Acute effects of diets rich in almonds and walnuts on endothelial function

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ABSTRACT

Objective: Omega-3 fatty acids, especially alpha-linolenic acid (ALA), which are present in nuts may reduce cardiovascular disease (CVD) risk, by changing vascular inflammation and improving endothelial dysfunction. The objective of the study was to evaluate the acute effects of two different diets, one containing walnuts and the other almonds on endothelial function.

Methods: Twenty-seven overweight volunteers underwent a randomized 2-period, crossover, controlled intervention study. The subjects were given either walnut or almond diets which varied in monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content. The walnut diet provided 23.3% energy from MUFA and the almond diet provided 7.6% energy from PUFA. Endothelial function was assessed physiologically by flow-mediated dilation (FMD) and biochemically by sVCAM (soluble vascular cell adhesion molecules).

Results: The walnut diet significantly improved FMD (p=0.004) and decreased sVCAM (p=0.009) whereas the almond diet tended to improve FMD (p=0.06) and significantly decreased sVCAM (p=0.004).

Conclusion: Both walnut and almond diets improved FMD and sVCAM and there was no significant difference in physiological and biochemical markers between the two diets.

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1. Introduction

Endothelial dysfunction is an early event in the development of atherosclerotic vascular disease. Recent studies indicate that coronary endothelial dysfunction predicts future cardiovascular disease (CVD) events. Endothelial function can be assessed noninvasively in the peripheral circulation by brachial artery ultrasound and it correlates well with coronary artery endothelial function.

Walnuts differ from other nuts by a high content of alpha-linolenic acid (ALA), a vegetable n-3 fatty acid, which might confer additional anti-atherogenic properties. Replacement of fatty foods in the diet with nuts reduces blood cholesterol and has other beneficial effects on the cardiovascular risk profile. Monounsaturated fatty acids (MUFA), mainly oleic acid, is predominant in almonds while walnuts are rich in polyunsaturated fatty acids (PUFA) like linoleic acid and ALA. Walnuts have a higher amount of n-6 and n-3 fatty acids as compared to almonds. Also, the n-6/n-3 ratio is lower in walnuts. A lower ratio of n-6/n-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases. The fatty acids from nuts have a beneficial effect on the cardiovascular system such as lowering blood cholesterol, reducing vascular inflammation and improving endothelial function.

Various studies have assessed endothelial function and inflammatory markers after supplementing diets with almonds or walnuts separately. However none of the studies have investigated the acute effect of a diet rich in both almonds and walnuts on the endothelium.
To test the hypothesis that walnut intake (high PUFA content) would improve endothelial function compared to almond intake (high MUFA content), we performed a randomized, crossover feeding trial with a walnut-enriched diet and compared it with an almond-enriched diet to access the brachial artery vasomotor function and a circulating marker of endothelial activation in over weight subjects.

2. Subjects and methods

2.1. Subjects

Twenty-seven asymptomatic over weight individuals (BMI > 25 kg/m²) were recruited into a protocol approved by the institutional review board and gave informed consent. Of the 27 participants in the study, 16 were women and 11 were men. All participants were non-smokers, had normal blood pressure and took no medications or antioxidant supplements. Health status of the subjects was evaluated by questionnaire. Subjects that did not fit the study criteria were excluded by questionnaire. Exclusion criteria included subjects with diabetes, coronary artery disease, allergy to nuts (walnuts and almonds), abnormal fasting blood glucose, thyroid, renal and hepatic function.

2.2. Study protocol

We conducted a randomized 2-period, crossover, controlled-feeding trial. All the diets were provided. The protocol was approved by the West Virginia University Institutional Review Board and the study was conducted in accordance with the institutional guidelines. Ethic Committee approval number was H-22811. All measurements and blood samples were collected in the vascular laboratory. Between 2 and 6 weeks before testing, the patients were interviewed over the phone for clinical history, had an interview with the dietitian and anthropometric and blood pressure measurements were noted. Participants were individually randomized in a crossover design between 2 diet sequences (high-fat diet with walnuts or almonds) and were studied on two separate days 2 weeks apart. The experiments were performed in the afternoon to avoid confounding by the early morning blunting in endothelial function. On each study day, participants were asked to eat a low-fat breakfast at 7:00 AM and to refrain from further food intake until 11:30 AM, when they reported to the vascular laboratory and had a blood sample taken. Blood pressure was measured with a standard mercury sphygmomanometer. At 12:00 PM a baseline ultrasound assessment of endothelial function in the brachial artery was performed after resting for half an hour. Therefore, participants ate 1 of the 2 diet under the supervision of a clinical investigator. The protocol was repeated 4 h postprandially, with a blood sample taken at 3:30 PM and a second endothelial function test at 4:00 PM after resting for half an hour. Previous studies have shown that 4 h is sufficient time to evaluate the acute effect of nuts. Also, the largest changes in triglycerides and endothelial function have been observed 3–4 h postprandially. During the 4-h interval, participants rested in a quiet room and were allowed to drink water.

The end points were the between-diet differences in changes from baseline of flow-mediated dilation (FMD) assessed as the percentage change in brachial artery diameter during reactive hyperemia and postprandial changes in FMD and plasma concentrations of soluble vascular cell adhesion molecules (sVCAM).

2.3. Test diets

The diets were prepared at the hospital’s kitchen and consisted of 1200 kcal with 62.3% fat (20% saturated fatty acids), 15% protein, 23% carbohydrate, and 122 mg cholesterol, for a total fat content of 80 g. In both diets, the total fat from walnuts and almonds was kept the same. The diets consisted of a sandwich with 100 g white bread, 75 g salami, and 50 g fatty cheese, 125 g fat-rich (10%) yogurt, and water. Additionally, participants consumed 77 g almonds (almond diet) or 60 g shelled walnuts (walnut diet). 77 g almond or 60 g shelled walnuts were chosen to match the fat content in walnuts and almonds (76 g fat each). The unsaturated fatty acid content of the almond and walnut diets differed: 28.3% and 15.2% MUFA, and 7.7% and 23.1% PUFA, respectively. Only the walnut diet contained ALA (5 g). The nutrient composition of the walnuts and almonds used in the study is listed in Table 1. The dietary analysis was performed using Nutritionist Pro version 5.0.0 licensed modules Core, Client Management, Food Labeling.

2.4. Endothelial function testing

The operator was unaware of diet sequences. Suitable measurements were obtained in all tests. Subjects abstained from tobacco smoking and coffee. After blood samples had been drawn, subjects were placed supine at rest for 15 min in a quiet room at normal room temperature. A continuous ECG was recorded for timing of measurements. The brachial artery was scanned longitudinally 2–5 cm above the anticubital crease. This location was marked on the skin and all subsequent measurements were performed at the same location. FMD measurement using brachial artery reactivity testing was performed by 2-dimensional gray-scale and color flow Doppler vascular imaging by a Philips Sonos 7500 ultrasound machine (HP, Andover, Massachusetts) with an 11-MHz vascular ultrasound probe at baseline and after 4 h. Baseline dimensions were recorded at the center of the vessel with optimal contrast between the anterior and posterior vessel walls and the lumen.

Table 1

Average fatty acid composition of walnut and almond diets.

<table>
<thead>
<tr>
<th></th>
<th>Walnut Diet</th>
<th>Almond Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole Diet</td>
<td>Walnuts only</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>1128</td>
<td>392</td>
</tr>
<tr>
<td>Fat, g</td>
<td>80</td>
<td>39</td>
</tr>
<tr>
<td>SFA, g</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>MUFA, g</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>18:1, Oleic</td>
<td>17.3</td>
<td>5</td>
</tr>
<tr>
<td>PUFA, g</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>18:2, Linoleic</td>
<td>21.7</td>
<td>22</td>
</tr>
<tr>
<td>18:3, Linolenic</td>
<td>5.0</td>
<td>5</td>
</tr>
<tr>
<td>Protein, g</td>
<td>42</td>
<td>9</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>122</td>
<td>0</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>66</td>
<td>8</td>
</tr>
</tbody>
</table>

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Doppler blood flow images were recorded from the center of the vessel. Then an arterial occlusion cuff, placed above the antecubital fossa was inflated to 300 mmHg for five minutes. Immediately after deflation of the cuff, a continuous 90 s recording of blood flow and vessel dimensions were obtained. The peak diameter of the artery was recorded 60 s after deflation of the cuff, and the percentage change from the baseline diameter was calculated. FMD percent diameter change was determined as follows: (diameter after reactive hyperemia — baseline diameter) ÷ baseline diameter × 100. To avoid the confounding effects of arterial compliance and its cyclic changes in dimensions, all measurements were obtained at the peak of the R-wave of the electrocardiogram. The mean diameter of the brachial artery was determined at baseline, then continuously up to 3 min after reactive hyperemia. These images were then stored on a digitized system (Cantronics Medical Systems, Hartland, Wisconsin) that has a caliper with 0.1 mm resolution. Average diameters (intima to intima) from 3 cardiac cycles were obtained. Brachial artery diameter measurements were done in a random order and the investigator was blinded to the experimental condition. Shear rate was calculated per standard protocol blood flow velocity divided by vessel diameter.20,27

2.5. Determination of sVCAM levels

For the analysis of soluble vascular cell adhesion molecules (sVCAM), blood samples were ethylenediamine tetraacetic acid (EDTA)-treated and centrifuged (2,000g, 15 min, 4 °C) immediately after collection. Plasma was rapidly frozen and stored at −80 °C. After thawing, sVCAM, catalog number DVC00, was quantified by ELISA (R & D Systems).

2.6. Statistical analyses

Baseline characteristics for the 2 groups were compared using Student’s t and chi-square tests as appropriate. Significance of changes from baseline to 4h post-intervention was assessed separately for the walnut and almond diet groups using matched-pair t-tests. Comparisons of the magnitude and direction of changes among groups was assessed by analysis of variance appropriate to the repeated measures nature of the experimental design. Changes in FMD and sVCAM were assessed by 2 factors with repeated measures: period (almond vs. walnut diets) and time (baseline vs. post-intervention), and all their interactions. Associations between changes in inflammatory marker and risk factors (age, sex, body mass index) with changes in FMD were evaluated by partial correlation analyses. Significance levels (p < 0.05) were used for all comparisons. No adjustment for multiple testing was employed at any point in the analysis.

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Almond Diet</th>
<th>Walnut Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Int</td>
</tr>
<tr>
<td>FMD (Diameter, mm)</td>
<td>0.33 ± 0.19</td>
<td>0.36 ± 0.2</td>
</tr>
<tr>
<td>FMD/shear rate</td>
<td>0.18 ± 0.11</td>
<td>0.23 ± 0.14</td>
</tr>
<tr>
<td>FMD (changes%)</td>
<td>10.8 ± 6.7</td>
<td>12.4 ± 7.4</td>
</tr>
<tr>
<td>AVP (cm/s)</td>
<td>199 ± 52.7</td>
<td>206 ± 26.2</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>118 ± 4.6</td>
<td>116.8 ± 4.5</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>73 ± 6.8</td>
<td>73.6 ± 6.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>70 ± 7.2</td>
<td>66.8 ± 8.1</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sVCAM (mg/L)</td>
<td>1380 ± 406</td>
<td>1257 ± 321</td>
</tr>
</tbody>
</table>

FMD = Flow mediated dilation, AVP = Average peak velocity, SBP = Systolic Blood pressure, DBP = Diastolic Blood pressure, HR = Heart rate.

Flow-mediated dilation = absolute change in diameter, Pi = p value pre and post almond diet intervention, Pi = p value pre and post walnut diet intervention, P = p value between change in almond and walnut intervention. sVCAM-1 = soluble vascular cell adhesion molecule 1.

3. Results

Of the 27 participants in the study, 16 were women and 11 were men. Average age of participants was 42.3 ± 6.8 years. The average BMI of the study population was 28.6 ± 2.2 kg/m². Clinical characteristics and parameters of endothelial function of the study population are presented in Table 2.

3.1. Flow-mediated dilatation

During almond diet intervention, the baseline brachial artery lumen diameter changed from 3.3 ± 0.18 mm to 3.6 ± 0.16 mm and the absolute change in diameter was 0.33 ± 0.19 mm. The mean endothelial function measured as FMD percent change was 10.08 ± 6.7. After intervention, the diameter changed from 3.3 ± 0.17 to 3.7 ± 0.2 mm and the absolute change in diameter was 0.36 ± 0.2 mm. The mean endothelial function measured as FMD percent change was 12.4 ± 7.4. The percent change in FMD pre-and post-intervention was not significantly different with p = 0.06 (Fig. 1). Normalized FMD did not change significantly pre- and post-intervention from 0.18 ± 0.11 to 0.23 ± 0.14, p = 0.08 (Table 2).

Similarly, during walnut diet intervention, the baseline diameter changed from 3.2 ± 0.16 mm to 3.57 ± 0.15 mm and the absolute change in diameter was 0.36 ± 0.17 mm. The mean endothelial function measured as FMD percent change was

![Fig. 1. FMD% change comparison between walnut and almond groups at baseline and 4h after diet. There was significant improvement in normalized FMD in the walnut diet group (p = 0.004). Almond diet group showed an upward trend but did not reach significance.](image-url)
11.7 ± 6. After intervention, the diameter changed from 3.23 ± 0.19 to 3.75 ± 0.17 mm and the absolute change in diameter was 0.52 ± 0.2 mm. The mean endothelial function measured as FMD percent change was 16.2 ± 5.8. The percent change in FMD pre-and-post intervention was significantly different with p = 0.004. However, the % change in FMD pre-and post-intervention between almond and walnut diet was not significant (p value = 0.34). Normalized FMD also changed significantly pre-and post-intervention from 0.2 to 0.31 (p = 0.005). Inter-observer variation assessment for brachial artery measurements was performed in the study participants using a correlation coefficient, and the variation was found to be small (r = 0.94).

3.2. sVCAM

The level of sVCAM decreased significantly with the almond diet from 1380 to 1257 mg/l (p = 0.004). Similarly, the level of sVCAM decreased significantly with the walnut diet from 1400 to 1241 (mg/l), (p = 0.009) (Table 2). However, there was no significant difference changes in sVCAM between the almond and walnut diets (p = 0.51).

Baseline vessel diameter in men and women was not significantly different (3.4 ± 0.17 vs 3.3 ± 0.18 mm, p = 0.36). Partial correlation analysis was performed between changes in FMD and changes in other variables (weight, systolic and diastolic blood pressures, and heart rate), and there was no significant relation seen in these groups. There was a significant inverse correlation between changes in FMD and changes in sVCAM in the walnut diet group (p = 0.000). This difference was not significant in the almond diet group.

The systolic blood pressure decreased with the almond diet (118–116.8 mmHg, p = 0.11). A similar change was seen with the walnut diet (119–117.9 mmHg, p = 0.17). Heart rate decreased with both almond (70–66 beats/min, p = 0.58) and walnut diets (69–63 beats/min, p = 0.17).

4. Discussion

In this study, there was acute and significant improvement in FMD with both walnut and almond diets. A similar interaction was seen between sVCAM and the walnut diet. For the almond diet, the absolute change in diameter was 0.33 ± 0.19 mm (baseline) and 0.36 ± 0.2 mm (after intervention), p = 0.11. Similarly, for walnut diet, the absolute change in diameter was 0.36 ± 0.17 mm (baseline) and 0.52 ± 0.2 mm (after intervention), p = 0.001. The FMD percent change with the walnut diet at baseline was 11.7 ± 6.5% and 15.2 ± 5.8% after intervention with p = 0.004. This improvement is consistent with the results of several studies in the past that have assessed endothelial FMD after a walnut diet.10–21 Although the FMD percent change tended to increase with almond diet (10.8 ± 6.7 to 12.4 ± 7.4), the change was not significant (p = 0.06). The FMD percent change between the almond and walnut diets was not significant (p = 0.34). Similarly, even though the change in normalized FMD with the walnut diet was significant (p = 0.005) and tended to improve with almond diet (p = 0.08), there was no difference between the two diets (p = 0.36).

Endothelial dysfunction is a critical event in atherosclerosis that is implicated both in early disease and in advanced atherosclerosis, where it relates to perfusion abnormalities and the causation of ischemic events.2,5 Disturbed endothelial function can also be assessed by levels of adhesion molecules like sVCAM-1.28 It has been postulated that abnormal levels of these molecules are associated with development of atherosclerosis and increased risk of major future cardiovascular events.29–30 n-3 PUFA have clearly demonstrated anti-thrombotic, anti-inflammatory and anti-arrhythmic effects, thus possessing specific anti-atherosclerotic properties.31 n-3 PUFA from nuts, mainly ALA in walnuts, may have a number of cardio protective actions including protection from fatal coronary heart disease and sudden death due to their anti-arrhythmic properties.32–34 De Lorezgil et al in the Lyon Diet Heart Study observed that n-3 PUFA provided significant protection from coronary death.34

The role of ALA in reducing inflammation is important because inflammatory events are central in the pathogenesis of atherosclerosis.35 Rallidis et al concluded that diets enriched with ALA from walnuts or other sources reduce circulating levels of cytokines and other inflammatory mediators involved in endothelial activation.36 Several clinical trials have assessed the effect of walnuts and almonds on VCAM-1 concentration.37–38 Charles-Dufet et al, using a crossover design, showed an increase in plasma VCAM-1 levels after a western diet compared to a walnut enriched diet, although no significant difference was seen between the olive oil and walnut diets.37 In our study the walnut diet significantly decreased the sVCAM (p = 0.009). Likewise, the almond diet also significantly decreased the sVCAM (p = 0.004). There was no significant difference between the two diets on sVCAM levels. These results are in accordance with results from a randomized controlled cross-over study by Ros et al who showed improvement in endothelial dependent vasodilation and reduced vascular cell adhesion molecule-1 levels compared to a Mediterranean control diet.39

Few studies have evaluated the effects of walnuts or other nuts on blood pressure, but most of these have shown either a beneficial effect or no effect.40–44 In our study there was no significant change in blood pressure with the walnut or almond diet even though it tended to decrease in both groups.

5. Conclusion

Both walnut and almond diets improved endothelial function as seen by significant improvement in FMD and another marker of endothelial dysfunction, the adhesion molecule (sVCAM). However, there were no significant differences between the two diets as regards to FMD and sVCAM. Larger studies with longer follow-ups are needed to further study the effect of walnut and almond diets on endothelial function.

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Conflict of interest
None.

References


