British Journal of Nutrition (2011), **106**, 123–129
doi:10.1017/S0007114510005829

Effects on markers of inflammation and endothelial cell function of three *ad libitum* diets differing in type and amount of fat and carbohydrate: a 6-month randomised study in obese individuals

Else-Marie Bladbjerg[1]*, Thomas M. Larsen[2], Anette Due[2], Steen Stender[3], Arne Astrup[2,4] and Jørgen Jespersen[1]

[1]*Unit for Thrombosis Research, Institute of Public Health, The University of Southern Denmark, c/o Department of Clinical Biochemistry, Hospital of South West Denmark, Finsensgade 35, DK-6700 Esbjerg, Denmark*
[2]*Department of Human Nutrition, Centre for Advanced Food Studies, Faculty of Life Sciences, University of Copenhagen, Frederiksberg, Denmark*
[3]*Department of Clinical Chemistry, Copenhagen University Hospital, Gentofte, Denmark*
[4]*Department of Clinical Nutrition, Copenhagen University Hospital, Gentofte, Denmark*

(Received 5 July 2010 – Revised 8 December 2010 – Accepted 13 December 2010 – First published online 15 February 2011)

Abstract

Diet is important for the prevention of CVD, and diets high in MUFA might be more cardioprotective than low-fat diets. We hypothesise that inflammation and endothelial cell function will be improved most favourably by a high-MUFA diet compared with a low-fat diet. This was tested in a parallel randomised intervention trial on overweight individuals (aged 28·2 (SD 4·6) years) assigned to a diet moderate in the amount of fat (35–45% of energy; >20% of fat as MUFA; MUFA diet, *n* 39), a low-fat (20–30% of energy) diet (LF diet, *n* 43) or a control diet (35 % of energy as fat, *n* 24) for 6 months after weight loss. Protein constituted 10–20 % of energy in all diets. Food was provided free of charge. Fasting blood samples were collected before and after the intervention and analysed for C-reactive protein (CRP), IL-6, intercellular adhesion molecule, von Willebrand factor (vWF) and tissue factor pathway inhibitor. vWF concentrations tended to fall on the LF diet (4·78 (SD 16·44) %; $P=0·07$). Concentrations of IL-6 were reduced by the MUFA (0·37 (SD 0·74) pg/ml; $P<0·01$) and LF (0·47 (SD 0·69) pg/ml; $P<0·001$) diets, and CRP was reduced on all diets (MUFA: 0·48 (SD 1·93) mg/l ($P<0·01$); LF: 1·46 (SD 2·89) mg/l ($P<0·001$); control: 1·20 (SD 1·97) mg/l ($P<0·01$)). No significant differences were observed between changes induced by the different diets. Our findings suggest that in overweight subjects after weight loss, the MUFA and LF diets have similar long-term effects on inflammation and endothelial cell function.

Key words: Diet interventions: Endothelial dysfunction: Inflammation: Long-term studies: MUFA: Randomised studies

Diet is an important factor in the prevention of CVD as demonstrated in the Nurses' Health Study, a large prospective study on 121 700 initially healthy nurses[1], in which high intakes of SFA, *trans*-fatty acids and carbohydrates with a high glycaemic index (GI) were associated with increased CVD risk[1,2], and high intakes of MUFA and PUFA were associated with reduced CVD risk[1].

An alternative dietary pyramid has been introduced in the USA by Willett[3], one of the Nurses' Health study investigators. The main difference between Willett's new pyramid and the US Department of Agriculture (USDA) 2004 Food Pyramid[4] is that the new pyramid has no restrictions on fat as long as it is of vegetable origin, and carbohydrates should have a high content of whole grains. Such a diet is assumed to have a more cardioprotective effect than a diet following the USDA 2004 Food Pyramid. Although the Nurses' Health Study is a very large and well-designed study, the study conclusions should not alone be used to change the established dietary recommendations without also having results from long-term controlled comparisons with existing dietary recommendations. Because a controlled randomised dietary study in healthy individuals with CVD as the end point will be practically infeasible, CVD risk markers can be studied as an alternative.

Inflammation and endothelial dysfunction are key factors in CVD as illustrated by the association between plasma variables and the risk of CVD in prospective studies. Associations have been observed for C-reactive protein (CRP)[5], IL-6[6],

Abbreviations: CRP, C-reactive protein; GI, glycaemic index; ICAM, intercellular adhesion molecule; TFPI, tissue factor pathway inhibitor; vWF, von Willebrand factor.

* **Corresponding author:** E.-M. Bladbjerg, fax +45 79182430, email ebladbjerg@health.sdu.dk

intercellular adhesion molecule (ICAM-1)[7], von Willebrand factor (vWF)[8,9] and tissue factor pathway inhibitor (TFPI)[9]. Intervention studies have reported an effect of specific dietary components on these CVD risk markers, e.g. effects of specific fatty acids on CRP and IL-6[10–12], and intervention studies based on dietary advice have observed long-term effects of complex dietary changes on CRP[13–15].

The aim of the present study was to compare the long-term effect on markers of inflammation and endothelial cell function of three different *ad libitum* diets in a 6-month strictly controlled dietary intervention in healthy obese subjects after weight loss. The diets compared were Willett's new Healthy Eating Pyramid (high in MUFA and low in GI), the Official Nordic Dietary Guidelines (low in fat and medium in GI) and the average Danish diet (high in SFA and high in GI). We hypothesise that inflammation and endothelial cell function will be improved most favourably by the diet high in MUFA and low in GI.

Experimental methods

Study population

The dietary intervention, Mono Unsaturated Fatty acids in Obesity (MUFObes), was conducted at the Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen (Frederiksberg, Denmark), and has been described in detail elsewhere[16,17]. Briefly, 131 obese individuals were randomly assigned to one of three diets. The inclusion criteria were age 18–35 years, BMI 28–36 kg/m^2, body-weight fluctuations of <3 kg over the previous 2 months and a non-smoking status. Subjects were healthy and took no regular medicine other than contraceptive pills. All subjects gave oral and written informed consent, and the Ethics Committee of the Municipalities of Copenhagen and Frederiksberg approved the study according to the Declaration of Helsinki. The trial was registered at clinicaltrials.gov as NCT00274729.

The population characteristics at study entry are described in Table 1. For various reasons, twenty-five participants dropped out during the intervention period, leaving thirty-nine participants in the MUFA group, forty-three participants in the LF group and twenty-four participants in the control group[16].

Study design

The study was a parallel intervention trial comparing the effect of three diets on body weight and cardiovascular risk after 6 months of intervention. The study design is presented in Fig. 1 and has been described in detail previously[18]. After an initial 8-week low-energy diet, only subjects who lost >8% of their body weight were randomised to one of the experimental diets. After randomisation, the participants completed a 3-week standardisation period (weight stabilisation and adaptation to the supermarket model) eating a diet resembling the average Danish diet, corresponding to the control diet. The 6-month intervention study started in May 2004 and ended in November 2004.

The experimental diets

The 6-month dietary intervention was based on an *ad libitum* design in order to mimic free-living conditions and to test the real appetite regulation of the diets. The three prescribed diets were as follows: (1) the MUFA diet designed to be moderate in fat (35–45% of energy), high in MUFA (>20% of energy) and moderate in low-GI carbohydrates (40–50% of energy), with a high content of vegetable oils, whole-grain food, nuts and legumes); (2) the LF diet low in fat (20–30% of energy) and high in mixed-GI carbohydrates (55–65% of energy); (3) the control diet corresponding to the average Danish diet moderate in fat (30–40% of energy), high in SFA (>15% of energy), moderate in high-GI carbohydrates (45–55% of energy) and low in fibre (<3% of energy). All three diets were moderate in protein (10–20% of energy). Foods recommended for the three dietary groups have previously been described[16]. The actual dietary composition (Table 2) was in accordance with the prescribed dietary composition, and the biopsy content of fatty acids (assessment of compliance) was in accordance with the prescribed diets[16].

Table 1. Population characteristics at study entry*
(Mean values and standard deviations)

	High-MUFA diet (*n* 54)		Low-fat diet (*n* 51)		Control diet (*n* 26)	
Variables	Mean	SD	Mean	SD	Mean	SD
Age (years)	29·2	4·5	27·3	4·9	27·6	5·1
Sex (*n*)						
Male	22		22		11	
Female	32		29		15	
Body weight (kg)	95·4	12·8	96·9	13·5	93·9	13·8
Height (m)	1·74	0·1	1·75	0·1	1·73	0·1
BMI (kg/m^2)	31·4	2·7	31·6	2·7	31·3	2·5
Waist circumference (cm)	102·9	8·8	104·4	8·9	103·8	8·7
Hip circumference (cm)	115·6	8·1	116·3	7·0	114·4	6·5

*Measurements were made at screening before weight loss. Mean values for the three diets were compared with an ANOVA. Sex was compared using the χ^2 test. No significant differences existed between the groups at study entry.

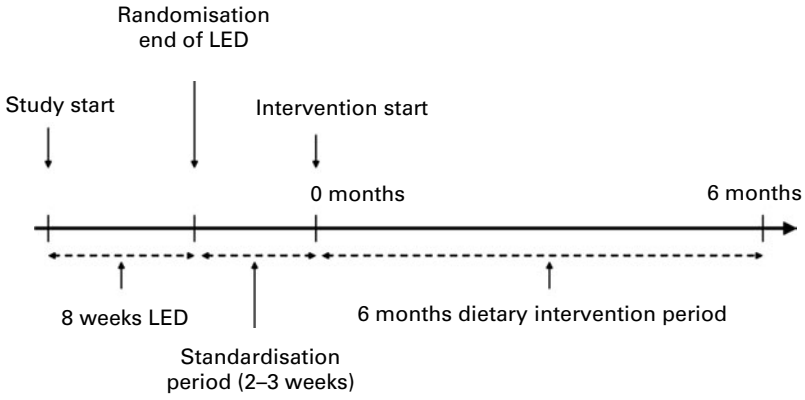

Fig. 1. Design of the Mono Unsaturated Fatty acids in Obesity study. LED, low-energy diet.

Supermarket foods, the computer program and shopping sessions

To provide the subjects with all necessary foods and to accomplish a total recording of the food consumed, a validated supermarket model[18] was established at the department. The supermarket foods, the computer program and the shopping sessions have been described extensively elsewhere[16,17,19]. Throughout the 6 months of intervention, the subjects obtained all foods and beverages at the study supermarket, free of charge, and they were instructed to consume only these foods.

Blood sampling

Venous blood samples were drawn after lying in a supine position for 10 min after at least 10 h fasting, and 24 h abstention from alcohol and strenuous physical activity.

Blood samples were collected with minimal stasis using siliconised vacutainers and 21 gauge needles in citrated tubes at room temperature for analyses of CRP, IL-6, ICAM, vWF, TFPI, TAG and total cholesterol.

Tubes were centrifuged at 20°C for 20 min at 2000 ***g***. Plasma was pipetted into plastic vials and stored at −70°C.

Blood analyses

Plasma samples were rapidly thawed in a water-bath at 37°C and analysed in one series for each subject. Cholesterol, HDL-cholesterol and TAG were determined enzymatically with a COBAS INTEGRA (Roche, Mannheim, Germany). LDL concentration was calculated as follows: LDL = total cholesterol − HDL − (TAG/2·2). Concentrations of CRP (mg/l) were determined by the Latex method (Roche). Commercial ELISA were used to measure concentrations of IL-6 (pg/ml) (R&D Systems, Abingdon, Oxon, UK), ICAM (ng/ml) (R&D Systems), vWF (%) (Liatest®VWF:Ag; Diagnostica Stago, Asnières, France) and total TFPI (ng/ml) (Asserachrom®Total TFPI; Diagnostica Stago). ELISA used were highly sensitive, and no results were below the detection limits. The inter-assay CV were <6 % for CRP, <7 % for IL-6, <7 % for ICAM, <3 % for vWF and <8 % for TFPI.

Statistics

Statistical analyses were done on completers only and performed using the SPSS program (version 16; SPSS, Inc., Chicago, IL, USA). The distribution of the results for TAG, CRP and IL-6 was skewed, and values were logarithmically

Table 2. Actual nutrient composition of the experimental diets
(Mean values and 95 % confidence intervals)

	High-MUFA diet (*n* 39)		LF diet (*n* 43)		Control diet (*n* 24)		
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	*P*‡
Energy intake (MJ/d)	11·5	10·6, 12·4	10·5	9·7, 11·3	10·9	9·6, 12·3	0·27
Total fat (E%)	38·4***†††	37·9, 39·0	23·6***	23·1, 24·1	32·1	31·4, 32·7	<0·001
SFA (E%)	7·1***†††	6·9, 7·4	7·9***	7·6, 8·3	15·1	14·7, 15·5	<0·001
MUFA (E%)	20·2***†††	19·8, 20·6	8·4***	8·1, 8·6	10·4	10·0, 10·8	<0·001
PUFA (E%)	7·8***†††	7·6, 8·1	5·2***	5·1, 5·4	4·0	3·7, 4·3	<0·001
Carbohydrates (E%)	43·3***†††	42·7, 43·9	57·6***	57·0, 58·2	49·8	49·2, 50·5	<0·001
Fibre (g/MJ)	4·2***†††	4·0, 4·4	4·0***	3·8, 4·2	2·9	2·6, 3·1	<0·001
Added sugar (E%)	5·4***†††	4·8, 5·9	7·2***	6·4, 7·9	9·7	8·7, 10·7	<0·001
Glycaemic index	Low		Moderate		High		
Protein (%)	15·3*†	14·9, 15·6	15·8	15·5, 16·2	15·9	15·4, 16·4	0·04
Alcohol (E%)	2·6	1·9, 3·3	2·6	2·0, 3·3	2·0	1·2, 2·8	0·43

LF, low fat; E%, percentage of energy.
Mean value was significantly different from that for control: *$P < 0·05$, ***$P < 0·001$ (pairwise analyses).
Mean value was significantly different from that for LF: †$P < 0·05$, †††$P < 0·001$ (pairwise analyses).
‡Mean values for the three diets were compared with an ANOVA.

British Journal of Nutrition

transformed before analysis. Fasting values were compared between the three diets at the start of the intervention (0 months) and at the end of the intervention (6 months) using an ANOVA. When significant between-diet effects were observed, *post hoc* analyses were performed for pairwise analyses and adjusted for multiple comparisons by the Bonferroni test.

For test for differences within groups, fasting values at 6 months were compared with fasting values at 0 months for each of the three diets separately using a paired *t* test. Furthermore, differences between the diet groups in changes from months 0 to 6 for all variables were analysed by a univariate ANOVA using dietary group as a fixed factor and baseline values, changes in BMI (or waist:hip ratio), and changes in total cholesterol and TAG as covariates in order to adjust for possible confounders. In the case of significance in the ANOVA, pairwise analyses were performed.

All results are presented as means or geometric means. A *P* value of less than 0·05 was considered statistically significant.

Results

Of the study participants, seven were excluded from the statistical calculations due to concentrations of CRP > 10 mg/l (*n* 6), indicating an inflammatory condition, or very high concentrations of ICAM (1434 ng/ml, *n* 1), signifying pronounced endothelial cell dysfunction. These seven participants were distributed as follows: three participants in the MUFA group; three participants in the LF group; one participant in the control group (four participants at 0 months and three participants at 6 months).

There were no significant differences in baseline values (month 0) between the three experimental diets except for significant difference in BMI between the MUFA diet and the LF diet (Table 3). This difference persisted after 6 months ($P<0·01$) with significant differences also between the MUFA diet and the control diet ($P<0·05$). No other significant differences were observed after 6 months between the three diets (Table 3).

When the effects of 6 months of intervention were analysed within each dietary group, all three diets resulted in a significant increase in BMI (MUFA, 1·28 (SD 1·45) kg/m^2; LF, 0·71 (SD 1·12) kg/m^2; control, 0·64 (SD 1·34) kg/m^2) and a significant decrease in CRP (MUFA, 0·48 (SD 1·93) mg/l; LF, 1·46 (SD 2·89) mg/l; control, 1·20 (SD 1·97) mg/l). Concentrations of IL-6 were reduced on the MUFA diet (0·37 (SD 0·74) pg/ml) and the LF diet (0·47 (SD 0·69) pg/ml). The LF diet also resulted in a significant decrease in TAG (0·16 (SD 0·37) mmol/l) and a tendency to a decrease in vWF (4·78 (SD 16·44) %), $P=0·07$). Concentrations of cholesterol were increased on the control diet (0·21 (SD 0·50) mmol/l; Table 3). No significant changes were observed in waist:hip ratio, LDL-cholesterol, ICAM and TFPI (Table 3).

Changes in vWF concentrations from months 0 to 6 tended to differ between the diet groups ($P=0·07$). This was due to significantly different vWF changes during 6 months (mo) between the MUFA diet (ΔvWF $0 \rightarrow 6\,\text{mo}_{\text{MUFA}} = 5·60$ (SD 23·8) %) and the LF diet (ΔvWF $0 \rightarrow 6\,\text{mo}_{\text{LF}} = -4·78$ (SD 16·4) %) ($P=0·022$), with a reduction on the LF diet and an increase on the MUFA diet. However, this difference was no longer significant ($P=0·21$) after adjustment for covariates due to a significant effect of baseline values and changes in TAG. No differences between the diet groups in changes from months 0 to 6 were observed for the other variables measured.

Discussion

In the present weight-loss maintenance study, CRP concentration was significantly lowered irrespective of the experimental diet, and the concentration of IL-6 was lowered by the MUFA diet and the LF diet (Table 3). We observed a tendency to reductions in vWF concentrations on the LF diet ($P=0·07$) and not on the MUFA diet. Changes in vWF concentrations during 6 months differed significantly between the LF and MUFA diet groups ($P=0·022$), but this difference disappeared after adjustment for covariates. No other significant differences were observed between changes induced by the different diets.

The observed effects on vWF may suggest a less favourable effect on the endothelium of the MUFA diet compared with the LF diet, but this cannot be confirmed by others. In one study, vWF concentrations decreased after 4 weeks on a high-MUFA diet compared with a low-fat diet[20]. Others found no difference in effects on vWF, when comparing a MUFA diet with either a PUFA diet[21] or a low-fat diet[22]. The significant between-diet effect (MUFA *v.* LF) observed in the present study seems to be due to (non-significant) differences in vWF baseline values. Also, we cannot exclude that the significantly higher body weight in the MUFA group may partly explain the less favourable vWF effect of the MUFA diet.

The two other markers of endothelial function, TFPI and ICAM, were not affected during 6 months by any of the three diets. Only a few dietary studies have measured TFPI, with either a decrease in TFPI concentrations after 4 weeks on a high-MUFA diet compared with a low-fat diet[20] or an increase in TFPI after 6 weeks on a diet high in marine *n*-3 fat *v.* a diet high in vegetable *n*-3 fat[23]. A cross-sectional study observed an association between high adherence to a Mediterranean diet and lower concentrations of ICAM[24]. Concentrations of ICAM are not affected by α-linolenic acid compared with linoleic acid in dyslipaemic men[11].

The most investigated inflammatory marker in dietary studies is CRP, which can be affected by various dietary components. In cross-sectional studies, low concentrations of CRP are associated with ingestion of a Mediterranean diet[24–26], nuts and whole-grain food[27], and low-GI diets[28]. In intervention studies based on dietary advice, CRP is lowered by a Mediterranean diet compared with a prudent diet in subjects with the metabolic syndrome[13], a low-GI diet *v.* a high-GI diet in patients with type 2 diabetes[15], a high-carbohydrate/low-fat diet compared with a low-carbohydrate/high-fat diet in overweight people[29] and a low-carbohydrate/high-MUFA diet compared with a low-carbohydrate/high-protein diet in overweight people[14]. Randomised controlled trials have demonstrated that concentrations of CRP are lowered by

British Journal of Nutrition

Table 3. Fasting concentrations of plasma variables before (0 months) and after (6 months) dietary intervention with a high-MUFA diet (MUFA; *n* 36), a low-fat diet (LF; *n* 40) or a control diet (*n* 23)
(Mean values and standard deviations)

	0 months		6 months		
Variables	Mean	SD	Mean	SD	*P*‡
BMI (kg/m^2)					
MUFA	28·42†	2·38	29·70*†	2·8	<0·001
LF diet	26·97	2·09	27·68	2·36	<0·001
Control	27·47	1·8	28·11	1·79	<0·05
WHR					
MUFA	0·87	0·07	0·87	0·08	0·53
LF	0·87	0·06	0·87	0·05	0·56
Control	0·88	0·06	0·88	0·08	0·59
TAG (mmol/l)§					
MUFA	0·93	1·48	0·82	1·48	0·12
LF	1·05	1·52	0·92	1·48	<0·05
Control	1·05	1·46	0·96	1·46	0·15
Cholesterol (mmol/l)					
MUFA	4·49	0·71	4·42	0·69	0·52
LF	4·52	0·93	4·52	1·04	0·98
Control	4·40	0·65	4·64	0·92	0·059
LDL-cholesterol (mmol/l)					
MUFA	2·78	0·62	2·68	0·63	0·2
LF	2·78	0·86	2·79	1·05	0·92
Control	2·76	0·66	2·96	0·98	0·11
ICAM (ng/ml)					
MUFA	185·6	31·3	191·1	31·2	0·17
LF	195·7	22·4	195·2	22·4	0·87
Control	200·1	21·7	199·3	23·0	0·81
vWF (%)					
MUFA	93·9	29·4	99·3	28·8	0·17
LF	105·2	32·1	100·4	30·6	0·07
Control	111·9	36·7	110·9	33·1	0·75
TFPI (ng/ml)					
MUFA	64·8	12·1	66·5	13·6	0·30
LF	63·8	15·6	62·0	14·7	0·25
Control	66·9	14·6	64·8	16·9	0·32
CRP (mg/l)§					
MUFA	1·52	2·19	0·85	3·19	<0·01
LF	2·13	2·76	0·89	3·51	<0·001
Control	2·35	2·34	1·55	2·53	<0·01
IL-6 (pg/ml)§					
MUFA	1·12	1·67	0·81	1·61	<0·01
LF	1·26	1·76	0·84	1·8	<0·001
Control	1·27	1·90	1·10	2·29	0·20

WHR, waist:hip ratio; ICAM, intercellular adhesion molecule; vWF, von Willebrand factor; TFPI, tissue factor pathway inhibitor; CRP, C-reactive protein.
* Mean value was significantly different from that for control ($P < 0·05$).
† Mean value was significantly different from that for LF ($P < 0·05$).
‡ Mean values at 0 and 6 months were compared using a paired *t* test within the diet groups. Between-diet comparisons at 0 and 6 months were performed using an ANOVA. When significant between-diet effects were observed, pairwise comparisons were performed using the Bonferroni test.
§ TAG, CRP and IL-6 were logarithmically transformed before analysis (geometric means and standard deviations).

almond-enriched high-MUFA diets compared with a healthy diet without nuts[30], and by α-linolenic acid compared with linoleic acid in dyslipaemic men[11–12]. Thus, many studies have suggested that a high-MUFA diet has a favourable effect on CRP. In the present study, CRP was lowered by all three dietary regimens. Most probably, this indicates that the controlled study in itself had a healthy effect on the study participants, and that all three experimental diets were healthier than the habitual diets eaten by the overweight study participants. Alternatively, the reduction in CRP might be due to an acute-phase reaction in the beginning of the study caused by a stress situation (substantial negative energy balance in the low-energy diet period, getting used to the experimental design and diets, etc.). However, the participants completed a 3-week standardisation period (weight stabilisation on the control diet and adaptation to the supermarket model) before the start of the study. A decrease over time can also be caused by a seasonal variation, but there seems to be no variation in CRP concentrations[31,32]. Whatever the reason for the decrease in CRP during the study period, the minor increase in body weight cannot counteract this effect, and there was no association between changes in BMI and changes in CRP (r 0·021, P=0·85) in the present study and in another study[33].

The dietary effect on CRP can be a direct effect of the experimental diets and/or reinforced by the observed reduction in IL-6, which can affect the hepatic synthesis of CRP[34]. In cross-sectional studies, low concentrations of IL-6 are associated with ingestion of a Mediterranean diet[24,25] and nuts and whole-grain food[27]. Randomised controlled trials demonstrate that concentrations of IL-6 are lowered by high-MUFA oleic acid compared with *tran*-fatty acid or stearic acid in healthy men[10], and α-linolenic acid compared with linoleic acid in dyslipaemic men may either lower IL-6 concentrations[12] or has no effect on IL-6[11]. Also, concentrations of IL-6 are not affected by almond-enriched high-MUFA diets compared with a healthy diet without nuts[30].

Thus, there seems to be no substantial difference between the three experimental diets (MUFA, LF and control) on the long-term effect on inflammation and endothelial cell function. Results from the same study showed that also several haemostatic variables (d-dimer, prothrombin fragment 1 + 2, factor VII coagulant activity and plasminogen activator inhibitor) were not affected by the three diets except for fibrinogen, which was significantly lowered by the LF diet and not by the MUFA diet, and this change over time was significantly different between the diet groups[35]. In contrast, the MUFA diet had a more favourable effect on glucose homeostasis than the two other diets[19].

Important strengths of the present study include the randomised design, the long-term observation, the number of subjects and the highly controlled supermarket model providing complex *ad libitum* diets for free-living individuals. It is extremely difficult to standardise dietary studies and to completely control what people are eating, and only a few long-term controlled dietary studies exist. The present study therefore adds important information to our present knowledge. The selected risk markers were not among the primary effect variables in the power calculation (which was based on changes in body weight)[19], but highly significant differences were observed for CRP and IL-6, and there was not even a trend towards differences in ICAM and TFPI. We cannot exclude that we were unable to detect a true vWF difference between the MUFA diet and the LF diet due to a type 2 error, or due to the significantly higher body weight in the MUFA group. A limitation of the study is the minor weight regain in all three dietary groups, which makes comparison with isoenergetic dietary studies more difficult. However, the aim of the study was to compare the long-term effect of three dietary regimens on inflammation and endothelial cell function during weight maintenance in relatively young subjects. An older (and more atherosclerotic or insulin resistant) study population might have caused a greater difference in risk markers within and between the diets.

In conclusion, the MUFA diet was not superior to the LF diet with respect to long-term effects on inflammation and endothelial cell function in overweight subjects after weight loss. Only the LF diet tended to lower plasma vWF, but this did not result in significant different vWF changes between the diets. The MUFA diet and the LF diet had similar favourable long-term effects on IL-6, and all three diets lowered CRP. The reduction in CRP on the average Danish diet also indicates that following a controlled study in itself has a beneficial effect.

Acknowledgements

The authors wish to thank the project staff of the Department of Human Nutrition. The technicians Kathrine Overgaard and Anette Larsen are kindly thanked for analysing the blood samples. Also, special thanks to Professor Walter Willett, Department of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA, USA and David Ludwig MD, PhD, Department of Medicine, Children's Hospital, Boston, MA, USA for participating in the design of the project. The work was supported by the HA Foundation, the Danish Heart Association, the Danish Diabetes Association, Centre for Advanced Food Research, the State Research Councils, and the Danish Pork Council foundations, associations and research councils: www.mufobes.dk/?show=sponsorer. Foods in the supermarket and low-energy diets were sponsored by Nutrillett, the HA Foundation, KGT/DEG, the Danish Pork Council, Danisco FDB, Arla Foods, the Danish Heart Association, and LMC Rådighedsfond. Food sponsors were as follows: 3-Stjernet, Aarhus United, Allara, Ardo/Frigodan, Beauvais, Bæchs Conditori, Bähncke, Cadiso, Cerealia, Daloon, Danisco, FDB, Flensted, Frisko, G-kartofler, Gamba Food, Jan Import, Kellogg's, Kims, Kraft Foods, Kryta, Københavns Engros Grønttorv, Kødbranchens Fællesråd, LCH catering, Lykkeberg, Malaco Leaf, Nutana, Nutrillett, Odense Marcipan, Polar Is, Puratos, Rose Poultry, Rynkeby, Saeby, Santa Maria, Schulstad, Svansø, Sønderjysk Kål, Tholstrup Cheese, Toms, Tulip, Unilever, Urtekram and Wasa. Analyses of blood samples were supported by the Faculty of Health Sciences, the University of Southern Denmark. The authors' contributions were as follows: A. A., T. M. L. and A. D. were involved in the study concept and design; A. D. and T. M. L. were involved in the collection of the data; E.-M. B., J. J. and S. S. were involved in the blood analyses; E.-M. B. performed the statistical analysis; E.-M. B. and J. J. were involved in the interpretation of the data; E.-M. B. performed the drafting of the manuscript; E.-M. B., A. D., T. M. L., J. J., S. S. and A. A. contributed to the critical revision of the manuscript for important intellectual content; A. A., T. M. L. and A. D. were involved in the obtained funding; A. D., T. M. L., E.-M. B., J. J., S. S. and A. A. were involved in the administrative, technical or material support; A. A. was involved in the study supervision. No sponsor participated in the analysis or interpretation of the data, manuscript preparation, review or approval, or the decision to publish. The authors declare no conflicts of interest.

References

1. Hu FB, Stampfer MJ, Manson JE, *et al.* (1997) Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* **337**, 1491–1499.
2. Liu S, Willett WC, Stampfer MJ, *et al.* (2000) A prospective study of dietary glycemic load, carbohydrate intake, and

risk of coronary heart disease in US women. *Am J Clin Nutr* **71**, 1455–1461.
3. Willett WC (2002) *Eat, Drink, and be Healthy: The Harward Medical School Guide to Healthy Eating*. New York, NY: Simon & Schuster.
4. US Department of Health and Human Services & US Department of Agriculture (2005) *Dietary Guidelines for Americans*, 6th ed. Washington, DC: US Government Printing Office.
5. Danesh J, Collins R, Appleby P, *et al.* (1998) Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA* **279**, 1477–1482.
6. Danesh J, Kaptoge S, Mann AG, *et al.* (2008) Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med* **5**, e78.
7. Ridker PM (1998) Intercellular adhesion molecule (ICAM-1) and the risks of developing atherosclerotic disease. *Eur Heart J* **19**, 1119–1121.
8. Martinelli I (2005) Von Willebrand factor and factor VIII as risk factors for arterial and venous thrombosis. *Semin Hematol* **42**, 49–55.
9. Morange PE, Simon C, Alessi MC, *et al.* (2004) Endothelial cell markers and the risk of coronary heart disease: the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study. *Circulation* **109**, 1343–1348.
10. Baer DJ, Judd JT, Clevidence BA, *et al.* (2004) Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr* **79**, 969–973.
11. Bemelmans WJ, Lefrandt JD, Feskens EJ, *et al.* (2004) Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis. *Eur J Clin Nutr* **58**, 1083–1089.
12. Rallidis LS, Paschos G, Liakos GK, *et al.* (2003) Dietary alpha-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients. *Atherosclerosis* **167**, 237–242.
13. Esposito K, Marfella R, Ciotola M, *et al.* (2004) Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA* **292**, 1440–1446.
14. Keogh JB, Luscombe-Marsh ND, Noakes M, *et al.* (2007) Long-term weight maintenance and cardiovascular risk factors are not different following weight loss on carbohydrate-restricted diets high in either monounsaturated fat or protein in obese hyperinsulinaemic men and women. *Br J Nutr* **97**, 405–410.
15. Wolever TM, Gibbs AL, Mehling C, *et al.* (2008) The Canadian Trial of Carbohydrates in Diabetes (CCD), a 1-y controlled trial of low-glycemic-index dietary carbohydrate in type 2 diabetes: no effect on glycated hemoglobin but reduction in C-reactive protein. *Am J Clin Nutr* **87**, 114–125.
16. Due A, Larsen TM, Mu H, *et al.* (2008) Comparison of 3 *ad libitum* diets for weight-loss maintenance, risk of cardiovascular disease, and diabetes: a 6-mo randomized, controlled trial. *Am J Clin Nutr* **88**, 1232–1241.
17. Rasmussen LG, Larsen TM, Mortensen PK, *et al.* (2007) Effect on 24-h energy expenditure of a moderate-fat diet high in monounsaturated fatty acids compared with that of a low-fat, carbohydrate-rich diet: a 6-mo controlled dietary intervention trial. *Am J Clin Nutr* **85**, 1014–1022.
18. Skov AR, Toubro S, Raben A, *et al.* (1997) A method to achieve control of dietary macronutrient composition in *ad libitum* diets consumed by free-living subjects. *Eur J Clin Nutr* **51**, 667–672.
19. Due A, Larsen TM, Hermansen K, *et al.* (2008) Comparison of the effects on insulin resistance and glucose tolerance of 6-mo high-monounsaturated-fat, low-fat, and control diets. *Am J Clin Nutr* **87**, 855–862.
20. Perez-Jimenez F, Castro P, Lopez-Miranda J, *et al.* (1999) Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* **145**, 351–358.
21. Turpeinen AM & Mutanen M (1999) Similar effects of diets high in oleic or linoleic acids on coagulation and fibrinolytic factors in healthy humans. *Nutr Metab Cardiovasc Dis* **9**, 65–72.
22. Thomsen C, Rasmussen O, Christiansen C, *et al.* (1999) Comparison of the effects of a monounsaturated fat diet and a high carbohydrate diet on cardiovascular risk factors in first degree relatives to type-2 diabetic subjects. *Eur J Clin Nutr* **53**, 818–823.
23. Goyens PL & Mensink RP (2006) Effects of alpha-linolenic acid versus those of EPA/DHA on cardiovascular risk markers in healthy elderly subjects. *Eur J Clin Nutr* **60**, 978–984.
24. Fung TT, McCullough ML, Newby PK, *et al.* (2005) Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* **82**, 163–173.
25. Panagiotakos DB, Dimakopoulou K, Katsouyanni K, *et al.* (2009) Mediterranean diet and inflammatory response in myocardial infarction survivors. *Int J Epidemiol* **38**, 856–866.
26. Pitsavos C, Panagiotakos DB, Tzima N, *et al.* (2007) Diet, exercise, and C-reactive protein levels in people with abdominal obesity: the ATTICA epidemiological study. *Angiology* **58**, 225–233.
27. Jiang R, Jacobs DR Jr, Mayer-Davis E, *et al.* (2006) Nut and seed consumption and inflammatory markers in the multiethnic study of atherosclerosis. *Am J Epidemiol* **163**, 222–231.
28. Liu S, Manson JE, Buring JE, *et al.* (2002) Relation between a diet with a high glycemic load and plasma concentrations of high-sensitivity C-reactive protein in middle-aged women. *Am J Clin Nutr* **75**, 492–498.
29. Rankin JW & Turpyn AD (2007) Low carbohydrate, high fat diet increases C-reactive protein during weight loss. *J Am Coll Nutr* **26**, 163–169.
30. Rajaram S, Connell KM & Sabate J (2010) Effect of almond-enriched high-monounsaturated fat diet on selected markers of inflammation: a randomised, controlled, crossover study. *Br J Nutr* **103**, 907–912.
31. Kelly GS (2005) Seasonal variations of selected cardiovascular risk factors. *Altern Med Rev* **10**, 307–320.
32. Rudnicka AR, Rumley A, Lowe GD, *et al.* (2007) Diurnal, seasonal, and blood-processing patterns in levels of circulating fibrinogen, fibrin D-dimer, C-reactive protein, tissue plasminogen activator, and von Willebrand factor in a 45-year-old population. *Circulation* **115**, 996–1003.
33. Belza A, Toubro S, Stender S, *et al.* (2009) Effect of diet-induced energy deficit and body fat reduction on high-sensitive CRP and other inflammatory markers in obese subjects. *Int J Obes* **33**, 456–464.
34. Robson SC, Shephard EG & Kirsch RE (1994) Fibrin degradation product D-dimer induces the synthesis and release of biologically active IL-1 beta, IL-6 and plasminogen activator inhibitors from monocytes *in vitro*. *Br J Haematol* **86**, 322–326.
35. Bladbjerg EM, Larsen TM, Due A, *et al.* (2010) Long-term effects on haemostatic variables of three *ad libitum* diets differing in type and amount of fat and carbohydrate: a 6-month randomised study in obese individuals. *Br J Nutr* **104**, 1824–1830.

British Journal of Nutrition