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# **ORIGINAL ARTICLE**

# The effect of an increased intake of vegetables and fruit on weight loss, blood pressure and antioxidant defense in subjects with sleep related breathing disorders

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**Objective:** To assess the effect of an increased consumption of vegetables and fruit on body weight, risk factors for cardiovascular disease (CVD) and antioxidant defense in obese patients with sleep-related breathing disorders (SRBD).

**Design:** Randomized, controlled trial of an intervention to increase the intake of vegetables to 400 g/day and fruit to 300 g/day. Dietary intake was calculated from a food frequency questionnaire. Antioxidant status was assessed with the ferric-reducing/antioxidant power (FRAP) assay. Plasma carotenoids were biomarkers for the intake of vegetables and fruit.

**Setting:** A hospital clinic preventing risk factors for CVD.

**Subjects:** Subjects were 103 men and 35 women with a body mass index of  $36.7 \pm 5.8 \, \text{kg/m}^2$  of which 57 (86%) in the control and 68 (94%) in the intervention group completed the study.

Intervention: Group-based behavioral program during 3 months.

Results: The mean between group differences in body weight was -2.0% (95% CI -3.6, -0.5), P < 0.0001. The mean between group difference in systolic and diastolic blood pressure (BP) was -7.1 mm Hg (95% CI: -11.6, -2.6), P = 0.0022 and -3.9 mm Hg (95% CI: -7.0, -0.9), P = 0.0120, respectively. The mean change in daily intake of vegetables and fruit was 12 g (95% CI: -33, 57) and -4 g (95% CI: -79, 71) versus 245 g (95% CI: 194, 296) and 248 g (95% CI: 176, 320) in the control and intervention groups, respectively. This was reflected in higher concentrations of α-carotene and β-carotene. No change in FRAP was seen. In a multiple regression analysis the change in intake of vegetables was a significant contributor ( $R_{\rm adj}^2 = 0.073$  (95% CI: 0.019, 0.214)) to the change in weight.

**Conclusion:** Targeted dietary advice to increase the intake of vegetables and fruit among subjects with SRBD contributed to weight reduction and reduced systolic and diastolic BP, but had no effect on antioxidant defense measured with FRAP. **Sponsorship:** None.

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Keywords: sleep apnea; treatment; dietary advice; risk reduction; carotenoids; FRAP (ferric reducing/antioxidant power)

# Introduction

Sleep-related breathing disorders (SRBD), including habitual snoring, increased upper airway resistance syndrome and obstructive sleep apnea (OSA) are increasingly common causes of morbidity and mortality among overweight and obese individuals. It is estimated that one of five white adults has at least mild disease and one of 15 has at least moderate disease (Caples *et al.*, 2005). There is a graded increase in OSA prevalence with increasing body mass index (BMI) and waist to hip ratio. Observational studies indicate that a decrease in body weight of 10% is associated with improvement in the apnea–hypoapnea index (AHI), a widely used measure of the frequency of disordered breathing events (Peppard *et al.*, 2000). Thus, weight loss is universally recommended to overweight or obese patients with SRBD. However, the response to weight loss is variable, weight loss is difficult to maintain long-term and SRBD may recur even after

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surgically induced weight loss (Pillar *et al.*, 1994; Strobel and Rosen, 1996). Furthermore, studies of weight loss are severely deficient in regard to their design and follow-up (Strobel and Rosen, 1996; Shneerson and Wright, 2001). As a result, the use of continuous positive airway pressure (CPAP), which appears to be the most effective therapy for symptoms of OSA, has become the treatment of choice. Treatment with CPAP may additionally cause weight loss (Loube *et al.*, 1997).

SRBD have been associated with hypertension and an increased risk of cardiovascular disease (CVD) in clinical and epidemiological reports for nearly three decades. This association is confounded by other comorbid conditions, most notably obesity, and to date the question of whether SRBD independently cause CVD remains controversial (Lavie, 2004; Stradling, 2004). Although the exact mechanisms of any atherogenic effects of SRBD have not been established, a number of hypotheses have been proposed. For example, increased sympathetic activity, endothelial dysfunction and inflammatory processes have been suggested to act as mediators of cardiovascular morbidity in patients with SRBD and have been shown to be associated with SRBD in clinical studies. Thus, patients with SRBD may exhibit evidence of sympathetic overactivity and increased oxidative stress, insulin resistance and a prothrombotic state (Shamsuzzaman et al., 2002; Fletcher, 2003; Lavie, 2003; Punjabi et al., 2004). Most convincing in this regard is evidence that treatment with CPAP reduces blood pressure (BP), at least in the short term (Becker et al., 2003).

Alhough the exact independent relation of SRBD to CVD is still not clear, there is widespread acceptance of the idea that patients with SRBD are at high risk of CVD because of concomitant factors, including the pervasiveness of male gender, age, central obesity, hypertension, diabetes and dyslipidemia (Newman et al., 2001). Dietary modification is a primary cornerstone of lifestyle changes that aim to prevent CVD, even though weight reduction sufficient to reduce the need of CPAP is probably very difficult to achieve in the long term. A diet rich in vegetables and fruits has been consistently associated with a reduced risk of cardiovascular disease (Dauchet et al., 2006), and is widely recommended to facilitate weight reduction (Krauss et al., 1996). The feasibility and efficacy of such a diet with the aim of CVD risk factor reduction in patients with SRBD has not been previously studied, to our knowledge. The specific purpose of this study was to test the effects of dietary advice given as a behavioral, group session-based intervention aimed to increase the consumption of vegetables and fruit compared with a single session of dietary advice, in free living overweight or obese subjects with SRBD. The primary outcome was weight change from baseline to 3 months. Changes in established risk factors for CVD including BP, lipids and glucose at 3 months were secondary outcomes. The third outcome was change in oxidative defense measured with ferric reducing/antioxidant power (FRAP) assays at 3 months.

# Subjects and methods

Subjects

Participants included 103 men and 35 women diagnosed with SRBD and a mean  $\pm$  s.d. age and BMI of  $48.2 \pm 9.0$  year and  $36.7 \pm 5.8 \,\text{kg/m}^2$ , respectively. The Ear, Nose and Throat department at the hospital or primary care physicians referred the subjects to the Department of Preventive Cardiology at Ullevål University Hospital for weight reduction. The diagnosis was verified during polysomnography in a sleep laboratory, mostly done at the Ullevål University Hospital. Of the total, 64 subjects had severe OSA (AHI > 30 mean episodes per hour of sleep), 34 subjects had moderate OSA (AHI 15-30), 23 subjects had mild OSA (AHI 5-15), five subjects only had SRBD (AHI <5) and AHI could not be calculated for one subject after the polysomnography owing to technical problems, but the oxygen saturation was 85% and the sleep pattern was in accordance with OSA. The actual AHI measurement could not be located for 11 subjects owing to moving of the Ear, Nose and Throat department to another location. Of the total, 93 subjects were treated with therapeutic or subtherapeutic continuous positive airways pressure CPAP and eight had undergone surgical treatments. No significant difference in AHI was seen between subjects treated with CPAP or who had undergone surgical treatment and subjects without treatment  $(32.3\pm25.7 \text{ versus } 39.8\pm$ 27.7, P = 0.1640).

# Study design

All participants underwent baseline examinations for eligibility, including a medical history and physical examination as well as CVD risk factor assessment (including cardiovascular risk factors, BP and blood chemistry). Inclusion criteria were men and women between the ages of 21 and 72 with BMI≥27 kg/m<sup>2</sup> and diagnosis of OSAS made during polysomnography in a sleep laboratory and based on standard criteria. Exclusion criteria were suspected non-compliance because of drug or alcohol abuse or lack of motivation, participating in another trial, any major non-cardiac disease expected to reduce life expectancy or interfere with the study and the use of appetite suppressants or weight-reducing medication within the last 3 months. The Ethical Committee (region 1) in Norway approved the protocol and all participants provided written informed consent. The study was conducted between May 2000 and December 2003.

The study design was a randomized, parallel group controlled comparison of a group-based nutritional and cognitive-behavioral intervention aimed to increase the intake of vegetables and fruit and simple dietary advice given only at baseline (control). The dietitian opened sealed, consecutively numbered, opaque envelopes containing the randomization number and group assignment (control or intervention). The dietitian then allocated the next available number to each screened and eligible subject. The randomization list was based on a table of random numbers set up by

an investigator, who had no contact with the subjects during the study period. Before randomization, all participants were given written individualized dietary advice aimed to achieve a 5% weight reduction. The advice was based on the subjects' usual diet assessed with a dietary interview based on a food frequency questionnaire (FFQ) as described below.

#### The control group

After the randomization, the participants in the control group were not given further dietary advice or counseling on behavior change. The subjects had no further contact with the dietitian until the 3 months examination.

#### The intervention group

Participants randomized to the intervention group were consecutively assigned to six groups with 10–18 participants in each group. Because of holidays, the meeting frequency for each group during the 3-month period was a minimum of six and a maximum of 10 sessions.

The primary goal of the intervention was to increase the consumption of vegetables (to at least 400 g/day) and fruit (to at least 300 g/day). The subjects were told the weights of various vegetables and fruit and encouraged to use a scaled weight at home to become familiar with the amount of vegetables and fruit that was necessary for goal achievement. Participants were instructed to use a plate model for hot meals and advised to fill one-half of the plate with vegetables, one-fourth with potatoes, rice or pasta and onefourth with meat, chicken, fish and/or legumes. When the plate model was used, the amount of vegetables increased and the portion size of meat and/or potatoes, rice and pasta decreased. Substitution of vegetables and fruit for high-fat, high-energy food was otherwise emphasized. The dietary sessions focused on how to implement an increased intake of vegetables and fruit in daily life settings and on problem solving issues. Recipes and tips to encourage compliance were provided.

#### Dietary assessments

The subjects participated in a dietary interview based on a FFQ before randomization and at the 3-month examination. The interview was done with a registered dietitian and lasted between 1 and 2 h. The FFQ was designed to assess the food intake during the last 3 months and has been validated and described in details elsewhere (Svendsen and Tonstad, 2006). In short, the questionnaire elicited frequencies and consumption of 174 food items (of these 28 vegetables and 29 fruits and berries). Portion sizes were estimated with the use of a photographic atlas, photographs, ordinary food items and standardized units. The FFQ was coded manually for the calculation of total energy, energy yielding nutrients and food items using a software program (Mat på data 3.0, 1996) based on the Norwegian food composition table (Rimestad et al., 1995). Two subjects in the control group and one

subject in the intervention group did not take part in the interview at the 3-month visit but completed the other examinations.

Use of supplements was assessed in the dietary interview. Fifteen subjects reported daily use of low-dose multivitamin supplements and two subjects used high-dose multivitamin supplements at baseline. As intake of vitamins and minerals from supplements was low, we did not include supplements in the calculated dietary intake or in the statistical analyses.

#### Anthropometry and blood pressure measurements

Subjects were weighed (in underwear) with a digital weight (Seca, Germany) to the nearest 0.1 kg. Height was measured with a standardized wall measuring stick scale to the nearest 0.5 cm. Waist circumference was measured at the umbilicus with the subject unclothed and in the standing position and hip circumference was measured at the greater trochanter.

After the subjects sat quietly for 5 min, BP was measured automatically in the right arm with a digital BP monitor (OMRON Hem-705 CP) and an appropriately sized cuff.

#### Laboratory measurements

Fasting (10 h overnight) blood samples were collected at baseline and after 3 months. All samples except those for the analysis of lipids and glucose were kept frozen at  $-70^{\circ}$ C for batch analyzes of the variables. Serum lipids and glucose were analyzed with conventional enzymatic methods and folate was analyzed with a chemoluminescence detection technology method (Advia Centaur immunoassay, Bayer AG, Leverkusen, Germany).

FRAP was determined as described elsewhere (Benzie and Strain, 1999). The carotenoids lutein, zeaxanthin,  $\beta$ -krytpoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene and lycopene were determined in plasma by high-performance liquid chromatography. Proteins were precipitated and removed by the addition of a 4.5 volume of isopropanol followed by centrifugation at 3000 g at 4°C for 15 min. The internal standard astaxanthin was added with the isopropanol and  $25 \,\mu l$  of the clear supernatant were used for analysis. The mobile phases consisted of A: 20% water and 24% acetone in ethanol and B: acetone. The gradient conditions were as follows: From 2 to 100% B within 20 min, followed by 100% B for 15 min. Detection was performed at 453 nm using a variable wavelength detector. Plasma calibrators quantified against the National Institute of Standards and Technology and 968c standard reference material were used as standards.

Values of the laboratory parameters (lipids and glucose) were missing for 1–5 in the control group and 2–4 subjects in the intervention group. Values of carotenoids and FRAP were missing for eight subjects and three subjects in the control and intervention group, respectively. Values of serum folate were missing for 13 subjects in the control group and 13 subjects in the intervention group. In all cases missing samples were due to insufficient material for analysis.



#### Statistical analysis

The results are presented as means  $\pm$  s.d., means (95% CI) or median and 25th, 75th percentiles. Mean differences within and between groups were tested with paired- and unpaired t-tests, respectively. Differences between the groups for skewed variables were tested with the Mann–Whitney two-sample rank test. The  $\chi^2$  test was used in comparisons of categorical variables. Changes were calculated as the difference between 3 months and baseline. Energy density was calculated for the whole diet minus all drinks (coffee, tea, milk, juice, soft drinks and alcoholic beverages).

The association between single, continuous variables was explored by Pearson or Spearman correlations as appropriate for normally distributed or skewed variables. We conducted multiple regression analyses to identify factors that determined weight change in all subjects as follows. To test for group homogeneity, we did a multiple regression analysis including the exposure factor  $(x_1)$ , treatment variable  $(x_2)$ and the product variable of  $x_1$  and  $x_2$ . When the product variable was found to be significant in the multiple regression analysis, the slopes of association were claimed to be different between treatment groups. In such a case simple regression analysis between change in weight and the exposure factor was done within each treatment group. Group homogeneity was tested for the presence of diabetes or CVD, use of antihypertensive or lipid-lowering medication, smoking habits and gender. The tests for homogeneity showed heterogeneity between the control and intervention group only in regard to the use of antihypertensive medication (P = 0.0165). Because of the heterogeneity, use of antihypertensive medication was not included in the final multiple regression analysis.

The tests were considered significant at P<0.05. Statistical analyses were performed using the Stat View 5.0.1 software (Abacus concepts, Berkeley, CA, USA).

# **Results**

A total of 125 subjects participated in the 3-month examination. There were nine dropouts in the control group and four in the intervention group. In the control group three subjects withdrew because of a high workload, three subjects did not show up after three reminders, one subject wanted another treatment, one subject was disappointed with the treatment and one moved to another city. In the intervention group one subject withdrew because of a divorce, one because of a hip operation, one because of alcohol abuse and one quit attending the group sessions and did not show up at the 3-month examination after three reminders.

The ranges for age and BMI were 28–72 years and 27.2–55.4 kg/m², respectively. Other characteristics of participants are shown in Table 1. There was no statistically significant difference in BMI between the groups.

At baseline, there were no statistically significant differences in weight, BP, serum concentrations of lipids, glucose and FRAP or dietary intake of vegetables and fruit between the groups (Tables 2 and 3). However, serum folate was higher in the intervention group.

#### Outcome effects

The mean  $\pm$  s.d. % weight loss was  $0.9\pm4.3\%$  in the control group and  $3.0\pm4.6\%$  in the intervention group (P<0.0001). In the control group eight subjects (14%) achieved a weight loss of  $\geq 5\%$  compared to 21 subjects (31%) in the intervention group (P=0.01).

Systolic and diastolic BP was reduced in the intervention group (Table 2). The mean change in systolic BP was 2.7% (95% CI: -0.3, 5.7) versus -3.2% (95% CI: -5.5, -1.0) and the mean change in diastolic BP was 1.3% (95% CI: -1.7, 4.3) versus -3.5% (95% CI: -5.8, -1.2) in the control group

 $\begin{tabular}{lll} \textbf{Table 1} & Baseline & characteristics & of participants & in the control & and intervention group \\ \end{tabular}$ 

	Control group	Intervention group
	(n = 57)	(n = 68)
Gender		
Male, n (%)	42 (74)	53 (78)
Female, <i>n</i> (%)	15 (26)	15 (22)
Age (years)	$49.2 \pm 10.3^{a}$	$47.1 \pm 7.5$
Cardiovascular disease, n (%)	7 (12)	9 (13)
Diabetes mellitus, n (%)	3 (5)	8 (12)
Hypertensive <sup>b</sup> , n (%)	31 (54)	38 (59)
Antihypertensive medication, n (%)	18 (32)	18 (26)
Lipid lowering medication, n (%)	11 (19)	15 (22)
Smokers, n (%)	19 (33)	17 (25)
CPAP <sup>c</sup> treatment, <i>n</i> (%)	34 (60)	43 (63)
Height (m)		
Male	$1.79 \pm 0.06$	$1.81 \pm 0.06$
Female	$1.66 \pm 0.07$	$1.64 \pm 0.06$
Weight (kg)		
Male	$120.0 \pm 23.7$	$115.5 \pm 17.6$
Female	$103.2 \pm 21.5$	$97.2 \pm 16.3$
BMI $(kg/m^2)$		
Male	$37.5 \pm 6.4$	$35.5 \pm 5.1$
Female	$37.6 \pm 7.5$	$36.0 \pm 5.5$
Waist (cm)		
Male	$124 \pm 16$	$119 \pm 12$
Female	$113 \pm 12$	$107\pm14$
Hip (cm)		
Male	$114 \pm 12$	111 <u>+</u> 9
Female	$114 \pm 12$	116±11
Waist-to-hip ratio	$1.1 \pm 0.1$	$1.0 \pm 0.1$

Abbreviations: BMI, body mass index; CPAP, continuous positive airway pressure

 $<sup>^{</sup>a}\chi^{-}\pm s.d.$  (all such values).

 $<sup>^</sup>bBlood\ pressure\ \geqslant 140/90\,mm\ Hg\ or\ current\ uses\ of\ antihypertensive\ medications.$ 



Table 2 Intervention outcomes at baseline and at 3 months

	Control group (n $=$ 57)		Intervention group ( $n = 68$ )	$P^a$	$P^{b}$
Weight (kg)					
Baseline	$115.6 \pm 24.2^{\circ}$	_	$111.5 \pm 18.8$	_	0.2867
3 months	114.7 + 25.2	_	108.1 + 18.0	_	_
Change	$-0.9 \pm 4.8$	0.1425 $-3.4 \pm 5.3$		< 0.0001	0.0074
Waist (cm)					
Baseline	$121 \pm 16$	_	116±14	_	0.0601
3 months	120+18	_	113+14		_
Change	$-1.6 \pm 5.5$	0.0365	$-3.0 \pm 5.4$	< 0.0001	0.1541
Systolic blood pressure (n	пт На)				
Baseline	127±18	_	$130\pm14$	_	0.2286
3 months	130±18	_	126±16	_	_
Change	$2.7 \pm 13.0$	0.1278	$-4.4 \pm 12.1$	0.0044	0.0022
Diastolic blood pressure (	ímm Ha)				
Baseline	81 ± 10	_	84+9	_	0.1692
3 months	82 <del>+</del> 11	_	80+9	_	
Change	$0.6 \pm 8.6$	0.5843	$-3.3\pm 8.3$	0.0019	0.0120
Total cholesterol (mmol/l	)				
Baseline	$5.5 \pm 1.0$	_	$5.6 \pm 1.2$	_	0.6390
3 months	5.4 <del>+</del> 1.1	_	5.4±1.2	_	_
Change	$-0.0 \pm 0.8$	0.7540	$-0.2 \pm 0.8$	0.0660	0.3219
HDL cholesterol (mmol/l)					
Baseline	$1.2 \pm 0.2$	_	$1.2 \pm 0.2$	_	0.7216
3 months	$1.2 \pm 0.2$	_	$-1.2 \pm 0.2$	_	_
Change	$0.0 \pm 0.1$		$0.0 \pm 0.1$ 0.1430		0.5853
Triacylglycerols (mmol/l)					
Baseline	$2.1 \pm 0.1$	_	$2.4 \pm 2.0$	_	0.3394
3 months	$2.2 \pm 1.2$	_	$2.0 \pm 1.5$	_	_
Change	_		$-0.4 \pm 1.4$	0.0260	0.0638
Glucose (mmol/l)					
Baseline	$6.1 \pm 1.1$	_	$5.9 \pm 2.1$	_	0.6520
3 months	$6.0 \pm 1.3$	_	$5.8 \pm 2.3$	_	
Change	$-0.1 \pm 1.1$	0.6587	$-0.2 \pm 1.6$	0.3905	0.6887
Serum folate (nmol/l)					
Baseline	$12.7 \pm 5.5$	_	$16.3 \pm 9.1$	_	0.0209
3 months	$13.6 \pm 6.0$	_	$16.9 \pm 7.7$	_	_
Change	$0.9 \pm 5.1$	0.2508	$0.6 \pm 6.2$	0.4792	0.8021
FRAP <sup>d</sup> (μmol/l)					
Baseline	$1369 \pm 209$	_	$1368 \pm 235$	_	0.9835
3 months	$1347 \pm 221$	_	$1376 \pm 243$	_	_
Change	$-22 \pm 111$	0.1692	$7 \pm 203$	0.7702	0.3596

<sup>&</sup>lt;sup>a</sup>Differences between baseline and 3 months within the control group and the intervention group (paired t-test).

and intervention group, respectively. The between-group difference in BP remained significant after adjustment for baseline BP and BMI (mean change systolic BP: -6.6 mm Hg (95% CI: -11.0, -2.2) and diastolic BP: -3.6 mm Hg (95% CI: -6.4, -0.8)).

No differences in lipid or glucose concentrations in between-group comparisons were seen, neither did we see

any statistically significant difference in antioxidant defense measured with FRAP within or between the groups (Table 2).

Effects of intervention on diet and serum biomarkers

The intake of vegetables and fruit (including berries and juice) was doubled in the intervention group which was a

<sup>&</sup>lt;sup>b</sup>Differences between the control group and the intervention group (unpaired *t*-test).

 $<sup>^{\</sup>rm c}\chi^-\pm{\rm s.d.}$  (all such values).

<sup>&</sup>lt;sup>d</sup>FRAP, ferric reducing/antioxidant power.





**Table 3** Intervention outcomes for vegetables, fruit and nutrients at baseline and 3 months

	Control group (n = 57)	Intervention group (n = 68)	P <sup>a</sup>
Vegetables (g,	/day)		
Baseline	226 ± 178 <sup>b</sup>	$223 \pm 159$	0.9717
3 months	$238\pm144$	$457 \pm 240$	
Change	12 (-33, 57) <sup>c</sup>	245 (194, 296)	< 0.000
	d berries (g/day)		
Baseline	$327 \pm 266$	$242 \pm 199$	0.0626
3 months	<u> </u>	$486 \pm 285$	
Change	-4 ( <del>-79, 71)</del>	248 (176, 320)	< 0.000
Energy (kJ/day			
Baseline	$11334 \pm 4220$	_	0.2322
3 months		$8982 \pm 2243$	
Change	-1563 (-2216, -910)	-1463 (-2300, -626)	0.8499
Energy density	/ (kJ/g) <sup>d</sup>		
Baseline	$6.62 \pm 1.25$	$6.63 \pm 1.48$	0.8081
3 months		$4.91 \pm 1.14$	
Change	-0.33 (-0.64, -0-01)	-1.76 (-2.08, -1.44)	< 0.000
Protein (% of	energy)		
Baseline	$16.3 \pm 2.8$	$16.7 \pm 2.6$	0.4118
3 months	$16.6 \pm 3.1$	$17.4 \pm 2.6$	
Change	0.4 (-0.5, 1.3)	0.6 (-0.2, 1.5)	0.6978
Fat (% of ene	rqv)		
Baseline	34.0±5.6	$33.4 \pm 7.0$	0.5997
3 months	$32.2 \pm 5.6$	$28.0 \pm 5.9$	
Change	-1.8 (-3.5, -0.1)	-5.2 (-7.1, -3.4)	0.0078
Saturated fat	(% of energy)		
Baseline	12.8 ± 3.1	$12.4 \pm 3.1$	0.4505
3 months	$11.8 \pm 2.9$	$8.9 \pm 2.9$	
	00/17 00	-3.4 (-4.2, -2.5)	. 0 000
Change	-0.9 (-1.7, -0.2)	-3.4 (-4.2, -2.3)	< 0.000
J		-3.4 (-4.2, -2.3)	<0.000
J	(% of energy)		
C <i>arbohydrate</i> Baseline	(% of energy) 45.3±6.4	44.8±7.1	
Carbohydrate	(% of energy)		0.6682
Carbohydrate Baseline 3 months Change	(% of energy) 45.3±6.4 47.3±6.0 1.8 (0.2, 3.3)	44.8±7.1 49.6±6.3	0.6682
Carbohydrate Baseline 3 months	(% of energy) 45.3±6.4 47.3±6.0 1.8 (0.2, 3.3) energy)	44.8±7.1 49.6±6.3 4.8 (3.2, 6.4)	0.6682
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$	0.6682
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$	0.6682 0.0091 0.3717
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$	0.6682 0.0091 0.3717
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$	0.6682 0.0091 0.3717 0.8432
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$	0.6682 0.0091 0.3717 0.8432
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$	0.6682 0.0091 0.3717 0.8432
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$	0.6682 0.0091 0.3717 0.8432
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1) ng/day)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 ( $-0.93$ , 0.66) $30 \pm 12$ $29 \pm 9$ -2 ( $-5$ , 1) ng/day) $337 \pm 133$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Change  Cholesterol (n Baseline	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1) ng/day)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Change  Cholesterol (m Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1) ang/day) $337 \pm 133$ $292 \pm 122$ -46 (-75, -18)	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (n Baseline 3 months Change  Cholesterol (m Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1) 10.00	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000 0.1547 0.3631
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (m Baseline 3 months Change  Cholesterol (m Baseline 3 months Change  Sodium (mg/c Baseline	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ $1.8 (0.2, 3.3)$ energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ $-0.13 (-0.93, 0.66)$ $30 \pm 12$ $29 \pm 9$ $-2 (-5, 1)$ $10g/day)$ $337 \pm 133$ $292 \pm 122$ $-46 (-75, -18)$ $1day)$ $3593 \pm 1674$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$ $3485 \pm 1278$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000 0.1547 0.3631
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (n Baseline 3 months Change  Cholesterol (m Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 ( $-0.93$ , 0.66) $30 \pm 12$ $29 \pm 9$ -2 ( $-5$ , 1) 10.09 $10.09$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$	<0.0000 0.6682 0.0091 0.3717 0.8432 0.1762 <0.0000 0.1547 0.3631 0.6847
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (n Baseline 3 months Change  Cholesterol (m Baseline 3 months Change  Sodium (mg/d Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 ( $-0.93$ , 0.66) $30 \pm 12$ $29 \pm 9$ -2 ( $-5$ , 1) 100 $100$ $10$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$ $3485 \pm 1278$ $2801 \pm 909$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000 0.1547 0.3631
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (n Baseline 3 months Change  Cholesterol (mg/c Baseline 3 months Change  Sodium (mg/c Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 (-0.93, 0.66) $30 \pm 12$ $29 \pm 9$ -2 (-5, 1) 10 + 10 = 10 = 10 = 10 = 10 = 10 = 10 =	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$ $3485 \pm 1278$ $2801 \pm 909$ $-609 (-1070, -380)$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000 0.1547 0.3631 0.6847 0.5208
Carbohydrate Baseline 3 months Change  Alcohol (% of Baseline 3 months Change  Fiber (g/day) Baseline 3 months Change  Cholesterol (n Baseline 3 months Change  Cholesterol (m Baseline 3 months Change  Sodium (mg/d Baseline 3 months Change	(% of energy) $45.3 \pm 6.4$ $47.3 \pm 6.0$ 1.8 (0.2, 3.3) energy) $3.2 \pm 4.4$ $3.0 \pm 3.6$ -0.13 ( $-0.93$ , 0.66) $30 \pm 12$ $29 \pm 9$ -2 ( $-5$ , 1) 100 $100$ $10$	$44.8 \pm 7.1$ $49.6 \pm 6.3$ $4.8 (3.2, 6.4)$ $3.9 \pm 4.0$ $3.9 \pm 4.5$ $-0.03 (-0.10, 0.63)$ $27 \pm 12$ $36 \pm 12$ $9 (6, 13)$ $304 \pm 125$ $239 \pm 95$ $-65 (-94, -36)$ $3485 \pm 1278$ $2801 \pm 909$	0.6682 0.0091 0.3717 0.8432 0.1762 <0.000 0.1547 0.3631

Table 3 Continued

	Control group (n = 57)	Intervention group (n = 68)	P <sup>a</sup>
Calcium (mg/	/day)		
Baseline	,, 1134±584	$1015 \pm 509$	0.2258
3 months	999±414	$900 \pm 463$	
Change	-138 (- <del>2</del> 55, -21)	-103 (- <del>221, 15)</del>	0.6763
Magnesium (	mg/day)		
Baseline	474±170	$437 \pm 144$	0.1899
3 months	$443 \pm 142$	$459 \pm 108$	
Change	-35 (-64, 6-)	25 (-5, 55)	0.0063
α-tocopherol	(mg/day)		
Baseline	$9.5 \pm 3.9$	$8.0 \pm 3.3$	0.0223
3 months	$8.7 \pm 3.5$	$9.0 \pm 3.3$	
Change	-0.9 (-1.9, 0.1)	1.1 (0.1, 2.1)	0.0065
Vitamin C (m	g/day)		
Baseline	215±438	$130 \pm 76$	0.1128
3 months	$151 \pm 83$	$240 \pm 107$	
Change	-68 (-190, 53)	113 (85, 141)	0.0019
Folate (μg/da	ay)		
Baseline	$303 \pm 115$	$272 \pm 101$	0.1053
3 months	$287 \pm 102$	$337 \pm 101$	
Change	-20 (-49, 8)	71 (42, 100)	< 0.0001
β-Carotene (μ	ıg/day)		
Baseline	2996±1870	$3037 \pm 2281$	0.9156
3 months	$3600 \pm 2729$	$7086 \pm 4528$	
Change	588 (-81, 1257)	4140 (2948, 5334)	< 0.000

Abbreviation: CI, confidence interval.

significant increase compared to controls and resulted in lower energy density of the diet, a reduction in energy percent from total and saturated fat, higher intake of energy percent from carbohydrate and higher intake of fiber, potassium, magnesium,  $\alpha$ -tocopherol, Vitamin C, folate and  $\beta$ -carotene (Table 3). No significant change was seen in the intake of calcium within the intervention group. However, the dietary intake of diary products was decreased (-62 g (95% CI -143, 19) and -77 g (95% CI -151, -3), P=0.8023 in the control and intervention group, respectively).

The increased intake of vegetables and fruit was reflected in increased plasma concentrations of  $\alpha$ - and  $\beta$ -carotene in between-group comparisons (Table 4). The mean between-group difference of the percentage change in serum levels of  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lycopene, lutein and zeaxanthin were 166.2% (95% CI 59.5, 273.0), 61.3% (95% CI 31.5, 91.1), 47.2% (95% CI 7.2, 87.86), 56.1% (95% CI -29.9, 142.0), 21.0% (95% CI -7.1, 49.9) and -3.0% (95% CI -65.7, 59.7), respectively.

 $<sup>^{\</sup>mathrm{a}}\mathrm{Differences}$  between the control group and the intervention group (unpaired  $t\text{-}\mathrm{test}$ ).

 $<sup>^{\</sup>rm b}\chi^-\pm{\rm s.d.}$  (all such values).

 $<sup>^{</sup>c}\chi^{-}$ ; 95% CI in parentheses (all such values).

<sup>&</sup>lt;sup>d</sup>Energy density was calculated for the whole diet minus tea, coffee, water and soft drinks.



Table 4 Changes in plasma carotenoid values from baseline to 3 months in the control and intervention group

	Control group (n = 49)	Intervention group (n $=$ 65)	P <sup>a</sup>
Lutein (µM)	-0.017 (-0.039, 0.005) <sup>b</sup>	0.005 (-0.013, 0.023)	0.0555
Zeaxanthin (μM)	-0.010 (-0.020, 0.005)	-0.002 (-0.062, 0.058)	0.0844
$\beta$ -Cryptoxanthin ( $\mu$ M)	0.026 (-0.004, 0.056)	0.055 (0.021, 0.089)	0.0516
α-Carotene (μM)	-0.002 (-0.016, 0.020)	0.046 (0.026, 0.066)	0.0023
β-Carotene (μM)	-0.029 (-0.077, 0.019)	0.086 (0.036, 0.150)	0.0031
Lycopene (μM)	0.014 (-0.004, 0.068)	0.073 (0.009, 0.137)	0.3015

Abbreviation: CI, confidence interval.

**Table 5** Correlations between antioxidant markers and change in intakes of vegetables and fruit between baseline and 3 months and intakes at 3 months in the intervention group

	Changes in intake $(n = 64)^a$				Intake at 3 mo (n = 65) <sup>b</sup>			
	Vegetables		Fruit		Vegetables		Fruit	
	R <sup>c</sup>	Р	r	Р	r	Р	r	Р
Lutein	0.190	0.1318	0.094	0.4569	-0.001	0.9971	0.045	0.7208
Zeaxanthin	-0.096	0.9609	0.023	0.8532	-0.127	0.3105	-0.016	0.8996
$\beta$ -Kryptoxanthin	0.108	0.3926	-0.041	0.7442	0.203	0.1038	0.270	0.0308
β-Carotene	0.072	0.5688	-0.018	0.8887	0.160	0.2010	0.233	0.0620
α-Carotene	0.323	0.0103	0.153	0.2238	0.213	0.0887	0.426	0.0006
Lycopene	0.038	0.7626	-0.004	0.9722	0.085	0.4973	-0.124	0.3219
FRAP <sup>d</sup>	0.135	0.2831	0.060	0.6322	-0.083	0.5092	0.001	0.9933

<sup>&</sup>lt;sup>a</sup>One subject was missing dietary data and three subjects were missing serum values for carotenoids and FRAP.

At 3 months, nine subjects (16%) in the control group and 37 subjects (55%) in the intervention group reported an intake of  $\geqslant$  400 g/day of vegetables (P = 0.001). Furthermore, 27 subjects (49%) in the control group and 46 subjects (69%) in the intervention reported an intake of fruit  $\geqslant$  300 g/day (P = 0.05). Compared to controls, the increased intakes of vegetables and fruit in the intervention group were also reflected in higher calculated intakes of  $\beta$ -carotene and folate (Table 3).

# Correlation analyses

Correlation analyses conducted among all subjects showed that changes in intake of vegetables and fruit were positively correlated to change in body weight ( $r_S$ =0.284, P=0.0015 and  $r_S$ =0.209, P=0.0207, respectively) but not to changes in systolic or diastolic BP (data not shown). Within the intervention group, weight changes were significantly correlated to changes in systolic BP (r=0.304, P=0.0137) not to diastolic BP (r=0.232, P=0.0631).

Tables 5 and 6 show correlations between antioxidant markers (lutein, zeaxanthin,  $\beta$ -kryptoxanthin,  $\beta$ -carotene,  $\alpha$ -carotene, lycopene and FRAP) and changes in the intake of

vegetables and fruit and the correlations between antioxidant markers and the intake at 3 months in the intervention group and among all subjects. The marker that showed best correlation in all analyses was  $\alpha$ -carotene (Tables 5 and 6). Changes in intake of  $\beta$ -carotene were significantly correlated to changes in serum concentrations of  $\beta$ -carotene ( $r_S = 0.234$ , P = 0.0135).

To explore the most important factor for weight reduction during the intervention we included change in the intake of vegetables, change in the intake of fruit and treatment group in a multiple regression analysis. As shown in Table 7 change in the intake of vegetables was the only significant factor in the model ( $R_{\rm adj}^2 = 0.073$  (95% CI 0.019, 0.214)). Including CPAP did not improve the model ( $R_{\rm adj}^2 = 0.062$  (95% CI 0.018, 0.210)) nor did the inclusion of age, gender and BMI at baseline ( $R_{\rm adj}^2 = 0.051$  (95% CI 0.020, 0.216)).

## Discussion

Our main finding was that an  $\sim 500\,\mathrm{g}$  increase in the total consumption of vegetables and fruit resulted in weight loss and BP reduction in the intervention group, but did not

<sup>&</sup>lt;sup>a</sup>Differences between groups (the Mann-Whitney two sample rank test).

<sup>&</sup>lt;sup>b</sup>χ<sup>-</sup>; 95% CI in parentheses (all such values). Eight subjects in the control group and three in the intervention group were missing values.

<sup>&</sup>lt;sup>b</sup>One subject was missing dietary data and two subjects were missing serum values for carotenoids and FRAP.

<sup>&</sup>lt;sup>c</sup>Spearmann correlations (all such values).

<sup>&</sup>lt;sup>d</sup>FRAP, the ferric reducing/antioxidant power.

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**Table 6** Correlations between antioxidant markers and change in intakes of vegetables and fruit between baseline and 3 months and intakes at 3 months in all subjects

	Changes in intake (n = 112) <sup>a</sup>					Intake at 3 mo	nths (n = 114) <sup>b</sup>	
	Veg	etables Fruit		Vegetables		Fruit		
	r <sup>c</sup>	Р	r	Р	r	Р	r	Р
Lutein	0.255	0.0073	0.121	0.2016	0.270	0.0041	0.156	0.0965
Zeaxanthin	0.103	0.2776	0.138	0.1490	0.126	0.1812	0.123	0.1911
$\beta$ -Kryptoxanthin	0.112	0.2366	0.067	0.4775	0.233	0.0133	0.319	0.0008
$\beta$ -Carotene	0.229	0.0159	0.134	0.1581	0.315	0.0008	0.235	0.0126
α-Carotene	0.422	< 0.0001	0.221	0.0198	0.399	< 0.0001	0.349	0.0002
Lycopene	0.117	0.2176	0.119	0.2115	0.207	0.0280	-0.054	0.5693
FRAP <sup>d</sup>	0.030	0.7484	0.046	0.6270	0.063	0.5005	0.069	0.4631

Abbreviation: FRAP, the ferric reducing/antioxidant power.

**Table 7** Multiple regression analysis of change in weight in all participants  $(n=122)^a$ 

	Standardized regression coefficients	Р
Changes in intake of vegetables	0.213	0.0443
Changes in intake of fruit	0.106	0.2813
Treatment	0.063	0.5565

<sup>&</sup>lt;sup>a</sup>Dietary data were missing for three subjects.

influence antioxidant defense measured with FRAP. Compared to the control group, the intervention group reported a doubled intake of vegetables and fruit, a reduced intake of saturated fat and increased intake of fiber, vitamins and minerals known to lower BP.

The effects that we observed in these obese subjects with SRBD were achieved with dietary advice to increase the intake of vegetables and fruit primarily. Even though no food was provided in our study, more than 50% of the participant reported achieving the dietary goals. A design with no food provided was also used in the PREMIER clinical trial (Appel et al., 2003). The study lasted for 6 months and tested the effect of established recommendations for BP reduction (weight loss, sodium restriction, increased physical activity and limited alcohol intake) compared to the effect of these recommendations plus the DASH diet among adults with above optimal levels of BP. As in our study the achieved weight reduction was about 1 kg per month and the reduction in systolic and diastolic BP was 4 and 3 mm Hg, respectively. In comparison, the DASH study, which was a feeding study, showed that a diet that was high in vegetables and fruit, low-fat dairy product, nuts, fish and otherwise low in total and saturated fat was associated with highly significant reductions of 6 and 3 mm Hg in systolic and diastolic BP, respectively, after 8 weeks (Appel et al., 1997). Furthermore, Nowson et al. (2005) showed that specific targets to increase the foods used in the DASH diet resulted in a greater decrease in BP than did an ordinary low-fat diet without dietary targets (Nowson *et al.*, 2005). As in the study by Nowson *et al.* (2005), we had specific targets for the intake of vegetables and fruit. Even more than seven servings of vegetables and fruit as we used in our study have been used to achieve BP reductions. For example, in the Optimal Macronutrient Intake Trial to Prevent Heart Disease trial the total servings of vegetables and fruit were nine to 11 servings per day (Appel *et al.*, 2005). However, this regimen may be difficult to achieve (Weinberger, 2005). In a daily life setting in which persons have to plan, purchase and prepare their own meals, a more moderate intake of vegetables and fruit will probably be more realistic and feasible.

In contrast to the DASH study (Appel *et al.*, 1997), the primary aim of our study which was performed in obese subjects with SRBD was to study the effect of increased intake of vegetables and fruit on weight reduction. We did not emphasize increasing the intake of low-fat diary products. Vegetables and fruit were recommended in exchange for other food groups. Therefore, in contrast to the DASH study we observed a small reduction in the intake of diary products in the intervention group.

The clinical relevance of the weight loss achieved in these obese subjects with SRBD is reflected in the BP reduction. In a study conducted in general practice among nearly 300 hypertensive subjects, prompt sheets for high fruit, vegetable, fiber and low fat resulted in a weight reduction of 1.2 kg during 6 months, but did not reduce blood pressure (Little et al., 2004). In our study, despite a 2% difference in body weight we did not see any difference in serum concentrations of lipids or glucose between the groups. Both the weight reduction and the reduced intake of total and saturated fat are expected to act favorably on concentrations of total and LDL cholesterol. In a recent controlled feeding study, the reduction in LDL cholesterol concentrations decreased as the percentage of body fat, BMI and insulin concentrations increased (Lefevre et al., 2005). Because our

<sup>&</sup>lt;sup>a</sup>Three subjects were missing dietary data and 10 subjects were missing serum values for carotenoids and FRAP.

<sup>&</sup>lt;sup>b</sup>Three subjects were missing dietary data and eight subjects were missing serum values for carotenoids and FRAP.

<sup>&</sup>lt;sup>c</sup>Spearmann correlations (all such values).

subjects were obese, this observation may partly explain the lack of effect of weight reduction on lipids in our study. Furthermore, the amount of weight loss needed to influence lipids or glucose concentrations are thought to be at least 5% (World Health Organization (1997)).

Obese subjects and subjects with SRBD may exhibit increased oxidative stress (Schultz et al., 2000; Dandona et al., 2001; Dyugovskaya et al., 2002), though a recent study questioned this notion (Svatikova et al., 2005). After dietary restriction and weight loss a decrease in reactive oxygen species generation by leukocytes and oxidative damage to lipids, protein and amino acids has been shown (Dandona et al., 2001). We measured fasting serum FRAP as a measure of antioxidant defense but this parameter was largely unaffected by our intervention. Similar findings were reported in a recent dietary study by Dragsted et al. (2004). This study showed that markers of oxidative damage, oxidative capacity or antioxidant defense were largely unaffected by an increase in vegetables and fruit. However, the diet was not based on vegetables and fruit known to have high FRAP values. For example, blue grapes, berries, dog roses, pomegranates, Brussels sprouts, kale, red cabbage, herbs, green tea, dark chocolate, seeds, nuts and oils have high FRAP values (Halvorsen et al., 2002; Dragland et al., 2003). This may also have been the case in our study. The participants chose their own vegetables and fruit based on preferences and primarily consumed carrots, tomatoes, apples and bananas (data not shown), which are not particularly high in FRAP. In contrast, increased levels of FRAP were demonstrated in 12 obese, hypertensive subjects that followed the DASH diet (Lopes et al., 2003). A limitation of our study was that we did not measure postprandial FRAP. It has been proposed that food items with very high amounts of specific plant phenols may have postprandial effects (Dragsted et al., 2004). In contrast to a number of other studies we did not include a depletion period and this may have minimized the effects of the diet. Furthermore, baseline concentrations of FRAP were high compared to other studies (Lopes et al., 2003; Dragsted et al., 2004). Indeed, we observed a small trend toward an increase in FRAP in the intervention group, but based on these small changes, we would require approximately 500 participants in each group to achieve statistical significance (data not shown) and the clinical significance of such a small change has not been proven.

#### Study limitations

We included all subjects in the analyses and did not separate subjects using CPAP and subjects not using CPAP due to reduced sample size and power. However, in the multiple regression analysis, CPAP treatment was not a significant contributor to the weight change observed.

In the multivariate analysis, changes in intake of vegetables only explained 7% of the variation in weight loss. This may be owing to weight reduction, which also depends on other factors such as motivation to reach goal weight and participants expectations of weight loss (Foster et al., 1997). These factors were not considered in the multivariate model. Because the intervention only lasted for 3 months the data do not address the feasibility and efficacy of the dietary advice for a longer time period. Another limitation is underreporting of energy, as commonly noted in dietary studies, particularly among obese individuals (Tooze et al., 2004). Despite a greater weight loss in the intervention group, there was no statistically significant difference in reported energy intake at 3 months between the control and the intervention groups. The intervention group may have been more aware of their dietary intake and reported more accurately. However, the primary goal of our intervention was to increase the consumption of vegetables and fruit. Over-reporting of vegetables and fruit may have occurred in the intervention group because of social desirability bias (Tooze et al., 2004) and because of the large number of vegetables, fruit and berries that were included in the FFQ (about 50). The reported difference in the intake of these items was highly statistically significant between the groups and was mirrored in increased serum concentrations of lutein,  $\beta$ -kryptoxanthin,  $\alpha$ -carotene and  $\beta$ -carotene in the intervention group. The relatively small correlation coefficients seen may be explained by the homogeneous population, the emphasis on all vegetables and fruit (not all vegetables and fruit are high in carotenoids) and the fact that concentration biomarkers often shows low correlation coefficients with reported dietary intake (Al-Delaimy et al., 2005).

We did not include specific targets to increase the intake of low-fat diary products in our intervention. In the DASH study a more beneficial effect was seen in the group that ate more low-fat diary products compared to the group that only increased the intake of vegetables and fruit. However, the subjects in the DASH study had a low baseline intake of calcium (Appel *et al.*, 1997). Our subjects had a high baseline intake of calcium. Further studies would be needed in obese subjects with SRBD to examine the effect of increasing low-fat diary products in the dietary intervention

A minor limitation is that we did not exclude subjects taking high doses of multivitamin supplements; however, only two subjects in the intervention group took such doses at baseline. This may be reflected in the difference in serum folate between the groups at baseline. These subjects stopped taking the supplements during the study. We have no reasons to believe that this has influenced our results.

## **Implications**

We have shown that simple dietary advice to increase intake of vegetable and fruit in a group session based behavior treatment was effective for moderate weight reduction and in reducing systolic and diastolic BP in a population of symptomatically treated subjects with SRBD. Vegetables and fruit are water-rich foods that are low in energy density and

hence allow big portion sizes while reducing energy intake. A behavioral program with targeted goals to increase the intake of vegetables appears to be important for weight loss and BP reduction. If no advice is given about the specific choice of food items known to be high in FRAP, antioxidant defense does not appear to be affected.

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