British Journal of Nutrition (2001), **86**, 181–188
DOI: 10.1079/BJN2001365

Flow-mediated vasodilation is not impaired when HDL-cholesterol is lowered by substituting carbohydrates for monounsaturated fat

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(Received 29 September 2000 – Revised 8 January 2001 – Accepted 30 January 2001)

Low-fat diets, in which carbohydrates replace some of the fat, decrease serum cholesterol. This decrease is due to decreases in LDL-cholesterol but in part to possibly harmful decreases in HDL-cholesterol. High-oil diets, in which oils rich in monounsaturated fat replace some of the saturated fat, decrease serum cholesterol mainly through LDL-cholesterol. We used these two diets to investigate whether a change in HDL-cholesterol would change flow-mediated vasodilation, a marker of endothelial function. We fed thirty-two healthy volunteers two controlled diets in a $2 \times 3{\cdot}5$ weeks' randomised cross-over design to eliminate variation in changes due to differences between subjects. The low-fat diet contained 59·7 % energy (en%) as carbohydrates and 25·7 en% as fat (7·8 en% as monounsaturates); the oil-rich diet contained 37·8 en% as carbohydrates and 44·4 en% as fat (19·3 en% as monounsaturates). Average (SD) serum HDL-cholesterol after the low-fat diet was 0·21 (SD 0·12) mmol/l (8·1 mg/dl) lower than after the oil-rich diet. Serum triacylglycerols were 0·22 (SD 0·28) mmol/l (19·5 mg/dl) higher after the low-fat diet than after the oil-rich diet. Serum LDL and homocysteine concentrations remained stable. Flow-mediated vasodilation was 4·8 (SD 2·9) after the low-fat diet and 4·1 (SD 2·7) after the oil-rich diet (difference 0·7 %; 95 % CI −0·6, 1·9). Thus, although the low-fat diet produced a lower HDL-cholesterol than the high-oil diet, flow-mediated vasodilation, an early marker of cardiovascular disease, was not impaired.

Lipoproteins: Cardiovascular disease: Diet

Diets low in saturated fats and high in carbohydrates are often advocated to reduce the risk of cardiovascular disease (CVD) because they lower serum total and LDL-cholesterol (Schaefer *et al.* 1995; Clarke *et al.* 1997; Turley *et al.* 1998). However, there has been a debate about whether to lower the intake of saturated fats by decreasing total fat intake or by replacing them with *cis*-unsaturated fats. Supporters of low-fat diets argue that replacement of fat by carbohydrates will not only decrease risk of CVD through lowering of serum cholesterol but will also help people lose weight (Connor & Connor, 1997) and thus prevent obesity (Bray & Popkin, 1998; Miller *et al*. 1998). However, others argue that low-fat diets might not be the wisest recommendation because these diets lower HDL-cholesterol (Katan *et al.* 1997), which may increase the risk of CHD (Pearson *et al.* 1979; Gordon *et al.* 1986, 1989; Huttunen *et al.* 1991; Castelli *et al.* 1992; Kitamura *et al.* 1994; de Backer *et al.* 1998; Ballantyne *et al.* 1999; Sharrett *et al.* 1999; Sorlie *et al.* 1999). HDL-cholesterol is not lowered when saturated and *trans* fatty acids are replaced by unsaturated vegetable oils, and therefore diets rich in vegetable oils might be a good alternative to low-fat diets (Katan *et al.* 1997).

To investigate whether the difference in HDL-cholesterol after a low-fat diet and a high-oil diet would affect risk of CVD, we used flow-mediated vasodilation (FMD) of the brachial artery as an outcome variable. FMD is a measure of endothelial function, which is believed to be an early stage of CVD (Kuhn *et al.* 1991; Clarkson *et al.* 1997). FMD of the brachial artery is mediated by nitric oxide released by the endothelial cells (Joannides *et al.* 1995) and can be measured non-invasively. We chose FMD because it appears to be predictive of cardiovascular events (Neunteufl *et al.* 1999).

In a previous study (de Roos *et al.* 2001) we showed that intake of *trans* fatty acids reduced serum HDL-cholesterol

Abbreviations: CVD, cardiovascular disease; en%, percentage of energy; FMD, flow-mediated vasodilation.

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and impaired FMD in healthy men and women. Although the results of that study seemed to be compatible with a causal relationship between HDL-cholesterol and FMD, a verification of the results was needed. Thus, we investigated whether the difference in HDL-cholesterol after a low-fat diet and an oil-rich diet affected FMD. We applied Bayesian methods to integrate the existing evidence for a protective effect of HDL-cholesterol with the present data.

Methods

The study was approved by the Medical Ethics Committee of Wageningen University. Each volunteer signed an informed consent form.

Subjects

We recruited thirty-nine non-smoking men and women by advertising in the university newspaper and by personally inviting subjects who had taken part in previous studies. We selected subjects on the basis of a medical questionnaire, serum cholesterol (<8 mmol/l) and triacylglycerols (<2 mmol/l), urinary protein (<0·3 g/l) and glucose (<5·5 mmol/l), and a good-quality ultrasound image of the brachial artery. We enrolled thirty-five subjects. One subject withdrew from the study after 1 week because he could not comply with the study protocol. The study was completed by thirty-four subjects; thirteen men and twenty-one women with mean age 27 (range 19–59) years. Their mean (SD) baseline body weight was 68 (SD 9) kg, BMI 22 (SD 2·3) kg/m^2, fasting total cholesterol 4·6 (SD 0·8) mmol/l and triacylglycerols 1·2 (SD 0·5) mmol/l.

Study design

Our aim was to test whether a difference in HDL-cholesterol induced by two different diets would result in a difference in FMD. To minimise the variation in the differences we chose a cross-over design. The order in which the two diets were given was randomly allocated.

We provided two controlled diets for 3·5 weeks each without a wash-out period. The diets were given in a 28 d menu cycle. On Mondays to Fridays subjects came to our dining room and ate a hot meal under our supervision. All other foods (bread; margarine; meat and/or cheese; honey, jam, or sprinkles; fruit; milk and/or yogurt) were provided in a package for consumption at home, as was food for the weekends.

On 2 d during the last week of each diet period, after subjects had consumed the diets for at least 22 d, we measured FMD and serum lipids in the subjects. The measurements were performed 1–2 d apart. Because not all subjects could be measured on the same day, they received the diets for 24–27 d (mean 25 d).

Diets

The two diets consisted of conventional food items. The composition of the two diets was calculated to change the concentration of HDL-cholesterol without changing LDL-cholesterol. Therefore, it was impossible to match the two diets for saturated and polyunsaturated fatty acid intake. We used a low-fat margarine, low-fat dairy products, and extra carbohydrate in the low-fat diet, and olive oil, margarine, and full-fat dairy products in the oil-rich diet (Table 1). The composition of the experimental diets was calculated using food composition tables (Anonymous, 1996; Hulshof *et al.* 1999). We checked the composition of the diets by collecting duplicates of all meals. The analysed values were similar to the calculated composition.

Habitual energy intake of the subjects was estimated from a food frequency questionnaire. We designed menus for fourteen levels of energy intake, ranging from 7 to 20 MJ/d. The subjects were allocated to an energy intake

Table 1. Food items (g/d) provided in a 11 MJ menu of the low-fat and unsaturated oil-rich diet

	Food item	Low-fat diet	Oil-rich diet
Food items that differed in amount and composition	Bread*	233·0	200·0
	Cookies*	30·0	45·0
	Sauce and gravy*	70·0	70·0
	Salad dressing†	15·0	15·0
	Dessert‡	250·0	125·0
	Table spread§	26·0	35·0
Foods that differed in amount only	Starch (potatoes, rice, pasta, bulgur)	270·0	180·0
	Vegetables	230·0	150·0
	Fruit	248·0	124·0
	Salad	38·0	38·0
	Meat	82·0	68·0
	Milk, 1·5 % fat	250·0	200·0
	Eggs	17·5	28·0
	Cheese, 31 % fat	16·0	32·0
	Meat (filling)	36·0	36·0
	Sweet fillings (honey, jam, sprinkles, etc.)	39·0	26·0
	Crisps	9·0	9·0

* Made with margarine (Blueband, Unilever, Vlaardingen, the Netherlands) and extra carbohydrates (Fantomalt, N.V. Nutricia, Zoetermeer Holland) in the low-fat diet and with olive oil (Carbonell, Cordoba, Spain) in the oil-rich diet.
† Made with low-fat salad dressing (5 g fat/100 g) and extra carbohydrates (Fantomalt, N.V. Nutricia, Zoetermeer Holland) in the low-fat diet and with olive oil in the oil-rich diet.
‡ Low-fat desserts in the low-fat diet and full-fat desserts in the oil-rich diet.
§ Low-fat margarine (35 g fat/100 g) in the low-fat diet and full-fat margarine (80 g fat/100 g) in the oil-rich diet.

level close to their habitual energy intake. We provided 90 % of energy (en%) and all food was weighed out for each subject. The remaining 10 en% had to be chosen from a list of low-fat food items. Subjects recorded their choice from this low-fat food list in a diary.

We measured body weight twice a week; if body weight changed more than 1 kg subjects were switched to a different energy intake level.

Blood lipids

We took fasting blood samples on two separate days after day 22 of each diet. All four blood samples of each subject were analysed in duplicate within one run. Total cholesterol and triacylglycerols (Cholesterol Flex™ and Triglycerides Flex™ reagent cartridge, Dade Behring, Newark, NJ, USA) and HDL-cholesterol (liquid N-geneous™ HDL-C assay, Instruchemie BV, Hilversum, the Netherlands) were measured, and LDL-cholesterol was calculated using the Friedewald formula. The coefficient of variation of sixty-four duplicate measurements was 0·4 % for total cholesterol, 1·5 % for triacylglycerols, and 1·1 % for HDL-cholesterol.

Brachial artery measurements

All brachial artery measurements were done in subjects after an overnight fast. We assessed endothelial function as FMD of the brachial artery as described elsewhere (Sorensen *et al.* 1995; Celermajer *et al.* 1996). We measured the diameter of the artery at rest and at maximum vasodilation, and calculated the FMD as the percentage increase. All measurements were done at end-diastole by the use of the R-wave of the electrocardiogram.

The ultrasound images were made with a 7·5 MHz linear array transducer of an Ultramark™ 9 HDI duplex scanner. All images were stored on super-VHS videotapes for off-line analysis.

All measurements were done by one technician in a temperature-controlled room (range 20–24°C). Subjects were lying down with the right arm in two arm support cushions. An inflatable cuff was placed around the forearm. The measurements were done at the site of the antecubital crease. The position of the transducer was held constant during the measurements with a specially developed transducer arm fixature (TAF® method developed by Meijer and colleagues, Vascular Imaging Center, The Julius Center for Patient Oriented Research, UMC Utrecht, the Netherlands).

We first obtained an optimal two-dimensional B-mode ultrasound image of the brachial artery at rest. The search was for a good trailing edge of the adventitia interface of the near wall and a leading edge of the media–adventitia interface of the far wall of the artery. Three optimal images were frozen at the R-wave of the electrocardiogram, at end-diastole, and stored on videotape. These images were used to calculate the resting diameter of the artery. We then inflated the cuff to 250 mmHg and kept this pressure constant for 5 min to induce ischaemia in the forearm and hand. After 5 min the cuff was deflated. The image of the brachial artery was again optimised and changes in the diameter of the artery were recorded during the next 5 min. Every 15 s a frozen image was stored on videotape. At the end of the second feeding period we also measured endothelium-independent vasodilatation after a sublingual dose of 400 μg of nitroglycerin.

All images were read at the Vascular Imaging Center of the University Medical Center in Utrecht by one reader who was blinded to the treatment. The reader rated the quality of the images as class 1 (perfect), class 2 (fair), class 3 (marginal) to class 4 (unfit for use). All thirty-four subjects were measured twice on both diets, so we had four measurements per subject. Of these 136 measurements, sixteen were rated as marginal and three as unfit. We only used measurements rated perfect or fair, which left us with thirty-two subjects for whom we had observations on both diets. At a mean FMD of 4·5 %-units, the within-subjects SD was 2·9 %-units so the corresponding coefficient of variation was 65 %. The biggest difference between duplicate FMD measurements was 9 %-units (measurements: −8·05 and 0·95 %-units); the smallest difference was 0·01 %-units (measurements: 2·83 and 2·82 %-units). The coefficient of variation of the diameter of the brachial artery at rest was 6·9 %.

Serum homocysteine

Total homocysteine concentrations in serum were measured with HPLC and fluorimetric detection (Ubbink *et al.* 1991; Ueland *et al.* 1993). The coefficient of variation was 3·2 % within and 8 % between runs.

Statistics

We averaged the duplicate measurements in each dietary period and then calculated for each subject the difference between treatments. We tested whether these differences were significantly different from zero with Student's *t* test for paired samples. We give two-sided 95 % CI for the differences. All statistical analyses were performed with the SAS System for Windows (SAS Institute Inc., Cary, NC, USA), release 6·12.

We used Bayesian statistics to combine existing evidence for a protective effect of HDL-cholesterol with the present data. The existing evidence was used to postulate an a priori probability (i.e. *before* the present study) for a direct effect of HDL-cholesterol on FMD. We postulated an a priori probability of 75 %. The effect size was estimated from data of our previous study (de Roos *et al.* 2001): we hypothesised that FMD would be 1 %-unit lower on the low-fat diet than on the high-oil diet. The rationale behind this hypothesis was that in the previous study a decrease in HDL-cholesterol of 0·36 mmol/l went together with a decrease in FMD of 1·8 %-units. In the present study we expected to see a decrease in HDL-cholesterol of 0·20 mmol/l on the low-fat diet and therefore a decrease in FMD of 1 %-unit ($1/0{\cdot}2 = 1{\cdot}8/0{\cdot}36$). We used the Bayes factor, which was derived from the *P*-value from the Student's *t* test, to evaluate whether the data from the present study changed the a priori probability (Goodman, 1999).

Table 2. Diet composition in the low-fat and in the unsaturated oil-rich period

Component	Low-fat diet	Oil-rich diet
Energy (MJ/d)	11·4	11·3
(kcal/d)	2702	2693
Carbohydrate (energy%)	59·7	37·8
Protein (energy%)	13·4	16·4
Total fat (energy%)	25·7	44·4
Saturated	10·3	15·5
Lauric acid (C12:0)	0·8	1·3
Myristic acid (C14:0)	0·9	1·5
Palmitic acid (C16:0)	5·7	7·9
Stearic acid (C18:0)	2·2	3·7
Monounsaturated, total	7·8	19·3
Polyunsaturated	6·9	8·8
Cholesterol (mg/MJ)	25·9	34·1
(mg/d)	294	386
Fibre (g/MJ)	2·6	2·4
(g/d)	29·5	27·1
Alcohol (energy%)	1·3	1·2

In both periods 90 % of energy was provided and duplicate meals were analysed. The remaining 10 % of energy was chosen from a list of low-fat food items and the composition of these was calculated.

Results

All results refer to eleven men and twenty-one women for whom data were complete (data for two men were incomplete). They had a mean age of 26·8 (SD 12·8) years, a mean pre-study weight of 68·5 (SD 8·6) kg, and a mean BMI of 22·1 (SD 2·2) kg/m^2.

Body weight and food intake

Body weight was fairly constant during the study and hardly differed between the two diet periods: the average body weight was 68·7 (SD 8·7) kg after the oil-rich diet and 68·6 (SD 8·7) kg after the low-fat diet. On average subjects consumed 10·2 MJ/d of the experimental diets that were provided by us. They consumed an additional 1·1 MJ of free-choice low-fat food items per day. The composition of the total daily food intake (provided food plus free-choice foods) in both periods is given in Table 2.

Blood lipids

Serum HDL-cholesterol was 0·21 mmol/l (8·1 mg/dl) lower after the low-fat diet than after the oil-rich diet (95 % CI, −0·26, −0·17). Serum total cholesterol was 0·14 mmol/l lower after the low-fat diet than after the oil-rich diet (95 % CI −0·27, −0·01). In contrast, serum triacylglycerols were 0·22 mmol/l higher after the low-fat diet than after the oil-rich diet (95 % CI 0·12, 0·32). Serum LDL-cholesterol remained stable (Table 3).

The order in which the two diets were taken barely affected the change in HDL-cholesterol: the mean change was −0·23 (SD 0·14) mmol/l in subjects who changed from the low-fat to the oil-rich diet and 0·20 (SD 0·13) mmol/l in subjects who received the diets in the reverse order.

Brachial artery measurements

The resting and maximum diameter of the brachial artery were hardly affected by the type of diet (Table 4). FMD was slightly better after the low-fat diet than after the oil-rich diet: 4·80 (SD 2·94) % *v.* 4·13 (SD 2·72) % ($P = 0·29$), which was a difference of −0·67 %-units (95 % CI, −1·94, 0·61). Subjects who changed from the oil-rich diet to the low-fat diet showed a bigger change in FMD (1·26 %-units) than subjects who received the diets in reverse order (0·08 %-units).

All subjects showed vasodilation after nitroglycerin (range 1·1–26·4 %), indicating that their smooth muscle cells were able to respond to nitric oxide. The type of diet had hardly any effect on nitroglycerin-mediated vasodilation, which was 10·0 (SD 5·1) % after the low-fat diet and 11·9 (SD 7·3) % after the oil-rich diet.

Table 3. Concentration of serum lipids (in mmol/l) after consumption of the two diets

(Values are means and their standard deviations for thirty-two subjects)

	Oil-rich diet		Low-fat diet		
	Mean	SD	Mean	SD	Difference (95 % CI)
Total cholesterol	4·48	0·87	4·34	0·84	−0·14 (−0·27, −0·01)
HDL-cholesterol	1·66	0·39	1·44	0·35	−0·21 (−0·26, −0·17)
LDL-cholesterol	2·45	0·65	2·42	0·67	−0·03 (−0·12, 0·07)
Triacylglycerols	0·81	0·41	1·03	0·52	0·22 (0·12, 0·32)

The thirty-two subjects consumed both diets for 3·5 weeks in random order. To convert values for total, HDL-, and LDL-cholesterol to mg/dl, multiply by 38·67. To convert triacylglycerols to mg/dl, multiply by 88·54.

Serum homocysteine measurements

Serum homocysteine concentrations were not affected by the difference in the two diets: concentrations after the low-fat diet were 10·0 (SD 2·5) μmol/l and after the oil-rich diet 10·1 (SD 2·7) μmol/l (difference 0·2 μmol/l, 95 % CI −0·3, 0·6).

Bayesian interpretation

Before the study we postulated that FMD would be 1 %-unit lower after the low-fat diet than after the oil-rich diet. We gave this hypothesis an a priori probability of 75 %,

Table 4. Brachial artery measurements after both diets

(Values are means and their standard deviations for thirty-two subjects)

	Oil-rich diet		Low-fat diet	
	Mean	SD	Mean	SD
Resting diameter (mm)	3·91	0·68	3·95	0·55
Maximum diameter (mm)	4·07	0·69	4·13	0·55
Absolute vasodilation (mm)	0·16	0·10	0·18	0·10
Flow mediated vasodilation (%)*	4·13	2·72	4·80	2·94
Endothelium-independent dilation (%)	11·9	7·3	10·0	5·1
Systolic blood pressure (mmHg)	121·9	11·2	120·4	11·5
Diastolic blood pressure (mmHg)	72·2	9·1	71·0	7·4

None of the measurements was statistically significant ($P < 0·05$) between the diets.

* Calculated for each subject as absolute vasodilation divided by resting diameter×100 %.

Table 5. Change in prior probabilities, ranging from weak to strong, to posterior probabilities using data of the present study

Prior probability	Prior odds	Posterior odds	Posterior probability
0·75 (strong)	$0{\cdot}75/(1-0{\cdot}75)=3$	3× Bayes factor* = 0·12	$0{\cdot}12/(1+0{\cdot}12)=0{\cdot}11$
0·50 (equivocal)	$0{\cdot}50/(1-0{\cdot}50)=1$	1× Bayes factor = 0·042	$0{\cdot}042/(1+0{\cdot}042)=0{\cdot}04$
0·25 (weak)	$0{\cdot}25/(1-0{\cdot}25)=0{\cdot}33$	0·33× Bayes factor=0·014	$0{\cdot}014/(1+0{\cdot}014)=0{\cdot}014$

A priori probabilities were first converted to a priori odds. The a priori odds were then multiplied by the Bayes factor to obtain a posteriori odds. Finally, the a posteriori odds were converted to a posteriori probabilities.

* Bayes factor = e to the power $z^2/2$, where z is the z-score of the P-value for obtaining a result as large as +0·67 %-units under the hypothesis that the result would be −1·0 %-units. P – value = 0·0119, z – score = 2·52.

which corresponds with a priori odds of $0{\cdot}75/(1-0{\cdot}75)=3$. From our data we calculated that the probability P of finding an effect of −0·67 %-units under this hypothesis was 0·29. A P-value of 0·29 corresponds with a z-score of 2·52 and a minimum Bayes factor of 0·04. This Bayes factor was used to correct the a priori odds into a posterior odds by multiplication. Thus, the a posteriori odds for the hypothesis was $0{\cdot}04\times 3=0{\cdot}12$, which corresponded with an a posteriori probability of the hypothesis of $0{\cdot}12/(1+0{\cdot}12)=0{\cdot}11$, or 11 % (Table 5). Consequently, smaller a priori probabilities of 50 or 25 % corresponded with even smaller a posteriori probabilities (Table 5).

Discussion

We found that a change in HDL-cholesterol induced by two different diets, one low in fat and one high in oil, did not change FMD, one of the markers of endothelial function. This suggests that the reduction in HDL-cholesterol by a low-fat, high-carbohydrate diet does not have an adverse effect on vascular functioning in individuals of the type studied here.

Does a reduction in HDL-cholesterol impair endothelial function? From prior to posterior probability

We expected to find a smaller FMD after the low-fat diet than after the high-oil diet. We based this on data of our previous study and on data of studies of others. In our previous study, a decrease in serum HDL-cholesterol of 0·36 mmol/l went together with a decrease in FMD of 1·8 %-units. We designed the diets in the present study in such a way that a difference in HDL-cholesterol of 0·20 mmol/l could be expected, and thus a difference in FMD of $0{\cdot}20/0{\cdot}36\times 1{\cdot}8$ %-units or 1 %-units. This expectation is based on a positive, linear relation between HDL-cholesterol and endothelial function. Indeed, many (Kuhn *et al.* 1991; Zeiher *et al.* 1994; Jensen-Urstad & Rosfors, 1997; O'Brien *et al.* 1997; Simons *et al.* 1998; Toikka *et al.* 1999; Zhang *et al.* 2000) but not all (Tawakol *et al.* 1997) cross-sectional studies showed a positive relation between serum HDL-cholesterol and endothelial function. Another reason why we expected to see a decrease in endothelial function after a decrease in HDL-cholesterol is that other studies showed changes in endothelial function when risk factors for CVD were changed. For example, lowering of elevated homocysteine by folic acid improved endothelial function after 6 weeks (Bellamy *et al.* 1999). Also lowering of LDL-cholesterol by statins (Anderson *et al.* 1995; Treasure *et al.* 1995; Vogel *et al.* 1996*a*; O'Driscoll *et al.* 1997; Dupuis *et al.* 1999) or diet and cholestyramine (Leung *et al.* 1993) was shown to improve endothelial function. Based on these previous studies, we hypothesised that a predicted decrease in serum HDL-cholesterol of 0·2 mmol/l would lower FMD by at least 1 %-unit. We gave this hypothesis an a priori probability of 75 %, but evidently different a priori probabilities may be postulated (Table 5). Based on our data, the hypothesis that a diet low in fat would decrease FMD by the postulated amount became less likely; the a posteriori probability was only 11 %. Moreover, a recent study in Australian men and women showed that a low-fat diet decreased serum HDL-cholesterol but did not affect arterial elasticity when compared to a diet high in monounsaturated fats (Ashton *et al.* 2000).

We did not measure HDL composition or particle size. However, it is possible that different diet-induced decreases in HDL-cholesterol have different effects on HDL composition or particle size. Indeed, studies in which fat was replaced by carbohydrates show a change in the composition of HDL particles, with a larger decrease in the antiatherogenic HDL2 subfraction than in the HDL3 subfraction (Berglund *et al.* 1999; Walden *et al.* 2000). In contrast, replacement of saturated fat by *trans* fat decreased serum HDL-cholesterol without changing the composition of the HDL particles (Lichtenstein *et al.* 1999) and with only a slight decrease in apolipoprotein A-1 (Aro *et al.* 1997; Müller *et al.* 1998). However, these differences point at a more atherogenic change in HDL induced by a low-fat diet than by a diet rich in *trans* fatty acids, and this is not reflected in the changes in FMD.

Other factors in the diets that might have affected endothelial function

The goal of the two study diets was to achieve a difference in HDL-cholesterol while keeping the diets as equal as possible. Although that goal was reached, there were a number of differences between the diets that might have counteracted an effect of HDL-cholesterol. First, there was a difference in fatty acid composition between the two diets because we wanted to keep serum LDL-cholesterol constant. If we had replaced 20 en% of carbohydrates with 20 en% of monounsaturated fatty acids, serum LDL-cholesterol would have decreased by 0·12 mmol/l (Mensink & Katan, 1992). Thus, the high-oil diet was higher in saturated fat (5 en%) and polyunsaturated fat (2 en%). The higher intake of saturated fat might have impaired endothelial function, but this is only suggested by short-term studies that compared high-fat with low-fat meals

(Vogel *et al.* 1997*b*; Ong *et al.* 1999). On the other hand, the higher intake of polyunsaturated fats might have improved endothelial function because these fats were shown to improve arterial compliance, although at higher intakes (Nestel *et al.* 1997). The mechanism by which fats might affect endothelial function is not clear, because not all studies show an impairment of endothelial function after a high-fat meal (Williams *et al.* 1999). It is possible that high concentrations of triacylglycerols in serum cause the impairment because intravenous dosing of triacylglycerols results in impaired endothelial function (Lundman *et al.* 1997). However, others suggest that, in particular, fats that have been used for deep-frying and are therefore rich in degradation products may impair endothelial function (Williams *et al.* 1999). Although in our study the concentration of fasting triacylglycerols in serum was higher after the low-fat diet than after the high-oil diet, it is unlikely that this had an effect on endothelial function (Schnell *et al.* 1999).

The two diets not only differed in fat and carbohydrate content: the intake of fruits and vegetables was also higher on the low-fat diet than on the high-oil diet. We could have kept the intake of fruits and vegetables equal on the two diets, but then the amount of starchy foods, such as potatoes, rich and pasta, would have been too bulky to be appetising. Thus, the intake of some vitamins was different between the diets. We estimate that the daily intake of folate from fruits and vegetables was 25–50 μg higher from the low-fat diet than from the high-oil diet (Brouwer *et al.* 1999). Consequently (Schorah *et al.* 1998; Brouwer *et al.* 1999), serum homocysteine concentrations were slightly (0·2 μmol/l) lower after the low-fat diet than after the high-oil diet. This decrease was probably too small to have improved FMD (Bellamy *et al.* 1999; Wilmink *et al.* 2000). Another difference between the two diets was vitamin C: the low-fat diet contained about 30 mg/d more vitamin C than the high-oil diet. This difference is not likely to have had an effect on endothelial function because studies that had showed an effect of vitamin C used amounts of 500–1000 mg/d (Plotnick *et al.* 1997; Duffy *et al.* 1999; Chambers *et al.* 1999; Gokce *et al.* 1999). In contrast to vitamin C and folic acid, which were higher on the low-fat diet, vitamin E intake was higher on the high-oil diet, mainly because we used olive oil. However, vitamin E does not appear to have strong effects on endothelial function (Neunteufl *et al.* 2000) and the difference between diets was only 10 mg/d, probably too small to have had any effect.

In conclusion, we showed that FMD, one of the markers of endothelial function, was not affected when HDL-cholesterol was lowered by substituting carbohydrates for monounsaturated oil. Thus, our data provide no evidence for an adverse effect of low-fat diets on vascular functioning.

Acknowledgements

We are indebted to the volunteers who took part in the study. We thank Els Siebelink for calculating the diets, Marieke Spaan and Kirsten van den Brink for preparing the diets, Jan Harryvan for the brachial artery measurements, Rudy Meijer (Radiology Department, University Medical Center Utrecht) for ultrasound training and support and Karin Duiser (Julius Center, University Medical Center Utrecht) for reading the images, Truus Kosmeyer for analysis of the duplicate diets, and Louise and Nadège Beyne, Anke Bongers, Irna Hertel, Marieke de Lange, Judith Manniën, and Karen Oberndorff for their assistance during the study.

This study was financially supported by the Dutch Dairy Foundation on Nutrition and Health.

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