Upsala Journal of Medical Sciences

ISSN: 0300-9734 (Print) 2000-1967 (Online) Journal homepage: informahealthcare.com/journals/iups20

Effects of exercise with or without blueberries in the diet on cardio-metabolic risk factors: An exploratory pilot study in healthy subjects

Sofia Nyberg, Edvard Gerring, Solveig Gjellan, Marta Vergara, Torbjörn Lindström & Fredrik H Nystrom

To cite this article: Sofia Nyberg, Edvard Gerring, Solveig Gjellan, Marta Vergara, Torbjörn Lindström & Fredrik H Nystrom (2013) Effects of exercise with or without blueberries in the diet on cardio-metabolic risk factors: An exploratory pilot study in healthy subjects, Upsala Journal of Medical Sciences, 118:4, 247-255, DOI: 10.3109/03009734.2013.825348

To link to this article: https://doi.org/10.3109/03009734.2013.825348

Upsala Journal of Medical Sciences. 2013; 118: 247–255

informa
healthcare

ORIGINAL ARTICLE

Effects of exercise with or without blueberries in the diet on cardio-metabolic risk factors: An exploratory pilot study in healthy subjects

SOFIA NYBERG, EDVARD GERRING, SOLVEIG GJELLAN, MARTA VERGARA, TORBJÖRN LINDSTRÖM & FREDRIK H NYSTROM

Department of Medical and Health Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden

Abstract

Background. The improvement of insulin sensitivity by exercise has been shown to be inhibited by supplementation of vitamins acting as antioxidants.

Objective. To examine effects of exercise with or without blueberries, containing natural antioxidants, on cardio-metabolic risk factors.

Methods. Fifteen healthy men and 17 women, 27.6 ± 6.5 years old, were recruited, and 26 completed a randomized cross-over trial with 4 weeks of exercise by running/jogging 5 km five times/week and 4 weeks of minimal physical activity. Participants were also randomized to consume 150 g of blueberries, or not, on exercise days. Laboratory variables were measured before and after a 5 km running-race at maximal speed at the beginning and end of each period, i.e. there were four maximal running-races and eight samplings in total for each participant.

Results. Insulin and triglyceride levels were reduced while HDL-cholesterol increased by exercise compared with minimal physical activity. Participants randomized to consume blueberries showed an increase in fasting glucose levels compared with controls, during the exercise period (blueberries: from 5.12 ± 0.49 mmol/l to 5.32 ± 0.29 mmol/l; controls: from 5.24 ± 0.27 mmol/l to 5.17 ± 0.23 mmol/l, $P = 0.04$ for difference in change). Triglyceride levels fell in the control group (from 1.1 ± 0.49 mmol/l to 0.93 ± 0.31 mmol/l, $P = 0.02$), while HDL-cholesterol increased in the blueberry group (from 1.51 ± 0.29 mmol/l to 1.64 ± 0.33 mmol/l, $P = 0.006$).

Conclusions. Ingestion of blueberries induced differential effects on cardio-metabolic risk factors, including increased levels of both fasting glucose and HDL-cholesterol. However, since it is possible that indirect effects on food intake were induced, other than consumption of blueberries, further studies are needed to confirm the findings.

Key words: *Blueberries, cholesterol, exercise, glucose, running, troponin*

Introduction

Common strategies in the primary prevention of cardiovascular disease include recommendations to perform physical exercise several times a week in order to counteract components of the metabolic syndrome and to increase physical fitness (1). Although exercise can lead to improved blood lipid levels, reduced blood pressures, and lower glucose levels, several studies have shown that strenuous long-distance races, such as marathons and triathlons, increase markers of inflammation (2-6). It has been proposed that this pro-inflammatory effect was caused by inclusion of subjects that were less physically fit than the actual exercise required, as inflammation seems to occur less frequently following a period of regular exercise (7), although this has not been specifically investigated in a randomized manner. Interestingly, several earlier trials have shown that highly strenuous exercise, such as running a

Correspondence: Professor Fredrik H. Nystrom, MD, PhD, Department of Medical and Health Sciences, Linköping University, SE 581 85 Linköping, Sweden. Fax: +46 13 145949. E-mail: fredrik.nystrom@lio.se

(Received 6 April 2013; accepted 10 July 2013)

ISSN 0300-9734 print/ISSN 2000-1967 online
DOI: 10.3109/03009734.2013.825348

marathon, can induce release of troponin-T (2,8,9), which is a protein that is used clinically to diagnose myocardial infarction since it is a specific marker for cardiac myocyte damage (10).

Oxidative stress is implicated in human disease, and in experimental studies antioxidants decrease oxidative damage (11). Many subjects are using antioxidant supplements, which are often marketed as to improve health and to prevent disease (12-14). Whether antioxidant supplements are indeed beneficial or harmful is uncertain, but a meta-analysis of randomized trials suggested that treatment with beta carotene, vitamin A, and vitamin E may increase mortality, while the effect of vitamin C was less clear (15). Ingestion of berries, with natural antioxidants, have, on the other hand, been demonstrated to improve many cardiovascular risk factors (16). However, antioxidants in the form of pills containing vitamin C and E specifically inhibited the increased insulin sensitivity following 4 weeks of exercise, and this effect was present in moderately as well as in highly fit participants (17).

We know of no study specifically designed to compare the effects of running a distance common in recreational exercise on components of the metabolic syndrome, in which the effects of natural antioxidants and vitamins in blueberries were analyzed. The aim of this randomized cross-over trial was to compare the effects of a 4-week exercise protocol with or without blueberries to inactivity on markers of cardio-metabolic disease in healthy subjects. The participants were randomized to starting with the exercise period or with the period of minimal physical activity, and also to consuming 150 g of blueberries on the day they exercised, or to keeping eating habits unchanged.

Material and methods

By local advertising at Linköping University we recruited 32 participants as volunteers for the study. The participants had to be free from major disease as judged by medical check-up and history, and they had to have some earlier experience of exercise in the form of running. The trial consisted of two main periods: 4 weeks of running (or jogging), and 4 weeks during which the participant should strive for minimal levels of physical activity, without any exercise whatsoever. The study period was from beginning of September 2010 to the end of December 2010, and the study organizers aimed for one month of return to individual regular exercise habits (wash-out) in between the running and minimal-physical-activity periods. However, if anything arose that affected the participants' ability to exercise or the evaluation in these two main periods, such as musculoskeletal problems or upper respiratory infections, the priority was set to achieve evaluation of the two main periods, and hence it was possible to prolong either period by up to a week, and thus the wash-out period could be shortened correspondingly. Participants were randomized to start with either the exercise period or with the 4 weeks of minimal physical activity. At the beginning and end of each trial period the participants ran 5 km at the fastest possible time in the evening (at 6 p.m.), here denoted 'running-race'. Venous blood was drawn for analysis of cardio-metabolic risk markers after an overnight fast (10 hours between 6 p.m. and 9 a.m.) on the morning of the day of the evening run, and the corresponding morning after, except for troponin-T which was analyzed only from samples after the races. Figure 1 shows a schematic presentation of the study design for a participant randomized to start with the exercise period when reading the figure from left to right. Each participant who completed the whole trial thus ran a total of 4 running-races at maximal speed, and venous blood was correspondingly drawn 8 times in the fasting state; thus he or she had one period of exercise, a wash-out period, and also a period of minimal physical activity in this cross-over trial.

The participants were instructed to exercise in the form of running or jogging 5 km five times a week during the exercise period. The randomized design to test effects of antioxidants on the presumed insulin-sensitizing effect of exercise was based on a study by Ristow et al. in which it was found that vitamin C and E in combination hinders benefits on insulin sensitivity of regular exercise in humans (17). However, in our study the participants were randomized to consume 150 g of frozen blueberries (berries that have a high natural content of several antioxidants (18-20)) on each day of running, or to keep eating habits unchanged, during the training period. No particular instructions were given on how or when to consume the blueberries on the exercise days, in order to make potential findings easily incorporated in regular eating habits. The blueberries had a content of 40 kCal/100 g and also had 0.5 g protein, 8 g carbohydrates, and 0.5 g fat per 100 g of the product. All blueberries were bought on the same occasion from a regular local grocery store, in order to assure that there were no changes in nutrient composition during the study period.

The participants were subjected to determination of body fat content with BodPod (Life Measurement, Inc., Concord, CA, USA) at the end of both main periods. At these time points resting blood pressures were measured in the seated position with manual technique.

All analyses of laboratory variables including high-sensitivity C-reactive protein (hs-CRP) were done at the Department of Clinical Chemistry,

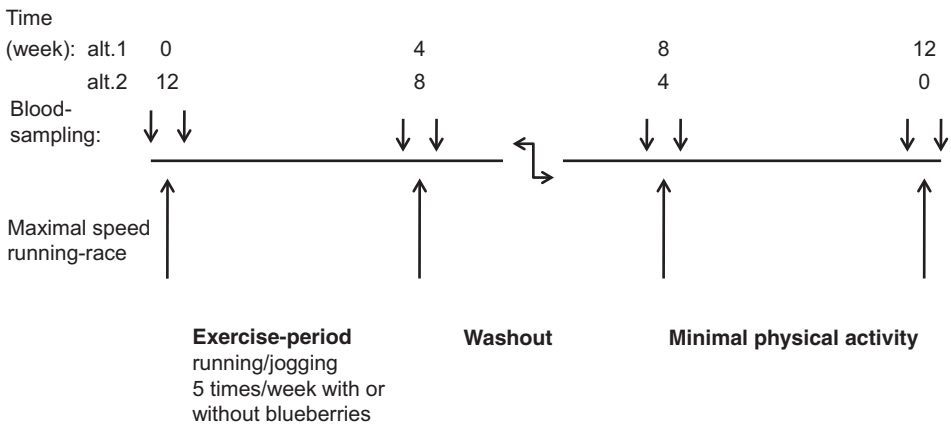

Figure 1. Study design description. The figure shows the design for a participant who started with the exercise period (when read from left to right). The subjects were randomized to whether they started with exercise or with the period of little physical activity. During the exercise period participants were randomized to consume blueberries on exercise days, or to be asked not to make any changes in regular eating habits. Blood sampling was performed in the fasting state in the morning; running-races at maximal speed were performed in the evening at 6 p.m.

Linköping University Hospital as part of their regular clinical routine analyses. Troponin-T was analyzed with an electrochemical luminescence method (Troponin-T STAT Cardiac T, Cobas E411 equipment, Roche, Basel, Switzerland). Serum-insulin was determined by an immunoassay (AutoDelfia, Perkin Elmer, Linköping, Sweden). Total-cholesterol, HDL-cholesterol, and triglycerides were determined by colorimetric analyses (Siemens, Liederbach, Germany), and LDL-cholesterol was calculated according to Friedewald (total-cholesterol – HDL-cholesterol – 0.456 × total triglyceride concentration). The methods for analyses of the other routine samples have been published (21,22).

Ethics

The study was approved by the Regional Ethics Committee of Linköping and performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participating subjects. The study was registered at ClinicalTrials.gov (NCT01274078).

Statistics

Statistical estimates were calculated using SPSS 19.0 software (IBM Corporation, Somers, New York, USA). Comparisons within and between groups were done with Student's paired and unpaired 2-tailed *t* test or as stated in the results section. Mean values and standard deviations are given. Statistical significance refers to 2-sided $P \leq 0.05$. Since the lower limit of detection of troponin-T was 5 ng/l, the results were set at 2.5 ng/l in calculations of non-detectable levels. Correspondingly, serum hs-CRP that was undetectable was set as 0.15 mg/l (limit of detection =0.3 mg/l) in calculations.

The sample size was based on the trial by Ristow et al. in which 20 untrained subjects performed regular exercise for 4 weeks and were randomized to consume antioxidants or not (17). The sample size in our study was increased 60% to allow for drop-outs during the longer total study period of three months. Analyses were performed per protocol.

Results

We recruited 32 subjects with a mean age of 27.6 ± 6.5 years (15 men and 17 women, age range 21–48 years) for the trial. Six subjects could not complete the whole trial (two because of upper airway infections and four because they found the trial to be too time-consuming). Three subjects were unable to run all the laps during the complete exercise period due to musculoskeletal problems and performed strenuous bicycling on training bikes instead. Thirteen subjects did the last running-race indoors ($n = 5$) or on a treadmill ($n = 8$), due to cold weather and snow which made the race-track difficult to run at a high speed in a secure way.

The participants who started with the exercise period shortened the times to finish the race from 1772 ± 371 s to 1621 ± 288 s ($P = 0.002$), and those who began with minimal physical activity required 1681 ± 341 s to finish the race after the four weeks of little physical activity compared with 1611 ± 348 s at the beginning of the study ($P = 0.032$). The race-times were not statistically different at baseline between the groups ($P = 0.21$). Since the particular track that was used for the maximal running-races became difficult to run on at high speed due

to large amounts of snow and slippery conditions, the corresponding race-times achieved at the end of the study period (late autumn 2010) were not used in these particular analyses of race-times.

Results in the mixed population

As seen in Table I, exercise compared with a minimal physical activity induced lower insulin and triglyceride levels, and higher HDL cholesterol levels, when data from all participants, with or without blueberry supplementation, were analyzed as one group. Levels of creatine kinase (CK) almost doubled on average after the running-races (levels before race 2.45 ± 3.2 μkat/l; levels after race 4.44 ± 4.7 μkat/l, $P < 0.0001$, $n = 112$). The levels of CK were elevated similarly after the running-race when levels at the end of the exercise period were compared with those after minimal physical activity, while body mass but not fat mass was lower at the end of the period with low physical activity (Table I).

Serum troponin-T was found to be present at a detectable level after 51 out of the total 112 running-races (45.5%) in the study, 7 of which (6.2%) were above the reference limit of 14 ng/l. Among participants who ran at least two running-races 75% showed detectable troponin-T levels in plasma after at least one race. There was no detectable change in post-race troponin-T when the individual levels after the races at the end of the exercise period and after that of minimal physical activity were compared in the mixed population. When analyzed according to gender, however, a reduction in post-race troponin-T after exercise was found in women (Table I).

The levels of hs-CRP increased after the running-races in general (data from all races accumulated, hs-CRP before race 0.49 ± 0.7 mg/l; after 0.58 ± 0.7 mg/l, $P = 0.017$, after exclusion of two subjects with hs-CRP > 5 mg/l, considered to be outliers, $n = 108$). Levels of hs-CRP tended to be lower after the race when results after the exercise period were compared with those at the end of the minimal physical activity period (Table I).

Acute effects of the running-races on metabolic markers

The design of the study allowed us to analyze effects of a single running-race on levels of cardio-metabolic risk markers. When fasting levels on the morning of the running-race were compared with the corresponding levels the morning after the running-race, glucose levels were found to be lowered by the races (data from all running-races accumulated, levels before 5.28 ± 0.44 mmol/l; after 5.11 ± 0.39 mmol/l, $P < 0.0001$), while corresponding insulin levels did not change statistically significantly (levels before 56 ± 23 pmol/l; after 53 ± 25 pmol/l, $P = 0.13$). Analyzed in the same manner, triglyceride levels also decreased the morning after a running-race (levels before 0.99 ± 0.52 mmol/l; after 0.88 ± 0.45 mmol/l, $P = 0.008$), while levels of HDL-cholesterol or LDL-cholesterol did not change significantly (not shown).

Effects of blueberries during the exercise period

Table II shows changes in fasting levels of markers of insulin sensitivity and lipids (mean values of the levels before and after the race) in the groups randomized to consume blueberries or to keep eating habits unchanged. Interestingly, fasting glucose levels tended to increase despite exercise in the blueberry group, and the difference in changes between the two groups was statistically significant ($P = 0.044$). Correspondingly, triglyceride levels decreased only in the control group. These changes were in contrast to effects on HDL- and LDL-cholesterol which improved in the blueberry-group, although these changes only bordered on statistical significance between groups. Effects of blueberries on the corresponding levels of apolipoprotein concentrations showed similar tendencies as on cholesterol levels (Table II). Ingestion of blueberries had no discernible effect on levels of hs-CRP (Table II). However, there was a diminution of troponin-T levels after the races at the end of the exercise period compared with baseline levels of the same period within the group randomized to blueberries (Table II).

Discussion

As expected, based on the study by Ristow et al. (17), the participants in our study improved cardio-metabolic risk markers by exercise, HDL-cholesterol increased, and triglyceride levels fell after exercise, when compared with a period when participants were asked to perform minimal physical activity. Fasting insulin levels also differed when comparing changes between these two main periods, suggesting that there indeed was a favorable effect of the exercise and/or an unfavorable effect of a sedentary life-style. A bit more concerning was that in participants who ran at least two races 75% displayed detectable troponin-T levels after at least one race. However, in women there was a diminution of the troponin-T levels when participants were relatively fit, at the end of the exercise period, as compared with less pronounced fitness at the end of the period with minimal exercise. It has earlier been reported that also a short bout of spinning exercise can induce detectable troponin-T in serum in healthy individuals (23), which

Table I. Anthropometrics, laboratory variables, and body fat content during the trial in the mixed population, i.e. without separate analysis depending on whether blueberries were consumed or not, $n = 26$. All laboratory variables, except S-CK and hs-CRP, were the mean of the analyses before and after the 5 km race that was performed at the beginning and at the end of both trial periods, i.e. the period of exercise or the period of minimal physical activity. There were no differences between the groups at baseline, i.e. baseline of exercise compared with baseline of exercise periods. *P* values correspond to Student's paired 2-tailed *t* test. The delta value is the level of a measurement at the end of the period minus the corresponding level at baseline for the same period.

Variable	Baseline before exercise period	After 4 weeks of exercise	Baseline before minimal physical activity period	After 4 weeks of minimal physical activity	*P* for difference between the end of the two periods	*P* for difference of delta values between the two periods
Weight (kg)						
All	–	70.3 ± 9.7	–	69.8 ± 9.7	0.010	–
Women	–	64.9 ± 11	–	64.6 ± 10	0.26	–
Men	–	73.7 ± 7.6	–	73.1 ± 7.9	0.022	–
Body fat (kg)						
All	–	14.4 ± 8.3	–	14.4 ± 8.4	0.8	–
Women	–	19.1 ± 7.7	–	19.3 ± 7.8	0.43	–
Men	–	11.5 ± 7.5	–	11.3 ± 7.4	0.26	–
Blood pressure (mmHg)						
All	–	116 ± 10/73 ± 7.2	–	116 ± 11/72 ± 6.5	0.7/0.9	–
Women	–	112 ± 11/72 ± 8.7	–	107 ± 11/68 ± 5.1	0.067/0.13	–
Men	–	119 ± 8.5/73 ± 6.6	–	121 ± 6.6/75 ± 5.8	0.25/0.30	–
S-LDL-chol (mmol/l)						
All	2.68 ± 0.73	2.62 ± 0.75	2.76 ± 0.69	2.77 ± 0.80	0.091	0.18
Women	2.46 ± 0.39	2.32 ± 0.33	2.77 ± 0.46	2.66 ± 0.40	0.042	0.063
Men	2.85 ± 0.89	2.80 ± 0.88	2.75 ± 0.85	2.85 ± 0.99	0.62	0.65
S-HDL-chol (mmol/l)						
All	1.50 ± 0.34	1.55 ± 0.38	1.50 ± 0.36	1.50 ± 0.35	0.003	0.039
Women	1.70 ± 030	1.76 ± 0.33	1.70 ± 0.34	1.73 ± 0.32	0.13	0.34
Men	1.34 ± 0.28	1.43 ± 0.36	1.35 ± 0.30	1.34 ± 0.29	0.017	0.071
S-triglycerides (mmol/l)						
All	0.94 ± 0.41	0.88 ± 0.31	0.91 ± 0.56	1.0 ± 0.45	0.046	0.036
Women	0.71 ± 0.29	0.70 ± 0.29	0.72 ± 0.23	0.85 ± 0.35	0.056	0.27
Men	1.1 ± 0.40	0.99 ± 0.27	1.1 ± 0.69	1.1 ± 0.48	0.20	0.087
S-ApoB (g/l)						
All	0.875 ± 0.19	0.854 ± 0.19	0.891 ± 0.20	0.891 ± 0.21	0.082	0.21
Women	0.814 ± 0.08	0.784 ± 0.12	0.872 ± 0.12	0.855 ± 0.13	0.11	0.73
Men	0.925 ± 0.24	0.898 ± 0.22	0.905 ± 0.24	0.915 ± 0.25	0.39	0.23
S-ApoA1 (g/l)						
All	1.38 ± 0.22	1.41 ± 0.23	1.35 ± 0.23	1.38 ± 0.23	0.058	0.71
Women	1.45 ± 0.25	1.50 ± 0.26	1.45 ± 0.25	1.49 ± 0.27	0.59	0.86
Men	1.32 ± 0.19	1.35 ± 0.20	1.28 ± 0.18	1.30 ± 0.16	0.063	0.76
FS-glucose (mmol/l)						
All	5.15 ± 0.40	5.25 ± 0.27	5.17 ± 0.37	5.24 ± 0.36	0.9	0.85
Women	4.91 ± 0.38	5.14 ± 0.22	5.02 ± 0.27	5.18 ± 0.36	0.95	0.38
Men	5.35 ± 0.30	5.31 ± 0.28	5.28 ± 0.41	5.29 ± 0.37	0.92	0.77

Table I. (*Continued*).

Variable	Baseline before exercise period	After 4 weeks of exercise	Baseline before minimal physical activity period	After 4 weeks of minimal physical activity	*P* for difference between the end of the two periods	*P* for difference of delta values between the two periods
FS-insulin (pmol/l)						
All	57 ± 25	49 ± 17	54 ± 17	58 ± 17	0.015	0.009
Women	54 ± 25	49 ± 20	58 ± 19	62 ± 17	0.16	0.14
Men	59 ± 26	49 ± 16	51 ± 16	55 ± 17	0.044	0.038
S-CK before race (μkat/l)						
All	2.50 ± 1.6	1.85 ± 0.71	3.00 ± 5.1	1.6 ± 0.62	0.024	0.48
Women	1.6 ± 0.98	1.8 ± 0.96	4.2 ± 7.7	1.9 ± 1.6	0.16	0.29
Men	4.2 ± 4.3	2.0 ± 0.55	2.4 ± 1.3	1.8 ± 0.49	0.094	0.26
S-CK after race (μkat/l)						
All	5.3 ± 5.0	3.1 ± 1.9	3.6 ± 4.2	3.6 ± 4.2	0.6	0.28
Women	3.0 ± 2.3	2.4 ± 1.4	4.4 ± 6.7	1.9 ± 1.6	0.014	0.66
Men	7.8 ± 6.5	3.5 ± 2.0	5.8 ± 5.0	4.7 ± 4.9	0.36	0.31
Hs-CRP before race (mg/l)						
All	0.49 ± 0.59	0.42 ± 0.51	0.46 ± 0.52	0.58 ± 0.94	0.28	0.48
Women	0.63 ± 0.56	0.56 ± 0.70	0.68 ± 0.72	0.74 ± 1.1	0.41	0.91
Men	0.38 ± 0.61	0.35 ± 0.39	0.32 ± 0.31	0.52 ± 0.88	0.46	0.45
Hs-CRP after race (mg/l)						
All	0.63 ± 0.58	0.45 ± 0.54	0.57 ± 0.62	0.72 ± 0.86	0.054	0.19
Women	0.73 ± 0.59	0.59 ± 0.79	0.82 ± 0.82	1.2 ± 1.2	0.058	0.48
Men	0.56 ± 0.59	0.37 ± 0.35	0.41 ± 0.42	0.48 ± 0.47	0.47	0.27
Troponin-T after race (ng/l)						
All	7.1 ± 5.7	5.3 ± 3.3	6.4 ± 6.7	5.2 ± 4.8	0.96	0.92
Women	6.8 ± 5.4	5.0 ± 2.5	3.6 ± 2.1	3.4 ± 1.8	0.038	0.68
Men	7.2 ± 6.1	5.4 ± 3.7	8.3 ± 8.1	6.3 ± 5.6	0.53	0.86

Chol = cholesterol; CK = creatine kinase; FS = fasting serum; hs-CRP = high-sensitivity C-reactive protein; S = serum.

suggests that it might be quite normal to detect troponin-T in serum after a relatively short period of exercise in people who are generally healthy. But we know of no earlier study that specifically was designed to study effects of increased physical fitness. More bothersome, detectable troponin-T levels, when measured in subjects without specific symptoms of ischemia, have been shown to be related to increased incidence of mortality (10), thus suggesting that a detectable troponin-T is useful marker of cardiovascular risk.

It has been suggested that it is the strain per se, and the ensuing increase in oxidation, that induces the increase in insulin sensitivity following exercise, as recently tested by Ristow et al. (17), although more recent studies in rats have not confirmed this (24). Our trial design was based on the study by Ristow et al., but we aimed to investigate the effects of blueberries rather than supplementation of vitamin C and E in combination. Blueberries contain antioxidants such as resveratrol and vitamins (18-20) and are naturally growing berries in Europe in late summer and autumn. Interestingly we extended the findings in the study by Ristow et al. and found that also blueberries seem to affect unfavorably the insulin-sensitizing effects of exercise as shown by the difference in delta-values of fasting glucose in the two groups. However, we are not able to pinpoint what particular component in the berries that affected fasting glucose levels, and we are also not able to discern whether these effects were indirect, i.e. that other changes in food intake or behavior were induced by the intake of blueberries per se, as no data on dietary changes were available. The reason for the lack of such data from dietary records was that we did not anticipate that it would give useful reproducible information. Although food records might seem to give exact and detailed information, we have earlier

reported that caloric intake based on food records does not match basal metabolic rate even when physical activity was similar in lean and obese subjects (25), and Lof et al. have confirmed bias of caloric intake from food records with regard to BMI (26). Finally, the need to weigh and make notes of all food consumed for several days could also by itself affect dietary habits as suggested by under-reporting of vegetable and fruit intake by food records (27). Data from 24-h recalls of food intake would also not have been sufficient in this study, in which blueberries were not consumed on every day.

Insulin levels, known to have large intra-individual variation, showed no statistically significant changes in relation to blueberry intake, but there was a trend towards lowered levels by blueberries. Triglycerides, which are related to many components of the metabolic syndrome, decreased only in the control group. In contrast, levels of LDL-cholesterol decreased and HDL-cholesterol increased in participants who had been randomized to blueberries. Indeed, as pointed out earlier, an increase in HDL cholesterol by intake of berries has previously been shown by Erlund et al. (16). Interestingly, we found that ingestion of blueberries was linked with lower troponin-T levels after exercise when levels after the races at baseline of the exercise period were compared with those at the end of the same study period. This would be in accordance with cardio-protective effects of blueberries. However, due to the small number of subjects of each gender, these data should be interpreted with some caution, also since the difference in changes between the groups (blueberries versus controls) regarding troponin-T was not statistically significant. It is also important to point out that, in a recent study of 12 weeks of exercise and the same dose of vitamin E but lower vitamin C dose (500 mg instead of 1000 mg/day), the inhibition of improved insulin sensitivity reported earlier by Ristow et al. (17) was not confirmed (28). This suggests that the specific doses of vitamins and/or the duration of the exercise are of importance for affecting the insulin-sensitizing consequences of exercise in humans.

The particular trial design, incorporating a total of four running-races in which fasting blood samples were drawn before and after each such race, allowed for a study of the effects of exercise in the evening on fasting levels of insulin and glucose the following morning with high statistical power. When all 112 running-races from the participants were pooled together (irrespective of the period of the study they were taken) it became apparent that fasting glucose is reduced 12 hours after a running-race in healthy subjects, while the insulin levels were unaffected. This finding is in line with known effects of muscular activity to increase levels of adenosine monophosphate (AMP) which is formed from adenosine diphosphate that follows from hydrolysis of adenosine triphosphate. AMP can induce insulin-independent glucose uptake in fat and muscle cells through activation of AMP-kinase (29), and that this indeed was the explanation of our findings was in line with the fact that insulin levels tended to be lower the day after the running-races. Hence, when screening for diabetes, the sensitivity to detect the disease is probably diminished if sampling is performed in the fasting state the day after strenuous physical exercise. It is of importance for the interpretation of the data reported in this paper to keep these semi-acute effects (after 12 hours) of exercise in mind, since most analyses were based on average values before and after the race at maximal speed.

The increase in inflammation following the maximal races in our study tended to be reduced by the end of the exercise period. This inflammatory activity, that was measured as hs-CRP, could emanate from several bodily tissues and is thus unspecific. We would assume that at least some part was derived from large muscle groups such as leg muscles, and thus it seems reasonable that the levels tended to be reduced when physical fitness was achieved at the end of the exercise period when the running technique probably improved in participants, leading to a shortening of the time required to finish the race. These findings are in line with an earlier observation in subjects training in preparation for a marathon run (7).

A limitation of our study was that we did not have control of the period of minimal physical activity. But, despite the need to rely on compliance with this presumed period of sedentary life-style, we did indeed find changes in race-times at the beginning of the study, and also changes in markers of the metabolic syndrome, such as HDL-cholesterol and insulin levels, when comparing exercise with the presumed sedentary life-style, which was indicative of compliance with the protocol. We also acknowledge the limitation that not all participants were able to follow the running protocol, either due to side-effects of the exercise or due to the cold weather at the end of the trial period. However, these are common culprits in recreational running in a country such as Sweden, and the sample size of the study had been adjusted to allow for several such non-completers. The choice for each participant to consume the blueberries in any way preferred is a limitation from a strict scientific viewpoint. The reason for this design, however, was to make the findings potentially applicable to ordinary life and to differing habits. It is important to note that the design of our study did permit analysis of long-

Table II. Change in fasting levels of laboratory variables in the groups when comparing the 4 week exercise period with ($n = 13$), or without ($n = 13$), intake of blueberries. Delta values denote difference from baseline to the end of the period.

Variable	Supplement	Baseline before exercise period	After 4 weeks of exercise	*P* for difference within groups	*P* for group difference of delta values
S-LDL-chol (mmol/l)	Blueberries	2.86 ± 0.73	2.67 ± 0.80	0.037	0.13
	Control	2.55 ± 0.70	2.56 ± 0.73	0.91	
S-HDL-chol (mmol/l)	Blueberries	1.51 ± 0.29	1.64 ± 0.33	0.006	0.088
	Control	1.43 ± 0.39	1.47 ± 0.42	0.20	
S-triglycerides (mmol/l)	Blueberries	0.81 ± 0.28	0.83 ± 0.30	0.87	0.072
	Control	1.1 ± 0.49	0.93 ± 0.31	0.017	
S-ApoB (g/l)	Blueberries	0.90 ± 0.21	0.86 ± 0.20	0.021	0.20
	Control	0.86 ± 0.21	0.85 ± 0.20	0.77	
S-ApoA1 (g/l)	Blueberries	1.38 ± 0.19	1.44 ± 0.17	0.061	0.29
	Control	1.36 ± 0.26	1.37 ± 0.28	0.44	
FS-glucose (mmol/l)	Blueberries	5.12 ± 0.49	5.32 ± 0.29	0.082	0.044
	Control	5.24 ± 0.27	5.17 ± 0.23	0.35	
FS-insulin (pmol/l)	Blueberries	51 ± 24	43 ± 15	0.13	0.47
	Control	57 ± 19	54 ± 18	0.42	
hs-CRP before race (mg/l)	Blueberries	0.64 ± 0.77	0.39 ± 0.45	0.33	0.20
	Control	0.33 ± 0.29	0.44 ± 0.59	0.33	
hs-CRP after race (mg/l)	Blueberries	0.80 ± 0.65	0.46 ± 0.52	0.21	0.31
	Control	0.48 ± 0.50	0.45 ± 0.60	0.81	
Troponin-T after race (ng/l)	Blueberries	8.6 ± 4.7	4.7 ± 2.8	0.029	0.058
	Control	5.6 ± 6.4	5.6 ± 3.7	0.97	

Chol = cholesterol; FS = fasting serum; hs-CRP = high-sensitivity C-reactive protein; S = serum.

term effects of exercise in combination with blueberries. Indeed, in contrast to lack of positive effects in most studies of high doses of vitamins with presumed antioxidant effects (15), several observations support beneficial health effects of blueberries in humans. These potential effects include reduction of neuronal and cardiovascular diseases (30,31). Blueberry extract has also been shown to possess direct antioxidant effects that can be detected in human serum (32). We acknowledge that the small number of participants that completed the study of the potential effects of blueberries on cardiometabolic risk factors, 13 in each group, is a limitation of the trial.

In conclusion, we found beneficial effects of blueberries on HDL- and LDL-cholesterol levels, and also that the troponin-T release after exercise was blunted in subjects who had been randomized to blueberry ingestion. These findings point to the complexity when analyzing effects in humans of a naturally occurring supplement containing several antioxidants and vitamins and were in contrast to unfavorable effects of blueberry ingestion on fasting levels of glucose, as was part of the main hypothesis tested in the study. It is important to note that we cannot discern whether the effects of ingestion of blueberries were direct, or indirect, due to other concomitant dietary changes, and thus we call for further studies on the subject.

Acknowledgements

The study was supported by research funds from the University Hospital of Linköping, Linköping University, and the County Council of Östergötland.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Orchard TJ, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, et al. The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. Ann Intern Med. 2005; 142:611–19.

2. Saravia SG, Knebel F, Schroeckh S, Ziebig R, Lun A, Weimann A, et al. Cardiac troponin T release and inflammation demonstrated in marathon runners. Clin Lab. 2010; 56:51–8.
3. Goussetis E, Spiropoulos A, Tsironi M, Skenderi K, Margeli A, Graphakos S, et al. Spartathlon, a 246 kilometer foot race: effects of acute inflammation induced by prolonged exercise on circulating progenitor reparative cells. Blood Cells Mol Dis. 2009;42:294–9.
4. Neubauer O, Konig D, Wagner KH. Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress. Eur J Appl Physiol. 2008;104:417–26.
5. Nakajima K, Kusuhara M, Yonemura A, Ayaori M, Saionji K, Tamai S, et al. Increasing physical fitness does not proportionally decrease circulating C-reactive protein level in men with varying fitness. Metabolism. 2008;57:650–7.
6. Margeli A, Skenderi K, Tsironi M, Hantzi E, Matalas AL, Vrettou C, et al. Dramatic elevations of interleukin-6- and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. J Clin Endocrinol Metab. 2005;90:3914–18.
7. Mattusch F, Dufaux B, Heine O, Mertens I, Rost R. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. Int J Sports Med. 2000;21:21–4.
8. Mingels AM, Jacobs LH, Kleijnen VW, Laufer EM, Winkens B, Hofstra L, et al. Cardiac troponin T elevations, using highly sensitive assay, in recreational running depend on running distance. Clin Res Cardiol. 2010;99:385–91.
9. Fu F, Nie J, Tong TK. Serum cardiac troponin T in adolescent runners: effects of exercise intensity and duration. Int J Sports Med. 2009;30:168–72.
10. de Lemos JA, Drazner MH, Omland T, Ayers CR, Khera A, Rohatgi A, et al. Association of troponin T detected with a highly sensitive assay and cardiac structure and mortality risk in the general population. JAMA. 2010;304:2503–12.
11. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr. 1996;16:33–50.
12. Nichter M, Thompson JJ. For my wellness, not just my illness: North Americans' use of dietary supplements. Cult Med Psychiatry. 2006;30:175–222.
13. Velicer CM, Ulrich CM. Vitamin and mineral supplement use among US adults after cancer diagnosis: a systematic review. J Clin Oncol. 2008;26:665–73.
14. Spencer EH, Bendich A, Frank E. Vitamin and mineral supplement use among US medical students: a longitudinal study. J Am Diet Assoc. 2006;106:1975–83.
15. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA. 2007;297:842–57.
16. Erlund I, Koli R, Alfthan G, Marniemi J, Puukka P, Mustonen P, et al. Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. Am J Clin Nutr. 2008;87:323–31.
17. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A. 2009;106:8665–70.
18. Lyons MM, Yu C, Toma RB, Cho SY, Reiboldt W, Lee J, et al. Resveratrol in raw and baked blueberries and bilberries. J Agric Food Chem. 2003;51:5867–70.
19. Rodriguez-Mateos A, Cifuentes-Gomez T, Tabatabaee S, Lecras C, Spencer JP. Procyanidin, anthocyanin, and chlorogenic acid contents of highbush and lowbush blueberries. J Agric Food Chem. 2012; Epub ahead of print.
20. Wang SY, Chen CT, Sciarappa W, Wang CY, Camp MJ. Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. J Agric Food Chem. 2008;56:5788–94.
21. Erlingsson S, Herard S, Dahlqvist Leinhard O, Lindstrom T, Lanne T, Borga M, et al. Men develop more intraabdominal obesity and signs of the metabolic syndrome after hyperalimentation than women. Metabolism. 2009;58:995–1001.
22. Kechagias S, Ernersson A, Dahlqvist O, Lundberg P, Lindstrom T, Nystrom FH. Fast-food-based hyper-alimentation can induce rapid and profound elevation of serum alanine aminotransferase in healthy subjects. Gut. 2008;57:649–54.
23. Duttaroy S, Thorell D, Karlsson L, Borjesson M. A single-bout of one-hour spinning exercise increases troponin T in healthy subjects. Scand Cardiovasc J. 2012;46:2–6.
24. Higashida K, Kim SH, Higuchi M, Holloszy JO, Han DH. Normal adaptations to exercise despite protection against oxidative stress. Am J Physiol Endocrinol Metab. 2011;301:E779–84.
25. Wallhuss A, Isik M, Nystrom FH. Comparison of the subjective sense of high or low metabolism and objectively measured resting metabolic rate. Scand J Clin Lab Invest. 2010; 70:334–7.
26. Lof M, Forsum E. Validation of energy intake by dietary recall against different methods to assess energy expenditure. J Hum Nutr Diet. 2004;17:471–80.
27. Kristal AR, Vizenor NC, Patterson RE, Neuhouser ML, Shattuck AL, McLerran D. Precision and bias of food frequency-based measures of fruit and vegetable intakes. Cancer Epidemiol Biomarkers Prev. 2000;9:939–44.
28. Yfanti C, Nielsen AR, Akerstrom T, Nielsen S, Rose AJ, Richter EA, et al. Effect of antioxidant supplementation on insulin sensitivity in response to endurance exercise training. Am J Physiol Endocrinol Metab. 2011;300:E761–70.
29. Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes. 1999;48:1667–71.
30. Basu A, Rhone M, Lyons TJ. Berries: emerging impact on cardiovascular health. Nutr Rev. 2010;68:168–77.
31. Shukitt-Hale B. Blueberries and neuronal aging. Gerontology. 2012;58:518–23.
32. Kay CD, Holub BJ. The effect of wild blueberry (Vaccinium angustifolium) consumption on postprandial serum antioxidant status in human subjects. Br J Nutr. 2002;88:389–98.