**Endothelial Dysfunction Induced by Post-Prandial Lipemia**

Complete Protection Afforded by High-Intensity Aerobic Interval Exercise

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**Objectives**
This study was designed to study the effect of exercise and a high-fat meal (HFM) on endothelial function.

**Background**
Post-prandial lipemia and exercise oppose each other in terms of cardiovascular risk; however, the mechanism of their interaction is not well understood.

**Methods**
Endothelial function was assessed by brachial artery flow-mediated dilation (FMD) in 8 healthy men before and after an HFM preceded (16 to 18 h) by rest, a single bout of continuous moderate-intensity exercise (CME), and high-intensity interval exercise (HIIE).

**Results**
Before the HFM, initial brachial artery diameters were similar in all trials (0.43 ± 0.04 cm), but after the HFM, basal diameter decreased only in the control (0.39 ± 0.03 cm) and CME (0.38 ± 0.04 cm) trials. Before the HFM, FMD/shear was improved by a single bout of CME (+20%, p < 0.01) and HIIE (+45%, p < 0.01; group differences, p < 0.01), with no effect in the control trial. After the HFM (30, 120, and 240 min), FMD decayed to a lesser extent with CME, but in a similar fashion to the control trial. In contrast, FMD in the HIIE trial remained elevated following the exercise despite a clear meal-induced lipemia. Although there were no correlations between vascular function and food-induced markers of cardiovascular risk, antioxidant status was strongly correlated with FMD (r = 0.9, p < 0.001).

**Conclusions**
These findings reveal a clinically relevant protective effect of acute exercise on the vasculature that is clearly exercise intensity dependent and tightly related to exercise-induced antioxidant capacity. (Endothelial Dysfunction Induced by Postprandial Lipemia: NCT00660491) (J Am Coll Cardiol 2009;53:200–6)

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Impaired endothelial function is central to the atherosclerotic disease process and serves as a strong independent risk factor for future cardiovascular disease and mortality (1,2). The ingestion of a high-fat meal (HFM) acutely changes the blood lipid profile and reduces endothelial function for many hours after the meal (3). Thus, as a significant proportion of life is spent in the post-prandial state, the factors leading to this transient impairment in endothelial function may well play a key role in the atherosclerotic disease process.

Interestingly, people who perform regular physical activity maintain low lipoprotein levels even after an HFM (4), but this ability is significantly attenuated when a 3-day period of inactivity precedes the HFM. Recently, Gill et al. (3) clearly demonstrated that exercising for 90 min at 50% of maximal oxygen uptake (VO\textsubscript{2max}) 16 to 18 h before HFM ingestion attenuated the reduction in endothelial function compared with the control situation without exercise. However, this study did not link the changes in endothelial function to either changes in blood lipid profile or a post-prandial inflammatory response and examined only continuous moderate-intensity exercise (CME) at 60% to 70% of maximal heart rate. Thus, the mechanism responsible for this exercise-induced improvement in post-prandial endothelial function, as well as the type of exercise from the *Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway; †Department of Cardiothoracic Surgery, St. Olav’s University Hospital, Trondheim, Norway; ‡Department of Medicine, Division of Geriatrics, and the ¶Department of Exercise and Sport Science, University of Utah, Salt Lake City, Utah; and the §Department of Geriatric Research Education and Clinical Center, Salt Lake City VAMC, Salt Lake City, Utah. Steven E. Nissen, MD, MACC, served as Guest Editor for this article.

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that best protects endothelial function in the face of post-prandial lipemia, have yet to be defined.

The coingestion of antioxidant vitamins with an HFM abolishes the post-prandial decrement in endothelial function (5), and it may be that exercise acts through a similar mechanism. Indeed, it is known that exercise training improves the antioxidant status in plasma (6), and, even acutely, there seems to be a mobilization of antioxidants into muscle itself (7). Recently, we reported that high-intensity interval exercise (HIIE) training with intervals at 85% to 95% of maximal heart rate was superior to CME training in terms of improving the cardiovascular risk profile, including endothelial function, and total antioxidant status in plasma, of patients with post-infarction heart failure (6). However, whether this link between oxidative stress, vascular function, and exercise intensity is apparent with a single bout of exercise in healthy subjects, as employed by Gill et al. (3), is currently unknown.

Therefore, this study sought to determine the efficacy of a single bout of 2 different, but clinically relevant, exercise regimens (6) in terms of their ability to attenuate the endothelial dysfunction induced by post-prandial lipemia. It was hypothesized that the 2 very distinct, but isocaloric, exercise sessions of either HIIE or CME would attenuate the post-prandial reduction in endothelial function in an intensity-dependent manner and that this would be related to changes in plasma antioxidant status.

Methods

Subjects. Eight healthy men participated in this study. Exclusion criteria were any known disease, orthopedic and/or neurological limitations to exercise, surgery during the intervention period, drug or alcohol abuse, or participation in another research study. The protocol was approved by the regional ethical committee for medical research, and the study conformed to the Declaration of Helsinki. Written informed consent was obtained from all subjects before inclusion in the study. For each subject, repeated tests were performed at the same time of day.

Study design. Nine days before the first exercise session, VO2max was determined during uphill treadmill running/walking as previously described (6). Each of the 8 volunteers participated in 3 randomized trials (HIIE, CME, and control [no exercise]) with 1 week between each trial, and the starting trial was randomized. The timeline for each trial is shown on the x-axis of Figure 1. Subjects were provided with standardized meals (Fjordland, Norway), which they consumed for 2 days before the 3 trials. Baseline-1 measurements were made in a rested (>48 h) and fasted state (>8 h) before performing the HIIE, CME, or control (resting) trial on the day preceding the HFM. For the 16-to 18-h period after exercise or the control trial, before baseline-2 (fasted state, >8 h) measurements, subjects abstained from exercise, caffeine, and alcohol. Following baseline-2 measurements, subjects ingested the HFM. Endothelial function was then assessed and blood samples taken 30 min, 2 h, and 4 h after finishing the HFM.

Exercise. The HIIE was performed on a treadmill and consisted of a 10-min warm-up period at 50% to 60% of maximal heart rate (HRmax) followed by 4 intervals of 4 min at an intensity that yielded 85% to 95% of HRmax. Between the intervals, the subjects performed 3 min of active recovery at 50% to 60% of HRmax. The exercise session concluded with a 5-min cool-down period. To achieve an isocaloric protocol, the CME involved walking continuously for 47 min on the treadmill at 60% to 70% of HRmax (8).

HFM. The HFM consisted of a vegetarian mozzarella pizza (Dr. Oetker) with a total weight of 335 g and the following nutritional composition: 48.3 g fat, 80.4 g carbohydrate, 38.5 g protein, and a total of 911.2 kcal. Pilot studies confirmed that in a rested state this meal produced a transient impairment in endothelial function.

Endothelial-dependent vascular function. Endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery using vascular ultrasound (14 MHz echo Doppler probe, Vivid 7 System, GE Vingmed Ultrasound, Horten, Norway) according to the current guidelines (9). Briefly, measurements were performed on the artery approximately 4.5 cm above the antecubital fossa. After 10 min rest in the supine position in a quiet, air-conditioned room with a stable temperature of 22 ± 1°C, the internal diameter of the brachial artery was assessed. Thereafter, a pneumatic cuff (SC10, Hokanson Inc., Bellevue, Washington) just distal to the elbow on the lower arm was inflated to 250 mm Hg for 5 min and deflated to create an ischemia-induced hyperemia. Blood velocity spectra were recorded 10 s after cuff release to measure peak blood velocity, and thereafter B-mode images were recorded for 5 min to assess artery diameter. To avoid confounding effects of arterial compliance and cyclic changes in arterial dimension, all measurements were obtained at the peak of the R-wave in the electrocardiogram (diastole). The mean of 3 diameter measurements (intima to intima) was recorded using calipers with a 0.1-mm resolution. Shear rate was calculated as blood velocity (cm·s−1) divided by vessel diameter (cm) as previously specified (10). All ultrasound images were analyzed in random order using EchoPAC (GE Vingmed Ultrasound AS, Horten, Norway) by an investigator who was blinded to the treatment.

Endothelial-independent vascular function. In an additional set of subjects (n = 8), the complete control protocol (no exercise) was reproduced, but endothelial-independent function was assessed by tracking brachial artery diameter with Doppler ultrasound, as described above, in response to
the sublingual administration of 500 μg of glycerol trinitrate.

**Blood profile measurements and analysis.** Blood samples were taken in a fasted state (>8 h) and plasma triglycerides, high-density lipoprotein cholesterol, total cholesterol, hemoglobin, high-sensitive C-reactive protein, glycosylated hemoglobin (HbA1c), glucose and insulin C-peptide, and total antioxidant status were analyzed as recently described (6).

**Statistics.** Repeated-measures analysis of variance (ANOVA) was performed to examine the differences between each measurement in each of the groups. One-way ANOVA and mixed factorial ANOVA were used to examine group differences. The Fisher least significant difference post-hoc test was used when appropriate. All data are presented as mean ± SD if not otherwise stated.

**Results**

**Subject characteristics.** Table 1 documents the subjects’ characteristics and reveals that, despite being slightly overweight, they exhibited a reasonable level of fitness as assessed by VO$_2$max. All blood parameters were within the normal range for normal healthy subjects of this age.

**Basal vessel diameter, blood velocities, and shear rates.** Initial brachial artery diameters were similar at baseline-1 and -2 in all trials (average 0.43 ± 0.04 cm). However, there was a significant decrease (p < 0.05) after the HFM in both the control and CME trials (0.39 ± 0.03 cm and 0.38 ± 0.04 cm, respectively), with no apparent effect in the HIIE trial (Fig. 1A). Consequently, average peak shear rates were similar at baseline-1 and -2 but increased in the control and CME trials and were unchanged in the HIIE trial at all time points after the HFM (Fig. 1A).

Average peak shear rate at baseline-1 and -2 in the control and CME trials were 435 ± 23 s$^{-1}$ and 437 ± 33 s$^{-1}$, respectively. Average peak shear rate for all time points after the HFM for the control and CME trials was elevated to 490 ± 23 s$^{-1}$. In contrast, average peak shear rate in the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject Characteristics</th>
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<tr>
<td>Age, yrs</td>
<td>42 ± 4</td>
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<tr>
<td>Height, cm</td>
<td>179 ± 3</td>
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<tr>
<td>Weight, kg</td>
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<td>BMI, kg/m$^2$</td>
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<td>HR$_{max}$, beats/min</td>
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<td>VO$_{2max}$ ml/kg/min</td>
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<td>FMD, %</td>
<td>7.1 ± 0.3</td>
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<td>FMD/shear</td>
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<tr>
<td>Glucose, mmol/l</td>
<td>5.01 ± 0.24</td>
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<td>Cholesterol, mmol/l</td>
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<td>HDL, mmol/l</td>
<td>1.25 ± 0.08</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>15.9 ± 0.14</td>
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The table presents baseline characteristics. Data are mean ± SEM.

BMI = body mass index; FMD = flow-mediated vasodilation; Hb = hemoglobin; HDL = high-density lipoprotein; HR$_{max}$ = maximal heart frequency; VO$_{2max}$ = maximal oxygen uptake.
HIIE trial was unaltered across all time points, averaging 433 ± 37 s⁻¹, and was significantly lower than the CME and control trial after the HFM (p < 0.03).

**Endothelial-dependent vascular function.** Both a single bout of CME and HIIE improved FMD measured 16 to 18 h after exercise, whereas FMD was unaltered in the control trial (baseline-2) (Figs. 1B and 1C). All subjects achieved the greatest FMD after performing high-intensity aerobic interval training (p < 0.01) (Figs. 1B and 1C). Continuous moderate exercise did not completely protect vascular function from the food-induced reduction in FMD; however, the post-prandial fall in FMD was significantly less than that observed in the control trial. In contrast, HIIE not only completely protected the vessel from the lipemia-induced reduction in FMD observed after the HFM in the 2 other trials (CME and control), but actually resulted in greater vascular function than initially assessed at baseline-1 (Figs. 1B and 1C).

**Endothelial-independent vascular function.** There was no impact of the HFM on endothelial-independent vascular function, and therefore, at all time points, sublingual glycerol trinitrate (NTG) resulted in approximately a 15% vasodilation in the rested state (Fig. 1B).

**Blood analyses.** Exercise-induced changes in plasma total antioxidant status revealed a pattern very similar to FMD, with a significant increase following both HIIE and CME from baseline-1 to -2 (Fig. 2A). Following the HFM, total antioxidant status in the exercise trials remained higher than the control trial at all remaining time points (Fig. 2A). Additionally, with all trials pooled, there was strong and significant correlation between total antioxidant status and FMD (Fig. 2B).

As there were no statistically discernable differences among the 3 trials (HIIE, CME, and control) in terms of the other food-ingestion–related blood parameters assessed, the group data were pooled to illustrate the time course of the intravascular effects of the HFM. Neither resting nor the performance of HIIE or CME (baseline-1 to -2) had an effect on the HFM induced changes in glucose, C-peptide, triglycerides, high-density lipoproteins, C-reactive protein, cholesterol, HbA1c, or high-sensitive C-reactive protein, and therefore, only the most responsive variables are illustrated in Figure 3. The HFM raised both blood glucose and C-peptide levels within 30 min of food ingestion, whereas triglycerides were not elevated until 2 h after a meal (Figs. 3A to 3C). In contrast, high-density lipoprotein tended to fall after the HFM, achieving significance at the 4-h after-meal time point (Fig. 3D).

**Discussion**

This study examined the efficacy of 2 forms of acute exercise to attenuate the endothelial dysfunction induced by post-prandial lipemia. In agreement with other studies (11), the current data confirm that a single bout of CME 16 to 18 h before the HFM reduced endothelial dysfunction when compared with the control condition without exercise. The novel finding in the present study was that HIIE not only completely prevented the normal post-prandial reduction in endothelial function but, in fact, augmented FMD despite the lipemia. Additionally, the spectrum of plasma total antioxidant status following the HFM, from attenuated in the control to maintained with CME and consistently elevated with HIIE, revealed a strong relationship between the available protection from oxidative stress and vascular function. In combination, these findings reveal a clinically relevant protective effect of exercise on the vasculature that is clearly exercise-intensity dependent and appears to be related to acute exercise-induced antioxidant capacity and therefore suggestive of a link to NO bioavailability.

**Exercise, antioxidant status, and vascular function.** In terms of the current data, exercise, antioxidants, and vascu-
lar function reveal an interesting paradox. Acutely, exercise augments circulating free radical levels in both blood and muscle (7,12–14), which could be presumed to inactivate large amounts of nitric oxide and negatively affect endothelium-mediated vasodilation. However, our current findings, as well as studies by others (15,16), reveal an increase in antioxidant capacity and endothelial function following acute exercise. Indeed, it was recently recognized (7) that even during exercise there seems to be a transfer of antioxidants into muscle itself, presumably from the vasculature. Thus, it appears that acute exercise tends to tip the pro- and antioxidant balance in favor of increased antioxidant status, producing an end result (maintained vascular function) similar to that previously observed by the co-ingestion of antioxidants and an HFM (5).

Lipemia, oxidative stress, and basal arterial diameter. Although the HFM used in the current study (vegetarian pizza) had dietary constituents similar to a meal commonly selected for this type of research (McDonald’s Corporation breakfast) (17–19) and resulted in parallel changes in blood chemistry markers following such a meal (Fig. 3), an unexpected difference was the impact on basal arterial diameter. Specifically, in the control condition there was a marked reduction (approximately 10%) in basal arterial diameter following the ingestion of the HFM, supporting the concept that this was purely an effect of the food ingestion. With the concomitant fall in total antioxidant status (Fig. 2A), it is tempting to surmise that this reduction in vessel diameter was the consequence of a lipemia-induced increase in oxidative stress and subsequent fall in NO bioavailability. The same reduction in basal vessel diameter was apparent in the CME trial. Using the same paradigm, one could infer that this reduction in basal diameter was apparently not protected by the preceding exercise intervention. In contrast, in the HIIE trial, which yielded the greatest increase in total antioxidant status and was the only
trial to maintain this elevation throughout the study (Fig. 2A), there was no such reduction in arterial diameter (Fig. 1B). Collectively, these findings support the concept that lipemia elevates oxidative stress and reduces NO bioavailability, as evidenced by reduction in basal vasodilatory tone, whereas HIIE can not only reverse these effects but can also increase NO bioavailability by increasing antioxidant status above and beyond the influence of lipemia. Additional support for the link between NO bioavailability and the current findings is provided by additional trials in which superphysiologic NO levels were promoted by sublingual NTG. Here, there was no impact of the HFM on endothelial-independent vascular function (a 15% vasodilation at all time points) (Fig. 1A), which supports the concept that NO bioavailability (alleviated by the NTG) was likely the mechanism responsible for attenuated endothelial-dependent vasodilation instigated by the HFM.

However, although a definitive explanation for this observation is still to be found, the current findings of differences in vascular function among the control, CME, and HIIE trials cannot be explained by these significant changes in baseline diameter. Specifically, the reduction in brachial artery diameter after the HFM in both the control and CME trials mathematically biases the calculation of %FMD toward a greater change from baseline. However, even in the face of this bias, the HIIE trial (which did not result in a reduction in baseline diameter) yielded a consistently greater %FMD. As shear stress is proportional to shear rate times viscosity, one could speculate that an HFM changes viscosity and thereby shares stimulus for FMD. However, this seems unlikely, as a study by Cicha et al. (20) specifically addressed this issue and found no change in whole-blood viscosity after an HFM.

**Vascular function, lipemia, and exercise.** Although the exact mechanism responsible for the recognized post-prandial attenuation in vascular function is not completely understood, the increased levels of plasma lipoproteins are thought to play a major role (21). As already recognized, engagement in regular exercise facilitates the maintenance of lower lipoprotein levels even after an HFM (4). However, in the current study, the performance of a single bout of either CME or HIIE had no impact on the multiple markers typically used to assess the impact of a meal, suggesting no effect of acute exercise on how the HFM was handled (Fig. 3). The exception to the selected assays was the assessment of total antioxidant status that differentiated the 3 trials (control, CME, and HIIE). In fact, in the trial that involved no exercise (control), and thus highlighted the role of the meal in isolation, there was a precipitous drop in antioxidant status 30 min after the food intake (Fig. 2A). This effect was lessens in the CME trial and abated following HIIE. Therefore, the current findings suggest that, unlike chronic exercise, which may have a significant effect on the direct “handling” of an HFM, acute exercise 16 to 18 h before the ingestion of the meal appears instead to be related in an exercise-intensity dependent manner to the plasma antioxidant status, with a greater protection from the oxidative stress of the HFM afforded by the highest intensity of activity before its consumption (Figs. 1B and 1C).

Why HIIE was more effective in improving antioxidant status is not known, but it seems reasonable to speculate that higher shear stress experienced during the exercise bout in the HIIE trial also yields a greater response at the cellular and molecular level. On the other hand, the HIIE response on FMD is similar to that observed when coingesting antioxidant vitamins with an HFM (5). It seems unlikely that ingestion of antioxidants will increase the shear stress of the vessel, and it may be, therefore, that the observed increase of antioxidant is just a marker and not the important underlying mechanism of the observed changes in FMD (5). The possibility also exists that antioxidant vitamins and exercise training act on FMD through different signaling pathways. This view is supported by the observation that exercise training, but not necessarily vitamins, improves survival in coronary artery disease. Therefore, the present study indicates, but does not prove, that the effects of different exercise regimens on post-prandial FMD are related to different effects on antioxidant status.

**Study limitations.** The number of subjects in our study was small and all were healthy men, and it is not known whether the present training protocol will give similar adaptations in other populations such as women or subjects with cardiovascular disease. Furthermore, the observed relation between antioxidant status and post-prandial FMD does not prove causation in explaining the different effects of exercise.

**Conclusions**

This study has revealed a clinically relevant protective effect of exercise upon the vasculature that is clearly exercise-intensity dependent and appears to be tightly related to acute exercise-induced antioxidant capacity and, therefore, strongly suggestive of a link to NO bioavailability. As much of human life is spent in a post-prandial state, these findings are of significance for the understanding and reduction of cardiovascular risk and offer insight into the central mechanisms by which exercise reduces these risks.

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