Markers of endothelial dysfunction in young non-overweight women - effect of serum lipids, body measures and nutrition

Witkowska AM.*, Zujko ME.

Department of Food Commodities Science and Technology, Medical University of Białystok, Białystok, Poland

ABSTRACT

Purpose: Since endothelial dysfunction can develop early in the adulthood, the purpose of the study was to determine how serum lipids, body measures and dietary habits affect serum markers of vascular activation in young women.

Materials and methods: Twenty five healthy women, aged 19-22 years, were enrolled in the study. Serum lipids profile (total cholesterol, HDL-cholesterol, triglycerides) was assayed with laboratory test kits. Concentrations of sICAM-1, sVCAM-1 and E-selectin were determined with the ELISA technique. Anthropometric measurements were taken including skinfold thickness and waist circumference. Food consumption data were collected using 3 repeats of 24-hour dietary recalls. Dietary habits of the women were assessed with a 9-point alternate Mediterranean Diet score (a-MED).

Results: Sixty eight percent of the subjects had their HDL-cholesterol levels below the desirable concentration, 20% had LDL-cholesterol elevated, and 32% demonstrated increased total triacylglycerols (TAG). The levels of serum TAG >199 mg/dL were associated with a significant rise in the VCAM-1 concentration. Dietary wholegrain products seem to reduce the serum E-selectin.

Conclusions: The results suggest that young women of normal body mass, but demonstrating increased levels of serum TAG, may be at risk of developing endothelial dysfunction. An implementation of the wholegrain products consumption into their dietary practices would possibly be of health benefit.

Key words: Endothelial dysfunction, cellular adhesion molecules, lipids, nutrition, body measures, women.
INTRODUCTION

Cell adhesion molecules (CAMs) - intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin are endothelial stress markers involved in the early atherosclerotic processes [1, 2]. These CAMs are located on the external surfaces of cellular membranes. They are involved in an array of interactions between cells, including leukocyte-endothelial cell signal transduction. ICAM-1, VCAM-1 and E-selectin are expressed on the cytokine-activated endothelial cells (EC). ICAM-1 and VCAM-1 are present on unstimulated EC, while E-selectins are expressed upon stimulation by cytokines. Cytokine molecules and CAMs work in concert allowing leukocyte homing and transmigration through the walls of blood vessels into the sites of inflammation. Proinflammatory cytokines TNF-α, IL-1, (IFN)-γ induce CAMs expression on EC [3]. Membrane E-selectin binds circulating leukocytes with low affinity which allows roll motion along the surface of endothelium. This initial step slows leukocyte in the blood stream and enables interactions between other adhesion molecules and cytokines and leukocyte. ICAM-1 and VCAM-1 facilitate monocyte recruitment and mediate leukocyte-endothelial adhesion and signal transduction [4, 5]. Certain portions of cell-bound molecules are shed from the cytoplasmatic membranes into the circulation. These emerging soluble forms of CAMs play diverse biological roles that include immunological response in the vascular environment. The factors of lifestyle play important roles in the initiation and the prevention of metabolic diseases. Previous studies prompted that soluble CAMs can be favorably modulated by dietary factors in subjects at risk of atherosclerosis [6]. Atherosclerosis as a result of a longstanding inflammatory process, is manifested in the middle-age adults by the formation of atherosclerotic plaques in the arterial walls. Though, subclinical symptoms can be observed in young adults [7] and even in the adolescents [8]. Since endothelial dysfunction can develop early in the adulthood, the purpose of the study was to find out how serum lipids, body measures and dietary habits affect serum markers of vascular activation in young women.

MATERIALS AND METHODS

Subjects and study design

Twenty five women, aged 19-22 years, were enrolled in the study. The women were recruited on the basis of non-overweight/non-obese body mass (BMI \(\leq 25\ \text{kg/m}^2\)) and the absence of inflammatory, autoimmune or metabolic diseases. The women referred their general subjective health status as good or very good. They reported non-vegetarian dietary habits (except for one subject who was pesco-lacto-ovo vegetarian). None of the subjects were taking medications known to affect lipid metabolism.

Blood sampling

Fasting blood samples were drawn from an antecubital vein into vacuum test tubes with clot activator and gel for serum separation (Becton, Dickinson and Company, France). The samples were allowed to clot within 30 minutes, then centrifuged for 10 minutes at approximately 1800 x g. Serum was removed and kept frozen at \(-20^\circ\text{C}\) until analysis. Serum samples were thawed before analyses.

Serum lipid profile

Serum lipid profile (total cholesterol, HDL-cholesterol, triglycerides) was assayed with commercially available test kits (Randox Laboratories Ltd., Crumlin, United Kingdom). Serum LDL-cholesterol values were calculated according to the Friedewald formula [9].

ELISA assays

Serum concentrations of sICAM-1, sVCAM-1 and E-selectins were determined with sandwich enzyme-linked immunosorbent (ELISA) assays (Bender MedSystems Diagnostics GmbH, Vienna, Austria) according to the manufacturer’s recommendations. Absorbance was read on a Rayto ELISA Reader 6100, Rayto, China.

Anthropometric measurements

Skinfold thickness measurements were performed with a Lange caliper (Fabrication Enterprises, Inc., New York, USA) over the triceps muscle of the upper arm. Body measurements were taken with a tape measure. Waist circumference was taken in the mid-way between the lowest rib and the anterior superior iliac spine. Body mass and height was reported by the participants. Body Mass Index (BMI) was calculated in kg/m². Central Fat Mass (CFM) was calculated according to Ketel et al. [10]. An equation \(18.53 + 0.20\ \text{waist} + 0.51\ BMI\) was used for these calculations [10].

Assessment of dietary intake and the alternate Mediterranean Diet Index (aMED)

Food consumption data were collected before blood taking on the basis of 24-hours dietary recalls performed in triplicate in the spring season. Dietary habits of the women were assessed using a 9-point alternate Mediterranean Diet score (a-MED) [11, 12]. The consumption of eight groups of food products (vegetables excluding the potatoes; legumes; fruits; nuts and seeds; wholegrain products; red meat; fish, and alcohol) and the ratio of monounsaturated to saturated fatty acids were
assessed. The fatty acids consumption was calculated using a Dieta 5.0 software (National Institute of Food and Nutrition, Warsaw, Poland). The frequency of consumption was counted for food portions as standard food volume measures. One point was assigned for consumption of individual food above the median value calculated for the consumption in the whole group of participants, except for the red meat consumption, which was counted 1, when red meats were eaten less frequently than the median value. Another 1 point was assigned when the alcohol consumption was in a range 5-25 g/day.

The study protocol was conducted according to the principles of the Helsinki Declaration and was approved by the Local Ethical Committee of the Medical University, Białystok.

**Statistical analysis**

Statistical calculations were performed with a Statistica 10.0 software (StatSoft, Inc.) using non-parametric tests. Median values were presented together with quartiles. The Kruskal-Wallis post-hoc test was used for multiple comparisons between the serum VCAM-1 concentrations within specific TAG levels.

Multiple paired Spearman rank order tests were performed to test correlations between adhesion molecules, lipids and anthropometric measures. A Bonferroni adjustment was applied to set the significance value ≤0.007 a priori.

The effects of a-MED score and individual variables included within the a-MED score on soluble E-selectin concentration were tested using Spearman rank order correlation. P values below 0.05 were considered statistically significant.

**RESULTS**

Table 1. Subjects’ body measures.

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.67</td>
<td>1.62</td>
<td>1.70</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>54</td>
<td>50</td>
<td>57</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>19.5</td>
<td>18.2</td>
<td>20.5</td>
</tr>
<tr>
<td>Skinfold thickness (mm)</td>
<td>14.7</td>
<td>11.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>69</td>
<td>65</td>
<td>72</td>
</tr>
</tbody>
</table>

Table 1. shows anthropometric measures of the women. The median height was 1.67 (lower and upper quartiles - Q1 and Q3 1.62-1.70 m), and the median body mass was 54 kg (Q1-Q3 50-57 kg). The body mass indices (BMIs) of the females were within the range 17-23.7. According to the WHO cut-offs for the BMI [13], eight women were underweight (<18.5 kg/m²), and seventeen women had regular weight (18.5-23.7 kg/m²).

The median skinfold thickness (ST) was 14.7 mm (Q1-Q3 11-18.0mm). ST is a rough measure of the body fat deposits. In fact, it measures subcutaneous adipose tissue, and allows further calculations of the body fat percentage. The mean calculated percentage of the body fat was ≤21.5 %. This value was made out of the Lange caliper’s operator manual. According to the WHO guidelines, essential fat in women should be 10-12%, it is acceptable to 32%, while the higher values are met in obese women.

The median waist circumference of the women was 69 cm, (Q1-Q3 65-72 cm, range 62-78 cm). According to the WHO criteria for the waist circumference measurements [13] all women had normal waist circumference (below 80 cm).

The serum concentrations of the assayed soluble endothelial CAMs and the selected lipid parameters are given in table 2. The median total cholesterol concentration was 167 mg/dl, but two women (8 %) had their cholesterol levels increased above value 199 mg/dl, what is considered as a borderline high risk factor for hypercholesterolemia and heart disease [14]. The HDL-cholesterol levels recommended for women should be at least 50 mg/dl. Only eight women (32 %) had the desirable level of HDL, but the great majority (68 %) had not. The desirable LDL-cholesterol level should be less than 100 mg/dl. [14]. In view of this recommendation, five women (20 %) had increased LDL concentration. The triacylglycerol level was elevated (>199 g/dl) in eight women (32 %). The median serum levels of sICAM-1, sVCAM-1 and sE-selectin were 242 ng/ml (Q1-Q3 214-283 ng/ml), 1004 ng/ml (Q1-Q3 760-1120 ng/ml) and 27.1 ng/ml (Q1-Q3 18.5-33.5 ng/ml), respectively.

Table 2. Serum concentrations of the lipid parameters and the endothelial soluble adhesion molecules.

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Q1</th>
<th>Q3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>167</td>
<td>159</td>
<td>179</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>44</td>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>85</td>
<td>63</td>
<td>99</td>
</tr>
<tr>
<td>Triacylglycerols (g/dl)</td>
<td>192</td>
<td>144</td>
<td>218</td>
</tr>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>242</td>
<td>214</td>
<td>283</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>1004</td>
<td>760</td>
<td>1120</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>27.1</td>
<td>18.5</td>
<td>33.5</td>
</tr>
</tbody>
</table>
Of the CAMs tested in this study, only the sVCAM-1 levels were nearly related to the one of the lipid parameters – the triacylglycerol concentration ($r=0.352$, $p=0.085$), but not related to the total cholesterol, LDL- and HDL-cholesterol (Table 3). This finding was subsequently tested against triacylglycerol levels (Figure 1).

**Table 3.** Correlations between the sICAM-1, sVCAM-1 and sE-selectin serum concentrations and the cholesterol and triacylglycerol serum concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Total cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>Triacylglycerols (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>$R=0.152$ $p=0.467$</td>
<td>$R=0.120$ $p=0.569$</td>
<td>$R=0.123$ $p=0.557$</td>
<td>$R=0.178$ $p=0.394$</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>$R=0.081$ $p=0.701$</td>
<td>$R=0.182$ $p=0.385$</td>
<td>$R=0.101$ $p=0.632$</td>
<td>$R=0.352$ $p=0.085$</td>
</tr>
<tr>
<td>sE-selectin (ng/ml)</td>
<td>$R=0.163$ $p=0.434$</td>
<td>$R=0.163$ $p=0.385$</td>
<td>$R=0.128$ $p=0.542$</td>
<td>$R=0.045$ $p=0.831$</td>
</tr>
</tbody>
</table>

The Kruskal-Wallis post-hoc test was used for multiple comparisons between the serum VCAM-1 concentrations within specific triacylglycerol levels. Figure 1. shows the serum sVCAM-1 mean concentration in relation to normal (<150 mg/dL), borderline high (150-199 mg/dL) and high TAG levels (>199 mg/dL). The serum sVCAM-1 concentration was significantly higher ($p<0.05$) in the subjects with the high TAG levels (1296 ± 468 ng/mL) (n=8) compared to the individuals with normal (868 ± 205 ng/mL) (n=7) or the borderline TAG levels (866 ± 188 ng/mL) (n=10).

**Figure 1.** The serum sVCAM-1 mean concentration values in the respective serum triacylglycerol levels

The serum sE-selectin concentration was inversely related to the consumption of whole grain products in Spearman's correlation test (Table 5). A correlation coefficient $R$ demonstrated mediocre value -0.500 (limits of absolute values 0.3≤$r$≤0.5) [15]. The serum VCAM-1 and ICAM-1 were not associated with the a-MED scores.

Within the CAMs and lipids tested, none was associated with body measures (Table 4).
Table 4. Correlations between the lipid parameters, sICAM-1, sVCAM-1 and sE-selectin serum concentrations and the body measures * calculated with the Pearson’s correlation test.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Height (m) R</th>
<th>Body Mass (kg) R</th>
<th>BMI (kg/m²) R</th>
<th>ST (mm) R</th>
<th>WC (cm) R</th>
<th>CFM R</th>
<th>CFM/ST R</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1 (ng/ml)</td>
<td>0.233 p=0.263</td>
<td>0.067 p=0.748</td>
<td>-0.070 p=0.737</td>
<td>0.042 p=0.839</td>
<td>0.002 p=0.991</td>
<td>-0.010 p=0.961</td>
<td>-0.096 p=0.655</td>
</tr>
<tr>
<td>sVCAM-1 (ng/ml)</td>
<td>0.216 p=0.300</td>
<td>0.110 p=0.585</td>
<td>0.000 p=0.997</td>
<td>-0.164 p=0.435</td>
<td>-0.285 p=0.168</td>
<td>0.216 p=0.831</td>
<td>0.242 p=0.254</td>
</tr>
<tr>
<td>sE-selectins (ng/ml)</td>
<td>-0.131 p=0.533</td>
<td>0.027 p=0.900</td>
<td>0.080 p=0.703</td>
<td>0.222 p=0.287</td>
<td>0.114 p=0.588</td>
<td>0.201 p=0.345</td>
<td>0.268 p=0.205</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>0.028 p=0.893</td>
<td>0.040 p=0.970</td>
<td>-0.012 p=0.956</td>
<td>-0.137 p=0.132</td>
<td>-0.309 p=0.296</td>
<td>-0.222 p=0.296</td>
<td>0.137 p=0.525</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>0.173 p=0.408</td>
<td>-0.091 p=0.667</td>
<td>0.233 p=0.262</td>
<td>0.001 p=0.434</td>
<td>0.004 p=0.987</td>
<td>-0.205 p=0.337</td>
<td>0.021 p=0.924</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>-0.166 p=0.427</td>
<td>-0.022 p=0.916</td>
<td>-0.142 p=0.500</td>
<td>-0.174 p=0.406</td>
<td>-0.368 p=0.070</td>
<td>-0.088 p=0.683</td>
<td>0.160 p=0.455</td>
</tr>
<tr>
<td>Triacylglycerols (g/dl)</td>
<td>0.118 p=0.573</td>
<td>0.181 p=0.388</td>
<td>0.030 p=0.887</td>
<td>-0.145 p=0.489</td>
<td>0.032 p=0.880</td>
<td>0.063 p=0.770</td>
<td>0.229 p=0.281</td>
</tr>
</tbody>
</table>

ST – skinfold thickness; WC – waist circumference; CFM/ST – Central Fat Mass to Skinfold Thickness Ratio

The serum sE-selectin concentration was inversely related to the consumption of whole grain products in Spearman’s correlation test (Table 5). A correlation coefficient R demonstrated mediocre value -0.500 (limits of absolute values 0.3≤r≤0.5) [15]. The serum VCAM-1 and ICAM-1 were not associated with the a-MED scores.

**DISCUSSION**

A great majority of the subjects (68%) had their HDL-cholesterol levels below the desirable concentration; 20% had LDL-cholesterol elevated, and 32% demonstrated increased total triacylglycerols, which altogether are regarded individual or collective risk factors of atherosclerosis. The triacylglycerol levels in these latter 32% were associated with the elevated levels of sVCAM-1. Earlier studies showed that fatty meals may increase sVCAM-1 levels for many hours as well as the levels of some inflammatory parameters [16]. A stimulation with postprandial triacylglycerol-rich lipoproteins (PP-sTAG) alone is not capable to elevate endothelial cell (EC) membrane expression of VCAM-1, but only the concurrent low-dose TNF-α stimulation may increase expression of this CAM [17]. In contrast to other studies, the present study shows that not only the postprandial TAG, but also the fasting serum TAG may affect sVCAM-1 concentration. The elevated levels of serum TAG>199 mg/dL at fasting were associated in our study with significant, more than 40% rise in the VCAM-1 serum concentration. We hypothesize that elevated sVCAM-1 may depend on the body fat distribution. Central Fat Mass (CFM) expresses visceral adiposity, and in young adults it is related to cardiovascular risk factors and metabolic syndrome.
One of the best methods to determine fat distribution is a dual energy X-ray absorptiometry (DEXA) method. Compared to DEXA, however, anthropometric measurements are easy to perform, not exposing subjects to irradiation and cost-saving. Recently, equations based on anthropometric measurements have been found to correlate with DEXA-CFM [10]. With respect to this, we tested correlations of the lipid parameters and CAMs against CFM calculated on a basis of WC and BMI [10]. These calculations showed no associations of the tested variables with CFM and CFM/ST ratio, most likely because the subjects were lean. The synthesis of E-selectin is regulated at the transcription level, and its expression on endothelial cells takes place only after stimulation by inflammatory cytokines. E-selectin allows leukocyte rolling motion along the membrane surface and its binding by other CAMs. These interactions promote leukocyte adhesion, trans endothelial migration and inflammation within vascular walls. With respect to this, prevention of E-selectin expression as an initial step of leukocyte extravasation would be of interest. Healthy eating patterns promote decreases in inflammatory parameters and E-selectin, allowing a reduction of risk of atherosclerosis and diabetes [20]. A diet which includes a combination of whole grain products, fish and bilberries may improve endothelial dysfunction in overweight/obese subjects with impaired glucose metabolism, and cause decreases in plasma E-selectin levels [21]. It has been postulated that Mediterranean diet could decrease a pro-inflammatory environment induced by LDL oxidation and lowered E-selectin expression on endothelial cells [22]. E-selectin can be released into the circulation and quantified in plasma and serum, reflecting by this an inflammatory processes within vascular cell walls.

In our study diets that included increased amounts of whole grain products (whole grain rye bread, buckwheat groats, breakfast cereals, wheat bran) were found to reduce the serum E-selectin.

CONCLUSIONS

Our observations concerning serum lipids demonstrate that about 70% of the women participating in the study had at least one abnormal lipid parameter and therefore, may be at risk of developing atherosclerosis. Another insight from our survey regards the fact that the serum sVCAM-1 concentration in the women was affected by the serum lipids. Fasting serum, VCAM-1 levels were significantly increased in the women demonstrating high serum triacylglycerol concentrations (above 199 mg/dL), but not affected by the normal and the borderline high TAG levels (150-199 mg/dL). Hence, we think that elevated TAG levels (TAG>199mg/dL) in young women may impact endothelial physiology and have adverse effects on vascular function.

In addition to these findings, the study also discovered some positive role of dietary practices on CAMs. The nutritional habits, which were associated with consumption of whole grain products were associated with the lowering effect on sE-selectin concentration.

In fact, these results suggest that even the non-overnight young women, but demonstrating elevated levels of serum triacylglycerols, may be at a greater risk of developing vascular dysfunction, and their lifestyles need intervention, which should encompass modification of dietary habits. An implementation of whole grain products consumption into the dietary practices of women, which are prone to develop endothelial dysfunction, would be of great benefit. This preliminary research throws a light on CAM’s behavior in young healthy individuals, which should be taken into account in the early prevention of atherosclerosis. The results of our study suggest that this somehow limited research need to be continued and extended to larger populations.

ACKNOWLEDGEMENTS

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Conflicts of interest

The authors state that no commercial associations or patent licenses that might result in a conflict of interest with the work are presented in the submitted paper.

REFERENCES


