Supplementation with a juice powder concentrate and exercise decrease oxidation and inflammation, and improve the microcirculation in obese women: randomised controlled trial data

Manfred Lamprecht1,2*, Georg Obermayer1,2, Kurt Steinbauer3, Gerhard Cvirn1, Lidija Hofmann4, Gerhard Ledinski1, Joachim F. Greilberger1,5 and Seth Hallstroem1

1Institute of Physiological Chemistry, Centre for Physiological Medicine, Medical University of Graz, Harrachgasse 21/II, 8010 Graz, Austria
2Institute of Nutrient Research and Sport Nutrition, Petersbergenstrasse 95b, 8042 Graz, Austria
3SportchirurgiePlus, Centre for Individual Sport Medicine and Surgery, Bertold Linderweg 15, 8047 Graz, Austria
4FH JOANNEUM, University of Applied Sciences, Eggenberger Allee 11, 8020 Graz, Austria
5Institute of Laboratory Sciences, Dr Greilberger GmbH, Hauptstrasse 140, 8301 Laßnitzhöhe, Austria

(Submitted 18 October 2012 – Final revision received 5 March 2013 – Accepted 5 March 2013 – First published online 16 April 2013)

Abstract

Obesity and sedentary lifestyle are associated with increased oxidative stress, inflammation and vessel dysfunction. Previous research has shown that an encapsulated fruit/berry/vegetable juice powder (FBV) supplement or controlled exercise training improve the markers of redox biology, low-grade inflammation and circulation. The aim of the present study was to assess the effects of 8 weeks of supplementation with FBV or placebo, and a single bout of controlled walking on the markers of oxidation, inflammation and skin capillary microcirculation in forty-two obese pre-menopausal women (41 (SD 5) years, non-smokers and BMI 34·5 (SD 3·8) kg/m2) using a randomised, double-blind, placebo-controlled design. All assessments were made before and after 8 weeks of capsule supplementation, and pre- and post-30 min of controlled treadmill walking at 70% of VO2max. Venous blood was collected for the determination of carbonyl proteins (CP), oxidised LDL (ox-LDL), total oxidation status (TOS) of lipids, malondialdehyde, TNF-α and IL-6. Capillary blood flow, O2 saturation of Hb (SO2Hb) and the relative concentration of Hb (rHb) were assessed at a 2 mm skin depth. Following 8 weeks of supplementation, compared with placebo, the FBV group had a significant (P<0·05) reduction in CP, ox-LDL, TOS and TNF-α, and a significant increase in blood flow, SO2Hb and rHb. Independent of supplementation, moderate exercise significantly increased blood flow and rHb, with a trend towards increased SO2Hb. Compared with placebo, 8 weeks of supplementation with FBV decreased the markers of systemic oxidation and inflammation. Both FBV supplementation and a single walking bout improved the markers of the microcirculation in these obese women.

Key words: Dietary supplements: Exercise and obesity: Oxidation and inflammation: Skin microcirculation

In recent decades, increasing prevalence of obesity has become a serious public health concern. Suboptimal dietary habits coupled with a sedentary lifestyle are thought to be major contributors to this situation. Overweight and obesity are associated with irregularities in redox homeostasis, imbalanced pro-inflammatory and anti-inflammatory states and microcirculatory dysfunction1–3. Reactive Oxygen and Nitrogen species and pro-inflammatory cytokines from both visceral and subcutaneous fat compartments are implicated in increased cardiometabolic disease risk4–5. Recent research has shown that obese people have structural and functional alterations in skin microcirculation, which are proportional to the increase in the degree of global and central obesity6. Hence, diets rich in antioxidants and anti-inflammatory nutrients, as well as physical exercise, are of interest to combat some of the detrimental side effects of overweight and obesity. It has been reported that increased consumption of fruits and vegetables improves the body’s antioxidant and anti-inflammatory capacities7–9. Nutraceuticals providing phytochemicals and vitamins, such as an encapsulated fruit and

Abbreviations: CP, carbonyl proteins; FBV, fruit, berry and vegetable juice powder concentrate; MDA, malondialdehyde; ox-LDL, oxidised LDL; rHb, relative Hb concentration; SO2Hb, O2 saturation of Hb; TOS, total oxidation status of lipids; VO2max, maximum O2 uptake.

*Corresponding author: Dr M. Lamprecht, fax +43 3163809610, email manfred.lamprecht@medunigraz.at
vegetable juice powder concentrate, have also demonstrated beneficial effects on the markers of oxidative stress, inflammation and skin microcirculation\(^{10-12}\).

Similar effects are also observed with exercise. Regular exercise training and single bouts of exercise improve redox biology, exert anti-inflammatory effects and are able to enhance the microcirculation in different populations, including overweight subjects\(^{13-16}\).

However, the influence of both exercise and nutrient supplementation on oxidation, inflammation and skin microcirculation in a target group of obese women has not been established.

Thus, the primary objective of the present study was (1) to explore the effects of a fruit, berry and vegetable juice powder concentrate (FBV) on oxidation, inflammation and skin microcirculation, compared with placebo. The secondary and tertiary goals were (2) to evaluate whether a single bout of defined walking exercise – with or without the FBV treatment – affects the capillary microcirculation from the skin surface to a 2 mm depth, and (3) to evaluate whether a defined model of walking exercise generates oxidative stress – with or without the FBV treatment – in a cohort of obese but otherwise healthy pre-menopausal women.

### Experimental methods

#### Study population

A total of forty-two overweight and obese pre-menopausal women participated in the present trial. Inclusion criteria were as follows: female; age 35–50 years; regular menses; able to participate in walking exercise; non-smokers; sedentary work and lifestyle; BMI between 28 and 40 kg/m²; no dietary or nutritional supplement use within the 4 weeks before the first exercise test. Exclusion criteria included the following: smokers; women who failed exercise eligibility testing – as described by the Austrian and German standards in sports medicine\(^{17}\); chronic or excessive alcohol consumption; pregnancy and/or lactation; recent surgery or illness; diabetes; dyslipidaemia; current participation in a weight management programme; diagnosis of osteoporosis or osteopenia; current use of any medication known to significantly influence inflammation, redox biology or haemostasis. In addition to these inclusion and exclusion criteria, a standard blood chemistry panel, exercise echocardiography and maximum O\(_2\) uptake (VO\(_{2\text{max}}\)) were determined in all women to confirm general health before study enrolment. All subjects also completed a medical history and a physical activity/well-being questionnaire.

The study focused on office workers and was announced in local newspapers. A telephone screening conducted by study staff resulted in fifty-nine volunteers for further eligibility testing. Among these volunteers, forty-four women met the inclusion and exclusion criteria and were enrolled.

Subjects were randomised in blocks of six and sequentially numbered (www.randomization.com). To guarantee a balanced BMI distribution between the groups (FBV or placebo), we conducted stratification via BMI rank statistics. The randomisation code was held by a third party (Union of Sport and Exercise Scientists Austria) and provided for statistical analyses of the complete dataset.

#### Study design and time schedule

This was a randomised, double-blind, placebo-controlled study. All eligibility testing was finalised 4 weeks before the baseline controlled walking bout. On that morning, a standardised breakfast (2–3 h before exercise) was provided. Then, each subject came to the laboratory to perform her 30 min exercise test at an intensity of 70% of individual VO\(_{2\text{max}}\). After the test, the investigator dispensed the randomised capsule supply according to the subject’s BMI ranking. Following 8 weeks of capsule supplementation as directed, they returned their remaining capsules and the same test procedure was repeated. All subjects were checked by a physician before each exercise test. The walking tests were scheduled between days 10 and 20 of the menstrual cycle.

#### Dietary parameters

Subjects were instructed to maintain their habitual diet and lifestyle during the 8-week study and to duplicate their diet before each exercise testing/blood collection appointment, as described below. Before the first 30 min walking test, subjects completed a 7 d food record to assess nutrient intake. Subjects subsequently received copies of their 7 d diet records and were instructed to replicate the diet before the second exercise test. The standardised breakfast was served 2–3 h before both exercise tests to limit nutrient variation due to self-selection on the morning scheduled for blood collection. The standardised breakfast consisted of 250 ml low-fat yogurt, 10 g butter, 20 g jam or honey, 50 g rye-wheat bread and 500 ml of plain water, providing 1500 kJ, 13 g protein, 47 g carbohydrate and 13 g fat. Diet records were analysed for total energy, protein, carbohydrate, fat, cholesterol, fibre, water, alcohol and several vitamins, minerals and fatty acids using Opti Diet software 5.0 (GOEmbH).

#### Study capsules

Women randomised to the FBV group (n 22) received capsules containing primarily a blended fruit, vegetable and berry juice powder concentrate derived from the following: acerola cherry, apple, bilberry, blackcurrant, blueberry, beetroot, broccoli, cabbage, carrot, Concord grape, cranberry, elderberry, kale, orange, peach, papaya, parsley, pineapple, raspberry, red currant, spinach and tomato (Juice Plus +\(^{\copyright}\)).

---

**Ethical aspects, recruitment and randomisation**

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Review Committee of the Medical University of Graz, Austria. All subjects provided written informed consent before participating in the present investigation. The trial was registered at www.clinicaltrials.gov (identifier no. NCT01476033).
Premium; NSA), as described previously\(^{(10)}\). Briefly, the FBV capsules provided 7.5 mg \(\beta\)-carotene, 200 mg vitamin C, 60 mg \(\alpha\)-tocopherol, 600 \(\mu\)g folate and 63 kJ/d. Those subjects randomised to the placebo group (\(n=22\)) received identically appearing opaque white capsules containing microcrystalline cellulose. All subjects were instructed to take three capsules twice daily with meals, in agreement with the label use instructions for the retail product, for a total of six capsules per d.

**Eligibility exercise test**

As part of eligibility testing, each subject performed an incremental exercise test on a treadmill ergometer (QUASARmed; HP Cosmos Sports & Medical GmbH) to check the heart and circulatory function and for the determination of \(V_{O_{2}\text{max}}\). A standard electrocardiogram was recorded throughout all exercise tests, which were supervised by a physician. Respiratory gas exchange variables were measured throughout the incremental exercise tests using a breath-by-breath mode (Metalyzer 3B; Cortex Biophysik GmbH).

**Endurance exercise test**

For the 30 min aerobic exercise tests, the walking speed was adjusted to 70 % of individual \(V_{O_{2\text{max}}}\) on the treadmill ergometer after the standardised breakfast described previously. All tests were performed on the same treadmill, with the same standardised room temperature (20°C) and humidity (60 %). Blood pressure was measured at the beginning and every 10 min until the bout was completed.

**Blood collection and sample preparation**

At each laboratory visit, two EDTA blood samples were collected from each participant, in a supine position, from a medical cubital vein: before exercise (pre) and immediately post-exercise (post). This venous blood was collected to determine the concentrations of carbonyl proteins (CP), oxidised LDL (ox-LDL), total oxidation status of lipids (TOS), malondialdehyde (MDA), TNF-\(\alpha\) and IL-6. After centrifugation for 10 min, plasma was removed and frozen at \(-70^\circ\text{C}\) until analysis.

**Biochemical analyses**

CP concentration was quantified using ELISA methods developed previously by Buss & Winterbourn\(^{(18)}\) and Alamdari et al.\(^{(22)}\). These methods are based on the antibody recognition of carbonyl protein-bound 2,4-dinitrophenylhydrazine.

For ox-LDL determination, a commercially available immunoisorbit kit (Mercodia AB) based on a direct sandwich technique was utilised.

The TOS assay determines total lipid peroxides (Immundiagnostik AG) by the detection of a coloured product from the reaction of a peroxidae with the peroxides in the sample, followed by the conversion of tetramethylbenzidine.

MDA concentration was determined according to a previously described HPLC method by Pilz et al.\(^{(20)}\) after derivatisation with 2,4-dinitrophenylhydrazine.

Both TNF-\(\alpha\) and IL-6 concentrations were analysed using commercially available ELISA kits with monoclonal antibodies (TNF-\(\alpha\): Immundiagnostik AG; IL-6: Invitrogen; LifeTech Austria).

**Microcirculation parameters**

Measurements were conducted in a supine position after a 10 min rest before and after the walking exercise bout, on the back of the hand, between the first and second metacarpal bone. This tissue photo spectrometry technology is also called ‘oxygen to see’ (Lea Instruments). All measurements were performed by the same technician. A laser Doppler effect, as described elsewhere\(^{(12,21)}\), was used to determine microcirculatory blood flow. For the determination of \(O_2\) saturation of Hb (\(SO_2\text{Hb}\)) and relative Hb concentration (\(r\text{Hb}\)), white-light tissue spectrometry was utilised: \(SO_2\text{Hb}\) is identified by the colour of Hb, as the degree of molecular \(SO_2\text{Hb}\) relates to a certain colour. \(r\text{Hb}\) was quantified using light absorption by the conversion of white light into red light, which is proportional to the concentration of Hb\(^{(22)}\). Blood flow and \(r\text{Hb}\) are expressed in arbitrary units, whereas \(SO_2\text{Hb}\) is expressed as a percentage of \(O_2\) on Hb. This technology measures the microcirculation of blood from the skin surface to a 2 mm depth.

**Blood chemistry panel**

Standard blood chemistry was determined for eligibility testing after an overnight fast using EDTA plasma from the peripheral venous blood using a routine clinical chemistry analyser (Abbott Diagnostics).

**Statistical analyses and sample size calculation**

Per-protocol analyses were performed using IBM SPSS for Windows software, version 19.0 (SPSS Inc.). Data are presented as means and standard deviations. Data for pre–post-comparisons were adjusted for plasma volume changes as described elsewhere (except for CP, as it is already expressed in relation to protein concentration)\(^{(23)}\). Statistical significance was set at \(P<0.05\). The Shapiro–Wilks test was used to determine a normal distribution. Baseline characteristics, performance data, nutrient and clinical chemistry data were compared using the unpaired Student’s \(t\) test. Data obtained for CP, ox-LDL, TOS, MDA, TNF-\(\alpha\), IL-6, \(SO_2\text{Hb}\), \(r\text{Hb}\) and blood flow were analysed using a univariate, three-factorial, repeated-measures ANOVA. Factors were as follows: treatment (FBV or placebo); exercise (pre- and post-exercise); session (walking test 1 and walking test 2). Significant interactions and main effects were analysed by Bonferroni correction.

The sample size estimate of seventeen subjects per group was based on previous data on oxidation and inflammation markers (markers of primary outcome) and subjected to a probability of error (\(\alpha=5\%\)) and to a test power (\(1-\beta=80\%\)). Concerning the mean values, we assumed to discover a difference of 20 % between the FBV and placebo groups after 8 weeks of treatment (and in comparison from pre- to post-exercise) and a standard deviation of 20 % for the oxidation markers CP and MDA. For the mean values of
TNF-α and IL-6, we assumed to discover a difference of 30% between the FBV and placebo groups after 8 weeks of treatment (and in comparison from pre- to post-exercise) and a standard deviation of 30%. Allowing for an anticipated attrition of 20% in each group, twenty-two subjects per group were recruited to discover the assumed differences.

**Results**

**Study population and nutrition**

A CONSORT (consolidated standards of reporting trials) diagram outlining participant recruitment is depicted in Fig. 1. Of the forty-four randomised women, forty-two completed the full programme and were included in the statistical analyses. There was one early termination in each study group: in the FBV group, one subject was disqualified at the follow-up visit due to weight loss > 3% of baseline body weight; in the placebo group, one person withdrew due to illness unrelated to the study.

The returned capsule count at the end of the study estimated a compliance > 85% in both groups. The groups did not differ in age, BMI, VO_{2max}, VO_{2max} related to body weight, maximum workload (P_{max}), clinical blood chemistry variables and habitual diet (P > 0.05; Table 1).

![Fig. 1. CONSORT (consolidated standards of reporting trials) diagram.](https://doi.org/10.1017/S0007114513001001)
30 min controlled exercise bout

The post-exercise analyses revealed that these women performed at 68·2 (SD 3·1) % of individual VO\textsubscript{2max}. The average walking performance was approximately 6 km/h (Table 1). There were no significant differences between the FBV and placebo groups for these parameters (\(P_{\text{T}x} > 0.05\)).

Carbonyl proteins

The mean values of both groups were comparable with healthy people of this age (reference interval 0·37–1·16 nmol/mg). There were no differences between the groups at baseline, pre- and post-exercise. After 8 weeks of supplementation, there was a significant difference between the FBV and placebo groups (\(P_{\text{T}x} = 0.022\); Fig. 2), both pre- and post-exercise. The FBV group had significantly lower CP concentrations compared with the placebo group. The model of exercise had no influence on CP concentrations.

Oxidised LDL

There were no differences between the groups at baseline, but a significant difference after 8 weeks of FBV or placebo supplementation (\(P_{\text{T}x} = 0.015\); Fig. 3). The FBV group showed lower concentrations compared with the placebo group. The model of exercise had no influence on ox-LDL concentrations. However, all concentrations were within the reference interval, at the beginning and end of the study, pre- and post-exercise (30–80 U/l).

Total oxidation status

There were no differences between the groups at baseline, pre- and post-exercise. After 8 weeks of supplementation, there was a significant difference between the groups (\(P_{\text{T}x} = 0.010\); Fig. 4). After the supplementation period, FBV supplementation had reduced the elevated baseline values (reference cut-off <350 \(\mu\)M-H\(_2\)O\(_2\)) from >900 \(\mu\)M-H\(_2\)O\(_2\) down to approximately 750 \(\mu\)M-H\(_2\)O\(_2\), which is still above the reference interval. The model of exercise had no influence on TOS.

Malondialdehyde

There were no differences between the groups at baseline and after 8 weeks of supplementation, pre- and post-exercise, with all concentrations within the reference interval (2·16 (SD 0·29) nmol/ml; data not shown). Also, the model of exercise had no influence on MDA concentrations.

TNF-\(\alpha\)

Despite the typically high standard deviation for TNF-\(\alpha\), due to the established cytokine inter-individual variability, the data were normally distributed. There were no differences between the groups at baseline, although pre- and post-exercise

Table 1. Baseline characteristics, performance data, clinical chemistry and nutrition data of the forty-two pre-menopausal, obese, but otherwise healthy, women

<table>
<thead>
<tr>
<th>Variables</th>
<th>Reference values(^{\ddagger})</th>
<th>FBV (n 21)</th>
<th>Placebo (n 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>40·8</td>
<td>41·3</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td></td>
<td>34·3</td>
<td>34·6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td>92·2</td>
<td>91·6</td>
</tr>
<tr>
<td>VO\textsubscript{2max} (ml)</td>
<td></td>
<td>2198</td>
<td>2259</td>
</tr>
<tr>
<td>(P_{68%\text{VO}_{2\text{max}}}) (km/h) + slope (%)</td>
<td></td>
<td>25·8</td>
<td>27·1</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td></td>
<td>5·1</td>
<td>5·0</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td></td>
<td>126</td>
<td>130</td>
</tr>
<tr>
<td>Fe ((\mu)mol/l)</td>
<td></td>
<td>16·4</td>
<td>17·6</td>
</tr>
<tr>
<td>Ferritin ((\mu)g/l)</td>
<td></td>
<td>78·4</td>
<td>89·4</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td></td>
<td>5·47</td>
<td>5·56</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td></td>
<td>1·27</td>
<td>1·11</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td></td>
<td>1·27</td>
<td>1·41</td>
</tr>
<tr>
<td>Energy (kJ/d)</td>
<td>7927</td>
<td>7745</td>
<td>7923</td>
</tr>
<tr>
<td>Fat &lt;30 % of kJ/d</td>
<td></td>
<td>5·1</td>
<td>5·0</td>
</tr>
<tr>
<td>Carbohydrates &gt;50 % of kJ/d</td>
<td></td>
<td>1·27</td>
<td>1·41</td>
</tr>
<tr>
<td>Alcohol (%)</td>
<td>&lt;3·5</td>
<td>1·27</td>
<td>1·41</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>2600</td>
<td>2162</td>
<td>2022</td>
</tr>
<tr>
<td>fibre (g)</td>
<td>30</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>(\beta)-Carotene (mg)</td>
<td>4·8</td>
<td>0·14</td>
<td>0·17</td>
</tr>
<tr>
<td>Fruit and vegetable portions/d</td>
<td>5</td>
<td>1–2</td>
<td>1–2</td>
</tr>
</tbody>
</table>

FBV, fruit, berry and vegetable juice powder concentrate; \(P_{\text{T}x}\), maximal performance; \(P_{68\%\text{VO}_{2\text{max}}}\), performance 68 % of maximal oxygen uptake.

\(^{\ddagger}\) Reference values for dietary intake (RDA) in Germany, Austria and Switzerland\(^{[55]}\).

\(^{\ddagger}\) Reference intervals and upper limits for clinical chemistry parameters\(^{[54]}\).
concentrations at baseline (mean value > 24 pg/ml) exceeded the upper reference limit (20 pg/ml; Fig. 5). Following 8 weeks of FBV supplementation, pre- and post-exercise, TNF-α concentrations were within the normal physiological range, whereas the values remained elevated in the placebo group. Hence, there was a significant difference between the FBV and placebo groups after 8 weeks of intervention. The model of exercise had no influence on TNF-α.

IL-6

There were no observed differences between the groups at baseline and after 8 weeks of capsule supplementation, both pre- and post-exercise. Also, the model of exercise had no influence. IL-6 concentrations for all subjects remained below the reference cut-off value (< 11.3 pg/ml; data not shown) throughout the investigation.

Fig. 2. Plasma concentrations of carbonyl groups bound on protein in overweight and obese women (n 42) before and after 8 weeks of supplementation, and pre- and post-30 min of walking exercise. Values are means (n 21 per group), with standard deviations represented by vertical bars. There was a significant effect of treatment after 8 weeks, with no influence of exercise (P = 0.022; ANOVA). FBV, fruit, berry and vegetable concentrate group.

Fig. 3. Plasma oxidised LDL concentrations in overweight and obese women (n 42) before and after 8 weeks of supplementation, and pre- and post-30 min of walking exercise. Values are means (n 21 per group), with standard deviations represented by vertical bars. There was a significant effect of treatment after 8 weeks, with no influence of exercise (P = 0.015; ANOVA). FBV, fruit, berry and vegetable concentrate group.
Skin microcirculation

All values were within the normal limits provided by the equipment manufacturer for healthy people. There were no significant differences between the groups at baseline with pre- and post-exercise blood flow. After the 8-week supplement period, blood flow was significantly higher in the FBV group compared with placebo ($P_{\text{TX}} = 0.029$). There was also a significant increase due to exercise from pre- to post-exercise ($P=0.004$) in both groups, at baseline and after 8 weeks.

There were no differences in $SO_2Hb$ between the groups at baseline, pre- and post-exercise. Following the 8-week

![Graph showing plasma total oxidation status of lipids in overweight and obese women](image1)

**Fig. 4.** Plasma total oxidation status of lipids in overweight and obese women (n 42) before and after 8 weeks of supplementation, and pre- and post-30 min of walking exercise. Values are means (n 21 per group), with standard deviations represented by vertical bars. There was a significant effect of treatment after 8 weeks, with no influence of exercise ($P=0.010$; ANOVA). FBV, fruit, berry and vegetable concentrate group.

![Graph showing plasma TNF-α concentrations in overweight and obese women](image2)

**Fig. 5.** Plasma TNF-α concentrations in overweight and obese women (n 42) before and after 8 weeks of treatment, and pre- and post-30 min of walking exercise. Values are means (n 21 per group), with standard deviations represented by vertical bars. There was a significant effect of treatment after 8 weeks, with no influence of exercise ($P=0.011$; ANOVA). FBV, fruit, berry and vegetable concentrate group.
capsule supplement period, SO2Hb was significantly higher in the FBV group compared with placebo ($P_{\text{Tx}} = 0.032$), pre- and post-exercise (Table 2). The model of exercise also affected the SO2Hb values, with increased values from pre- to post-exercise in both groups at both time points, but this effect did not reach significance ($P_{\text{Ex}} = 0.075$).

As with SO2Hb, there were no differences between the groups at baseline with pre- and post-exercise for rHb. After the 8-week supplement period, rHb was significantly higher in the FBV group compared with placebo ($P_{\text{Tx}} = 0.041$), pre- and post-exercise (Table 2). There was also a significant effect of exercise to increase rHb from pre- to post-exercise ($P_{\text{Ex}}=0.021$) in both groups, at baseline and after 8 weeks of treatment.

### Discussion

Increased protein and lipid oxidation, as well as low-grade inflammation, are conditions associated with increased cardiovascular and chronic disease risk\(^1\,^2\,^5\). Obese people are at a higher risk of developing chronic medical conditions, thus making an interesting study population. The present investigation was primarily focused on the responses of oxidation, inflammation and capillary microcirculation markers in obese pre-menopausal women, after an 8-week intervention with FBV or placebo capsules, followed by a single bout of aerobic exercise. The resulting data show, after the 8-week study period, (1) compared with placebo, the FBV group had a reduction in the markers of protein oxidation, ox-LDL and total lipid oxidation, and lower concentrations of the chronic inflammation marker TNF-$\alpha$. The study also revealed that (2) FBV supplementation increased the microcirculation in the skin. (3) A 30 min walking exercise at 70 % of VO2max increased blood flow and rHb in the skin in both groups (secondary outcome) and (4) the walking exercise did not generate additional oxidative stress in these sedentary obese women (tertiary outcome).

### Oxidative stress markers

In the present study, protein oxidation (as assessed by CP) was decreased after the 8-week FBV supplementation period. The decrease, or attenuation, of CP in healthy and trained subjects in response to this FBV supplementation has been reported previously\(^10,24–26\), and this effect is also now demonstrated in these obese women. The bioavailability of FBV antioxidant vitamins and phytonutrients has been reported previously\(^31,27,28\) and may be the explanation for the consistent observation of decreased or attenuated protein oxidation. However, all CP values remained within the reference interval throughout the investigation in all participants.

Similar to CP, ox-LDL was reduced after 8 weeks of FBV supplementation. Ox-LDL is one of the few recognised parameters of the European Food Safety Authority to estimate oxidative damage to lipids\(^29\). It is also a recognised cardiovascular risk factor associated with obesity\(^30–34\). Over the study period, the FBV group had a 12 % reduction in ox-LDL concentrations (from 58 to 51 U/l). A similar reduction has been reported in heavy smokers using the same FBV capsules for a 3-month
period. This consistent finding may be due to the antioxidant activity of FBV, protecting LDL from oxidation.

TOS, another marker of lipid peroxidation, was elevated at all measured time points, indicating higher concentrations of total lipid peroxides in this cohort of obese women. This surrogate marker is a comprehensive indicator of lipid peroxidation, and thus not as specific for the oxidation of certain molecules, unlike ox-LDL or MDA. The elevated TOS values observed here might reflect a higher oxidation state of MUFA or oxidation derived from other sources, such as advanced glycation end products. These substances originate from a fat-rich diet, which was consumed by the women in the present study (Table 1). The antioxidant functions of FBV decreased TOS concentrations; however, the values remained elevated at the end of the present 8-week study. It would be interesting to observe the response of this marker after long-term FBV supplementation.

MDA is a commonly used marker to estimate lipid peroxidation. It is an indicator of damage to PUFA. Protein-bound MDA was assessed in the present investigation. Neither the capsule treatment nor exercise was distinctive enough to effect changes in MDA concentrations, which remained within the reference interval throughout the study.

Inflammatory markers

Low-grade chronic systemic inflammation has commonly been reported in obese populations, which is accompanied by increased systemic levels of cytokines including TNF-α and IL-6. The changes in TNF-α concentrations in the FBV group were remarkable. At baseline, both study groups had elevated concentrations, exceeding the reference cut-off value (20 pg/ml). After 8 weeks of supplementation, TNF-α in the FBV group was within the reference limit. This is an important finding in the context of the frequently postulated involvement of TNF-α in obesity and insulin resistance (40–42). It has been postulated that adipose tissue, which produces TNF-α, is the main source of circulating TNF-α. However, it has also been observed that a low intake of β-carotene, found in fruits and vegetables, is inversely related to TNF-α. In our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max exercise for 40 min in trained men.

IL-6 concentrations, and obviously, the model of exercise was not exhaustive enough to generate IL-6 from muscle inflammation.

Microcirculation markers

The maintenance of microvascular integrity is related to intact endothelial NO metabolism and protective against adiposity-linked CVD. As NO metabolism is also dependent on redox biochemistry, we hypothesised that FBV supplementation might exert the effects on the microcirculation assessed on the easily accessible skin surface.

All skin microcirculation values were within the reference limit at all the time points assessed; however, these values did increase with both FBV supplementation and exercise. The observed increases in blood flow, SO2, and rHb indicate reduced O2 extraction and vasodilation of the blood vessels. In theory, perhaps the constituents of FBV would have stimulated NO metabolism, for better oxygenation in the capillaries near the skin surface. Plotnick et al. demonstrated that this FBV provides dietary nitrate, resulting in an increase in nitrate/nitrite levels after a 4-week study of FBV supplementation in healthy volunteers, accompanied by improved flow-mediated dilatation after a high-fat meal. Once NO is generated via nitrate and nitrite reductase, it reduces O2 extraction from Hb and also the O2 cost in the tissue. In addition to the nutrients from FBV, it is presumable that exercise affected NO generation via increased blood flow, exerting shear stress on endothelial cell membranes. It has been well established that an increase in blood flow stimulates vascular endothelial cells and promotes the production of various vasodilator substances including NO or prostacyclin.

Effect of exercise on oxidation

A null effect was observed in these women with regard to exercise-generated oxidation. The 30 min exercise bout at close to 70 % of VO2max did not result in increased protein and lipid oxidation (indicated here via CP, ox-LDL, TOS and MDA). This is in line with the recent findings that demonstrated a lack in the increase of CP concentrations with 70 % VO2max exercise for 40 min in trained men. However, to our knowledge, the present investigation demonstrated for the first time that obese, but otherwise healthy, women can perform walking exercise at 70 % VO2max over 30 min without generating oxidative stress. This suggests that exercise regimens for basically healthy obese women should also include aerobic exercise of higher intensity than is usually applied to accelerate improvements in energy consumption capacity and aerobic fitness.

Conclusions

The overall result from the present study is that 8 weeks of supplementation with an encapsulated fruit, berry and vegetable concentrate decreased the oxidation of proteins and lipids in plasma and reduced low-grade inflammation in overweight and obese women compared with the placebo group.
Further, FBV supplementation and 30 min of aerobic walking exercise complemented each other to promote the microcirculation in the skin. In addition, we demonstrated that this population does not suffer oxidative damage or inflammation after 70% VO2max walking intensity over 30 min.

Acknowledgements

The present study was funded by a competitive research grant from NSA LLC (Collierville, Tennessee, USA) to the Institute of Nutrient Research and Sport Nutrition (Graz, Austria). The authors wish to thank Dr Anita M. Boddie for improving the English of the manuscript.

M. L. was the principal investigator and contributed to the development of the overall research plan and study protocol, project management and study oversight, statistical analyses and the preparation of the manuscript; G. O. contributed to the microcirculation measurements, data collection, statistical analyses and manuscript revision; K. S. was involved in the performance diagnostics, data collection and manuscript revision; G. C. participated in the blood sampling, laboratory logistics, data collection and manuscript revision; L. H., G. L. and J. F. G. contributed to the laboratory analyses, data collection and manuscript revision; S. H. was responsible for the laboratory analyses, data collection and the preparation of the manuscript.

M. L. and G. O. are affiliated with the Institute of Nutrient Research and Sport Nutrition. The rest of the authors declare no conflict of interest.

References

Supplementation and exercise in obese women


