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Addition of glucose to a fatty meal delays chylomicrons and suppresses VLDL in healthy subjects

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Abstract

Background Postprandial lipemia has been shown in a number of studies to be associated with atherosclerosis. However, the test meals used in these studies were heterogeneous particularly in their carbohydrate content, which may be important for the resulting lipemia and which makes comparison between different studies difficult. We studied the effect of 75 g glucose added to a fatty meal on various lipoproteins and on gastric emptying.

Materials and methods Fourteen healthy young volunteers were studied in the fasting state and until 7 h postprandially. In a crossover design, each subject received an oral fat load (1 g fat kg^{-1} body weight) with or without 75 g glucose. Triacylglycerol (TG) and free fatty acids (FFA) were then measured in whole blood and lipoproteins were separated off by ultracentrifuging. Gastric emptying was determined by the ^{13}C breath test.

Results The addition of 75 g glucose to a fatty meal had two different effects. Gastric emptying was delayed by about 2 h and the chylomicron response was consequently postponed. In addition, the postprandial increase in VLDL triacylglycerol was reduced by 40%, which may be due to the pronounced FFA depression during the glucose-induced rise in insulin.

Conclusions 75 g glucose added to an oral fat load causes a delay of the chylomicron response and a marked suppression of the postprandial increase in VLDL.

Keywords Chylomicrons, free fatty acids, gastric emptying, glucose, postprandial, VLDL.
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Introduction

Postprandial lipemia has attracted scientific interest after it had been suggested that it promotes atherosclerosis [1]. Retrospective and prospective studies have in fact confirmed that elevated postprandial lipemia is associated with coronary heart disease [2–6]. The mechanisms by which postprandial lipemia is atherogenic are still under investigation. They are complex, comprising direct effects on endothelial cells [7] and on macrophages [8], exchange processes generating small dense LDL and low HDL cholesterol [9], increased atherothrombosis [10] and impaired fibrinolysis [11]. Finally, it has also been proposed that elevated postprandial free fatty acids (FFA) increase the risk of atherosclerosis [12].

The test meals used to induce experimental postprandial lipemia differed considerably between the studies in the amount of fat administered and also in the use of additional constituents like carbohydrates, proteins, etc. Fat alone was used frequently in the form of cream and ranged from around 0·2 g to around 2 g fat per kg body weight [13,14]. Fat combined with other nutrients was usually employed in the form of specially prepared meals with a varying carbohydrate moiety. One group of investigators even used a preparation containing alcohol in combination with fat, carbohydrates and protein [15]. However, these additional constituents of the test meals may exert a considerable influence on the resulting lipemia and may make comparison between different studies difficult. Insulin in particular can be expected to modify postprandial lipemia by inhibiting hormone-sensitive lipase in adipocytes [16] and/or by activating lipoprotein lipase [17]. Addition of carbohydrates to fat loads has been studied a few times in the past but the results have been variable. An aggravation of postprandial

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lipemia was reported in [18] but also no effect at all [19] and even decreases [20]. If experimental postprandial lipemia is to be used in further clinical studies, a standardised procedure is mandatory.

Against this background, the present study was undertaken to investigate the influence of glucose added to a fatty meal on postprandial lipemia, and in particular, on various triacylglycerol-rich lipoprotein fractions. In addition, as it is known that gastric emptying can be affected by glucose [21] and by insulin [22], we studied not only quantitative effects on various lipoproteins but also gastric kinetics by the ^{13}C breath test.

Methods

Subjects and the oral fat load

Fourteen students (seven males, seven females) took part in this study. The subjects were clinically and metabolically healthy, not obese, and nonsmokers [Table 1]. Each subject was studied twice with an interval of at least 3 days between the two oral fat loads. The test meals consisted of 30% whipping cream, 3 mL (1 g fat) being given per kg body weight. 100 mL of the cream contained 30 g fat (18·2 g saturated and 9·04 g monounsaturated fatty acids), 3·5 g carbohydrates, and 2·5 g protein. The cream was mixed, in randomised order, with 300 mL of water containing or not containing 75 g of a mono-/oligosaccharide mixture (Dextro® O.G-T., Hoffmann-La Roche AG, Gußnzach, Germany). The meals were eaten within 15 min between 7 : 30 and 8 : 00 am. To test whether the effects of a glucose addition would also be observed if common food components were used, a substudy was carried out as follows: four individuals received a typical German fat-rich meal, comprising 200 g of sausage (0·6 g carbohydrate, 50 g fat, 26.8 g protein per 200 g) with 200 g of potato salad (30 g carbohydrate, 10 g fat, 8 g protein per 200 g) with and without the glucose test drink. All test meals were well tolerated and no gastrointestinal symptoms were reported.

The first blood sample was withdrawn after a fasting period of 12 h. Further blood samples were taken immediately before and 0·5, 1, 1·5, 2, 3, 4, 5, 6 and 7 h after the oral fat tolerance test. No other source of energy was provided, but water was allowed. The participants did not engage in any physical activity during the test and exercise had been avoided during the 24 h before the tests. Venous blood samples were collected under standardised conditions and serum was separated from the blood cells by centrifuging for 10 min at 3000 g. Analyses of the lipoproteins and the metabolic parameters were carried out within 24 h.

Table 1 Characteristics of the subjects

n (male/female)	14 (7/7)
Age (years)	22 ± 2
BMI kg m^{-2}	22 ± 1
Glucose (mmol L^{-1})	5·20 ± 0·24
Insulin (pmol L^{-1})	27± 14
Triacylglycerol (mmol L^{-1})	1·07 ± 0·27
Cholesterol (mmol L^{-1})	5·02 ± 0·37
LDL-cholesterol (mmol L^{-1})	3·16 ± 0·36
HDL-cholesterol (mmol L^{-1})	1·5 ± 0·36
Free fatty acids (mmol L^{-1})	0·51 ± 0·15

Mean ± SD; BMI, body mass index; HDL-cholesterol, high-density-lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol.

Determination of lipids and conventional lipoproteins

To isolate chylomicrons, 1 mL plasma was layered under 2 mL of saline (9 g NaCl L^{-1}, d = 1·006 g mL^{-1}) and ultracentrifuged in polycarbonate tubes (Beckman Instruments, Beckman Coulter, Unterschleißheim, Germany) at 20 000 r.p.m. in a 50·3 Ti rotor, for 20 min at 10 °C. Chylomicrons were carefully isolated from the supernatant. To determine the TG in VLDL, serum was ultracentrifuged for 18 h under the same conditions and the supernatant containing VLDL plus chylomicrons was aspirated off. The TG in VLDL were calculated by subtracting chylomicron TG from total TG in this fraction. All values were corrected for different yields by weighing the tubes before ultracentrifugation and after removal of the supernatant.

The TG concentrations were determined by commercial enzymatic methods in a random-access analyzer (Hitachi 911, Roche Diagnostics, Mannheim, Germany). All reagents and calibrators were from Roche Diagnostics. Plasma glucose was determined by a commercial enzymatic method (GOD, Roche Diagnostics), insulin by a commercial radioimmunoassay (BI-Insulin IRMA, BIO-RAD, France), and FFA by a commercial enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

Gastric emptying [23]

All gastric emptying tests were done in combination with the test meals, 150 mg of ^{13}C-sodium acetate being dissolved in the fatty meal. Breath samples were collected before and then every 15 min for 240 min after the test meal, and were analysed for isotopic enrichment using an isotope ratio mass spectrometer with an on-line gas-chromatographic purification system. The half-time of gastric emptying was calculated after curve fitting of the ^{13}C exhalation to a modified power exponential function.

Calculation and statistics

Data are presented as medians with 25th and 75th percentiles. Differences between different test meals were checked for significance by the *t*-test for paired samples, taking $P = 0·05$ as the significance threshold for primary endpoints. SPSS for WINDOWS (Version 7·5; SPSS Inc., Chicago IL, USA) was used for the analyses. To evaluate the overall FFA response during the 7 h postprandial

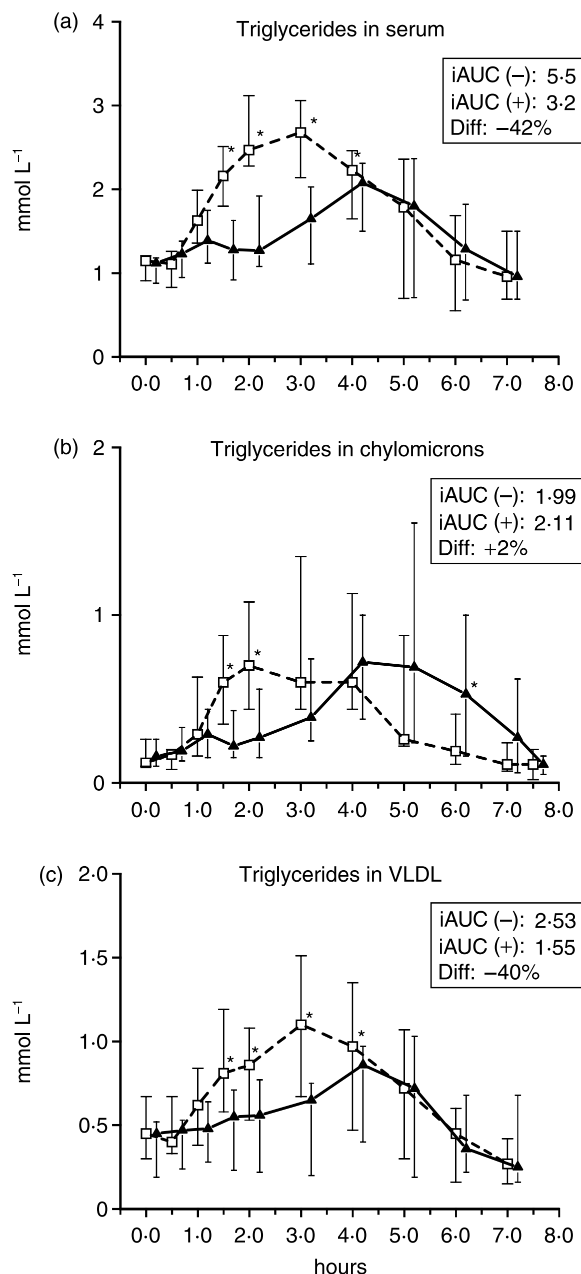

Figure 1 Kinetics of postprandial triacylglycerol (TG) in (a) serum, (b)chylomicrons and (c) VLDL in 14 normolipidemic subjects after meals containing 1 g fat kg^{-1} body weight in combination with 75 g glucose (continuous line), or 1 g fat kg^{-1} body weight alone (broken line). $^{*}P < 0{\cdot}05$. Medians with 25th and 75th percentiles

period, the area under the postprandial curve (AUC) was calculated by the trapezoid rule. The incremental area under the postprandial curves (iAUC) of total triacylglycerol, triacylglycerol in VLDL and triacylglycerol in chylomicrons were calculated from the differences between the postprandial triacylglycerol concentration of different time point and the fasting triacylglycerol in total serum and in VLDL and chylomicrons.

Results

Figure 1(a) shows the increase in total serum triacylglycerol after ingestion of fat alone and after fat plus glucose. When fat alone is ingested, an increase of 40% occurs after 1 h. After 3 h the increase reaches its maximum of 130%. When glucose is added to the fat drink, however, the increase in TG is almost completely absent during the first 2 h and only reaches 40% as late as 3 h. The maximum increase occurs 4 h after the meal and is now only 80%. All in all, addition of glucose caused a delay of the increase in triacylglycerol and a lower maximum. The reduction of postprandial lipemia, expressed as the incremental AUC (iAUC), is 42% ($P = 0{\cdot}017$).

Figures 1(b) and 1(c) show the triacylglycerol in chylomicrons and in VLDL. When glucose has been added to the test meal the initial increase is largely suppressed in both fractions during the first 2 h. Thereafter, glucose addition acts differently on chylomicrons and on VLDL. Chylomicrons (Fig. 1b) reach equally high peak values after either meal, but the maximum is delayed by 2–3 h when glucose had been added. The chylomicron iAUC remains unchanged. In the case of VLDL, in contrast, the major difference between the two test meals is that the VLDL triacylglycerol increases by 130% after fat alone, but only by 90% after fat plus glucose (Fig. 1c). The iAUC of VLDL triacylglycerol shows the total reduction to be 40% ($P = 0{\cdot}010$). This reduction in VLDL triacylglycerol is accompanied by a postponement of the peak maximum, as in the case of chylomicrons, but the shift delay is only 1 h. Taking all these results together, the major effect of the glucose addition on chylomicrons is a delay of the peak. In contrast, the effect on VLDL triacylglycerol is predominantly a reduction, the delay of the peak being moderate.

With the glucose-induced reduction of postprandial lipemia there is a simultaneous pronounced suppression of FFA during the first 4 h (Fig. 2c). From hours 5–7, the FFA are equally elevated above the baseline after both meals, reflecting the postabsorptive release of FFA from adipocytes. The AUC of the FFA is 30% smaller after a glucose addition ($P = 0{\cdot}001$). This effect is paralleled by the expected increases in glucose and insulin (Fig. 2a and 2b).

The effect of the addition of glucose to the fat drink on gastric emptying was studied in all participants using the breath test. After fat alone, the half-time of gastric emptying was 127 min, after fat plus glucose it was 193 min ($P = 0{\cdot}01$).

In an additional part of the study we investigated the reproducibility of these findings by giving four individuals a typically German fat-rich meal. Figure 3 shows the serum triacylglycerol and serum FFA after a meal consisting of sausage with potato salad, which was consumed twice: once with and once without the glucose test drink. Although the differences between the two meals were not of the same magnitude as in Figures 1 and 2, it is evident that the effects of glucose on postprandial lipemia also occur under these everyday conditions.

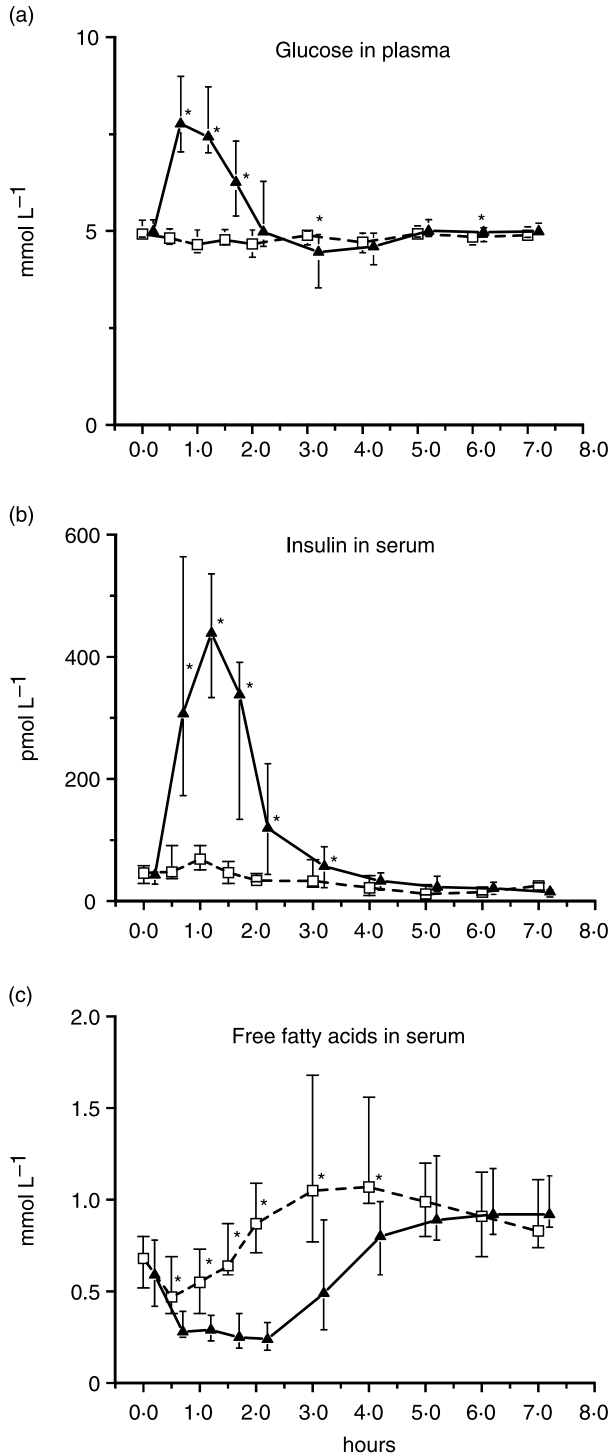

Figure 2 Kinetics of postprandial glucose in (a) plasma, (b) insulin in serum, and (c) free fatty acids in serum in 14 normolipidemic subjects after meals containing 1 g fat kg^{-1} body weight in combination with 75 g glucose (continuous line), or 1 g fat kg^{-1} body weight alone (broken line). $^{*}P < 0{\cdot}05$. Medians with 25th and 75th percentiles.

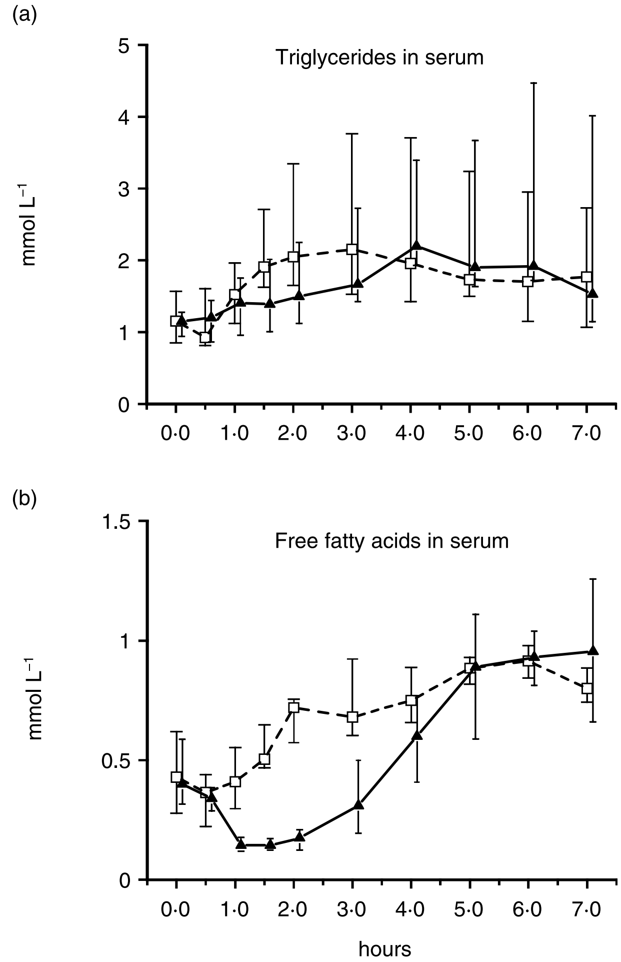

Figure 3 Kinetics of postprandial triacylglycerol (TG) in (a) serum, and (b) free fatty acids in serum in 4 normolipidemic subjects after a typically German fat-rich meal of sausage and potato salad with (continuous line) and without (broken line) the glucose test drink. $^{*}P < 0{\cdot}05$. Medians with 25th and 75th percentiles.

Discussion

The increase in total triacylglycerol after a fatty meal is well known. The acute influence of a concomitant carbohydrate intake on postprandial lipemia has been investigated to a smaller extent, and the results have been inconclusive. An increase in postprandial lipemia was observed when 50 g fructose was given together with an oral fat load to healthy adults [18,24]. Using 17·5 g glucose as a sweetener, Singleton *et al.* reported an increase in postprandial lipemia [25]. No effect on postprandial lipemia was observed by Nicholls and Cohen [26] when they administered 50 g glucose orally or intravenously 1.5 h after a fatty meal. Similarly, no effect was found by Cohen and Schall [19] after the addition of 50 g glucose to 40 g fat. Decreases in postprandial lipemia were found in two studies, in which 50 g or more glucose had been used. A dose dependent effect was observed by

Cohen and Berger [20], with a 25% decrease in the triacylglycerol response after 50 g glucose and a 39% decrease after 100 g glucose. Complete prevention of postprandial lipemia was achieved by Albrink *et al.* who added 100–250 g glucose to the fatty meal [27]. Summarising, the changes in the postprandial lipemia due to the carbohydrate additions depend on both the nature and amount of the carbohydrate added: fructose, which does not stimulate insulin secretion, aggravates postprandial lipemia, while glucose suppresses it if used in doses of 50 g or more.

In the present study, the addition of 75 g glucose caused a pronounced decrease in postprandial lipemia, by 40%, confirming the results of Cohen *et al.* [20] and of Albrink *et al.* [27]. Our data also extend current knowledge by showing that different lipoproteins are affected in different ways: the chylomicron response is delayed and the VLDL response is suppressed. These findings emphasize the necessity of standardizing the test meal composition in studies of postprandial lipemia, or of using specific compositions according to the metabolic problem being investigated.

As chylomicron triglycerides were delayed but not lowered, a change in the lipid absorption is unlikely. This delay is caused by a delay in gastric emptying, as shown by the ^{13}C breath test. Several mechanisms may be operating together: the osmotic activity of the glucose solution [28] and the postprandial rise in glucose [21] and insulin [22]. Another reason for the observed postponement of postprandial lipemia may be a direct effect of insulin on the small intestine. Thus, Loirdighi *et al.* [29] cultured jejunal explants with ^{14}C-oleic acid, and when insulin was added to the medium the triacylglycerol synthesis remained unchanged, with triacylglycerol secretion into the medium decreasing by 20% ($P < 0{\cdot}05$). If this also occurs in humans, triacylglycerol secretion will resume only when the insulin level has fallen off, which might add to the observed delay of the chylomicron response.

The 40% lowering of the postprandial VLDL is most probably caused by insulin acting via several mechanisms. One mechanism concerns the release of FFA from adipose tissue which is markedly suppressed by insulin, resulting in a 30% reduction in the AUC of FFA (Fig. 2c). As the rate of hepatic VLDL production is strongly dependent on the FFA supply [30], the hepatic triacylglycerol synthesis and VLDL secretion are consequently slowed down. A second mechanism of insulin may be direct inhibition of VLDL synthesis. In cultured rat hepatocytes, insulin inhibits hepatic secretion of apo-B-containing lipoproteins [31,32]. This insulin effect on hepatic VLDL production has also been demonstrated in humans [33]. Insulin decreases the expression of microsomal triglyceride transfer protein (MTP), which is essential for biosynthesis of apo B-containing lipoproteins [34]. A further possibility may be an inhibition of VLDL secretion leading to increased triglyceride storage in the liver. Finally, insulin might stimulate lipoprotein lipase [35], resulting in an accelerated clearance of triacylglycerol-rich lipoproteins [36]. This possibility was addressed by Cohen and Berger, who compared the kinetics of triacylglycerol after infusions of Intralipid with and without prior ingestion of 50 g glucose. As the kinetics of the triacylglycerol decreases were the same in both cases, the authors concluded that increased triacylglycerol clearance may not be the reason for the observed reduction in postprandial lipemia. Although our data do not allow an assessment of the contribution made by each mechanism, we believe that insulin-stimulated rerouting of FFA to the fat deposits, thus depriving the liver of its fuel for VLDL synthesis, is of major importance in this context.

In a consideration of the biological significance of the observed effects, it is worth recalling that in European and Arab cuisine a rich meal is commonly completed by a sweet dessert. As our data describes short-term effects, we can only speculate about any potential long-term effects of this custom. One immediate disadvantage is that the additional carbohydrates add to the calorie intake and may lead to a gain in weight. If, as is assumed, the insulin-stimulated rerouting of FFA toward adipose tissue is the predominant effect, a second disadvantage would be an enforced replenishment of fat cells, which is cosmetically and metabolically unwelcome. An advantage, on the other hand, may be that the postprandial concentration of atherogenic VLDL becomes markedly lower, which reduces both their interaction with vascular cells and the consecutive exchange effects causing small dense LDL and low HDL. On balance, addition of glucose to a fatty meal prevents the postprandial rise in atherogenic VLDL and might be beneficial, as long as the calorie addition is compensated by calorie restriction in other meals. However, this question warrants long-term studies on control of the body weight, body fat and the effects on lipoproteins.

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