Effect of variation in the apo A-IV gene on body mass index and fasting and postprandial lipids in the European Atherosclerosis Research Study II

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Abstract The aims of the study were to investigate associations of the apolipoprotein (apo) A-IV polymorphisms Thr347Ser and Gln360His with anthropomorphic measurements and fasting and postprandial lipids in subjects participating in the European Atherosclerosis Research Study II (EARS II). The allelic frequencies of Ser347 and His360 were 0.185 and 0.067, respectively, in the sample as a whole. There were no significant differences in rare allele frequency between cases (offspring of fathers who suffered a myocardial infarction before the age of 55 years) and controls. Control subjects who were carriers of Ser347 had significantly higher body mass indices (BMIs), waist:hip ratios, total and low density lipoprotein cholesterol and triacylglycerol (TG) concentrations (all $P \leq 0.02$) than control subjects who were non-carriers, but these effects were not seen in the cases. Control subjects who were carriers of His360 had lower BMIs ($P = 0.04$), cholesterol and TG concentrations (both $P \leq 0.07$) compared to non-carriers, but these effects were not seen in the cases. After consumption of an oral fat load, carriers of His360 who were most obese (subjects in the third tertile of BMI) had significantly reduced postprandial lipemia ($P < 0.03$, as assessed by area under the curve).—Fisher, R. M., H. Burke, V. Nicaud, C. Ehnholm, and S. E. Humphries. **Effect of variation in the apoA-IV gene on body mass index and fasting and postprandial lipids in the European Atherosclerosis Research Study II. *J. Lipid Res*. 1999.** 40: **287–294.**

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Apolipoprotein (apo) A-IV, a 46 kD, 376 amino acid glycoprotein synthesized in and secreted by enterocytes, is a structural component of intestinally derived triacylglycerol (TG)-rich chylomicron particles (1). Within the plasma, apoA-IV is also found in high density lipoproteins (HDL) and as "free" apoA-IV that is not associated with lipoproteins (2). The precise physiological role of apoA-IV is unclear, but it has been shown in vitro to be a cofactor for the enzyme lecithin:cholesterol acyl transferase (LCAT) (3, 4), to enhance cholesteryl ester transfer protein (CETP)-mediated cholesteryl ester (CE) transfer (5), to promote the efflux of cholesterol from cells (6–10), to be involved in remodelling of HDL particles (11), and to modulate the activity of lipoprotein lipase (LPL) (12). Human apoA-IV expressed in the liver of mice prone to the development of atherosclerosis appeared to protect against atherosclerosis (13). Although this protection was thought to be independent of HDL-cholesterol (HDL-C) concentrations, plasma from the transgenic mice had an increased capacity (compared to plasma from control mice) to promote cholesterol efflux from hepatoma cells (13). Transgenic mice overexpressing mouse apoA-IV were also found to be protected against the formation of diet-induced aortic lesions and this appeared to be mediated through apoA-IV influencing the metabolism and anti-atherogenic properties of HDL (14). Furthermore, experiments on apoA-IV knockout mice indicated that apoA-IV played a role in increasing HDL-C concentrations by decreasing the fractional catabolic rate of HDL-CE (15).

ApoA-IV has been implicated as a regulator of satiety, particularly in the postprandial period when its synthesis and secretion by the small intestine is increased (16, 17). In rats, cerebrospinal apoA-IV concentrations increased significantly as a result of fat feeding (18), and physiological doses of apoA-IV injected either intravenously or intracerebroventricularly caused dose-related suppression of fat feeding (18, 19). A suppressive effect of chylomi-

Abbreviations: apo, apolipoprotein; TG, triglyceride; HDL, high density lipoprotein; HDL-C, HDL-cholesterol; LDL, low density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; NIDDM, non-insulin-dependent diabetes mellitus; CE, cholesteryl ester; MI, myocardial infarction; PCR, polymerase chain reaction; MADGE, microplate array diagonal gel electrophoresis; AUC, area under the curve; EARS, European Atherosclerosis Research Study; LPL, lipoprotein lipase.

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crons on food consumption has been attributed to apoA-IV (20). Furthermore, apoA-IV inhibited gastric acid secretion (21, 22), indicating that it could affect gut functions centrally. However, the overexpression of human apoA-IV (primarily in the intestine) in mice had no effect on intestinal absorption of fat and fat-soluble vitamins or on feeding behavior (23). Whilst apoA-IV knockout mice showed no alterations in weight gain or routine food consumption, male but not female mice had increased food consumption after an 18-h fast (15).

The apoA-IV gene is located in a complex with the apoA-I and apoC-III genes on chromosome 11 (24–26). ApoA-I and apoA-IV genes are transcribed in one direction, whilst the apoC-III gene located between them is transcribed in the opposite direction. Two common polymorphisms within exon 3 of the apoA-IV gene have been identified, Thr347Ser (27) and Gln360His (28, 29). A number of studies have looked for associations between these polymorphisms and fasting plasma lipids, but results have been inconsistent (see Discussion below). One study has evaluated these polymorphisms in relation to non-fasting lipid parameters and found significant associations with total and low density lipoprotein (LDL)-cholesterol and TG concentrations, suggesting an important role for apoA-IV in regulating post prandial lipoprotein metabolism (30). The response of total and LDL-cholesterol to changes in dietary intake of fat has been associated with Thr347Ser genotype (31), whilst HDL-C and apoA-I response was associated with Gln360His status (32). Kinetic studies showed that the His360 isoform had a slower fractional catabolic rate compared to the Gln360 isoform (33). This was attributed to an increased fraction of HDL-associated apoA-IV His360, probably due to its greater α-helical content and higher affinity for phospholipids compared to apoA-IV Gln360 (34). However, carriers of His360 may be more susceptible to heart disease, as was shown in obese patients with NIDDM (35), and they may develop a more atherogenic lipoprotein profile on exposure to increased smoking and saturated fat intake (36).

The aims of this study were to investigate associations of the apoA-IV polymorphisms Thr347Ser and Gln360His with anthropomorphic measurements and fasting and postprandial lipids in subjects participating in the European Atherosclerosis Research Study II (EARS II).

MATERIALS AND METHODS

Subjects and protocols

Participants of EARS I were recruited in 1990 from 14 university student populations from 11 European countries (Austria, Belgium, Denmark, Finland, France, Germany, Italy, Spain, Sweden, Switzerland and the United Kingdom) as described previously in detail (37). Male and female native-born university students between 18 and 26 years whose father had a documented acute myocardial infarction (MI) before the age of 55 years were recruited for the study and represent the cases. Two age- and sex-matched control subjects were recruited by random selection from the same university population. The control students are representative of the total student population as they were randomly chosen irrespective of their parental history of heart disease. Details of lifestyle, medical history, and physiological measurements were established using standardized protocols and biochemical analyses were performed as described previously (37). Venous blood was collected after a 14-h fast.

EARS II was carried out in 1993. Male students between 18 and 28 years with a paternal history of MI before age 55 (cases) were recruited from 14 university student populations from 11 European countries which were divided into four regions: Baltic (Estonia and Finland); United Kingdom; Middle Europe (Belgium, Denmark, Germany and Switzerland); and South Europe (Greece, Italy, Portugal and Spain). For each case a single age-matched control was randomly selected from the same university population. Recruitment and data collection were carried out using the same protocols as for EARS I. Venous blood samples were collected after a 14-h overnight fast. Subjects also underwent an oral fat tolerance test consisting of 66 g fat (42 g saturated), 22 g protein, 56 g carbohydrate, and 417 mg cholesterol, total energy 6186 KJ. Blood samples were taken at 2, 3, 4, and 6 h after the lipid load. Biochemical analyses are described elsewhere (37–40).

Polymerase chain reaction (PCR) amplification

A 149 base pair fragment of exon 3 of the apoA-IV gene was amplified from genomic DNA (predried onto a microplate) using PCR modified according to Hixon and Powers (41). Oligonucleotide primers were synthesized according to the published apoA-IV gene sequence (26). The reverse primer introduced a *PvuII* site (mismatch underlined) that distinguished the Gln360 and His360 alleles (41).

Forward primer:
5′-GCTTCCTGGAGAAGGACCTGAGGGACAAGG-3′
Reverse primer:
5′-CATCTGCACCTGCTCCTGCTGCTGCTCCAG-3′

Each amplification reaction contained 0.1 pmol/μl of each primer, 0.03 U/μl of *Taq* polymerase (Pharmacia) in a final volume of 30 μl and a final $MgCl_2$ concentration of 1.75 mm/l. Each reaction was heated to 95°C for 5 min, and then subjected to 5 cycles of 95°C (1 min) and 72°C (2 min), and 30 cycles of 95°C (1 min), 68°C (1 min), and 70°C (1 min) on a DNA Engine Tetrad thermal cycler (MJ Research).

Restriction enzyme digestion and genotyping

For genotyping of the Gln/His polymorphism at position 360, 10 μl of each PCR reaction was digested at 37°C in a final volume of 15 μl containing 1% BSA and 3 U of *PvuII* in the appropriate buffer. Introduction of a *PvuII* site resulted in the digestion of the 149 bp PCR product into fragments of 119 and 30 bp in amplifications of the Gln360 allele, but this site was abolished in the His360 alleles. For genotyping of the Ser/Thr polymorphism at position 347, 10 μl of each PCR reaction was digested at 37°C in a final volume of 15 μl containing 3 U of *HinfI* in the appropriate buffer. DNA amplified from the Thr347 allele were cut into fragments of 77 and 72 bp, but this site was abolished in the Ser347 alleles. Fragments were separated by 7.5% polyacrylamide gel electrophoresis using microplate array diagonal gel electrophoresis (MADGE) (42) and visualized directly after staining with ethidium bromide.

Statistical analysis

Results are presented for different numbers of individuals within the two genotype groups as a result of differences in the numbers of samples genotyped because of PCR failure or incomplete digestion such that genotyping was not possible. Overall in EARS II, 91% and 94% of the samples were genotyped for the

Thr347Ser and Gln360His polymorphisms, respectively. Statistical analysis was performed with SAS statistical software (SAS Institute Inc., Cary, NC). Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium was tested by chi-square analysis with one degree of freedom. Pairwise linkage disequilibrium coefficient between Thr347Ser and Gln360His was estimated using log-linear analysis (44) and was expressed as the ratio of the unstandardized coefficient to its minimal value ($-D'$). The association of lipid concentrations with apoA-IV genotypes were tested by analysis of variance (SAS-PROC GLM) controlling for age and region, and further adjusted for sex in EARS I. TG concentrations were log-transformed to remove positive skewness, but geometric means ± SEM are presented for ease of understanding. For the postprandial analysis of TG measured at five time points in EARS II, three parameters were calculated: the area under the curve (AUC) above the fasting concentration, calculated by the trapezoid rule; the peak (highest value minus the fasting value); and the time at peak (time at which the highest value was observed).

RESULTS

Frequencies

The frequencies of the rare alleles at positions 347 and 360 within the apoA-IV gene are shown in **Table 1**. Genotype distributions were as expected according to Hardy-Weinberg equilibrium. There were no significant differences in frequencies of either polymorphism between cases and controls, although the frequency of the Ser347 allele was non-significantly higher in the controls ($P < 0.07$). There was a significant difference ($P < 0.02$) in the His360 allele frequency among the four European regions, the frequency tending to increase from the south to the north of Europe, with the highest frequency observed in control subjects from the UK (Table 1). Linkage disequilibrium between the two polymorphisms was established (**Table 2**). Excluding the 16 individuals who were heterozygous for both polymorphisms (where phase could not be determined unambiguously), the His360 allele occurred only on a Thr347 allele and the Ser347 allele occurred only on a Gln360 allele.

TABLE 1. Distribution according to region in cases and controls of Thr347Ser and Gln360His genotypes of the apoA-IV gene in EARS II

	Ser347		His360	
Region	Number Genotyped	Ser Allele Frequency	Number Genotyped	His Allele Frequency
Baltic				
Cases	84	0.137	84	0.066
Controls	88	0.136	89	0.079
UK				
Cases	75	0.167	77	0.078
Controls	79	0.215	83	0.121
Middle				
Cases	115	0.170	119	0.071
Controls	114	0.228	121	0.058
South				
Cases	96	0.188	99	0.030
Controls	94	0.223	100	0.050
All				
Cases	370	0.166	379	0.061
Controls	375	0.203	393	0.074

The frequencies of both genotypes were in accordance with Hardy-Weinberg equilibrium. Case/control difference in Ser347 frequency, adjusted for region: $P < 0.07$. Region difference in allele frequencies, adjusted for status: $P < 0.07$ for Ser347 and $P < 0.02$ for His360.

TABLE 2. Linkage disequilibrium between the Thr347Ser and Gln360His polymorphisms in the apoA-IV gene in EARS II

	Gln360His		
Thr347Ser	Gln/Gln	Gln/His	His/His
Thr/Thr	414	82	1
Thr/Ser	195	16	0
Ser/Ser	30	0	0

Numbers are for cases and controls combined. Linkage disequilibrium between Thr347Ser and Gln360His : $D' = -1.00$, $P < 0.01$.

Obesity and fasting lipids

The characteristics of subjects participating in EARS II have been described elsewhere. Control subjects and cases were no different with respect to anthropometric and lifestyle characteristics and blood pressure except that controls were on average 1 cm taller than the cases ($P < 0.02$). Controls also had significantly lower fasting concentrations of total cholesterol, LDL-cholesterol, apoB, and apoE (all $P \leq 0.05$).

Control subjects who were carriers of the Ser347 allele had significantly greater BMIs than non-carriers (**Table 3**), while control subjects who were His360 carriers had significantly lower BMIs than Gln360 homozygotes (**Table 4**). Control Ser347 carriers also had significantly greater waist:hip ratios than non-carriers. Subdivision of EARS II subjects above and below the center-specific median BMI revealed the frequency of His360 to be greater in control subjects with BMI below the median, but this was not observed in the cases. Similarly for Ser347, the frequency was greater in control subjects with BMI above the median, but frequencies above and below the median BMI were the same in the cases (**Fig. 1**).

Effects of both polymorphisms on fasting lipids appeared to be greater in control subjects than in the cases. Ser347 carriers who were controls had significantly higher concentrations of cholesterol, TG, and apoB, but the same trends were not apparent in the cases. His360 carriers appeared to have lower cholesterol and TG concentrations than non-carriers, although these effects were of only borderline significance in control subjects and were not significant in the cases. There was no effect of either polymorphism on CETP activity. When the analysis was repeated in only those individuals who were homozygous for Thr347, control subjects who were His360 carriers no longer had lower BMIs, cholesterol or TG concentrations compared to non-carriers. In those individuals who were homozygous for Gln360, the effect of the Ser347 allele in the control subjects was maintained.

To investigate further the relationship of His360 with BMI, the EARS I sample was analyzed. EARS I had previously been phenotyped for the apoA-IV Gln360His polymorphism at the protein level by isoelectric focusing and

TABLE 3. Baseline characteristics of subjects according to apoA-IV Thr347Ser genotypes and case-control status in EARS II

	Thr347Ser					
	Cases			Controls		
	Thr/Thr (n = 258)	Ser+ (n = 112)	*P*	Thr/Thr (n = 242)	Ser+ (n = 133)	*P*
BMI	23.4 ± 0.2	23.3 ± 0.3	NS	23.0 ± 0.2	23.8 ± 0.2	<0.004
Waist:Hip	0.850 ± 0.003	0.852 ± 0.004	NS	0.847 ± 0.003	0.861 ± 0.004	<0.004
Cholesterol	4.56 ± 0.05	4.46 ± 0.08	NS	4.23 ± 0.05	4.42 ± 0.07	<0.02
TG	1.02 ± 0.03	0.99 ± 0.05	NS	0.90 ± 0.03	1.03 ± 0.04	<0.003
HDL-C	1.19 ± 0.01	1.17 ± 0.02	NS	1.19 ± 0.02	1.19 ± 0.02	NS
LDL-C	2.92 ± 0.05	2.85 ± 0.07	NS	2.63 ± 0.05	2.77 ± 0.07	<0.08
ApoB	74.1 ± 1.0	72.5 ± 1.6	NS	67.5 ± 1.1	71.7 ± 1.5	0.01
ApoA-I	100.1 ± 1.1	99.6 ± 1.6	NS	100.6 ± 1.1	101.2 ± 1.5	NS
CETP[a]	94.0 ± 1.3	93.4 ± 1.9	NS	91.5 ± 1.3	91.5 ± 1.7	NS

Values expressed as mean ± SEM; kg/m^2 for BMI, mmol/l for lipids, mg/dl for apolipoproteins, and nmol/h/ml for CETP activity. TG concentrations were log-transformed prior to analysis. There was significant heterogeneity between cases and controls in the effect of genotype on BMI, cholesterol, TG, HDL-C, and apoB (all $P \leq 0.03$) and of borderline significance for waist:hip ratio and LDL-C (both $P \leq 0.09$).

[a] Number of subjects for whom CETP data was available: cases Thr/Thr n = 238, Ser+ n = 101; controls Thr/Thr n = 236, Ser+ n = 132.

immunoblotting (45). The molecular bases for the phenotypes apoA-IV-1 and apoA-IV-2 have been identified as Gln360 and His360, respectively (29). Genotyping for the Thr347Ser polymorphism was not carried out in EARS I. **Table 5** shows the baseline characteristics of His360 carriers and non-carriers in EARS I. The effect of the His360 allele in EARS I was similar to that in EARS II. When all subjects were considered (i.e., cases and controls combined) carriers of His360 had lower BMIs than non-carriers in both EARS I ($P < 0.03$) and EARS II ($P < 0.07$). In EARS I this effect of His360 on BMI was confined to the male subjects ($P = 0.054$ for males and $P = 0.26$ for females).

Postprandial response

There were no differences in postprandial plasma TG responses to the oral fat load as assessed by area under the curve (AUC), peak TG concentration, or time of peak between Ser347 carriers and non-carriers in either cases or controls. For carriers of His360 there was no effect on postprandial lipemia either in the cases or in the controls when the groups were considered as a whole, but when subjects were grouped into tertiles of BMI to investigate the effect of obesity, His360 carriers who were controls in the highest tertile of BMI had a significantly lower AUC ($P < 0.03$) than non-carriers (**Fig. 2**). This effect was not observed in the cases.

DISCUSSION

The frequencies of the Ser347 and His360 alleles in EARS II (in cases and controls combined) were 0.185 and 0.067, respectively, similar to those published previously, with neither polymorphism showing significant differences between the cases and controls. For the polymorphism at position 347, rare allele (Ser347) frequencies

TABLE 4. Baseline subject characteristics according to apoA-IV Gln360His genotypes and case-control status in EARS II

	Gln360His					
	Cases			Controls		
	Gln/Gln (n = 333)	His+ (n = 46)	*P*	Gln/Gln (n = 336)	His+ (n = 57)	*P*
BMI	23.4 ± 0.2	23.2 ± 0.4	NS	23.3 ± 0.2	22.6 ± 0.4	0.04
Waist:Hip	0.849 ± 0.003	0.864 ± 0.007	0.05	0.853 ± 0.006	0.843 ± 0.006	NS
Cholesterol	4.55 ± 0.05	4.41 ± 0.12	NS	4.32 ± 0.04	4.12 ± 0.11	0.06
TG	1.02 ± 0.03	0.95 ± 0.07	NS	0.96 ± 0.03	0.86 ± 0.06	0.07
HDL-C	1.18 ± 0.01	1.21 ± 0.04	NS	1.19 ± 0.01	1.17 ± 0.03	NS
LDL-C	2.91 ± 0.04	2.77 ± 0.11	NS	2.70 ± 0.04	2.55 ± 0.10	NS
ApoB	74.1 ± 0.9	70.4 ± 2.5	NS	69.4 ± 0.9	66.0 ± 2.2	NS
ApoA-I	99.8 ± 0.9	100.3 ± 2.5	NS	101.1 ± 0.9	98.2 ± 2.3	NS
CETP[a]	93.9 ± 1.1	93.8 ± 3.0	NS	92.2 ± 1.1	89.5 ± 2.6	NS

Values expressed as mean ± SEM; kg/m^2 for BMI, mmol/l for lipids, mg/dl for apolipoproteins, and nmol/h/ml for CETP activity. TG concentrations were log-transformed prior to analysis. There was significant heterogeneity between cases and controls in the effect of genotype on waist:hip ratio ($P < 0.02$).

[a] Number of subjects for whom CETP data was available: cases Gln/Gln n = 306, His+ n = 43; and controls Gln/Gln n = 329, His+ n = 57.

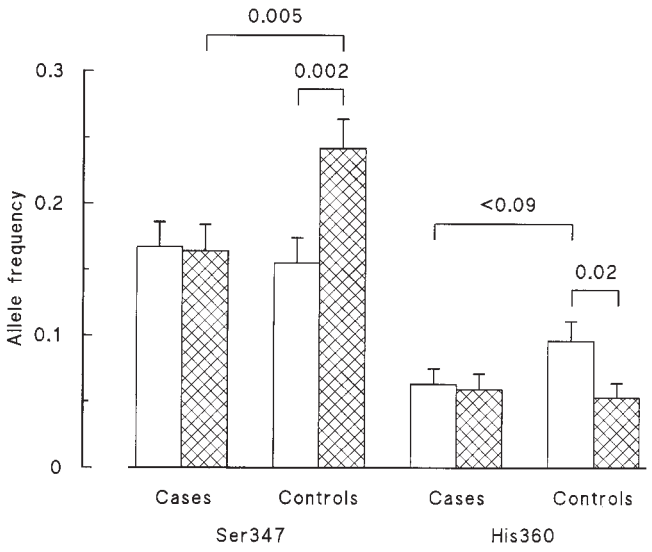

Fig. 1. Allele frequencies (mean ± SD) of Ser347 and His360 in cases and controls above (▨) and below (□) the center-specific median BMI in EARS II. The mean BMIs for subjects grouped below and above the center-specific median BMI were 21.2 and 25.3 kg/m^2, respectively.

from 0.15 to 0.22 have been reported (41, 45, 46) and published frequencies for His360 range from 0.03 to 0.10 (41, 44, 47, 48). In EARS I the frequency of the A-IV-2 allele (His360) in 1890 individuals (cases and controls combined) was 0.077 and there was no significant difference in His360 frequency between cases and controls (44). In contrast to the trend observed in EARS II, the frequency of the His360 allele in EARS I increased from the north to the south of Europe (44). This difference in His360 allele distribution across the regions of Europe between EARS I and EARS II may reflect chance alone, differences in subject selection criteria, or differences in country selection. The linkage disequilibrium between the two sites, with the His360 allele occurring on a Thr347 allele and the Ser347 allele occurring on a Gln360 allele (where phase could be determined unambiguously), was in accordance with previous observations (45).

Previous reports on the effect of His360 on plasma lipids have been inconsistent. Some reports have demonstrated significantly higher HDL-C concentrations (49, 50) and lower TG concentrations (51, 52) in Gln/His360 heterozygotes compared to Gln/Gln homozygotes, but other studies have failed to show these effects (45, 53–58). One study showed higher plasma TG concentrations in His360 carriers, although these subjects were non-fasting (30). A similar lack of effect of the Gln360His polymorphism on plasma lipids was observed in EARS I (44). An effect of this polymorphism on cholesteryl ester transfer protein (CETP) activity in both male and female coronary heart disease patients has been reported, with Gln/His360 heterozygotes having significantly lower activities than Gln/Gln homozygotes (47). In EARS II this effect of the His360 allele on CETP activity was not observed and there was no effect of the Ser347 allele on CETP activity. Previously, the Thr347Ser polymorphism has shown associations with decreased plasma (30) and LDL-cholesterol concentrations (30, 45) and lower plasma apoB concentrations (45). Other studies have failed to confirm these associations (46, 51), but control subjects in EARS II who were carriers of Ser347 had significantly increased cholesterol and TG concentrations and an increase in LDL-C of borderline significance.

The major novel finding of this study was the association between different alleles of the apoA-IV polymorphisms and BMI and waist:hip ratio. This was confirmed to some extent in EARS I subjects, but needs further confirmation in other studies. There is now considerable evidence from animal studies implicating apoA-IV as a satiety signal (18–22). It is possible that it is through this as yet poorly understood pathway that an association of apoA-IV genotype with BMI could be explained. However, this association was only seen in EARS control subjects and was lost in the offspring of men who had suffered a premature MI. This relationship with BMI in the controls may explain the associations of the Ser347 and His360 alleles with pro- and anti-atherogenic lipoprotein profiles, respectively, observed in EARS II given the known associations of obesity with adverse plasma lipid concentrations (59, 60). Beneficial associations of the His360 allele with anthropometric measurements and fasting plasma lipids were lost in Thr/Thr347 homozygotes, but unfavorable as-

TABLE 5. Baseline subject characteristics according to apoA-IV Gln360His genotypes and case-control status in EARS I

	Gln360His					
	Cases			Controls		
	Gln/Gln (n = 543)	His+ (n = 86)	*P*	Gln/Gln (n = 1074)	His+ (n = 187)	*P*
BMI	22.1 ± 0.1	21.7 ± 0.3	NS	21.9 ± 0.1	21.6 ± 0.2	<0.08
Waist:Hip	0.793 ± 0.002	0.783 ± 0.005	NS	0.789 ± 0.002	0.787 ± 0.004	NS
Cholesterol	4.60 ± 0.04	4.39 ± 0.09	<0.04	4.42 ± 0.03	4.29 ± 0.06	<0.04
TG	0.95 ± 0.02	0.89 ± 0.04	NS	0.87 ± 0.01	0.83 ± 0.03	NS
HDL-C	1.41 ± 0.01	1.42 ± 0.03	NS	1.43 ± 0.01	1.41 ± 0.02	NS

Values expressed as mean ± SEM; kg/m^2 for BMI and mmol/l for lipids. TG concentrations were log transformed prior to analysis. There was no significant heterogeneity between cases and controls in the effect of genotype.

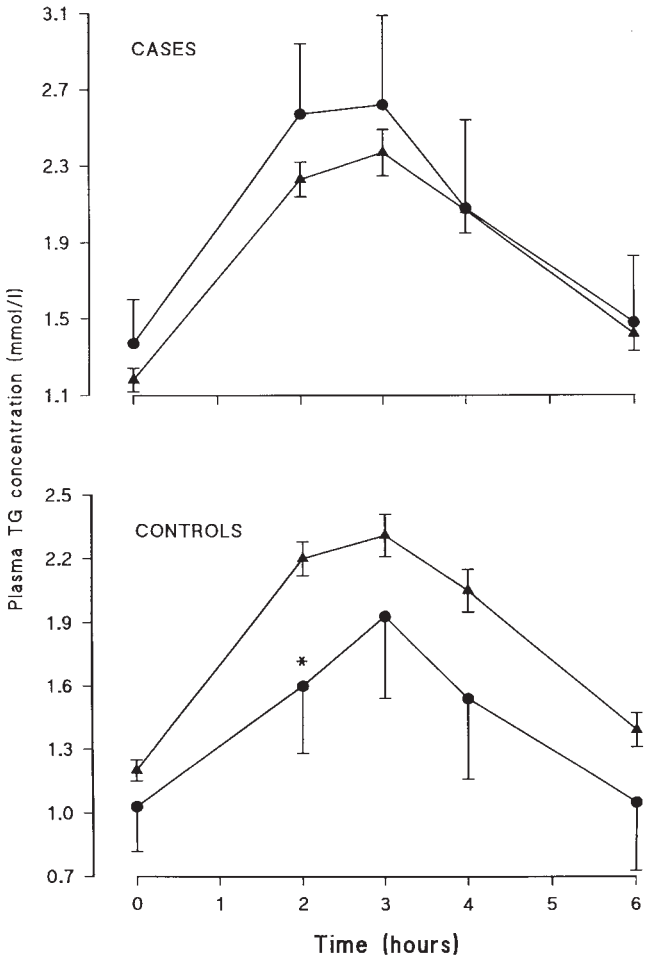

Fig. 2. Plasma TG concentrations (mean ± SEM) after an oral fat tolerance test in cases (upper panel) and controls (lower panel) in EARS II subjects in the third tertile of BMI in carriers and non-carriers of His360. Control subjects in the upper tertile of BMI who were carriers of the His360 allele had significantly lower TG concentrations at 2 h ($P = 0.04$) and reduced total postprandial lipemia, as assessed by area under the curve ($P < 0.03$). ▲Gln/Gln; ● His+; *$P < 0.05$ for Gln/Gln vs. His+. Cases: Gln/Gln n = 116, His+ n = 8. Controls: Gln/Gln n = 104, His+ n = 8.

sociations of the Ser347 allele were maintained in Gln/Gln360 homozygotes. This indicates that Thr347Ser is more strongly associated with these parameters than Gln360His. Although the absolute differences in BMI, waist:hip ratio, and fasting plasma lipid concentrations between control subjects with different apoA-IV genotypes were small, larger and more clinically significant differences might be expected in different subject groups. Participants of the EARS studies were young, healthy, lean individuals where a 3.5% increase in BMI was unlikely to increase significantly their disease risk. However, older, more obese subjects or subjects with diabetes (those at higher risk of heart disease) might be affected more adversely by a similar percentage increase in their BMI.

ApoA-IV has been suggested to have an important role in the postprandial period (30), thus variation in the apoA-IV gene might be expected to influence postprandial lipemia. In the EARS II subjects there was no association of the Thr347Ser polymorphism with plasma TG concentrations after an oral fat load. However, control subjects in the upper tertile of BMI who were carriers of His360 had significantly reduced postprandial lipemia. This suggests that any protective effect of apoA-IV His360 only becomes apparent when the lipolytic system is being stressed, i.e., by both increasing obesity and fat consumption (similar to the situation observed in carriers of the LPL variant Asn291Ser (61)). This beneficial association was lost in the offspring of MI patients (as was observed for the effect on fasting lipid concentrations). The data were not re-analyzed in only those individuals homozygous for the Thr347 allele due to the small sample size of the subgroups. It cannot be ruled out that this association of the His360 allele with postprandial lipemia is a chance finding and it will be of interest to see whether the observation is reproduced in another study. However, differences in the protein structures of the Gln360 and His360 apoA-IV proteins have been predicted (34). ApoA-IV His360 has been shown to adopt a more tightly folded conformation and to have an increased affinity for phospholipid in vitro. The increased α-helical content of apoA-IV His360 in its native form (75% compared to 56% for apoA-IV Gln360) was postulated to allow deeper penetration of the protein into the phospholipid monolayer which could account for its greater activation of LCAT (34). Therefore it is likely that the apoA-IV Gln360 and His360 proteins function differently in vivo and this might explain the observations in subjects of different genotype.

The results from the EARS studies demonstrate an association of apoA-IV gene variation with BMI in control subjects and suggest that the mechanisms behind this association may occur in the postprandial period. The loss of these associations in the cases may be one factor that is associated with an increased risk of MI. It is possible that these observations could be related to apoA-IV's putative role as a satiety signal, but further research is required in this area.

APPENDIX

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