This is an accepted manuscript of an article published by Springer-Verlag in European Journal ofApplied Physiology, published online 02. March 2017. Available from;
https://link.springer.com/article/10.1007%2Fs00421-017-3576-2

Acute effects of post-absorptive and postprandial moderate exercise on markers of inflammation in hyperglycemic individuals

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Acknowledgments

We acknowledge Tine SA and Fjordland AS for supplying us with food for dietary standardization. Thanks to Kathrine Kroken and Olav Andreas Tuterud Nordølum for their contribution to the data sampling.

Abstract

Purpose

Systemic inflammation is involved in the development of several diseases, including cardiovascular disease and type 2 diabetes. It is known that vigorous exercise affects systemic inflammation, but less is known about exercise at lower intensities. Hyperglycemia can also entail pro-inflammatory responses, however postprandial hyperglycemia is blunted if the meal is followed by exercise. Hypotheses were: 1) Moderate physical exercise acutely affects levels of C-reactive protein (CRP) and serum soluble vascular cell adhesion molecule 1 (sVCAM-1) in hyperglycemic individuals, and 2) The effect depends on whether the activity is performed in a post-absorptive or postprandial state.

Methods

Twelve participants diagnosed with hyperglycemia, but not using anti-diabetic medication, underwent 3 test days in a randomized cross-over study; one control day without exercise, one

day with 60 min of treadmill walking ending 30 min before breakfast and one day with an identical bout of activity 30 min after the start of breakfast. Food intake was strictly standardized and venous blood for CRP and sVCAM-1 analysis was sampled at standardized time points during the first 3.5 hours after breakfast and once 24 hours later.

Results

Merged data from the two exercise days showed that sVCAM-1 increased from baseline (4 ± 16 ng/mL) compared to the control condition (-28 ± 47 ng/mL, ES=0.7, p=0.024). There was no statistically significant difference in changes in sVCAM-1 levels between the two exercise test days. Exercise did not affect CRP values.

Conclusion

Moderate exercise increases sVCAM-1 in hyperglycemic individuals, whereas it does not affect CRP.

Keywords

Physical activity, blood, CRP, sVCAM-1, atherosclerosis, life style

Abbreviations

BrEx	Test day with exercise after breakfast
CON	Control day
CRP	C-reactive protein
ES	Effect size
ExBr	Test day with exercise before breakfast
HbA1c	Glycosylated hemoglobin
HDL	High density lipoprotein

INT	Intervention group
LDL	Low density lipoprotein
RPE	Rate of perceived exertion
sVCAM-1	Soluble vascular cell adhesion molecule 1

Introduction

Systemic inflammation is involved in the development of a wide range of diseases, including cardiovascular disease, and in the progression of mild hyperglycemia into type 2 diabetes (Goldberg 2009). Exercise affects inflammation, and one bout of vigorous exercise initiates a cascade of both pro-inflammatory and anti-inflammatory events. These acute responses to exercise may be vital for the long-term adaptations to training, including the anti-inflammatory response associated with regular exercise (Allen et al. 2015). In contrast to vigorous exercise, less is known about the effect of moderate exercise on systemic inflammation. In addition, the inflammatory response to exercise in hyperglycemic persons may be related to “time since food intake” since hyperglycemia itself results in systemic inflammation (Nappo et al. 2002), and exercise after carbohydrate ingestion reduces postprandial hyperglycemia, while exercise prior to a meal does not (Colberg et al. 2009; Derave et al. 2007). It would therefore be interesting to investigate the acute effects of moderate physical exercise before and after a carbohydrate meal on inflammatory markers.

Numerous markers involved in the inflammatory process related to development of cardiovascular disease have been extensively studied, including C-reactive protein (CRP) and soluble vascular cell adhesion molecule 1 (sVCAM-1) (Goldberg 2009). The former is an acute-phase protein synthesized in response to homeostatic disturbances (Semple 2006),

which predicts the degree of cardiovascular risk, even in apparently healthy individuals (Emerging Risk Factors et al. 2010; Libby and Crea 2010; Semple 2006). The adhesion molecule VCAM-1 is crucial for leucocyte migration into tissues, facilitating adhesion to endothelial cell membranes (Cook-Mills et al. 2011; Price and Loscalzo 1999). A portion of the membrane expressed VCAM-1 is cleaved from the endothelial cells after cytokine activation, and can be measured in plasma as sVCAM-1 concentration (Pigott et al. 1992), which predicts cardiovascular disease in hyperglycemic persons (Goldberg 2009). In the present study, we tested the hypothesis that moderate exercise acutely affects sVCAM-1 and CRP levels in hyperglycemic individuals, and that these effects differ between exercise performed in the post-absorptive and the postprandial state.

Methods

Participants

The study population consisted of individuals diagnosed with hyperglycemia, i.e. previously measured fasting venous plasma glucose ≥6.1 mmol/L and/or 2 hr glucose tolerance ≥7.8 mmol/L, who were not using glucose-lowering medications and did not have autoimmune disease, cancer or other diseases directly and significantly affecting inflammatory status or metabolism, except for diabetes. Sample size calculations for a fixed effect model showed that n=8 would be enough to obtain a p value <0.05, with power=0.80, and an expected change in sVCAM-1 of 20 ± 20 ng/mL. Regarding the uncertainty in this calculation we included 13 participants, of which one was excluded from the data set because further examination of her patient journal after enrollment showed that she did not meet the inclusion criteria. Four women and eight men, all of European descent, completed the study and are included in the results. Their characteristics are summarized in Table 1. Dosage and timing of intake of all

drugs were kept stable during the study period, i.e. Lipid-lowering therapy (n=4), antithrombotic agents (n=4), angiotensin II receptor antagonists (n=2) and ACE inhibitors (n=1). Median time from diagnosis of hyperglycemia to participation in the study was 8.5 months (IQR: 31 months). Seven of the participants had at least one parent or one sibling with diabetes. All women were postmenopausal. The participants reported that they had performed (mean ± SD): 219 ± 237 min of endurance or strength training, 177 ± 143 min of walking and 122 ± 49 min of lighter activity (like gardening and housework) per week for the last three months prior to study enrollment. Eight participants reported via questionnaire about their dietary habits, to be very conscious, and four somewhat conscious about their food intake.

Ethics statement

The Regional Ethics Committee (REK Sør-Øst, Norway) approved the study, and all participants gave their written informed consent.

General design

The study was performed using a randomized crossover design. Each subject carried out three test days (Figure 1) in a balanced order, with at least six days and no more than 21 days between each: one test day with physical exercise performed before breakfast (ExBr), one with identical exercise performed after breakfast (BrEx), and one day without exercise (CON). All experimental days were identical, except for the different exercise regimes or the lack thereof. Participants were sedentary on test days except for the exercise sessions, and all meals were standardized. All test days started in the morning, approximately at the same time for each participant (within 1 hr), and blood samples were taken at standardized time points in the postprandial period after breakfast (until 3.5 hrs after start of breakfast) and after 24 hrs.

Exercise and nutrition

The participants were instructed not to perform any physical exercise during the last three days leading up to test days, and any eventually light activity during the first of these three-day periods were recorded and repeated before the second and third test day. The exercise sessions in both ExBr and BrEx consisted of 60 min of treadmill walking at an individually standardized speed at 8% inclination. The individual speed was decided during a familiarization session >6 days before the first test, and defined as the speed corresponding to 12 at the Borg 6-20 RPE scale (Borg 1982) after 30 min of walking at 8% inclination. At the end of exercise at test days the rating of perceived exertion was 12.0 ± 0.2 and 12.4 ± 0.2 ($p=0.021$) on Borg scale, and blood lactate levels were 1.4 ± 0.2 and 1.7 ± 0.2 mmol/L ($p=0.038$), for ExBr and BrEx respectively. All other physical activity was limited to what was absolute necessary, like walking to the car and moving between living room, toilet, kitchen and bedroom.

Use of antioxidants or anti-inflammatory agents was not allowed during the last month leading up to study participation. We instructed the participants to standardize their diet three days prior to each test by writing down food intake in the days leading up to the first test day and repeating this regimen before the second and third test day. An absolute dietary standardization was performed from the evening 10 hrs before breakfast on each test day until 22 hrs after the breakfast. Standardization was achieved by repeating the diet eaten on the first test day both on the second and third test day. The Participants were instructed to eat and register a self-chosen meal containing >30 g carbohydrate in the evening 10 hrs preceding breakfast. The breakfast contained 250 mL semi-skimmed milk and cornflakes corresponding to 1g carbohydrate per kg body weight (1371 ± 966 KJ, 12 ± 6 g protein, 10 ± 4 g fat and 43 ± 27 g carbohydrate). Lunch (3.5 hrs after breakfast) contained a yogurt and self-chosen

amounts of wholegrain crispbread, butter, cheese and water. The participants could choose between several boil-in-bag dinner packages (salmon with rice and vegetables, chicken casserole, meatballs potatoes and creamed peas or minced steak with stewed cabbage and potatoes; Fjordland AS, Norway) for dinner (7 hrs after breakfast). Leftovers were registered, and the corresponding food was removed on the second and the third test day. The evening meal (11 hrs after breakfast) consisted of a self-chosen amount of whole meal bread, butter, cheese and skimmed milk that also was carefully registered and repeated. Macronutrient intake for each meal is given in online resource 1. The research team provided the subjects with all food for breakfast, lunch, dinner and evening meal on test days.

The experiments were undertaken in our laboratory until the lunch meal was ingested, whereupon the participants were transported to their homes to stay for the remaining 22 hrs of the protocol. They had a checklist with details about the standardization, such as instructions about timing and amounts of food intake. Prior to test days, individual sessions were arranged with the participants, teaching them the importance of standardization and all procedures necessary for exact standardization. At the end of each test day, we had a dialog with each participant about how the standardization had been carried out with no deviations being reported.

Data sampling

Blood samples were drawn from an antecubital vein at baseline and thereafter 1.5, 2.5, 3.5 and 24 hrs after breakfast. In addition, blood was sampled at the end of the ExBr exercise bout (Figure 1). Blood was drawn into EDTA tubes and centrifuged immediately at 2600g for 12 min, before freezing. The plasma samples were thawed and freezed again prior to analysis. However, the results from the samples were found to be reliable, see online resource 2. Glucose and triglycerides were analyzed at Furst Medical Laboratories, Oslo, (Advia 2400

Chemistry System, Siemens Healthcare Diagnostics Inc). High-sensitivity CRP was determined by a solid-phase, chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, USA). In 35 out of 192 samples, CRP levels were below the minimum range of the assay, i.e. 0.2 mg/L. These samples were taken from 4 different subjects (6 + 5 + 16 + 8), and were set at the minimum range of the assay; 0.2 mg/L. We analyzed sVCAM-1 with commercially available ELISA kits (Human sVCAM-1/CD106 immunoassay Quantikine ELISA, R&D systems Inc, Minneapolis, USA). All analyses of CRP and sVCAM-1 from any particular subject were analyzed intra-assay. Intra-assay coefficients of variation were 7 % for CRP and 1 % for sVCAM-1.

Data on oxygen consumption, respiratory exchange ratio (Oxycon Pro, Erich Jaeger, Hoechberg, Germany) and heart rate were retrieved for 10 min at several standardized time points until 3 hrs after breakfast (Figure 1), and mean values of the last 2 min of each sampling were used in the analyses. Blood lactate concentration (Biosen C-line, EKF-diagnostic GmbH, Germany) was measured from capillary blood 55 min into the exercise bouts and Borg 6-20 RPE (Borg 1982) was determined 5, 30 and 55 min into the exercise bouts.

Data analysis

Expenditure of energy and carbohydrate and fat utilization were calculated from VO_2 and RER values using a table given in McArdle, Katch and Katch textbook of exercise physiology (p. 188) (McArdle et al. 2010), based on Zuntz et al. (1901). To examine the effect of exercise independent of timing between exercise and food intake, we used merged data from the two exercise interventions. An effect was defined as a between test-day difference in change from baseline to subsequent measures. We did the statistical analysis with IBM SPSS statistics, version 22.0, using a linear mixed model. We utilized absolute values, used participant number as the repeated “subjects” variable and included random intercept in the model. Test

day and time (baseline vs. subsequent sample) were used as fixed factors, and the residuals were checked for normality and homogeneity. The α-level was set at 0.05 and a p value <0.1 was considered as a tendency towards statistical significance. We calculated effect sizes (ES) for the effect of intervention and between interventions by using Cohen`s d_z (Lakens 2013), and interpreted the result according to Hopkins et al. (Hopkins et al. 2009): d>0.2=small effect, d>0.6=moderate effect, d>1.2=large effect. Data are presented as means ± standard deviation. Figures were made using SigmaPlot 12.0, Systat Software Inc.

Results

There were no differences in baseline values between the three test days for any of the measured variables (Table 2). Heart rate, energy expenditure, carbohydrate utilization and fat utilization increased during exercise (p<0.001 for all, Figure 2). These increases did not differ between ExBr and BrEx, except for carbohydrate utilization which tended to be higher for BrEx than ExBr, 0.83 ± 0.23 and 0.67 ± 0.32 g/minute, respectively (p=0.057).

The mean change from baseline in blood glucose and triglycerides concentrations did not differ between test days (p=0.870 and p=0.585 respectively). However, at 1.5 h, the blood glucose increase in BrEx (1.1 ± 1.0) was lower than in CON (3.0 ± 1.8 mmol/L, p=0.004) and ExBr (3.1 ± 1.7 mmol/L, p=0.001, Figure 3A). Triglyceride values increased from baseline to end of exercise within both ExBr (p=0.005) and BrEx (p=0.001, Figure 3B).

Merged data from ExBr and BrEx showed that the change in sVCAM-1 from baseline to all of the subsequent values was 32 ± 47 ng/ml higher after exercise compared to CON (p=0.024, Figure 3C). This was a result of a nonsignificant increase after exercise of 4 ± 16 ng/mL and a decrease in CON of 28 ± 47 ng/mL (p=0.014 within CON). The effect size analysis showed

a moderate effect of exercise on sVCAM-1 values (ES=0.7). The concentration of sVCAM-1 increased with 28 ± 23 ng/mL from baseline to the end of exercise (p=0.011, within exercise interventions). Compared to CON sVCAM-1 values were also increased as a result of exercise at 3.5 h (13 ± 22 vs. -46 ± 46 ng/mL from baseline in CON, p=0.007) and 24 h (9 ± 24 vs. -33 ± 52 ng/mL in CON, p=0.0016). No increase was present at 2.5 h compared to CON.

Mean change in sVCAM-1 from baseline to subsequent measures for all three test days separately are presented in Figure 3C. The difference between ExBr (-5 ± 19 ng/mL) and BrEx (16 ± 32 ng/mL) did not reach statistical significance (p=0.193 for the difference of 21 ± 40 ng/ml). There was however, a significant difference in change from baseline to the subsequent measures between CON and BrEx (p=0.020) and a tendency towards significant difference between CON and ExBr (p=0.099). The effect size analysis showed that the effect of the difference between the ExBr and BrEx was small (ES=0.5). Individual sVCAM-1 results and absolute values for glucose, triglycerides, sVCAM-1 and CRP are shown in online resource 1.

There were no effect of intervention on CRP values, neither when exercise days were merged together and compared to CON (p=0.921) nor when exercise interventions were compared to each other (p=0.666). Mean changes in CRP from baseline to subsequent measures in all three test days are presented in Figure 3D. The effect size analysis showed a trivial effect of exercise on CRP values (ES=0.1 vs CON), and the effect of the difference between the ExBr and BrEx was small (ES=0.3).

Discussion

The main finding in this study was that the levels of sVCAM-1 was increased after exercise compared to the control condition in hyperglycemic participants, but this was not the case for CRP, which remained unchanged. The latter is in agreement with a few previous studies exploring the effect of moderate exercise on CRP in healthy persons (Davis et al. 2008; Markovitch et al. 2008; Mendham et al. 2011). In contrast, CRP was found to increase in blood after 50 min cycling at 65% of VO_{2max} in subjects with coronary artery disease (Lara Fernandes et al. 2011). The response of severe exercise on CRP has been more extensively studied, with both vigorous eccentric and non-eccentric muscle exercise leading to increased levels (Semple 2006), peaking around 24 hours post exercise (Semple et al. 2004; Weight et al. 1991).

A decrease in the sVCAM-1 level during the control condition seems to be the main cause of the observed difference between the exercise and the control condition. It is plausible that the decrease in sVCAM-1 during the control condition was a result of inactivity. With regard to the self-reported activity level prior to study, the control condition represented a decrease in activity level, and the exercise test days may have reflected “real life” more closely than the control condition for those participants. The differences in sVCAM-1 levels can be explained by production of reactive oxygen species in the mitochondria when metabolism is altered, since increased oxidative stress augments inflammatory processes (Allen et al. 2015).

Our findings concerning sVCAM-1 are in accordance with previous studies on healthy and diseased persons. Different types of exercise have entailed increased sVCAM-1 levels, ranging from relatively short bouts of moderate intensity (Lara Fernandes et al. 2011) and high intensity (Brevetti et al. 2001) to a 246 km running competition (Bartzeliotou et al. 2007). However, some conflicting results exist (Gabriel et al. 2012; Smith et al. 2000).

Hyperglycemic excursions, which typically occur after a carbohydrate rich meal in individuals with reduced glucose tolerance or diabetes, potentially increases markers of systemic inflammation, sVCAM-1 included, via oxidative stress (Ceriello 2005; Nappo et al. 2002; Sampson et al. 2002; Standl et al. 2011). However, the test day with highest numeric sVCAM-1 values had the lowest postprandial glycemia after breakfast, i.e. the day with postprandial exercise. It is therefore likely that the sVCAM-1 increase in our study was mediated primarily by other factors than the hyperglycemia per se. Indeed, the results from the control day indicate that the carbohydrate rich breakfast did not increase s-VCAM1 levels. This could be because the carbohydrate content of the breakfast was too low or the participants in our study had only mild or moderate hyperglycemia, since the inflammatory response depends on the severity of hyperglycemia (Nappo et al. 2002; Quagliaro et al. 2005). Therefore, the current results cannot be generalized to persons with more severe dysregulation of glucose metabolism. It is also important to keep in mind that insulin levels and insulin sensitivity might influence responses to exercise. Insulin has been reported to have anti-oxidative (Monnier et al. 2010) and anti-inflammatory effects (Dandona et al. 2009), and there are large differences in insulin action among different hyperglycemic individuals.

The increase in sVCAM-1 after postprandial exercise was numerically larger than after post-absorptive exercise, but the difference did not reach statistical significance. We cannot rule out that this might be a type 2 error caused by a larger than expected variation in the sVCAM-1 response. Alternatively, the lower baseline value followed by numerically larger sVCAM response after postprandial exercise could suggest a regression towards means.

Exercise mediated cortisol secretion, which suppress several parts of the immune system, is exaggerated by glycogen depletion and attenuated by carbohydrate ingestion (Nieman 1999), which might explain why post-absorptive exercise eventually entails lower sVCAM-1 responses than postprandial exercise. However, the observed carbohydrate and fat utilization

does not support any effect via glycogen depletion and cortisol. If glycogen stores were depleted as a result of the exercise bout in the post-absorptive but not the postprandial state, a lower carbohydrate utilization should have occurred after the post-absorptive exercise (Devlin and Horton 1985). Therefore, one hour of moderate exercise may be too light and/or short to entail a substantial immunosuppressive effect of cortisol, even if it is performed in the post-absorptive state.

It is important to gain knowledge about how physical activity affects systemic inflammation and health, especially in individuals prone to cardiovascular disease, like the hyperglycemic persons in the present study. It is however hard to interpret the observed post-exercise increases in sVCAM-1 with regard to the clinical significance, since the present study only considered acute effects of exercise. Nevertheless, the acute inflammatory response to exercise may be vital for the adaptations to exercise training and also the anti-inflammatory response to exercise in the long-term (Allen et al. 2015). Since the lack of statistical significance between sVCAM-1 responses to post-absorptive and postprandial exercise might be a type 2 error, we cannot conclude that such difference do not exist. Future studies on exercise and inflammation should therefore still pay attention to dietary standardization and realize that dietary intake may affect the results. As the results of the present study entailed only trivial to moderate effect sizes, future related studies should also address more extreme dietary challenges, participants with severe hyperglycemia and/or higher doses of exercise. The finding that CRP is not affected acutely by prior moderate exercise might also be useful in the interpretation of "borderline" CRP results, if e.g. the patient was walking to the clinic.

Conclusion

Moderate exercise increases sVCAM-1 in hyperglycemic individuals, whereas it does not affect CRP. It appears that there are no substantial differences in the sVCAM-1 response depending on whether the exercise is performed in the post-absorptive or postprandial state.

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

References

Allen J, Sun Y, Woods JA (2015) Exercise and the Regulation of Inflammatory Responses. Prog Mol Biol Transl Sci 135:337-354. doi:10.1016/bs.pmbts.2015.07.003

Bartzeliotou AI, Margeli AP, Tsironi M, Skenderi K, Bacoula C, Chrousos GP, Papassotiriou I (2007) Circulating levels of adhesion molecules and markers of endothelial activation in acute inflammation induced by prolonged brisk exercise. Clin Biochem 40:765-770. doi:10.1016/j.clinbiochem.2007.01.013

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377-381.

Brevetti G et al. (2001) Exercise increases soluble adhesion molecules ICAM-1 and VCAM-1 in patients with intermittent claudication. Clin Hemorheol Microcirc 24:193-199.

Ceriello A (2005) Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 54:1-7.

Colberg SR, Zarrabi L, Bennington L, Nakave A, Thomas Somma C, Swain DP, Sechrist SR (2009) Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. J Am Med Assoc 10:394-397.

Cook-Mills JM, Marchese ME, Abdala-Valencia H (2011) Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. Antioxid Redox Sign 15:1607-1638. doi:10.1089/ars.2010.3522

Dandona P, Chaudhuri A, Ghanim H, Mohanty P (2009) Insulin as an anti-inflammatory and antiatherogenic modulator. J Am Coll Cardiol 53:S14-20. doi:10.1016/j.jacc.2008.10.038

Davis J, Murphy M, Trinick T, Duly E, Nevill A, Davison G (2008) Acute effects of walking on inflammatory and cardiovascular risk in sedentary post-menopausal women. J Sports Sci 26:303-309. doi:10.1080/02640410701552906

Derave W, Mertens A, Muls E, Pardaens K, Hespel P (2007) Effects of post-absorptive and postprandial exercise on glucoregulation in metabolic syndrome. Obesity (Silver Spring, Md) 15:704-711. doi:10.1038/oby.2007.548

Devlin JT, Horton ES (1985) Effects of prior high-intensity exercise on glucose metabolism in normal and insulin-resistant men. Diabetes 34:973-979.

Emerging Risk Factors C et al. (2010) C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 375:132-140. doi:10.1016/S0140-6736(09)61717-7

Gabriel B, Ratkevicius A, Gray P, Frenneaux MP, Gray SR (2012) High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress. Clin Sci (Lond) 123:313-321. doi:10.1042/CS20110600

Goldberg RB (2009) Cytokine and cytokine-like inflammation markers, endothelial dysfunction, and imbalanced coagulation in development of diabetes and its complications. J Clin Endocrinol Metab 94:3171-3182. doi:10.1210/jc.2008-2534

Hopkins WG, Marshall SW, Batterham AM, Hanin J (2009) Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc 41:3-13. doi:10.1249/MSS.0b013e31818cb278

Lakens D (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front Psychol 4:863. doi:10.3389/fpsyg.2013.00863

Lara Fernandes J et al. (2011) Acute and chronic effects of exercise on inflammatory markers and B-type natriuretic peptide in patients with coronary artery disease. Clin Res Cardiol 100:77-84. doi:10.1007/s00392-010-0215-x

Libby P, Crea F (2010) Clinical implications of inflammation for cardiovascular primary prevention. Eur Heart J 31:777-783. doi:10.1093/eurheartj/ehq022

Markovitch D, Tyrrell RM, Thompson D (2008) Acute moderate-intensity exercise in middle-aged men has neither an anti- nor proinflammatory effec.t J Appl Physiol 105:260-265. doi:10.1152/japplphysiol.00096.2008

McArdle WD, Katch FI, Katch VL (2010) Exercise physiology. 7 edn. Wolters Kluwer, Lippincott Williams & Wilkins,

Mendham AE, Donges CE, Liberts EA, Duffield R (2011) Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. Eur J Appl Physiol 111:1035-1045. doi:10.1007/s00421-010-1724-z

Monnier L, Colette C, Mas E, Michel F, Cristol JP, Boegner C, Owens DR (2010) Regulation of oxidative stress by glycaemic control: evidence for an independent inhibitory effect of insulin therapy. Diabetologia 53:562-571. doi:10.1007/s00125-009-1574-6

Nappo F et al. (2002) Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol 39:1145-1150.

Nieman DC (1999) Nutrition, exercise, and immune system function. Clin Sports Med 18:537-548.

Pigott R, Dillon LP, Hemingway IH, Gearing AJ (1992) Soluble forms of E-selectin, ICAM-1 and VCAM-1 are present in the supernatants of cytokine activated cultured endothelial cells. Biochem Biophys Res Commun 187:584-589.

Price DT, Loscalzo J (1999) Cellular adhesion molecules and atherogenesis. Am J Med 107:85-97.
Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, Zuodar G, Ceriello A (2005) Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: the distinct role of protein kinase C and mitochondrial superoxide production. Atherosclerosis 183:259-267. doi:10.1016/j.atherosclerosis.2005.03.015
Sampson MJ, Gopaul N, Davies IR, Hughes DA, Carrier MJ (2002) Plasma F2 isoprostanes: direct evidence of increased free radical damage during acute hyperglycemia in type 2 diabetes. Diabetes Care 25:537-541.
Semple SJ (2006) C-reactive protein - biological function, cardiovascular disease and physical exercise. South African Journal of Sports Medicine 18:24-28.
Semple SJ, Smith LL, McKune AJ, Neveling N, Wadee A (2004) Alterations in acute-phase reactants (CRP, rheumatoid factor, complement, Factor B, and immune complexes) following an ultramarathon. South African Journal of Sports Medicine 16:17-21.
Smith LL, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D (2000) Cytokines and cell adhesion molecules associated with high-intensity eccentric exercise. Eur J Appl Physiol 82:61-67. doi:10.1007/s004210050652
Standl E, Schnell O, Ceriello A (2011) Postprandial hyperglycemia and glycemic variability: should we care? Diabetes Care 34 Suppl 2:S120-127. doi:10.2337/dc11-s206
Weight LM, Alexander D, Jacobs P (1991) Strenuous exercise: analogous to the acute-phase response? Clin Sci (Lond) 81:677-683.
Zuntz N (1901) Ueber die Bedeutung der verschiedenen Nahrstoffe als Erzeuger der Muskelkraft. Archiv fur die gesamte Physiologie des Menschen und der Tiere 83:557-571. doi:10.1007/BF01746509

Table 1 Characteristics of the participants

n	12
Age (yrs)	65 ± 8
Body weight (kg)	73.3 ± 9.7
Height (m)	1.73 ± 0.08
Body mass index (kg/m^2)	24.5 ± 1.9
HbA1c (%)	6.1 ± 0.6
Diagnosed with diabetes (n)	4
Total cholesterol (mmol/L)	5.1 ± 1.3
HDL cholesterol (mmol/L)	1.4 ± 0.6
LDL cholesterol (mmol/L)	3.0 ± 1.0
Systolic blood pressure (mmHg)	133 ± 18
Diastolic blood pressure (mmHg)	74 ± 6

462 **Table 2** Baseline values. Mean baseline values from the control day without exercise (CON),
463 the day with exercise before breakfast (ExBr) and the day with exercise after breakfast (BrEx)

	CON	ExBr	BrEx	p
Heart rate (beats/min)	58 ± 7	55 ± 7	55 ± 8	0.151
Energy expenditure (KJ/min)	4.4 ± 0.7	3.8 ± 0.8	4.0 ± 1.2	0.146
Carbohydrate utilization (g/min)	0.08 ± 0.07	0.06 ± 0.08	0.08 ± 0.07	0.637
Fat utilization (g/min)	0.08 ± 0.03	0.07 ± 0.03	0.06 ± 0.04	0.332
Glucose (mmol/L)	6.3 ± 1.1	6.3 ± 1.2	6.4 ± 1.1	0.752
Triglycerides (mmol/L)	1.0 ± 0.3	1.2 ± 0.5	1.2 ± 0.5	0.116
sVCAM-1 (ng/mL)	647 ± 152	641 ± 139	619 ± 148	0.172
CRP (mg/L)	1.4 ± 1.0	1.2 ± 1.7	0.8 ± 0.7	0.451

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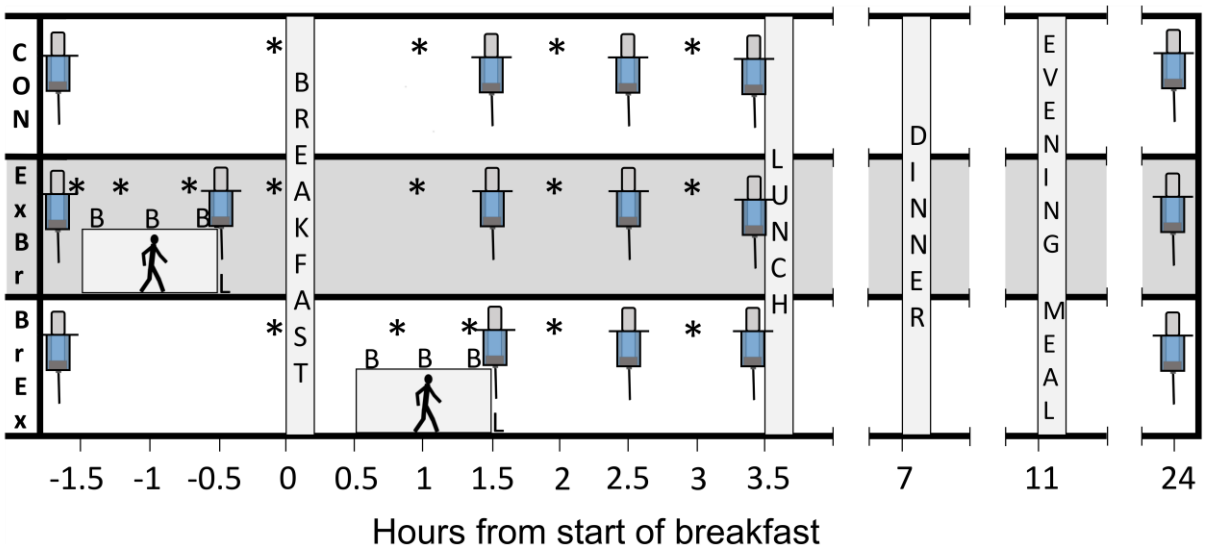

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471 **Fig. 1** Outline of the test protocol. The control day (CON) in the upper row, the day with

472 exercise in the post-absorptive state (ExBr) in the middle row and the day with exercise in the

473 postprandial state (BrEx) in the bottom row. Syringe = blood sample. * = Measure of heart

474 rate, oxygen consumption and respiratory exchange ratio values. B = Measure of perceived

475 exertion (Borg scale). L = measure of blood lactate

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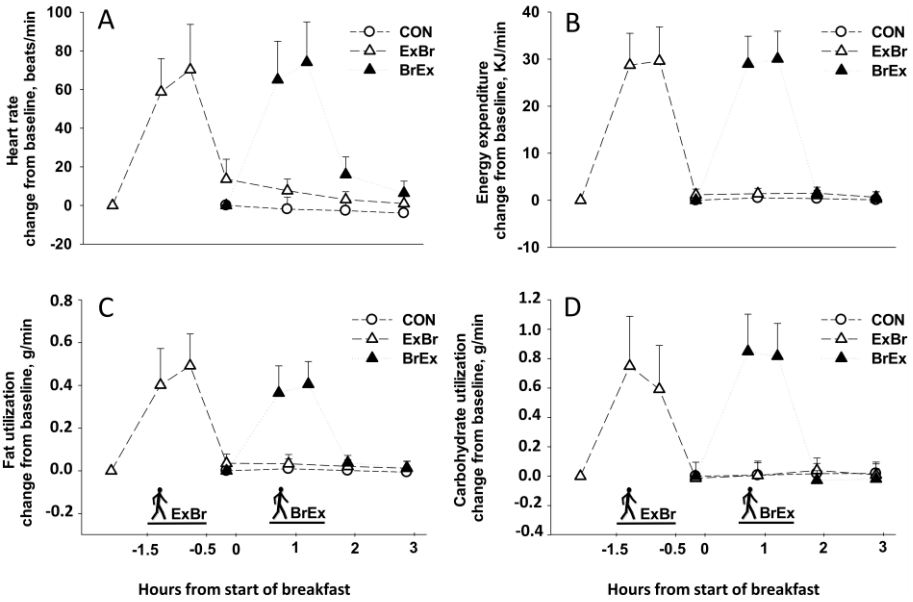

477

478 **Fig. 2** Heart rate and metabolism during and after exercise. Changes in heart rate (A), total
479 energy expenditure (B), fat utilization (C) and carbohydrate utilization (D) from baseline on
480 the three test days; The control day (CON), the day with post-absorptive exercise (ExBr) and
481 the day with postprandial exercise (BrEx)

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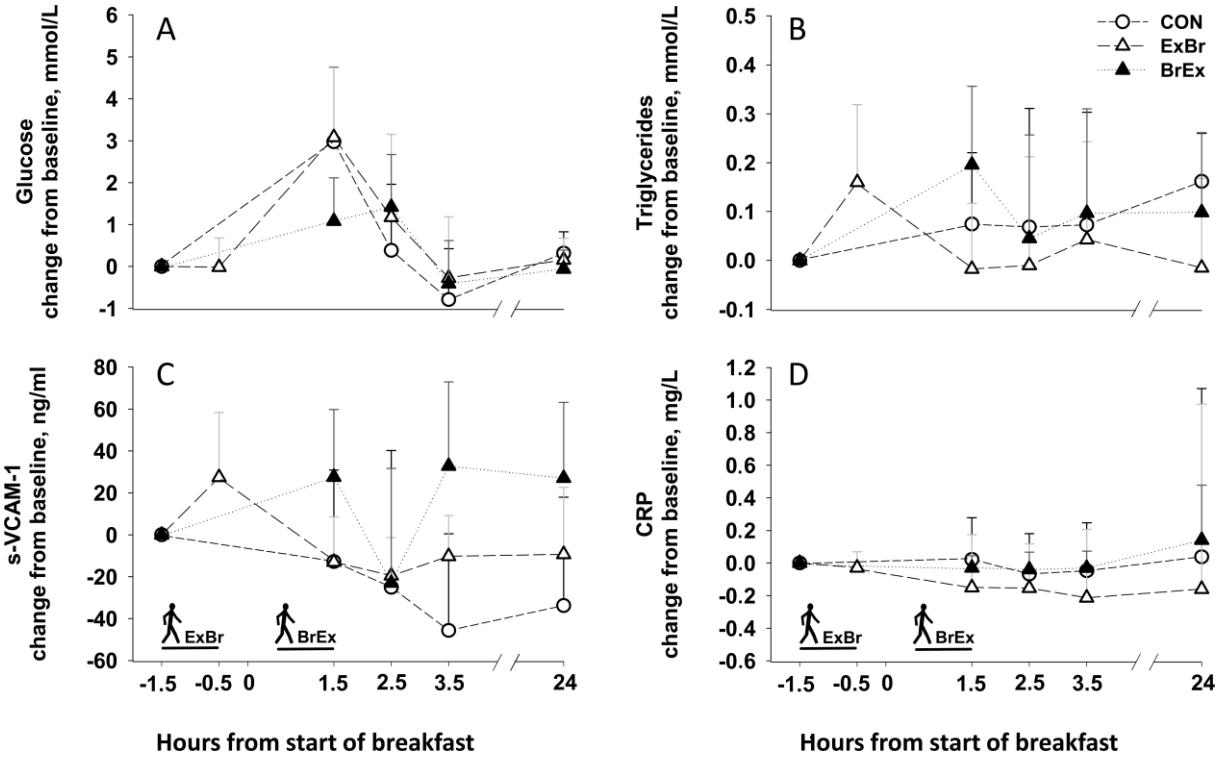

483

484 **Fig. 3** Blood values of nutrients and markers of inflammation. Glucose (A), triglycerides (B),
485 sVCAM-1 (C) and CRP (D) during the three test days; the control day (CON, open circles),
486 the day with exercise in the post-absorptive state (ExBr, open triangles) and the day with
487 postprandial exercise (BrEx, black triangles)

488