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Post-prandial lipaemia and endothelial function among healthy men

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Summary

Background: There is evidence that elevated post-prandial lipoproteins adversely affect progression and outcome of cardiovascular disease. Traditional risk factors are associated with impaired endothelium-mediated vasodilatation. However, studies regarding the relationship between post-prandial lipaemia and endothelial function are divergent.

Methods: Twelve healthy non-smokers were included in this study. Before and after intake of a lipid cocktail rich in dairy fat, we tested endothelial-dependent (acetylcholine 0.8–160 mg/min per 100 ml forearm tissue) and -independent (sodium nitroprussid 0.6 µg/min) vascular function in the forearm vascular bed with plethysmography. Moreover, we tested the effect of l-NMMA, a competitive inhibitor of the NO synthetase, on baseline flow. Extent of post-prandial lipaemia was assessed with the increases in triglycerides and retinyl-palmitate, a marker for intestinally derived lipoproteins.

Results: Baseline flow was higher after the test meal than during fasting (preprandial 6.5 ± 0.5 ml/min* 100 ml tissue, post-prandial 8.0 ± 0.5, p = 0.03), but similar after l-NMMA (p = 0.85). Before and after intake of the test meal, there was no significant difference in acetylcholine-induced endothelium-dependent vasodilatation (repeated measurement ANOVA, p = 0.22). At the highest acetylcholine dose, forearm flow was very similar (fasting 18.4 ± 1.9, post-prandial 17.9 ± 1.9, p = 0.75). At maximum acetylcholine dose, there was a weak inverse but non-significant correlation between forearm flow and post-prandial triglyceridaemia (r = –0.38, p = 0.23) and intestinally derived lipoproteins (chylomicrons r = –0.29, p = 0.35, chylomicron remnants r = –0.15, p = 0.63). However, at the lowest acetylcholine dose there was a suggestion for a positive correlation between change in flow and post-prandial lipaemia (triglyceridaemia, r = 0.53, p = 0.07; chylomicrons, r = 0.41, p = 0.18 and remnants, r = 0.51, p = 0.09). Endothelium-independent vasodilatation in response to sodium nitroprusside did not significantly change (p = 0.23).

Conclusion: Our results suggest that among healthy men post-prandial lipaemia is not associated with a notable impairment of endothelium-mediated vascular function in forearm resistance vessels.

Keywords: triglycerides; chylomicrons; remnants; vascular function; lipoproteins; post-prandial; endothelium

Introduction

In the post-prandial state, triglyceride-rich lipoproteins are markedly increased by both, hepatic and intestinally derived lipoproteins (very low-density lipoproteins and chylomicrons) [1]. Basic research suggests that post-prandial triglyceride-rich lipoproteins have atherogenic properties, in particular partially hydrolysed remnant lipoproteins of chylomicrons [2]. Post-prandial lipaemia was a cardiovascular risk factor in several case-control studies [3–5], and was found to be associated with progression of coronary atherosclerosis [6]. Because post-prandial triglyceride-rich lipoproteins are associated with fasting lipoproteins, glucose metabolism and life-style factors [7], it is not entirely clear whether post-prandial lipaemia is an independent risk factor.

Impairment of endothelial function is thought to be an early indicator of atherosclerosis. Traditional risk factors are associated with impairment of endothelium-mediated vasodilatation [8–11]. It has been shown that smoking acutely impairs endothelial function [12]; conversely, among subjects with high low-density lipoproteins, LDL-apheresis was associated with an acute increase in en-

dothelium-mediated forearm blood flow of ~100% [13], indicating that the endothelium may rapidly respond to changes in risk factor levels. An acute post-prandial decrease of endothelium function would support the concept that post-prandial lipaemia is an independent cardiovascular risk factor. Recent studies, however, have provided divergent results [14, 15]. We therefore tested this hypothesis among healthy male volunteers using forearm plethysmography.

Methods

Twelve healthy male non-smokers were included into this study (mean age was 29.5 ± 1.5 years, BMI 23.2 ± 1.2 kg/m^2). After an over-night fast of 12 hours blood was drawn for determination of lipids, glucose and insulin. Following this, vascular function was tested. Subsequently, the participants ingested a lipid cocktail, and after 4 hours, lipid parameters, glucose and insulin as well as vascular function were re-assessed.

The test meal consisted of a blend of 180 ml dairy cream, 2 g of lean milk powder, 20 mg sacharose and 10 g chocolate powder per square meter body surface area (BSA), providing 700 kcal energy/m^2 BSA. The composition of fat, carbohydrate and protein calories was 83%, 14% and 3%, respectively. 50 000 IU retinyl palmitate/m^2 BSA was supplemented to the lipid cocktail as a marker for intestinally derived lipoproteins [16]. Retinyl-palmitate does not affect oxidative susceptibility of lipoproteins [17–19].

To separate retinyl palmitate-labeled large chylomicrons and small chylomicrons/remnants, plasma was centrifuged for 1.7×10^6 g × min at d = 1.006 g/ml in a Cetrikon TST 60.4 swing-out rotor (Kontron, Zurich, Switzerland). Retinyl palmitate was measured by HPLC[5] in the fraction containing lipoproteins with Sf >1000 (chylomicrons) and Sf <1000 (small chylomicrons/remnants). Other lipoproteins and glucose were measured with standard methods as described elsewhere [20], and insulin was measured with a RIA (DPC, Los Angeles, USA).

Forearm vascular function was assessed as described in detail elsewhere [21]. Briefly, following baseline measurement the endothelium-independent vasodilator sodium nitroprusside was infused (0.6 µg/min per 100 ml forearm tissue for 3 minutes) through a brachial catheter. After washout, the endothelium-dependent vasodilator acetylcholine was applied (0.8, 10, 40 and 160 mg/min per 100 ml for 3 minutes each). Finally, l-NMMA (200 µg/min per 100 ml for 5 minutes), a competitive inhibitor of NO synthetase was administered. Forearm blood flow was measured with strain gauge plethysmography with the venous occlusion technique.

The primary hypothesis of the study was that intake of a fatty meal significantly affects acetylcholine-induced vasodilatation as demonstrated by a difference in the dose-resonse relationship between acetylcholine dose and flow before and after intake of the test meal, as well as at maximum acetylcholine dose. Further analyses are regarded as exploratory. Continuous variables are expressed as mean ± SEM. Data were checked for distribution patterns including visual inspection and taking account of kurtosis and skewness. The results provided no indication for a gross violation of the Normality assumption. Therefore, parametric tests were used. Paired variables were compared with the t-test for paired samples. The dose-response curve of acetylcholine-induced dilatation before and after intake of the test meal was compared with repeated measurement ANOVA applying mixed linear models using compound symmetry as covariance structure. Comparison of the dose-response curves before and after the test meal were done with a likelihood ratio test, comparing a model with the predictors time (before or after the fat meal), acetylcholine dose, and a time*dose interaction, and a model including only the acetylcholine dose. The primary outcome variable for acetylcholine-stimulated flow was crude forearm blood flow. Because baseline flow changed, we additionally compared incremental flow over baseline as well as percent incremental flow. The association of change in vascular function with changes in lipid fractions during endothelial stimulation with acetylcholine was tested with the Spearman rank correlation coefficient. All reported p-values are two-sided.

Results

Triglycerides increased from 1.00±0.13 mmol/l to 3.04±0.35 mmol/l. Retinyl palmitate in the chylomicron fraction (Sf >1000) rose from 0 to 1395±701 ng/ml, and in the fraction containing small chylomicrons/remnants (Sf <1000) from 36±16 ng/ml to 759±365 ng/ml. The mean post-prandial insulin level was 90 ±39 mIU/ml compared with 39 ±12 mIU/ml (p = 0.001) in the fasting state. Serum glucose was similar (5.0±0.5 mmol/l vs. 4.9±0.5 mmol/l, p = 0.68).

Mean blood pressure and heart rate were unchanged (89 mm Hg ±2 vs. 88 mm Hg ±2 and 66/min ±2 vs. 68/min ±2, respectively, p >0.3). Baseline flow was significantly higher after intake of the lipid cocktail (fasting 6.5±0.5, post-prandial 8.0±0.5, p = 0.03). However, this difference was no longer present after l-NMMA (preprandial 5.1±0.4, post-prandial 5.1±0.4, p = 0.85). Edothelium-independent dilatation following sodium nitroprusside was not significantly different before and after intake of the fat meal (fasting 18.3±2.0, post-prandial 20.8±1.9, p = 0.23).

Figure 1 depicts results of the main study endpoint, forearm blood flow during endothelium-mediated vasodilatation before and after intake of the lipid cocktail. Pre- and post-prandial acetylcholine-stimulated flow did not differ (p = 0.22). At the highest acetylcholine dose, blood flow was very similar before and after intake of the lipid-rich meal (before 18.4±1.9, after 17.9±1.9, p = 0.75). When acetylcholine-stimulated flow was expressed as incremental flow over baseline or percent flow increase, the conclusions remained unchanged. There was no significant difference in the

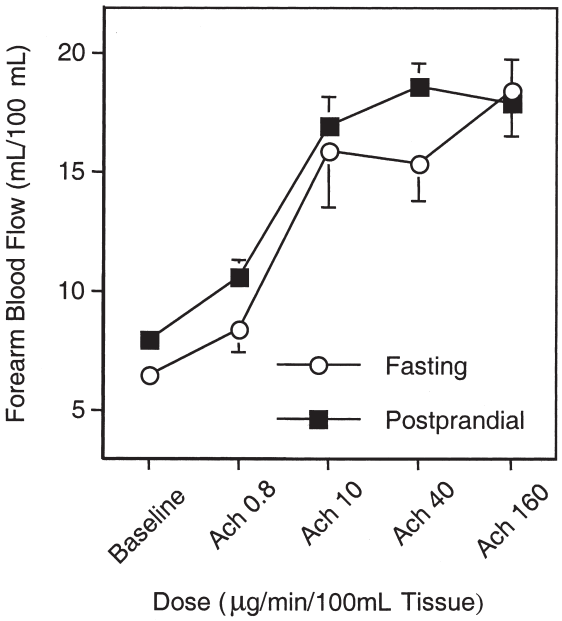

Figure 1

Dose-response curve of forearm blood flow during endothelium-mediated stimulation with acetylcholine (Ach) while fasting and after intake of a fat meal.

dose response curves (p = 0.58 and p = 0.12). The incremental flow at the highest acetylcholine dose was not significantly different (11.8±2.1 vs. 9.9±1.9, p = 0.15); however, the percent incremental flow over baseline at the maximum acetylcholine dose was smaller after the test meal than while fasting (fasting 210±41% vs. post-prandial 131±26%, p = 0.03).

There was no significant correlation at the highest acetylcholine dose between the change in endothelial-mediated flow and the increase in triglycerides (r = –0.38, p = 0.23), chylomicrons (r = –0.29, p = 0.35) or chylomicron remnants (r = –0.15, p = 0.63). However, there was a suggestion for a positive correlation between change in flow and extent of post-prandial lipaemia at the lowest acetylcholine dose (triglyceridaemia, r = 0.53, p = 0.07; chylomicrons, r = 0.41, p = 0.18 and remnants, r = 0.51, p = 0.09).

Discussion

In our study among healthy men, endothelium-dependent vascular function was not decreased after intake of a meal rich in dairy fat. Moreover, we found no significant correlation between increase in post-prandial triglyceride-rich lipoproteins and change in endothelial function.

In agreement with previous studies [14, 22], we observed a higher baseline blood flow after intake of the fat meal than during fasting, and post-prandial insulin levels were increased. After l-NMMA infusion, a competitive inhibitor of NO synthetase, forearm blood flow was very similar before and after intake of the test meal. This suggests that post-prandial baseline NO synthesis was activated, and is compatible with the finding that insulin-mediated vasodilatation is reversed by inhibition of NO synthesis [23, 24].

Some recent studies have suggested that acute triglyceride increase is associated with a reduced endothelium-mediated vasodilatation, but others could not confirm this. Three studies induced hypertriglyeridaemia with intravenous application of triglyceride emulsions [25–27]. In one study, a decrease in endothelium-dependent and -independent function was found with respect to brachial artery diameter changes [25]. Two other studies using endothelium-dependent flow as outcome variable, however, found no impairment of endothelial function [26, 27]. In one of the latter studies, a 15-fold increase in triglyceride levels was even associated with an increase in endothelium-dependent vasodilatation [27]. None of the studies reported a correlation between triglyceride levels and endothelium function. Because post-prandial lipaemia is associated with an increase in distinct lipoprotein particles, it is not clear how these studies compare with post-prandial triglyceride increase. Vogel et al reported that post-prandial endothelium-dependent dilatation of the brachial artery was reduced after a high fat meal compared with a low fat meal [14, 22]. Moreover, they described that post-prandial triglyceride increase was inversely correlated with flow-mediated vasodilatation. In these studies, however, post-prandial endothelium-dependent blood flow was higher than during fasting which does not suggest that endothelial function of resistance vessels was grossly affected. In a recent study, Williams [15] found a decrease in endothelium-dependent dilatation following a meal rich in used cooking fat, but not after intake of unused fat. Although the effect of the meal with used fat was highly significant, there was no association with the endothelium-dependent flow (p = 0.93), and incremental triglyceride levels were not correlated with vasomotion. In our study, post-prandial increase in triglycerides was more pronounced than in other studies, but endothelium-dependent and -independent blood flow as determined by forearm plethysmography was unaltered. At the highest acetylcholine dose, there was a very weak and non-significant negative correlation between post-prandial lipid increases and vascular function. In contrast, at stimulation of the endothelium with the lowest acetylcholine dose, higher post-prandial lipid increase even tended to be associated with a higher increase in flow.

The inconsistency of results might be due to limited power of the studies. However, there are several other potential explanations. Firstly, composition of chylomicrons depends on the nutritional fats, and this may be associated with different effects on the endothelium [28–30]. We used a lipid cocktail with a high content of unprocessed dairy fat, which might have a smaller untoward acute effect on endothelial function than oxidised cooking fat [15] in some fast food products as used

in other studies [14, 22]. Secondly, post-prandial lipoproteins are a heterogenous group of triglyceride-rich particles with respect to origin and size [1], and the post-prandial increment in triglycerides may not be a sensitive measure for subgroups of potentially atherogenic particles. Among the post-prandial lipoproteins, remnants of intestinally derived lipoproteins are most likely to be atherogenic [2, 6]. However, we failed to find a notable negative association between chylomicron remnants and endothelial function. Thirdly, there might be a discrepancy in the effect of acute triglyceride increase on endothelial function between conduit and resistance vessels. Including the current study, 3 studies assessed pharmacological response of resistance vessels as the criterion variable [26, 27]; 3 studies used flow-mediated change in brachial artery diameter [14, 22, 25]; and one tested both endothelium-mediated brachial artery diameter changes and flow [15]. Among those, all studies using arterial diameter changes as the criterion variable described an impairment of post-prandial endothelial function; conversely, none of the studies that measured endothelium-mediated flow reported a significant association. This discrepancy may be due to chance, but deserves further attention. Lastly, the association between endothelium-mediated flow and post-prandial lipaemia might even depend on the extent of endothelial stimulation.

A final consideration is whether measurements during endothelial stimulation should be standardised relative to baseline flows. The incremental flow over baseline may heavily depend on the extent of post-prandial baseline stimulation of NO synthesis by insulin or other vasoactive mediators. Therefore, we feel that within-subject comparisons of crude flow before and after intake of a test meal are best suited to providing meaningful information about the integrity of endothelial function.

In conclusion, our study provides no evidence that post-prandial lipaemia induced by a meal rich in unprocessed dairy fat impairs endothelial function. Additional studies using different fats and methods for assessing endothelial function will be needed to clearly define the effects of post-prandial lipaemia on vascular function.

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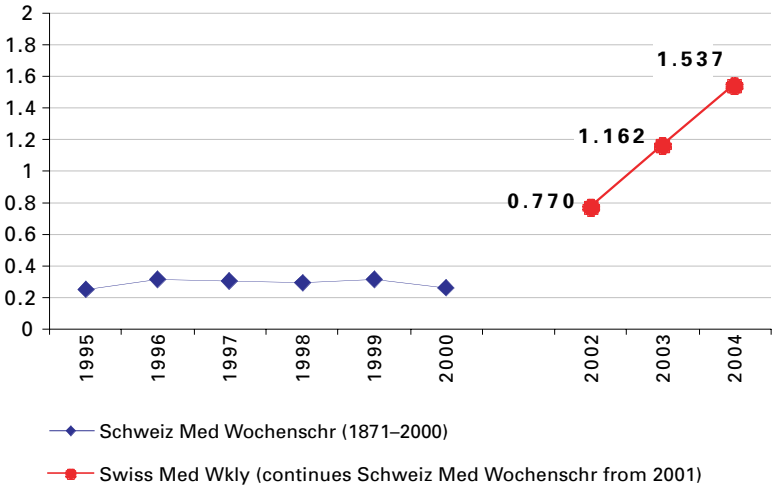

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