

Postprandial lipoprotein, glucose and insulin responses after two consecutive meals containing rapeseed oil, sunflower oil or palm oil with or without glucose at the first meal

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There is increasing evidence that the degree of postprandial lipaemia may be of importance in the development of atherosclerosis and IHD. Postprandial lipid, lipoprotein, glucose, insulin and non-esterified fatty acid (NEFA) concentrations were investigated in eleven healthy young males after randomized ingestion of meals containing rapeseed oil, sunflower oil or palm oil with or without a glucose drink. On six occasions each subject consumed consecutive meals (separated by 1.75 h) containing 70 g (15 g and 55 g respectively) of each oil. On one occasion with each oil 50 g glucose was taken with the first meal. One fasting and fifteen postprandial blood samples were taken over 9 h. There were no statistically significant differences in lipoprotein and apolipoprotein responses after rapeseed, sunflower and palm oils, whereas insulin responses were lower after sunflower oil than after rapeseed oil (ANOVA, $P=0.04$). The NEFA and triacylglycerol concentrations at 1.5 h were reduced when 50 g glucose was taken with the first meal (ANOVA, $P<0.0001$ and $P<0.05$ respectively), regardless of meal fatty acid composition. In conclusion, the consumption of glucose with a mixed meal containing either rapeseed, sunflower or palm oil influenced the immediate triacylglycerol and NEFA responses compared with the same meal without glucose, whereas no significant effect on postprandial lipaemia after a subsequent meal was observed. The fatty acid composition of the meal did not significantly affect the lipid and lipoprotein responses, whereas an effect on insulin responses was observed.

Fatty acid composition: Postprandial lipaemia: Glucose

Results from epidemiological and experimental studies suggest that non-fasting lipid metabolism plays a role in the development of atherosclerosis and IHD. Concentrations of non-fasting plasma triacylglycerols (TAG) and TAG-rich lipoproteins are elevated in patients with IHD (Simpson *et al.* 1990; Groot *et al.* 1991; Patsch *et al.* 1992), and a raised non-fasting serum TAG concentration was found to be an independent risk factor for mortality from IHD in 24 500 middle-aged women in Norway (Stensvold *et al.* 1993). This association may be causal because increased concentrations of remnants of chylomicrons and VLDL may be atherogenic (Zilversmit, 1979; Karpe, 1997). The production and clearance of lipoproteins and lipoprotein-derived remnants are affected by diet composition. The quantity of dietary fat increases postprandial lipaemia in a dose-dependent manner (Cohen *et al.* 1988; Dubois *et al.* 1994, 1998), while the effect of dietary fat quality on

postprandial lipaemia has been addressed in only a few well-designed studies. Weintraub *et al.* (1988) and Harris *et al.* (1988) found no differences in the postprandial TAG metabolism after meals differing in fatty acid composition, while Zampelas *et al.* (1994) showed a decreased non-fasting TAG concentration in plasma after meals containing large amounts of long-chain *n-3* polyunsaturated fatty acids compared with a meal containing a mixed oil representing the UK dietary fatty acid intake.

Several studies have demonstrated an effect of meal carbohydrate composition on postprandial lipaemia. Addition of glucose and soluble fibre to a meal resulted in lower postprandial lipoprotein concentrations (Cohen *et al.* 1988; Cohen & Berger, 1990; Cara *et al.* 1992; Sandström *et al.* 1994; Dubois *et al.* 1995), while addition of fructose augmented postprandial lipaemia (Nikkila & Pelkonen, 1966; Cohen & Schall, 1988). The effect of glucose is

Abbreviations: AUC, area under the curve; NEFA, non-esterified fatty acids; PO, palm oil; RO, rapeseed oil; SO, sunflower oil; TAG, triacylglycerols.

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thought to be mediated in part by the increased insulin secretion (Nilsson-Ehle *et al.* 1975) stimulating lipoprotein lipase (EC 3.1.1.34) activity and the clearance of lipids after meals (Sadur & Eckel, 1982). The lipid-lowering effect of some fibres is thought to be, in part, a consequence of decreased fat and cholesterol absorption in the small intestine (Lairon, 1996). Furthermore, it has been observed that the fatty acid composition of the diet affects the glucose and insulin responses after meals (Rasmussen *et al.* 1996; Joannic *et al.* 1997). As insulin plays a central role in lipid metabolism, carbohydrate metabolism and lipid metabolism are strongly interrelated.

Most investigations of postprandial lipaemia have studied the response to a single fat-rich meal (Weintraub *et al.* 1988; Demacker *et al.* 1991; Zampelas *et al.* 1994), or to liquid-formula and lipid emulsions (Harris *et al.* 1988; de Bruin *et al.* 1993). However, in a more realistic meal setting the composition of one meal may affect the glucose and insulin responses to the next meal (Jenkins *et al.* 1982; Trinick *et al.* 1986; Wolever *et al.* 1988). Consequently, non-fasting non-esterified fatty acid (NEFA) and TAG concentrations may be influenced by the carbohydrate composition of the previous meal, since their regulation is under the influence of insulin (Sadur & Eckel, 1982; Frayn, 1993; Coppack *et al.* 1994). Similarly to elevated postprandial TAG concentrations (Simpson *et al.* 1990; Groot *et al.* 1991; Patsch *et al.* 1992) elevated plasma NEFA concentration, especially in the postprandial period has been proposed to be a risk marker of IHD (Frayn *et al.* 1996). Thus, for a better understanding of the role of diet in development and prevention of IHD, investigations of the effect of diet composition under realistic meal conditions on postprandial NEFA and TAG metabolism are needed.

The aims of the present study were to evaluate the effect of dietary fatty acid composition (rapeseed, sunflower and palm oils) on postprandial lipid, glucose and insulin responses after intake of two consecutive solid test meals. A further aim was to study to what extent a glucose load at the first meal affects the postprandial NEFA and TAG responses after the first and a subsequent meal.

Subjects and methods

Subjects

Twelve young males were recruited for the study by local advertisement. One dropped out before the start due to an event unrelated to the study, one subject performed three and one performed five of the six tests. The eleven subjects who participated were aged 21–27 years (mean 24 years), weighed from 69 to 92 kg (mean 78 kg) and had BMI from 20 to 26 kg/m² (mean 23 kg/m²). All subjects were apparently healthy and had no family history of atherosclerotic disease or hypertension. They were non-smokers and did not use any medication. All subjects performed moderate amounts of physical activity (cycling to work and/or training a maximum of 1–2 h/week). The aim of the study was fully explained to the subjects who gave their written consent. The research protocol was approved by the Scientific Ethics Committee of the municipalities of Copenhagen and Frederiksberg (01-272/95).

Experimental design

The study was performed as a double-blind crossover experiment. Subjects were randomized to six different tests separated by a minimum of 3 weeks. They were instructed not to change dietary habits or physical activity level during the study period. The evening before the day of meal testing subjects consumed a standardized low-fat meal (4.8 MJ, 26.5 g fat) provided by the department. The subjects were instructed to avoid any heavy physical exercise and abstain from alcohol 48 h before the meal testing.

On the morning of each study day body weight was measured, and following the insertion of an indwelling cannula and supine rest for 10 min, a fasting blood sample was taken. The test meals were consumed at 09.00 hours (time 0 h) and at 10.45 hours (time 1.75 h). Postprandial blood samples were taken every 15 min for 1.5 h after the first meal, every 30 min for 2.5 h after the second meal, and thereafter hourly until the last blood sample 9 h after the consumption of the first meal (fifteen blood samples).

The six different test meals were consumed under supervision. On three of the test days test meals containing 15 and 55 g (breakfast and lunch respectively) of either rapeseed oil (RO), sunflower oil (SO) or palm oil (PO) were consumed. On the remaining three test days identical test meals with the three test oils were taken, and in addition 50 g glucose (dissolved in 185 ml water) was taken together with the breakfast: RO+glu, SO+glu and PO+glu respectively. Subjects consumed nothing but water, kept their physical activity to a minimum and did not leave the department during the day of meal testing (08.00–18.00 hours).

Meals

Test meals consisting of rice, beef and vegetables were prepared in one batch, weighed into breakfast and lunch portions, and kept at –20° until the day of consumption. After addition of 15 g oil (breakfast) and 55 g oil (lunch) to the test meals, they were heated carefully before being served with white bread. Breakfast, including 200 ml water was consumed in 10 min and lunch, including 400 ml water, in 20 min. RO, SO and PO were chosen to represent vegetable oils relatively rich in monounsaturated fatty acids, polyunsaturated fatty acids and saturated fatty acids respectively.

Food analysis

Duplicate portions of the non-glucose test meal (without test oils) were chemically analysed. N was determined according to the principle of Dumas (Kirsten & Hesselius, 1983) on an automatic N analyser (NA 1500; Carlo Erba Strumentazione, Milan, Italy). Fat content was measured after extraction according to the procedure by Folch *et al.* (1957), and the content of dietary fibre was determined enzymically (Asp *et al.* 1983) (Table 1). The fatty acid composition of the test oils was analysed by GC (HP 6890, Hewlett Packard, Germany) after methylation with methanolic KOH (Christopherson & Glass, 1969) (Table 2).

Table 1. Nutrient contents of the test meals

	Breakfast	Breakfast + glucose	Lunch
Energy (MJ)*	1.5	2.4	5.6
Fat (g)†	17.4	17.4	63.6
Protein (g)†	9.1	9.1	32.3
Carbohydrate (g)‡	42.8	92.8	152.8
Fibre (g)†	4.6	4.6	16.4

* Calculated as the sum of the energy contributions from fat, protein and carbohydrate.

† The analysed values are means of measurements of two duplicate portions.

‡ The nutrient content of the test oils was calculated and added to the results of the chemical analyses.

‡ Calculated as weight of dry matter minus the sum of protein, fat and ash.

Blood sampling

Blood samples were collected in tubes without additives for the analysis of C-reactive protein, in tubes containing NaF for the analysis of glucose, and in tubes containing EDTA for the analysis of insulin, NEFA, cholesterol, TAG, apolipoprotein (apo) A-1, apoB and lipoprotein fractionation. All plasma samples were immediately placed on ice and centrifuged at 3000 g for 15 min at 4°. Samples for serum C-reactive protein, plasma glucose, cholesterol, TAG, apoA-I and apoB were kept at -20°, samples for plasma insulin and NEFA at -80°, and samples for lipoprotein fractionation were kept at 4° until the next day. All subjects had serum C-reactive protein concentrations below 5 mg/l indicating no acute-phase response that could have influenced the analysis of other blood variables.

Blood analysis

All samples from each subject were analysed within a single run, except for the determination of cholesterol and TAG in lipoproteins which was done in fresh plasma after every test day. Serum C-reactive protein and plasma apoA-I and apoB levels were assessed by commercial immuno-turbidimetric methods (Roche, Basel, Switzerland), plasma glucose by a commercial enzymic method (Gluco-quant, Roche), plasma insulin by a standard radioimmunoassay kit (Insulin RIA 100; Pharmacia AB, Uppsala, Sweden) and plasma NEFA with a commercial enzymic colorimetric method (Wako Chemicals GmbH, Neuss, Germany).

Chylomicrons, VLDL + chylomicron remnants, and LDL + HDL fractions were separated by ultracentrifugation. Chylomicrons were isolated by carefully overlaying 3 ml plasma

Table 2. Analysed fatty acid compositions of the test oils (mol/100 mol)

	Rapeseed oil	Sunflower oil	Palm oil
SFA, total	7.0	11.1	39.2
palmitic acid (16:0)	4.9	6.8	33.9
stearic acid (18:0)	1.7	4.1	3.3
MUFA, total	63.0	21.4	46.8
oleic acid (18:1n-9)	58.5	20.3	45.0
PUFA, total	30.0	67.5	14.1
linoleic acid (18:2)	26.2	66.6	13.7
α -linolenic acid (18:3n-3)	3.8	0.7	0.3

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

with 2.5 ml saline of density 1.006 kg/l according to the nomogram of Dole & Hamlin (1962). The ultracentrifugation tubes (Quick-seal polyallomer; 13 × 64 mm, Beckman Instruments, Palo Alto, CA, USA) were centrifuged for 23 min at 20° at 100 000 g in an ultracentrifuge (L7-55; Beckman Instruments) using a fixed-angle rotor (50.4 Ti; Beckman Instruments). The tubes were sliced 45 mm from the bottom, and the top fraction ($S_f > 400$) was transferred and adjusted to a total volume of 5 ml with saline. The bottom fraction was transferred to another ultracentrifugation tube, adjusted to 5.5 ml with saline of density 1.006 kg/l and centrifuged for 16 h at 4° at 105 000 g. After tube-slicing 35 mm from the bottom, the top fraction containing chylomicron remnants and VLDL and the bottom fraction containing LDL + HDL were transferred to separate tubes and adjusted to a final volume of 5 ml.

Total, chylomicron, VLDL, LDL + HDL-cholesterol and TAG concentrations were assessed by commercial enzymic methods (Boehringer Mannheim GmbH, Mannheim, Germany) on a Cobas Mira analyser (Roche). HDL-cholesterol and HDL-TAG concentrations were determined after precipitation with polyethylene glycol (Quantolip, Immuno AG, Vienna, Austria). LDL-cholesterol concentration was calculated as the difference between HDL-cholesterol concentration and the cholesterol concentration in the LDL + HDL fraction, similarly for LDL-TAG concentration.

Control sera HK91 (DEKS, Clinical Biochemistry, Copenhagen County Herlev Hospital/University of Copenhagen, Denmark) and Precinorm L (Boehringer Mannheim GmbH) were used for quality control of the analysis. The intra-assay CV for the determinations were: glucose 1.6, insulin 2.0, NEFA 1.5, cholesterol 1.0, HDL-cholesterol 1.8, TAG 0.4. The inter-assay CV for the determinations were: glucose 2.2, insulin 5.0, NEFA 2.2, cholesterol 1.0, HDL-cholesterol 3.2, TAG 0.8.

Statistical analysis

Results are presented as mean values and 95 % CI (in text) and means with their standard errors (in figures), unless otherwise stated. Fasting values from the six test days were analysed by ANOVA. Paired *t* tests were used to analyse differences in postprandial and fasting concentrations. Summary measures relevant to the individual variables were calculated and data were checked for normal distribution. Summary measures for glucose and insulin were areas under the postprandial response curve and peak concentrations. Areas under the curves (AUC) were divided into 'first' AUC: time 0 h to time 1.5 h, 'second' AUC (glucose): time 1.5 h to time 4.75 h, and 'second' AUC (insulin): time 1.5 h to 8.75 h. Peak concentrations were 'first' peak: maximum value after time 0 h to time 1.5 h, 'second' peak (glucose): maximum value in the time period 1.5 h to 4.75 h, and 'second' peak (insulin): maximum value in the time period 1.5 h to 8.75 h. NEFA minimum postprandial level (minimum after time 0 h to time 8.75 h) was used and summary measures for TAG were AUC (time 0 h to time 8.75 h), and peak concentration (maximum value from time 0 h to time 8.75 h). To analyse for differences of the summary measures a two-way ANOVA was performed with subject, oil, glucose and the interaction between oil

and glucose as independent variables and the fasting value as covariate in the model. If the ANOVA indicated significant differences, it was followed by *post hoc* statistical analysis using modified *t* tests according to the Bonferroni method. *P* values > 0.1 were not Bonferroni corrected. Power calculations (power 0.85, significance level $P < 0.05$) showed that with a sample size of eleven subjects it should be possible to detect a difference in peak values of plasma TAG of 0.7 mmol/l, of plasma glucose of 0.8 mmol/l, of plasma insulin of 70 pmol/l and in minimum values of plasma NEFA of 0.05 mmol/l (Altman, 1991).

Results

No significant changes in the body weights of the subjects were observed during the study period. There were no significant differences on the six study days in fasting plasma glucose, insulin, cholesterol or TAG concentrations.

Lipid and lipoprotein response

The plasma total and chylomicron TAG concentrations reached a maximum 3 h after the second meal (4.75 h from start) and had returned to preprandial levels 4 h later (8.75 h) (Fig. 1).

After RO and SO the heights of the postprandial total TAG peaks were larger than after PO (mmol/l: RO 2.38 (95 % CI 1.91, 2.85); SO 2.43 (95 % CI 2.12, 2.73); PO 1.98 (95 % CI 1.48, 2.48)). However, this difference was not statistically significant (ANOVA, $P = 0.7$) (Fig. 1). Postprandial lipoprotein and apolipoprotein responses were not significantly different after RO, SO and PO. Intake of 50 g glucose resulted in lower increases of total and chylomicron TAG concentrations after the first meal regardless of oil quality (TAG at 1.5 h minus TAG at 0 h; ANOVA, $P < 0.0001$) (total TAG mmol/l; RO + glu *v.* RO: 0.006 (95 % CI -0.08, 0.09) *v.* 0.15 (95 % CI 0.05, 0.25); SO + glu *v.* SO: -0.06 (95 % CI -0.11, 0.01) *v.* 0.17 (95 % CI 0.04, 0.30); PO + glu *v.* PO, -0.01 (95 % CI -0.12, 0.09) *v.* 0.16 (95 % CI 0.08, 0.24). LDL-, HDL- and VLDL-TAG concentrations

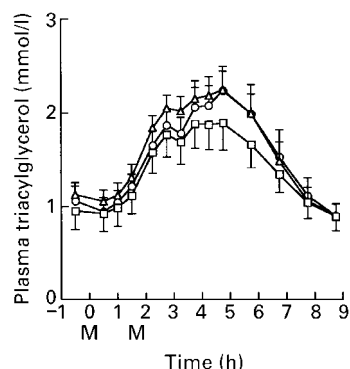


Fig. 1. Total plasma triacylglycerol concentrations after consumption of two consecutive test meals containing 15 and 55 g of a test oil respectively. (□), Palm oil; (○), rapeseed oil; (△), sunflower oil. Values are means for eleven healthy male subjects, with their standard errors represented by vertical bars. M, time of meal consumption.

(results not shown) were not significantly influenced by glucose in the first meal. VLDL-TAG increased significantly above fasting levels, peaked at 4.75 h and decreased below fasting levels at 8.75 h. LDL-TAG concentrations were significantly higher at 8.75 h compared with fasting values, while HDL-TAG decreased significantly below fasting levels at time 1.5 h, after which concentrations increased above fasting levels. After the meals RO + glu and SO + glu peak HDL-TAG concentrations were similar to fasting levels.

After the second meal there were no significant differences in the TAG responses between the six test meals. Eight out of ten subjects (one subject did not have PO + glu) had higher TAG peaks when they ingested glucose with PO than PO alone (mmol/l; PO + glu *v.* PO: 2.54 (95 % CI 1.99, 3.08) *v.* 1.98 (95 % CI 1.49, 2.47), Fig. 2). However, this difference was not statistically significant (ANOVA, $P = 0.19$, PO + glu *v.* PO, $P = 0.11$). The three groups combined, *i.e.* oil with glucose *v.* oil, resulted in no statistically significant difference in TAG concentrations (mmol/l; oil + glu *v.* oil: 2.38 (95 % CI 2.02, 2.74) *v.* 2.28 (95 % CI 1.86, 2.70).

There were no differences between RO, SO and PO with or without glucose in the meal with respect to the cholesterol content of the lipoproteins in the postprandial period. The pooled values from all tests are shown in Fig. 3. Chylomicron-cholesterol concentrations increased significantly and peaked at 4.75 h, while VLDL-cholesterol concentrations were unchanged, until the concentrations decreased at 8.75 h. LDL- and HDL-cholesterol concentrations decreased postprandially to a minimum at 4.75 h, except for the meal RO, after which the LDL-cholesterol level was similar to fasting values.

Plasma glucose response

Plasma glucose concentrations showed an initial rise 30 min after both meals and returned to fasting levels 3 h after lunch (4.75 h) (Fig. 4). There were no significant differences between RO, SO and PO regarding postprandial glucose responses. The plasma glucose concentrations were higher

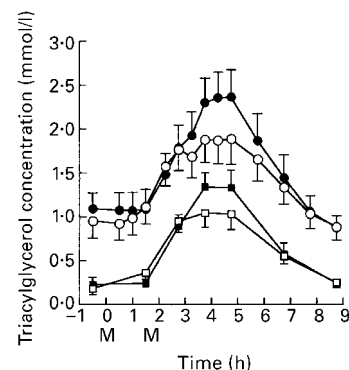


Fig. 2. Total plasma (●, ○) and chylomicron (■, □) triacylglycerol concentrations after consumption of two consecutive test meals containing 15 and 55 g palm oil respectively. (●, ■), Test meals containing palm oil plus a 50 g glucose drink at the first meal; (○, □), test meals containing palm oil. Values are means for eleven healthy male subjects, with their standard errors represented by vertical bars. M, time of meal consumption.

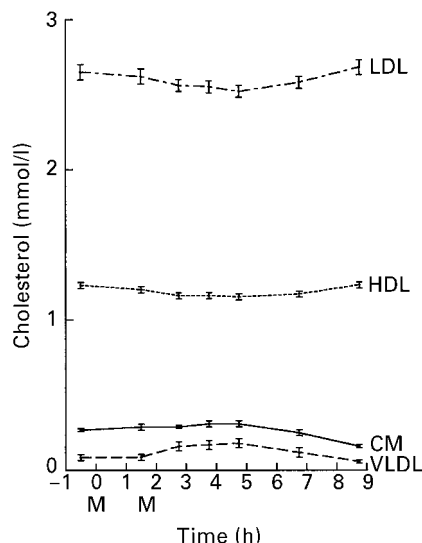


Fig. 3. Chylomicron (CM), VLDL, LDL and HDL cholesterol concentrations after consumption of two consecutive test meals containing 15 and 55 g test oil respectively. Results are pooled values from six different tests (rapeseed oil, sunflower oil or palm oil with and without a 50 g glucose drink at the first meal) and are presented as mean values for eleven healthy male subjects, with their standard errors represented by vertical bars.

(‘first’ peak and ‘first’ AUC) when glucose was ingested in the first meal (ANOVA, $P < 0.0001$) irrespective of the oil. The plasma glucose responses to the second meal (2.25 h) did not differ between any of the tests and were thus not influenced by ingestion of glucose in the previous meal.

Plasma insulin response

At 30 min after both meals (time 0.5 h and 2.25 h) insulin responses to SO were lower than those to RO-containing meals, with insulin responses to PO-containing meals intermediate (mmol/l; time 0.5 h, SO+glu: 266 (95% CI 212, 319), RO+glu: 351 (95% CI 241, 460); time 2.25 h, SO+glu: 241 (95% CI 144, 339), RO+glu: 323 (95% CI 172, 474); ANOVA, $P = 0.04$; Figs. 4 and 5). Insulin concentrations (‘first’ peak) were higher and ‘first’ AUC were larger when glucose was consumed at the first meal ($P < 0.0001$) irrespective of the oil quality, and similarly to plasma glucose, the plasma insulin responses to the second meal were not influenced by ingestion of glucose in the previous meal.

Plasma non-esterified fatty acid response

The NEFA responses to the meals are shown in Fig. 6. The fasting NEFA values showed a large intra-individual variation, and there was a borderline significant difference between RO and RO+glu (ANOVA, $P = 0.05$). There were no differences between RO, SO and PO with respect to the postprandial plasma NEFA responses. Ingestion of glucose in the previous meal compared with no glucose resulted in lower plasma NEFA concentrations at 1.5 h regardless of dietary fatty acid composition (mmol/l; time 1.5 h, oil+glu: 0.06 (95% CI 0.05, 0.07), oil: 0.12 (95% CI 0.11, 0.14); ANOVA, $P < 0.0001$).

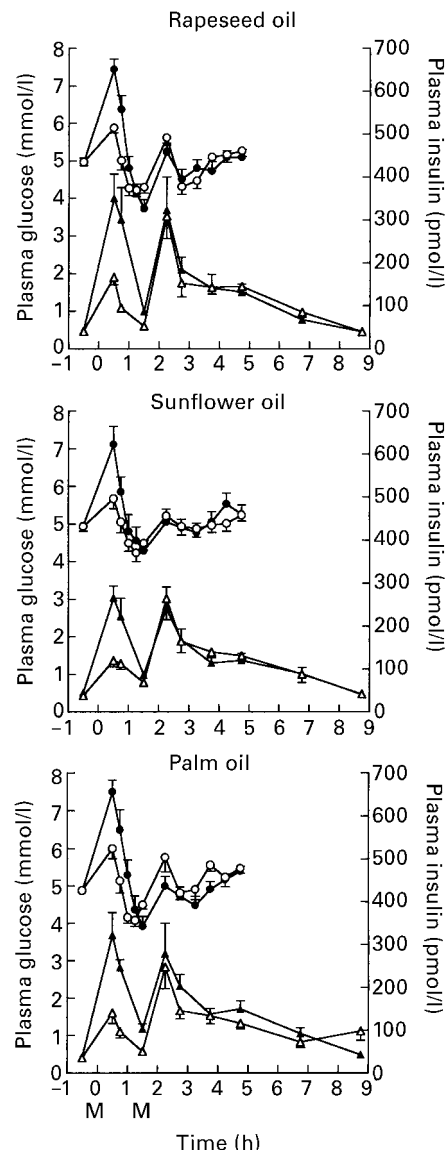


Fig. 4. Plasma glucose (●, ○) and insulin (▲, △) concentrations after consumption of two consecutive test meals containing 15 and 55 g oil respectively. (●, ▲), Test meals containing a test oil plus a 50 g glucose drink at the first meal; (○, △) test meals containing a test oil. Values are means for eleven healthy male subjects, with their standard errors represented by vertical bars. M, time of meal consumption.

Discussion

The present study was designed to evaluate the acute effect of consecutive solid meals rich in monounsaturated, polyunsaturated and saturated fatty acids, represented by RO, SO and PO, on postprandial lipid, glucose and insulin responses. Furthermore, the study aimed at evaluating to what extent a glucose load affected the immediate postprandial lipid, lipoprotein and NEFA responses, and any possible second-meal effect.

Total TAG peak concentration after ingestion of PO was 0.40 and 0.45 mmol/l lower than TAG peak concentrations after RO and SO respectively (Fig. 1). This difference was not statistically significant, probably because of the limited number of participants. At a power of 0.85 this study was

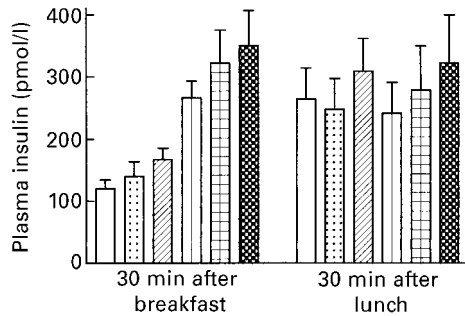


Fig. 5. Plasma insulin responses 30 min after consumption of two consecutive test meals containing 15 and 55 g oil respectively. (□), Sunflower oil; (▨), palm oil; (▧), rapeseed oil; (▩), sunflower oil with a 50 g glucose drink at breakfast; (▪), palm oil plus a 50 g glucose drink at breakfast; (▫), rapeseed oil plus a 50 g glucose drink at breakfast. Values are means for eleven healthy male subjects, with their standard errors represented by vertical bars.

only able to detect a significant difference in peak values of plasma TAG equal to or larger than 0.7 mmol/l as described on p. 100. However, in similar studies no effect on postprandial lipid concentrations was observed after meals differing in fatty acid composition (Harris *et al.* 1988; Weintraub *et al.* 1988). Only meals containing large amounts of *n*-3 long-chain polyunsaturated fatty acids seem to have an acute effect on postprandial lipaemia (Zampelas *et al.* 1994; Williams, 1997). The postprandial changes of the lipoproteins during the day seen in the present study were similar to results from other studies (Cohn *et al.* 1988; De Bruin *et al.* 1991; Dubois *et al.* 1994, 1998). The inverse relationship between the increase in chylomicron-cholesterol and the decrease in HDL-cholesterol concentrations is probably a result of the cholesteryl ester transfer protein-mediated transfer of cholesteryl esters from HDL to TAG-rich lipoproteins (Tall, 1993). The reverse transfer of TAG from TAG-rich lipoproteins to HDL may explain the postprandial increase in HDL-TAG concentrations observed in the present study.

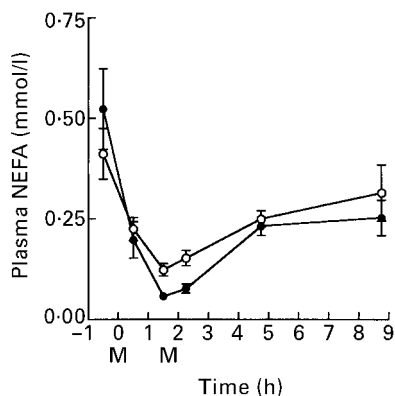


Fig. 6. Plasma non-esterified fatty acid (NEFA) concentrations after consumption of two consecutive test meals containing 15 and 55 g oil respectively. (●), Pooled values from test meals containing rapeseed oil, sunflower oil or palm oil plus a 50 g glucose drink at the first meal; (○), pooled values from test meals containing rapeseed oil, sunflower oil or palm oil. Values are means for eleven healthy male subjects, with their standard errors represented by vertical bars. M, time of meal consumption.

The addition of 50 g glucose to the first meal lowered the plasma total and chylomicron TAG responses to this meal, irrespective of the type of oil. This is consistent with an early study of nine subjects in which addition of 50 g glucose to a mixed meal containing 60 g dairy fat reduced alimentary lipaemia (Albrink *et al.* 1958). Later Cohen & Berger (1990) confirmed this finding in a larger study in which the addition of 50 g (*n* 55) or 100 g (*n* 18) glucose to a liquid formula containing 40 g fat as dairy cream, resulted in suppression of postprandial lipaemia. The decrease in postprandial lipaemia caused by glucose may be explained by the stimulation of lipoprotein lipase by insulin, leading to increased catabolism of TAG in TAG-rich lipoproteins and thereby reduction of plasma TAG concentrations. Added glucose might also reduce postprandial lipaemia via delayed gastric emptying (Cohen & Berger, 1990). However, not all studies find that addition of glucose to fat-containing meals results in less postprandial lipaemia (Mann *et al.* 1971; Nicholls & Cohen, 1985; Cohen & Schall, 1988). The lack of effect of ingestion of glucose on postprandial lipaemia despite a rise in insulin has been explained by the use of low glucose doses (50 g) as in the studies of Cohen & Schall (1988) and Nicholls & Cohen (1985). Cohen & Berger (1990) found that when 50 g glucose doses were used, the decrease in postprandial lipaemia (20%) was small and similar to the intra-individual variation seen after oral fat tolerance tests containing 40 g fat (Cohen *et al.* 1988), whereas with 100 g glucose doses the TAG reduction was greater than the variation. Additionally, early studies suggest that the amount of glucose required to lower postprandial TAG concentrations varies considerably between subjects (Albrink *et al.* 1958). In the present study we observed a TAG-lowering effect in young healthy subjects of 50 g glucose after ingestion of a meal containing only 15 g fat, suggesting that the proportion of rapidly absorbable carbohydrates in relation to fat is of importance for the TAG response.

In the present study, there were no significant effects of a glucose load on postprandial lipaemia after the second meal. However, when glucose was consumed at the first meal in combination with PO, 80% of the subjects had higher TAG peaks compared with PO alone (Fig. 2). The peak difference of 0.56 mmol/l was not significant (ANOVA, $P=0.19$, PO + glu *v.* PO, $P=0.11$) probably due to the limited number of participating subjects resulting in insufficient statistical power of the study. When the TAG value before the second meal (time 1.5 h) was used as a covariate in the model instead of the fasting value, the TAG peak difference reached significance for PO-containing meals with and without glucose, while there were no significant differences after RO- and SO-containing meals (ANOVA, $P<0.006$, RO + glu *v.* RO, $P=0.2$; SO + glu *v.* SO, $P=0.4$; PO + glu *v.* PO, $P=0.005$). However, when the fasting value was included in the statistical model, the TAG peak difference was insignificant, indicating that the effect of glucose addition on TAG concentration might be a function of the fasting TAG concentration. Anyway, as this tendency of a higher postprandial TAG peak after glucose addition to a meal is in contrast with what would be expected, it needs to be confirmed in larger studies.

The lower insulin responses 30 min after meals rich in SO

than after meals rich in RO seen in the present study, are in accordance with results obtained recently by Joannic *et al.* (1997). In a study of eight subjects, they found that glucose and insulin responses after 30 min were significantly lower after meals containing polyunsaturated fatty acids than after meals containing monounsaturated fatty acids, when the data from rice and potato meals were pooled. Recently Rasmussen *et al.* (1996) showed that insulin release was stimulated by butter but not olive oil in patients with non-insulin-dependent diabetes mellitus. The authors explained this result in part by differences in fatty acid chain length. In addition, the amount of fat in a meal seems to be able to affect glucose and insulin responses. Fat doses of 0, 15, 30 and 40 g have been shown to produce similar insulin responses, while 50 g fat produced significantly higher responses (Dubois *et al.* 1998). It is noteworthy that the present study showed differences in insulin responses to RO and SO meals containing only 15 g oil (ANOVA, $P=0.04$).

In the present study there was no effect of meal fatty acid composition on plasma NEFA concentrations. The addition of glucose to the meal led to significantly lower NEFA values, which may be explained by enhanced suppression of hormone-sensitive lipase (EC 3.1.1.3) activity by insulin (Frayn *et al.* 1994). The second meal did not decrease plasma NEFA further, in spite of the rise in insulin. This may be due to the opposing effect of overspill of NEFA into plasma during lipoprotein lipase-mediated degradation of TAG-rich lipoproteins (Binnert *et al.* 1996).

A limitation of this study is a lack of measurement of remnant particles (Karpe *et al.* 1995), which would have helped to differentiate between native chylomicrons and remnant particles, and to reveal any difference in absorption rates between the fats. Ideally this should have been done by measurements of apoB-48, a structural protein of the chylomicron. Simultaneous administration of retinyl palmitate and measurements of retinyl palmitate in plasma and lipoproteins have also been used to follow the postabsorptive fate of chylomicrons. An earlier study (Sandström *et al.* 1994) in young healthy subjects showed a good correlation between retinyl palmitate and total TAG, and the chylomicron fraction accounted for most of the rise in retinyl palmitate, while only small amounts of retinyl palmitate were found in the non-chylomicron fraction, indicating that in young healthy subjects most of the chylomicron remnants are found in the chylomicron fraction. With the consecutive meal design in the present study this technique was not judged to be applicable. Furthermore, measurements of remnant particles are presumably more important in older, overweight subjects or those with established disease.

Only a few studies of postprandial lipid and carbohydrate metabolism after consecutive meals have been published. One report in middle-aged subjects by Frape *et al.* (1997) showed that glucose and insulin responses were influenced by the fat content of a previous meal and that the different responses after the first and second meals were not related to an overnight fast or the time of day. In the present study of young healthy subjects, where the fat content in the first meal was held constant, consumption of glucose with a mixed meal lowered the immediate TAG and NEFA concentrations compared with the same meal without glucose. An effect of fatty acid composition on the insulin response

was observed, but the composition of the first meal did not affect the metabolic response to the second meal. We find that the acute influence of dietary fat quality on insulin responses and the impact of meal composition and pattern on postprandial lipid metabolism need to be studied further. The increased risk of IHD in individuals with insulin resistance and elevated non-fasting TAG underlines the suggestion that these issues may be of great importance.

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