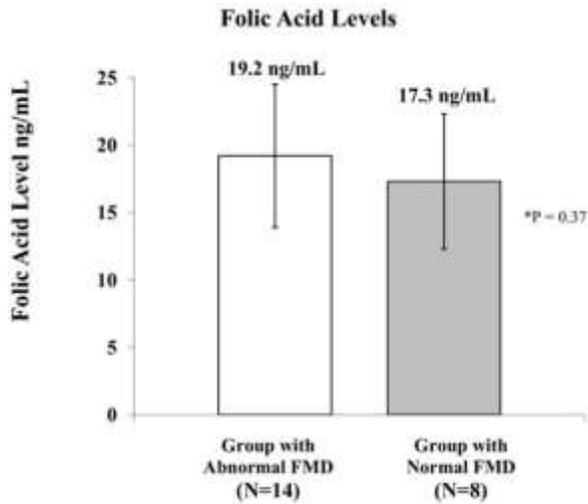Table 2. *Brachial artery studies before and after folic acid supplementation (A priori <5%)*

Ballet Dancers (N = 14)	Before Supplementation	After Supplementation	P Value
Heart rate (beats/min)	56.1 ± 9.9	58.4 ± 14.9	.33
Mean arterial pressure			
Systolic (mm Hg)	101.2 ± 10.0	102.1 ± 9.6	.50
Diastolic (mm Hg)	60.6 ± 8.9	63.7 ± 8.2	.12
Temperature (°F)	69.3 ± 1.3	69.8 ± 0.6	.09
Humidity	21.2 ± 4.5	21.3 ± 4.7	1.00
Baseline brachial artery diameter (mm)	2.8 ± 0.3	2.7 ± 0.3	.35
Peak brachial artery diameter (mm)	2.9 ± 0.3	2.9 ± 0.4	.69
Flow-mediated dilation (%)	2.9 ± 1.5	7.1 ± 2.3	<.0001
Peak change in flow velocity (%)	67.2 ± 41.7	69.1 ± 25.5	1.00

Values are mean ± standard deviation unless otherwise indicated.

Serum Folic Acid Levels

No difference was found in serum folic acid between the abnormal FMD group (<5%; n = 14; 19.2 ng/mL ± 5.3) and the normal FMD group (>5%; n = 8; 17.3 ng/mL ± 5.0) prior to supplementation (*P* = .37) ([Figure 3](#)). Folic acid levels increased in the folic acid treatment group after supplementation, but the exact amount is difficult to quantify because the highest value our laboratory reports is 24.0 ng/mL; however, all post-treatment levels were above the cut-off value of 24 ng/mL. In fact, the proportion of subjects above
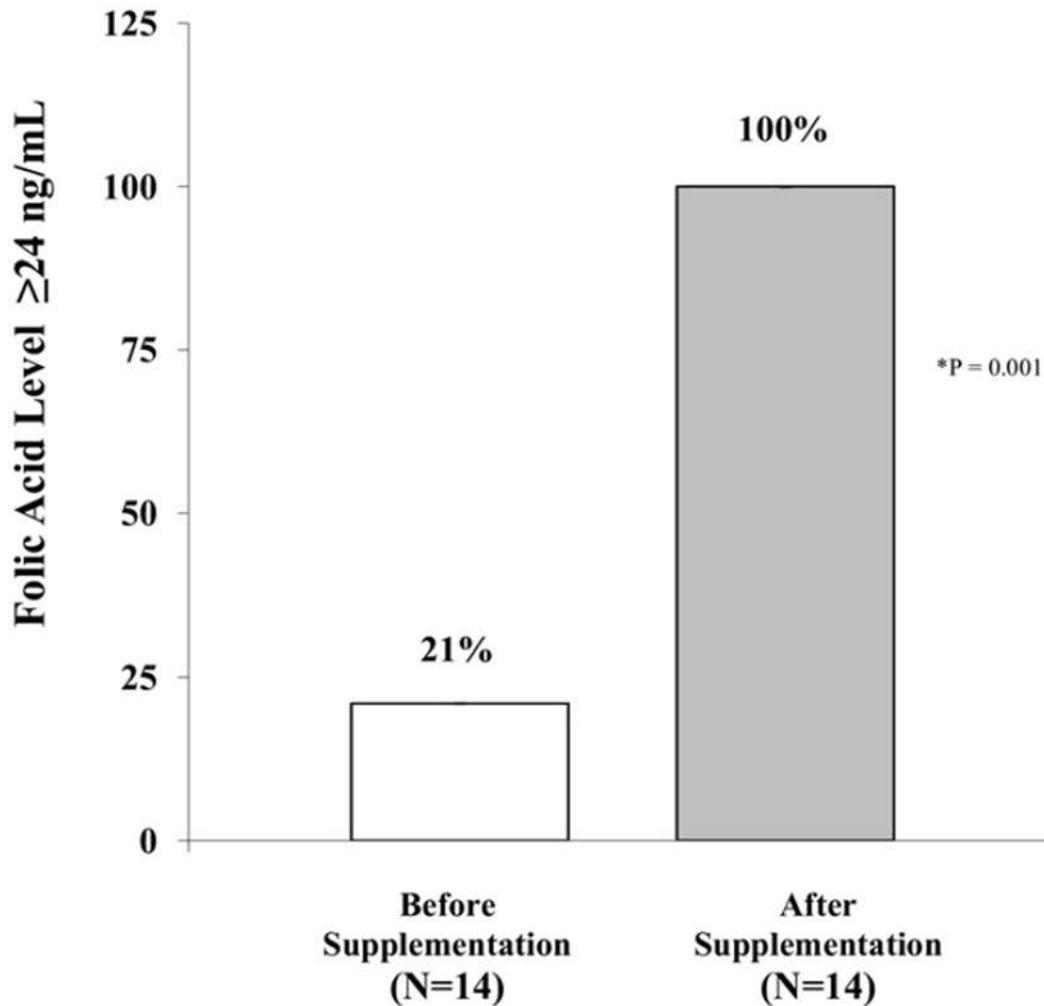
24 ng/mL increased significantly (21% versus 100%, *P* = .001 by exact McNemar's test) after supplementation (Figure 4).

Figure 3. Folic acid levels before supplementation at start of study between group with normal flow-mediated dilation (N = 8) and reduced flow-mediated dilation (N = 14). FMD = flow-mediated dilation.

Folic Acid Levels
Before & After Supplementation

Figure 4. Percentage of subjects who were above the laboratory threshold of 24 ng/mL before and after folic acid supplementation (10 mg/day for 4 weeks).

Dietary Intake

No difference was found in recorded dietary antioxidants (vitamin C and vitamin E), folic acid, or L-arginine (co-factor eNOS),

all which can affect NO levels. In addition, no differences in vitamin D, vitamin B12, calories, protein, fat, or carbohydrates were found between the abnormal FMD group and normal FMD group (P = .19) at the start of the study ([Table 3](#)).

Table 3. *Nutritional data at start of study*

	Group With Abnormal FMD (N = 14)	Group With Normal FMD (N = 8)	P Value
Vitamin D (IU)	503.8 ± 433.1	433.8 ± 303.8	.70
Vitamin C (mg)	601.6 ± 1057.5	729.1 ± 421.6	.21
Vitamin E (IU)	239.7 ± 482.1	149.8 ± 187.4	.88
L-arginine (g)	3.3 ± 1.1	4.1 ± 1.4	.32
Folic acid (mg)	0.8 ± 0.4	0.7 ± 0.4	.56
Protein (g)	62.0 ± 23.2	77.5 ± 26.1	.19
Fat (g)	63.1 ± 21.9	73.5 ± 29.5	.44
Carbohydrates (g)	259.2 ± 85.3	230.1 ± 46.2	.37
Energy (kcal)	1809.6 ± 572.3	1883.8 ± 479.2	.70
Vitamin B12 (μg)	26.9 ± 48.2	23.9 ± 11.8	.28

Values are mean ± standard deviation unless otherwise indicated.

Amenorrhea/Oligomenorrhea

At the time of the study, 2 dancers were amenorrheic (9%) (2.0 ± 0.1 years) and 6 dancers (27%) were oligomenorrheic (menstrual cycles >35 days for at least 12 months). Collectively, 36% of 22 dancers self-reported current menstrual dysfunction, and they all had an FMD of less than 5%. Three dancers (14%) were taking contraceptive hormones because of a history of secondary amenorrhea. The relationship between FMD and menstrual status is reported in a previous study [9].

Discussion

The main finding from this study is that 64% (n = 14) of the dancers in a professional dance company had evidence of abnormal FMD and that FMD significantly improved with folic acid supplementation (10 mg/day) for 4 weeks. The baseline FMD of this group was 2.9%, and it improved to 7.1% after supplementation.

Several studies in older men and women with chronic diseases have shown an increase in FMD with folic acid supplementation [30,44,45]. However, this is the first study to reveal that folic acid supplementation improves FMD in young professional dancers with endothelial dysfunction.

These findings are consistent with a previous study in young amenorrheic runners of similar age (mean ± SD, age 22.1 ± 4.8 years) with a similar reduction in FMD, who showed similar improvement (3.0% to 7.7%) after folic acid supplementation (10 mg/day) for 4 weeks [35]. However, a significant methodological difference exists between these two studies. In the previous study of young amenorrheic women runners, altered menstrual status (ie, amenorrhea) was the primary inclusion criteria for the study group. In the present study of ballet dancers, the inclusion criteria for the experimental group were dancers with reduced FMD (<5%) regardless of menstrual status.

In the present study of ballet dancers, all subjects with reported amenorrhea and oligomenorrhea had reduced FMD (<5%). Two of the 3 subjects were taking synthetic hormones for amenorrhea, and 4 eumenorrheic subjects had reduced FMD (<5%). Although abnormal FMD in amenorrheic dancers is consistent with previous research [1,2], the relationship between eumenorrhea and brachial artery FMD is not as clear. A 2009 study [36] evaluated eumenorrheic runners and brachial artery FMD. In this study, subjects were randomly assigned to placebo or folic acid (10 mg/day; 4 weeks) in a blinded fashion. Folic acid levels were measured before and after supplementation. Both groups had normal folic acid levels at the start of the study. After the supplementation period, the group taking folic acid had a significant increase in folic acid levels, whereas there was no change in folic acid levels in the placebo group. The group taking folic acid had a significant increase (3%) in FMD compared with the placebo group (0.11%), which had no increase. This finding is similar to that of the current study of ballerinas, which showed an increase in FMD in 4 eumenorrheic subjects with an initial FMD <5%. However, the data in eumenorrheic runners contradicts a 2010 study [35] which did *not* show an increase in FMD in eumenorrheic runners, only in amenorrheic runners, with folic acid supplementation. Possible explanations for the discrepancy in brachial artery FMD in eumenorrheic runners is most

likely multifactorial. The laboratory for these studies only reported an upper threshold value of 24 ng/mL for serum folic acid levels. It may be that the eumenorrheic group in the 2010 study had a significantly lower folic acid level compared with the other studies. Aerobic fitness is also known to affect FMD values, and this variable was not measured in any of the studies. Finally, subjects may have *incorrectly* reported eumenorrhea, but they could truly have anovulatory cycles that could affect estrogen levels and FMD. Future studies with a larger cohort with more precise measurement of serum folic acid and estrogen levels are needed to further quantify the effect of folic acid in eumenorrheic athletes.

The risk of toxicity from folic acid intake from supplements and/or fortified foods is low [46]. Folic acid is a water-soluble vitamin that is regularly eliminated in the urine. No adverse effects of folic acid have been reported at 10 mg/day [15,46–49], and the Food and Drug Administration MedWatch system does not list any adverse effects of folic acid that apply to this study [47]. Higher doses (>20 mg/day) can cause adverse effects such as upset stomach, sleep disturbances, and skin problems. The recommended daily allowance for folate for women ages 19–50 years is 400 µg/day, and the tolerable upper intake level (UL) is 1 g/day. For women who have risk factors for neural tube defects, 4 mg is recommended. It is important to recognize that the upper intake level refers to the amount of synthetic folate (folic acid) being consumed per day from fortified foods and/or supplements. There is no health risk and no UL for natural sources of folate found in food.

Despite the benefits of folic acid, some evidence indicates that high levels of folic acid can pose a variety of health risks. High doses of folic acid (>15 mg/day) can provoke seizures in patients taking anticonvulsant medications [49]. None of our subjects had a known seizure disorder. The National Institutes of Health [50] also states that caution regarding supplementation with folic acid should be used in patients with a vitamin B12 deficiency, because folic acid supplementation may hide the deficiency and silently exacerbate the condition. None of our subjects had low vitamin B12 levels as determined by their 3-day food record.

A recent study raises the possibility that folic acid supplementation might increase the risk of colorectal adenoma in patients with a known history of colorectal adenomas [51], but these data have not been confirmed in prospective trials. In this study [51], the rates of colon cancer recurrences were slightly higher at the first and second colonoscopic follow-up (44.1% and 41.9%, respectively) when compared with the placebo control group (42.4% and 37.2%, respectively). This small observed increase may be related to more widespread screening and better detection. The evidence linking folic acid supplementation and an increase in colon cancer rates is inconclusive and merits further investigation. Folic acid has not been shown to directly cause colon cancer; however, if colon cancer is present, folic acid *may* fuel its growth [51]. The risks of folic acid supplementation are small and may be inconclusive with regard to increased colon cancer risk. In many patient populations, the benefits of folic acid supplementation likely outweigh the risks. From a clinical perspective, it would seem logical that if FMD was determined to be low, clinicians could potentially recommend folic acid on a daily basis to athletes; however, length of time and optimal dosage is still to be determined.

Study Limitations

These results must be interpreted in light of several limitations. The small sample size and cross-sectional design are methodologic shortcomings of this study. In addition, because of the inability of our laboratories to quantify serum folic acid above 24 ng/mL, true folic acid levels were unknown. Finally, aerobic fitness was not determined, which is known to affect FMD. Future studies should include a larger cohort, with precise folic acid measurements in addition to metabolic testing.

Conclusion

In summary, this study showed that folic acid supplementation (10 mg/day for 4 weeks) significantly improved brachial artery FMD in professional dancers with reduced FMD. This finding may have clinical importance because reduced FMD is an accepted early sign of accelerated development of atherosclerosis, which may have an

adverse health impact in this asymptomatic population of athletic professionals. Further studies should test this hypothesis in a larger cohort of dancers with and without endothelial dysfunction.

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Footnotes

Peer reviewers and all others who control content have no relevant financial relationships to disclose.

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