Daylong triglyceridaemia in healthy Mediterranean and Northern European subjects

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ABSTRACT

Background: A Mediterranean eating pattern and diet enriched in monounsaturated fatty acids may result in a favourable daylong lipid profile.
Methods: 19 Spanish males (aged 32 ± 8 years) and 28 females (34 ± 8 years) were matched to Dutch subjects on the basis of fasting capillary triglycerides (TGc), gender and age. TGc were self-measured at six fixed time points over three days. Daylong TGc profiles were calculated as areas under the curve (TGc-AUC).
Results: Anthropometric parameters and fasting plasma lipids were comparable between Spanish participants and Dutch subjects. Insulin sensitivity (expressed as HOMA) was highest in the Dutch females (1.41 ± 1.09 *vs* 2.09 ± 1.23 in the Spanish females, p<0.05). Daylong TGc values were not different between Spanish and Dutch participants. Male Spanish subjects showed the largest daylong TGc increase after lunch, while in the Dutch males, the largest TGc increase was seen after dinner. Total daytime dietary energy and total fat intake were comparable when analysed by gender. However, the Spanish participants had a higher intake of monounsaturated and polyunsaturated fatty acids as percentage of energy.
Conclusion: There are no major differences in daylong triglyceridaemia between Dutch and Spanish subjects, despite different eating habits and a diet enriched in monounsaturated and polyunsaturated fat in the latter.

INTRODUCTION

Coronary heart disease (CHD) is the major cause of death in Western populations.[1] Due to different lifestyles and genetic background, there are large geographical differences in CHD mortality.[2] Dyslipaemia plays an important role in the development of atherosclerosis. However, approximately 40 to 50% of all premature atherosclerosis develops in fasting normolipidaemic individuals.[3-6] Since triglycerides (TG) are highly variable during the day due to food intake, and humans are in a postprandial state for the most part of the day, postprandial triglyceridaemia could be a concealed risk factor for CHD. Indeed several studies have demonstrated delayed clearance of TG-rich particles and their direct relation with atherosclerotic disease in different patient groups.[5,7-10]
Recently it was shown that CHD patients on a Mediterranean diet had a 50 to 70% reduction of cardiac endpoints when compared with people who did not receive dietary recommendations, and that this effect was independent of fasting plasma lipids.[11] A Mediterranean diet, which is enriched in unsaturated fatty acids, could have beneficial effects on postprandial TG when compared with a Northern European diet.[12-14] This may be either indirectly via reduction of fasting TG and therefore less remnants of TG-rich lipoproteins, or directly by improved metabolism of postprandial lipoproteins containing unsaturated fatty acids.[14] On the other hand, there are also studies showing undesirable effects of the Mediterranean diet on postprandial TG.[15,16] In addition, it has been shown that, after a similar test meal, people from Mediterranean countries have accelerated postprandial TG clearance when compared with Northern Europeans.[17] This may suggest increased lipolytic activity or decreased intestinal absorption of lipoproteins.[17] Furthermore, the different eating patterns of people from Mediterranean countries, e.g. the main meal in the afternoon instead of the evening,

could be beneficial with regard to postprandial TG. It is known that TG from an oral fat load given in the evening are cleared at a slower rate compared with the same fat load given in the morning.[18]

All the above-mentioned studies have assessed postprandial triglyceridaemia after a standardised oral fat load. In real life there are generally three eating occasions throughout the day with lower food intakes than the single oral fat-loading test. Recently, ambulant self-measurement of capillary TG (TGc) was described to study postprandial lipaemia in a free-living situation.[19-23] Using this technique we have confirmed reports in metabolic ward conditions showing postprandial hyperlipidaemia in males compared with females,[19] in obesity,[24] in diabetes mellitus type 2[25] and in patients with premature CHD.[26] We have previously shown that diurnal TGc profiles correlate well with standardised oral fat-loading tests that are regarded as the golden standard for testing of TG metabolism.[19] Major determinants of diurnal TGc profiles are gender, insulin sensitivity and age, besides fasting TGc. Furthermore, we have described positive associations between increments in diurnal TGc and the carbohydrate, protein and total energy content of the diet, whereas fat intake determined the total but not incremental TGc response.[19,22,23]

Geographical differences, including a different genetic background, lifestyle and diet, may affect daylong triglyceridaemia. We studied daylong TGc in healthy normolipidaemic subjects from Spain and the Netherlands in an uncontrolled out-of-hospital setting. Since fasting TGc have been shown to be the best predictor of daylong triglyceridaemia,[19,22] Spanish and Dutch subjects were matched for fasting TGc. In addition, determinants of the daylong TGc profiles were evaluated.

METHODS

Subjects

Healthy normolipaemic volunteers from the Departments of Internal Medicine in Utrecht (the Netherlands) and Valencia (Spain), aged 20 to 55 years, were recruited by advertisement. Exclusion criteria were fasting plasma cholesterol concentration >6.5 mM, fasting plasma TG concentration >2.3 mM, body mass index (BMI) >30 kg/m^2, smoking, renal or liver disease, diabetes mellitus, use of lipid-lowering medication, menopause or a postmenopausal state, and a family history of premature myocardial infarction (males <55 years, females <65 years) or type 2 diabetes mellitus. On the morning of inclusion, anthropometric measurements were performed using standard techniques. The Spanish subjects were matched to Dutch subjects on the basis of gender, age and fasting TGc. All subjects gave written informed consent before participating. The study was approved by the Independent Ethics Committee of Institutional Review Board of Utrecht University Medical Centre (the Netherlands) and Valencia University Hospital (Spain).

Self-measurements of TGc

TGc was self-measured with a TG-specific point-of-care testing device (Accutrend GCT; Roche Diagnostics, Mannheim, Germany)[19,21-23] after the subjects had received instructions from the same investigator. Subjects were instructed to wash and dry their hands thoroughly before each measurement. A drop of blood (30 µl) obtained from the finger using a lancing device was applied to the test strip in the device. Subsequently, TGc was measured by a process of dry chemistry and colorimetry. If there was not enough blood on the test strip, subjects were asked to repeat the measurement. The reference range for TGc is 0.80 to 6.86 mM. In a previous study, the coefficients of variation for different TGc concentrations ranged from 3.3 to 5.3%.[21] The correlation coefficient between TGc using the device and plasma TG according to enzymatic methods is 0.94.[21] Similar results were obtained in our laboratory.[19,22]

Subjects were instructed to measure their TGc concentrations on three different days (preferably Monday, Wednesday, and Friday; not in weekends) at the following six time points: fasting, before and three hours after lunch and dinner, and at bedtime. The three-hour postprandial measurements were performed exactly three hours after the meals, regardless of the intake of snacks, and the results were recorded in a diary. Subjects were requested to refrain from heavy physical activity, although normal daily activities such as riding a bike to work, were allowed. When one or more measurements were missing for a day, the data for that particular day were not used to create an average daylong TGc profile. The mean daytime TGc profile was used for statistical analysis.

Dietary intake

Dietary intake was recorded in the same diary in which the TGc concentrations were written. Subjects received no recommendations concerning the frequency and composition of the meals and were requested to consume their usual diet during the study. Quantities of intake were estimated according to instructions given by a dietician and by using a table with standardised portion sizes.[27] Other details, such as illness, were also recorded in the diary. The diaries were evaluated by a trained physician together with each subject. Foods consumed were converted into nutrients by using the Dutch Nutrient Database[28] and nutrition tables for Spain.[29] Dietary intakes were compared with the average diet in the Netherlands[27,28] and in Spain.[30] Dietary intakes were calculated per day and as an average of two or three days.

Analytic determinations

On the morning of inclusion, after an overnight fast of at least ten hours, blood was collected for measurement of plasma lipid, insulin and glucose concentrations. Total cholesterol, HDL cholesterol obtained after precipitation with Phosphotungstate/$MgCl_2$ and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits, respectively (Roche diagnostics, Germany).[7,31] LDL cholesterol was calculated using the Friedewald formula. Glucose was measured by glucose oxidase dry chemistry and colorimetry (Vitros GLU slides; Johnson & Johnson, Clinical Diagnostics, Rochester, NY), insulin was measured using a competitive radioimmunoassay with polyclonal antibodies. The HOMA index (homeostasis model assessment = glucose*insulin/22.5) was calculated to estimate insulin sensitivity.[32] All clinical chemistry determinations were performed at the laboratory of clinical chemistry of Utrecht University Hospital.

Statistics

Data are given as mean ± SD in the text and tables and as mean ± SEM in the figure. Daytime TGc profiles were calculated as total and incremental (after correction for fasting TGc) areas under the curve (TGc-AUC and dTGc-AUC, respectively). Dietary intakes and AUCs were calculated by using averages over two or three days. Differences in dietary intakes or TGc-AUC between three separate days were tested by paired *t* test. Differences between the study groups were tested with an unpaired Student's *t* test. Individual time points of daylong TGc were compared back to baseline by a 1-factor RM ANOVA, using time as within-subject factor, with Bonferroni adjustment for multiple comparisons. All comparisons were performed by gender because, after fasting TGc, this is the major determinant of daylong TGc.[19] To study variables associated with TGc-AUC and dTGc-AUC, univariate correlations were calculated using Pearson's correlation coefficients. Stepwise multiple regression analysis was performed with TGc-AUC and dTGc-AUC as dependent variables and with the significantly associated variables identified by univariate regression analysis as independent variables. Plasma TG, insulin and the HOMA index were analysed after logarithmic transformation because of the nonparametric distribution. SPSS version 10.0 (SPSS Inc, Chicago) was used for the statistical analysis. Areas under the TGc curve were calculated with PRISM version 3.0 (Graph Pad Software, San Diego) by using non-logarithmically transformed TGc concentrations. Statistical significance was set at $p<0.05$ (two-sided).

Table 1

Baseline characteristics (mean (SD)) and daylong triglycerides of the study group (20 to 55 years, n=94)

	SPANISH MALES (N=19)	DUTCH MALES (N=19)	SPANISH FEMALES (N=28)	DUTCH FEMALES (N=28)
Age (years)	32 (8)	33 (11)	34 (8)	34 (9)
Length (m)	1.76 (0.05)	1.83 (0.08) †	1.63 (0.05)	1.69 (0.06) ‡‡
Weight (kg)	78 (11)	79 (10)	60 (8)	65 (8) ‡
BMI (kg/m²)	24.9 (3.0)	23.4 (2.8)	22.7 (2.5)	22.7 (2.5)
Waist (m)	0.87 (0.10)	0.83 (0.08)	0.75 (0.08)	0.74 (0.08)
WH	0.90 (0.05)	0.86 (0.07)	0.79 (0.06)	0.77 (0.05)
Plasma TG (mM)	1.17 (0.65)	1.33 (0.48)	0.76 (0.35)	0.75 (0.37)
Cholesterol (mM)	4.73 (1.01)	5.01 (0.76)	4.90 (0.92)	4.32 (0.89) ‡
LDL cholesterol (mM)	3.00 (0.91)	3.17 (0.89)	3.16 (0.80)	2.46 (0.64) ‡
HDL cholesterol (mM)	1.19 (0.18)	1.24 (0.29)	1.42 (0.24)	1.52 (0.38)
Glucose (mM)	5.4 (0.5)	4.9 (0.8) †	4.9 (0.5)	5.3 (0.6) ‡
Insulin (iU/l)	7.1 (3.1)	8.4 (3.8)	6.3 (4.7)	8.9 (4.9) ‡
HOMA	1.72 (0.78)	1.85 (0.90)	1.41 (1.09)	2.09 (1.23) ‡
TGc-fasting (mM)	1.44 (0.52)	1.42 (0.49)	1.15 (0.29)	1.12 (0.29)
TGc-AUC (mM*h/l)	27.2 (8.9)	26.7 (9.3)	19.9 (5.8)	17.3 (6.0)
dTGc-AUC (mM*h/l)	7.0 (7.5)	7.7 (5.2)	3.9 (4.0)	3.2 (3.9)
dTGc pre-3h postlunch (mM)	0.92 (0.82)	0.48 (0.81)	0.13 (0.59)	0.15 (0.65)
dTGc predinner-bedtime (mM)	0.12 (0.85)	0.44 (0.83)	0.08 (0.86)	-0.03 (0.31)

WH = waist-to-hip ratio, HOMA = homeostasis model assessment, TGc = capillary triglycerides, TGc-AUC and dTGc-AUC = total and incremental area under the TGc curve, dTGc pre-3h postlunch = TGc change from lunch to three hours after lunch, dTGc predinner-bedtime = TGc change from dinner to bedtime, Student's t test = † p<0.05, †† p<0.005 Spanish vs Dutch males, ‡ p<0.05, ‡‡ p<0.005 Spanish vs Dutch females.

RESULTS

Subject characteristics

In total 100 subjects (50 in each country) were screened for inclusion. Six subjects were excluded due to elevated BMI (n=2), smoking (n=2) and a positive family history for premature atherosclerosis (n=2). Data are shown per gender and ethnicity (*table 1*). The Dutch participants were taller than the Spanish; however, body mass indexes were not different. Fasting plasma lipid values were within normal limits and comparable between the groups, except for a higher fasting total plasma cholesterol due to higher LDL cholesterol in the Spanish females when compared with Dutch females. As a result of differences in fasting glucose and insulin, insulin sensitivity was higher in the Dutch females when compared with the Spanish females. Four of the Spanish females and 12 of the Dutch females were on oral contraceptives.

Self-measurements of TGc and dietary intake

Mean fasting TGc values were not different between Dutch and Spanish participants (*table 1*). In the males all daylong TGc values, except TGc before lunch, were higher than at baseline (*figure 1*, upper panel). In the Spanish females, all postprandial TGc measurements were higher than at baseline, while in the Dutch females only TGc's after dinner were higher than at baseline (*figure 1*, lower panel). In the Spanish males, the largest TGc increment was observed after lunch (*table 1*); however, this increase was not higher than that in the Dutch males (p=0.1), while in the Dutch males the largest TGc increment was seen after dinner (*table 1*). In both groups of males, there was no TGc decline at bedtime. The differences in daylong TGc increments in the males did not result in different total and incremental AUCs (*table 1* and *figure 1*). In both groups of females, small gradual daylong TGc increments were seen that did not result in different total and incremental AUCs (*table 1* and *figure 1*). Subanalysis of TGc-AUC and dTGc-AUC according to the use of contraceptives did not show significant differences in the Spanish or Dutch women (data not shown). Both total and incremental TGc-AUC were higher in males compared with females in the Spanish as well as in the Dutch participants (p<0.05 for all comparisons, *table 1*). When fasting TGc, TGc-AUC and dTGc-AUC were compared between the two ethnic groups; this did not result in statistically significant differences (data not shown). In the males the total energy intake was comparable; however, the Spanish males had a higher monounsaturated and polyunsaturated fat intake and ingested more cholesterol, when compared with Dutch males (*table 2*). The females showed a comparable total energy intake. Similarly to the Spanish males, the Spanish females had a higher intake of monounsaturated fat and cholesterol when compared with the Dutch females (*table 2*).

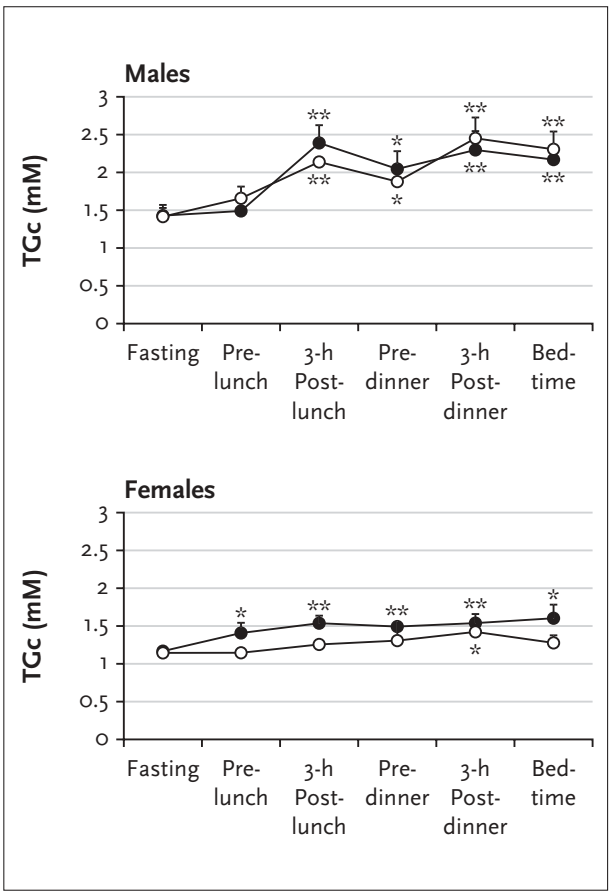

Figure 1

*Mean (± SEM) daylong capillary triglycerides (TGc) in Spanish (n=19, closed circle) and Dutch males (n=19, open circle), [upper panel] and in Spanish (n=28, closed square) and Dutch females (n=28, open square), [lower panel]. Between group differences (unpaired Student's t test for total and incremental AUCs): p=ns for both figures. Differences back to fasting (repeated measures ANOVA): *p<0.05, ** p<0.005.*

Determinants of daylong TGc

When all subjects were analysed together, TGc-AUC was significantly related to fasting TGc and plasma TG (r=0.73 and 0.65 respectively, p<0.001 for each), waist-to-hip ratio (r=0.43, p<0.001), cholesterol (r=0.31, p<0.005), HDL cholesterol (r=-0.30, p<0.005) and HOMA (r=0.22, p<0.05). From all dietary parameters, total energy intake (r=0.29, p<0.01), total intake of carbohydrates (r=0.32, p<0.005), total MUFA intake (r=0.26, p<0.01), total alcohol intake (r=0.28, p<0.01) and protein intake as percentage of energy (r=-0.28, p<0.01) were significantly related to TGc-AUC. Stepwise multiple regression revealed fasting TGc as best predictor (standardised β=0.72) explaining 51% of the TGc-AUC (p<0.001), the model improved significantly when carbohydrate intake, ethnicity and gender were entered (adjusted r^2=0.62, p<0.001).

Table 2
Mean (SD) dietary intake of the study group (20 to 55 years, n=94)

	SPANISH MALES (N=19)	DUTCH MALES (N=19)	SPANISH FEMALES (N=28)	DUTCH FEMALES (N=28)
Energy (kJ)	10832 (1950)	11288 (2485)	7954 (2414)	8577 (1766)
Total fat (g)	108 (25)	96 (24)	81 (29)	83 (26)
(% of energy)	37.4 (5.1)	32.4 (4.8) ††	38.1 (4.0)	36.2 (6.4)
Saturated fat (g)	41 (14)	36 (11)	30 (12)	32 (10)
(% of energy)	14.2 (3.3)	12.0 (2.8) †	14.1 (2.4)	13.9 (3.0)
MUFA (g)	45 (9)	38 (9) †	35 (13)	31 (9)
(% of energy)	16.0 (2.8)	12.8 (2.1) ††	16.9 (3.1)	13.4 (2.8) ‡‡
PUFA (g)	18 (6)	14 (4) †	11 (5)	14 (8)
(% of energy)	6.2 (1.8)	4.9 (1.2) †	5.5 (1.9)	6.0 (2.3)
Carbohydrates (g)	294 (65)	314 (64)	215 (73)	235 (56)
(% of energy)	45.5 (6.4)	47.8 (5.5)	45.0 (5.9)	47.0 (7.2)
Protein (g)	101 (23)	105 (22)	76 (19)	79 (16)
(% of energy)	15.7 (2.8)	15.9 (1.9)	16.5 (2.8)	15.9 (2.6)
Cholesterol (mg)	456 (213)	217 (92) ††	345 (179)	183 (67) ‡‡
Alcohol (g)	6.7 (8.6)	19.8 (28.5)	2.4 (5.2)	6.8 (7.3) ‡

MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, Student's t-test = † p<0.05, †† p<0.005 Spanish vs Dutch males, ‡ p<0.05, ‡‡ p<0.005 Spanish vs Dutch females.

The dTGc-AUC of the total study group was significantly associated with fasting TGc and plasma TG (r=0.20 and 0.40 respectively, p<0.05 for each), waist (r=0.21, p<0.05), cholesterol (r=0.26, p<0.01), HOMA (r=0.23, p<0.05) and diastolic blood pressure (r=-0.36, p<0.05). From all dietary parameters, total carbohydrate and total alcohol intake (r=0.27 for both, p<0.01 for each) and protein intake as percentage of energy (r=-0.25, p<0.05) were significantly related to dTGc-AUC. The best model to predict dTGc-AUC included gender only (standardised β=-0.66), predicting 42% of variation (p<0.001).

DISCUSSION

A Mediterranean diet consists of a higher amount of monounsaturated fatty acids when compared with a Northern European diet,[11] as was also observed in the present study, in particular in the male subjects. In the Lyon Diet Heart Study, the Mediterranean diet had an impressive beneficial effect on cardiovascular complications, despite unchanged fasting lipids, suggesting an alternative mechanism as for instance postprandial lipaemia.[11] However, there is controversy about the beneficial effects of unsaturated fatty acids on postprandial triglyceridaemia. Some studies have shown a reduction of postprandial TG,[12-14] whereas others have shown the contrary.[15,16] In all these studies, unphysiological oral fat-loading tests were used to study postprandial lipaemia. In the present study, in a nonstandardised setting reflecting the normal daily situation, we were not able to detect differences in daylong triglyceridaemia between Spanish and Dutch participants, despite a higher monounsaturated and polyunsaturated fat intake in the Spanish groups, while anthropometric and baseline laboratory values were similar and the dietary intakes reflected that of the general population.[27,28,30] Therefore, the effects of this diet on postprandial lipaemia in real life may be questioned. It could be quite possible that the beneficial effects of unsaturated fatty acids on the process of atherosclerosis depend on other mechanisms than postprandial lipaemia. In this regard, inhibition of endothelial activation by unsaturated fatty acids has been described[33] and others have shown improvement of postprandial endothelial function by antioxidant-rich components of the Mediterranean diet.[34] On the other hand, certain polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are believed to be beneficial with regard to CHD[35] and lipid metabolism,[36] may be unequally distributed among the two ethnic groups that we have studied. Unfortunately, we were not able to calculate these fatty acids separately in the present study due to the software used. It was, however, remarkable that the intake of dietary cholesterol was higher in the Spanish subjects than in the Dutch participants. A high dietary cholesterol may increase plasma cholesterol levels,[12] but effects on triglyceridaemia are unlikely since in another study no TG change was observed in type 2 diabetes patients after cholesterol supplementation.[37] Furthermore, as we already observed, we have never found a correlation between dietary cholesterol and

daylong triglyceridaemia in our previous studies.[19,22,23] Daylong triglyceridaemia was similar in both groups; however, the male Spanish participants showed the largest TGc responses after lunch while in the Dutch males this was seen after dinner. It is known that in Mediterranean countries most people have their main (hot) meal at noon, while this is uncommon in West European countries. The timing of the major meal could be of importance for the total daylong triglyceridaemia.[18] Assuming a difference in eating pattern between the Spanish and Dutch subjects, we did not observe beneficial effects on daylong triglyceridaemia. This suggests that the number of atherogenic chylomicron remnants generated by each meal may have been similar in both groups.
Daylong triglyceridaemia was relatively low and comparable in the Spanish and Dutch premenopausal females. However, in the Spanish females there was a more pronounced postprandial TGc increase. The lower insulin sensitivity in the Spanish females may have caused the small difference in daylong TGc. However, in previous studies in Dutch subjects, we have shown that insulin sensitivity affects daylong TGc more in males than in females.[19] In the present study we did not correct for the phase of the menstrual cycle, since it is unlikely that the oestrogen status of the premenopausal women influenced our results. It has previously been shown that in premenopausal women, despite fluctuations in plasma TG during the menstrual cycle, overall intraindividual TG variability was comparable with that of men.[38] It should be underlined that a subgroup of the female participants were on oral contraceptives. We can not rule out that this may have affected daylong triglyceridaemia. However, we do not believe this to be the case since a subanalysis did not show differences in daylong triglyceridaemia.
Table 1 suggested that fasting plasma TG were lower than capillary TG. We have previously shown that in a direct comparison TGc are slightly higher than plasma TG.[22] Secondly, plasma TG given in *table 1* represented a single measurement, whereas TGc comprised the average of three measurements at different days. Since TG are highly variable within individuals, repetitive measurements may have reduced the variation. Thirdly, plasma TG was determined on the day of inclusion after an overnight fast of at least ten hours, whereas TGc was self-determined without prior overnight restrictions since this measurement was intended to represent real-life fasting TG.
Ethnicity was one of the predictors of daylong TGc in the present study; however, the predictive value was much weaker than that of fasting TGc, and total and incremental triglyceridaemia were not different between Spanish and Dutch subjects. Nevertheless, with the present study we cannot exclude that genetic differences may have influenced the results. It is well known that the apolipoprotein E gene and many other genes can influence postprandial lipoprotein metabolism.[39] In this regard a novel gene, the apolipoprotein AV gene, has very recently been linked to daylong TGc.[40] In addition, differences in the activity of lipolytic pathways such as lipoprotein lipase and hepatic lipase could have influenced the data. Unfortunately we were not able to study differences in genotypes and activity of lipolytic enzymes. Furthermore, it is known that physical activity enhances lipolysis.[41] In the present study only normal daily activities were allowed on the days of TGc self-measurement. We did not quantify separately the daily activity.
In conclusion, there are no major differences in daylong triglyceridaemia between Dutch and Spanish subjects, despite different eating habits and a diet enriched in monounsaturated fat in the latter.

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AvO designed the study, was involved in the data collection and analysis and drafted the manuscript. JTR, RC and JFA contributed to the study design, interpretation and analysis of the data and were involved in the writing of the manuscript. MCC devised the study, supervised the data collection and analysis and contributed to the writing of the manuscript. MCC received educational grants from Merck, Pfizer, Novo Nordisk and AstraZeneca. The other authors had no conflict of interest.

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REFERENCES

1. World Health Statistics Annual 1996. WHO, Geneva. Interprint, Malta 1998.
2. Sans S, Kesteloot H, Kromhout D. The burden of cardiovascular diseases mortality in Europe. Task Force of the European Society of Cardiology on Cardiovascular Mortality and Morbidity Statistics in Europe. Eur Heart J 1997;18(8):1231-48.
3. Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. N Engl J Med 1997;337(19):1360-9.
4. Genest JJ, McNamara JR, Salem DN, Schaefer EJ. Prevalence of risk factors in men with premature coronary artery disease. Am J Cardiol 1991;67(15):1185-9.
5. Karpe F, Steiner G, Uffelman K, Olivecrona T, Hamsten A. Postprandial lipoproteins and progression of coronary atherosclerosis. Atherosclerosis 1994;106(1):83-97.

6. Miller M, Seidler A, Moalemi A, Pearson TA. Normal triglyceride levels and coronary artery disease events: the Baltimore Coronary Observational Long-Term Study. J Am Coll Cardiol 1998;31(6):1252-7.
7. Castro Cabezas M, Bruin TW de, Jansen H, et al. Impaired chylomicron remnant clearance in familial combined hyperlipidemia. Arterioscler Thromb 1993;13(6):804-14.
8. Groot PH, Stiphout WA van, Krauss XH, et al. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. Arterioscler Thromb 1991;11(3):653-62.
9. Patsch JR, Miesenbock G, Hopferwieser T, et al. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992;12(11):1336-45.
10. Weintraub MS, Grosskopf I, Rassin T, et al. Clearance of chylomicron remnants in normolipidaemic patients with coronary artery disease: case control study over three years. BMJ 1996;312(7036):936-9.
11. Lorgeril M de, Salen P, Martin JL, et al. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 1999;99(6):779-85.
12. Grundy SM, Denke MA. Dietary influences on serum lipids and lipoproteins. J Lipid Res 1990;31(7):1149-72.
13. Thomsen C, Rasmussen O, Lousen T, et al. Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. Am J Clin Nutr 1999;69(6):1135-43.
14. Weintraub MS, Zechner R, Brown A, Eisenberg S, Breslow JL. Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. J Clin Invest 1988;82(6):1884-93.
15. Bruin TW de, Brouwer CB, Linde-Sibenius TM, Jansen H, Erkelens DW. Different postprandial metabolism of olive oil and soybean oil: a possible mechanism of the high-density lipoprotein conserving effect of olive oil. Am J Clin Nutr 1993;58(4):477-83.
16. Mekki N, Charbonnier M, Borel P, et al. Butter differs from olive oil and sunflower oil in its effects on postprandial lipemia and triacylglycerol-rich lipoproteins after single mixed meals in healthy young men. J Nutr 2002;132(12):3642-9.
17. Zampelas A, Roche H, Knapper JM, et al. Differences in postprandial lipaemic response between Northern and Southern Europeans. Atherosclerosis 1998;139(1):83-93.
18. Hadjadj S, Paul JL, Meyer L, et al. Delayed changes in postprandial lipid in young normolipidemic men after a nocturnal vitamin A oral fat load test. J Nutr 1999;129(9):1649-55.
19. Castro Cabezas M, Halkes CJ, Meijssen S, Oostrom AJ van, Erkelens DW. Diurnal triglyceride profiles: a novel approach to study triglyceride changes. Atherosclerosis 2001;155(1):219-28.
20. Luley C, Ronquist G, Reuter W, et al. Point-of-care testing of triglycerides: evaluation of the Accutrend triglycerides system. Clin Chem 2000;46(2):287-91.
21. Moses RG, Calvert D, Storlien LH. Evaluation of the Accutrend GCT with respect to triglyceride monitoring. Diabetes Care 1996;19(11):1305-6.
22. Oostrom AJ van, Castro Cabezas M, Ribalta J, et al. Diurnal triglyceride profiles in healthy normolipidemic male subjects are associated to insulin sensitivity, body composition and diet. Eur J Clin Invest 2000; 30(11):964-71.
23. Wijk JP van, Castro Cabezas M, Halkes CJ, Erkelens DW. Effects of different nutrient intakes on daytime triacylglycerolemia in healthy, normolipemic, free-living men. Am J Clin Nutr 2001;74(2):171-8.
24. Halkes CJ, Castro Cabezas M, Wijk JP van, Erkelens DW. Gender differences in diurnal triglyceridemia in lean and overweight subjects. Int J Obes Relat Metab Disord 2001;25(12):1767-74.
25. Wijk JP van, Halkes CJ, Erkelens DW, Castro Cabezas M. Fasting and daylong triglycerides in obesity with and without type 2 diabetes. Metabolism 2003;52(8):1043-9.
26. Wijk JP van, Halkes CJ, Jaegere PPTh de, et al. Normalization of daytime triglyceridemia by simvastatin in fasting normotriglyceridemic patients with premature coronary sclerosis. Atherosclerosis 2003; in press.
27. Breedveld J, Hammink J, Oosten HM van. The Dutch food composition table. The Hague 1998. Netherlands Centre for Nutrition Education 2002.
28. Netherlands Centre for Nutrition Education. The Dutch national food consumption survey 1997-1998. The Hague: Netherlands Centre for Nutrition Education 1998-2002.
29. Mataix J. Tabla de composición de alimentos españoles. 3rd Edition. Granada: Universidad de Granada, 1998.
30. Capita R, Alonso-Calleja C. Intake of nutrients associated with an increased risk of cardiovascular disease in a Spanish population. Int J Food Sci Nutr 2003;54(1):57-75.
31. Bruin TW de, Brouwer CB, Gimpel JA, Erkelens DW. Postprandial decrease in HDL cholesterol and HDL apo A-I in normal subjects in relation to triglyceride metabolism. Am J Physiol 1991;260(3 Pt 1):E492-8.
32. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28(7):412-9.
33. De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. Am J Clin Nutr 2000;71(1 Suppl):213S-23S.
34. Vogel RA, Corretti MC, Plotnick GD. The postprandial effect of components of the Mediterranean diet on endothelial function. J Am Coll Cardiol 2000;36(5):1455-60.
35. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2(8666):757-61.
36. Moreno JJ, Mitjavila MT. The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (review). J Nutr Biochem 2003;14(4):182-95.
37. Romano G, Tilly-Kiesi MK, Patti L, et al. Effects of dietary cholesterol on plasma lipoproteins and their subclasses in IDDM patients. Diabetologia 1998;41(2):193-200.
38. Reed RG, Kris-Etherton P, Stewart PW, Pearson TA. Variation of lipids and lipoproteins in premenopausal women compared with men and postmenopausal women. DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity) Investigators. Metabolism 2000;49(9):1101-5.
39. Ordovas JM. Genetics, postprandial lipemia and obesity. Nutr Metab Cardiovasc Dis 2001;11(2):118-33.
40. Masana L, Ribalta J, Salazar J, et al. The apolipoprotein AV gene and diurnal triglyceridaemia in normolipidaemic subjects. Clin Chem Lab Med 2003;41(4):517-21.
41. Duncan GE, Perri MG, Theriaque DW, et al. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes Care 2003;26(3):557-62.