

# Intestinal fatty acid binding protein polymorphism at codon 54 is not associated with postprandial responses to fat and glucose tolerance tests in healthy young Europeans. Results from EARS II participants<sup>☆</sup>

Esa Tahvanainen<sup>a,\*</sup>, Mika Molin<sup>a</sup>, Saara Vainio<sup>a</sup>, Laurence Tiret<sup>b</sup>, Viviane Nicaud<sup>b</sup>, Eduardo Farinaro<sup>c</sup>, Luis Masana<sup>d</sup>, Christian Ehnholm<sup>a</sup>

<sup>a</sup> Department of Biochemistry, National Public Health Institute, Mannerheimintie 166, 00300 Helsinki, Finland

<sup>b</sup> INSERM U525, Epidemiologic and Molecular Genetics of Cardiovascular Diseases, 17 rue du Fer à Moulin, 75005 Paris, France

<sup>c</sup> Department of Preventive Medical Sciences, University of Naples 'Federico II', Via Pansini 5, 80131 Naples, Italy

<sup>d</sup> Unitat Recerca Lipids, Facultat Medicina Reus, Universitat Barcelona, c/Sant Llorenç, 21, 43201 Reus, Spain

Received 16 July 1999; received in revised form 8 November 1999; accepted 6 December 1999

## Abstract

Polymorphism Ala54Thr of the intestinal fatty acid-binding protein 2 (FABP2) has been reported to have an effect on the protein's affinity for long chain fatty acids and to be associated with serum lipid and insulin levels in fasting and especially postprandial states. We wanted to test whether this genetic variation is associated with fasting and postprandial glucose, insulin or lipid levels in 666 male university students participating in the second European Atherosclerosis Study (EARS II). We also studied whether the subgroup of 330 students with paternal history of myocardial infarction (MI) before the age of 55 have different genotype distribution than 336 matched controls. *Results*: No difference in genotype distribution was observed between offspring with and without paternal history of MI or between populations from 11 European countries. The frequency of the threonine encoding allele was 0.276 in cases and 0.266 in controls. There were no differences in fasting or postprandial serum lipid, glucose or insulin levels between subjects having different genotypes. *Conclusions*: In this study FABP2 Ala54Thr polymorphism was not associated with lipid or glucose metabolism. In addition to environmental and genetic factors, selection of study population also may explain the difference between this and earlier studies. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

*Keywords*: Polymorphism; Intestinal fatty acid binding protein; Atherosclerosis; European Atherosclerosis Research Study

## 1. Introduction

Intestinal fatty acid binding protein (FABP2, I-FABP) belongs to a large family of lipid binding proteins, some of which are postulated to capture fatty

acids at the plasma membrane and others to carry long chain fatty acids from the plasma membrane to cytosolic compartments for esterification or oxidation [1–3]. These proteins are needed especially in cells that either have a large flux of fatty acids, such as intestine and liver fat cells, or in cells that have a high demand for fatty acids as substrates for energy, such as heart, striated muscle, kidney, retina and brain [4–7]. Other functions suggested for these proteins include regulation of fatty acid mediated signal transduction and regulation of enzymes participating in fatty acid metabolism [7]. Since the description of the first FABP [8,9] in 1972 almost twenty members of fatty acid

<sup>☆</sup> On behalf of the European Atherosclerosis Research Study (EARS) group.

\* Corresponding author. Present address: Merck, Sharp and Dohme, Pyyntitie 5, 02230 Espoo, Finland. Tel.: +358-9-80465119; fax: +358-9-80465438.

E-mail address: esa\_tahvanainen@merck.com (E. Tahvanainen).

binding protein family have been discovered [7]. Many of these proteins are tissue specific. Examples of FABPs found in the cytoplasm of various cell types include, FABP1 (L-FABP) in liver [10], FABP3 (H-FABP) in striated muscle and heart [11,12], FABP4 (A-FABP) in adipose tissue [13], FABP5 (PA-FABP) in keratinocytes [14], FABP6 (ILBP) in ileum [15], and FABP7 (B-FABP) in brain and retina [16–18]. FABP2 is expressed at high levels only in the epithelial cells of small intestine; combined FABP1 and FABP2 represent 1–2% of total cytosolic protein and 3% of total mRNA of these cells [19]. The gene for FABP2 has been assigned [20] to chromosome 4q28–q31.

In the search for type 2 diabetes loci in Pima Indians, Prochazka et al. [21] found linkage between insulin resistance and a region on chromosome 4q near the FABP2 locus. This finding is supported by a positive linkage between postchallenge insulin levels and FABP2 in Mexican–Americans [22]. Because glucose and fatty acid metabolism are related FABP2 soon became an important candidate gene for type 2 diabetes. The threonine encoding allele of a polymorphism at codon 54 (Ala54Thr) was found to associate with an increased mean fasting plasma insulin concentration, a low mean insulin-stimulated glucose uptake rate, a high insulin response in an oral glucose test, and a high mean fat oxidation rate [23]. It has been shown by titration microcalorimetry that the threonine-containing FABP2 has greater affinity for oleate (C18:1) and arachidonate than the isoprotein having alanine at that codon [23]. It has also been reported that the threonine encoding allele is associated with increased BMI, body fat, and fasting plasma triglyceride concentration in aboriginal Canadians [24,25] and with higher fasting lipid oxidation rate, higher fasting plasma HDL and LDL triacylglycerols, and increased postprandial lipemic response in Finns [26,27].

The European Atherosclerosis Study (EARS) was designed to assess the involvement of genetic and environmental factors on the development of coronary heart disease [28]. The study population consists of university students from 11 European countries. In earlier studies it has been found that the levels of apoB and serum triacylglycerol concentration were the strongest discriminators between offspring of fathers with premature myocardial infarction and controls [29]. We found it important to study whether this difference could be partially explained by genetic variation in the FABP2 gene. The fasting and postprandial glucose, insulin and lipid levels were compared between individuals with different Ala54Thr genotypes. The study setting also allowed us to test whether the Ala54Thr genotypes are associated with paternal history of myocardial infarction and whether there are any differences between European populations.

## 2. Materials and methods

### 2.1. Study population

A detailed description of the design of EARS I has been outlined previously [28]. The EARS II was carried out using the same protocol, except that in EARS II the numbers of cases and controls were equal, the subjects of EARS II were all male and that EARS II also included oral glucose (GTT) and fat (FTT) tolerance tests. In short, the participants were university students aged between 18 and 28 years representing 14 centers in 11 European countries (see appendix). As study population students have roughly similar lifestyles and are exposed to similar environmental factors in each country. The countries were divided into four groups according to their ischaemic heart disease mortality, their geographical location, and by language. The purpose of that grouping was to increase sample size and thus statistical power over the country level. The Baltic group consisted of Finland and Estonia; United Kingdom-group had patients from Northern Ireland, Scotland and England; middle Europe included Denmark, Germany, Belgium and Switzerland; and southern Europe included Portugal, Italy, Spain and Greece. The index (case) group consisted of 353 sons of fathers who had had a documented premature acute myocardial infarction (MI) before the age of 55 years and the control group consisted of 363 men who were selected from the student register with closest birth date to the index case. We genotyped 330 cases and 336 controls whose DNA sample was available. All subjects were examined and blood samples were taken during 1993. The study has been approved by review committees of collaborating centers and the subjects have given informed consents.

### 2.2. Biochemical analyses

The sample handling and analyzing methods have been described [28–30]. In short, the venous blood samples were collected after overnight fast, frozen in dry ice and sent through the EARS center in Nancy to other participating laboratories where the actual biochemical analyses were done. In GTT the participants were given 75 g glucose and serum glucose and insulin values were measured at 30 min intervals. Oral fat tolerance test (FTT) was carried 1 week after GTT. In FTT the participants drank a liquid consisting of 42 g saturated fat, 22 g protein, 56 g carbohydrate, and 417 mg cholesterol. The total energy content of that formula was 6186 kJ. Serum triacylglycerol values were measured from samples taken at baseline

and at 2, 3, 4, and 6 h afterwards. Plasma total cholesterol, total triglycerides, and HDL cholesterol (HDL-C) were measured according to the Lipid Research Clinic's Manual of Laboratory Operations. The low-density lipoprotein cholesterol (LDL-C) was calculated by using Friedewalds formula. The apoA-I and apoB concentrations were measured by immunonephelometry. Blood glucose was measured after protein removal by glucose dehydrogenase method and insulin level by radioimmunoassay (RIA).

### 2.3. Solid-phase minisequencing

The genomic DNA from white blood cells was isolated using the 'salting-out' procedure [31]. Genotyping was done using solid-phase minisequencing method [32]. Three different primers were used. First the exon 2 of FABP2 was amplified by PCR using a forward primer 5'-ACAGGTGTTAATATAGTGAA-AAGG-3' and a reverse biotinylated primer 5'-TACCCTGAG-TTCAGTTCCTGCTGC-3'. The product was analyzed using a detection primer 5'-TCACAGTCAAAGAAT-CAAGC-3' in a single step extension reaction in avidine coated wells with a radiolabeled nucleotide (either G or A). The PCR conditions were: denaturing at 95°C, annealing at 60°C and extension at 72°C, each for 30 s in total of 30 cycles. The PCR reaction volume was 40 µl. The reaction temperature for detection was 50°C.

### 2.4. Statistical analysis

The database containing results obtained from various centers is stored in Paris on an IBM Risc 6000 computer. Statistical analyses were performed with the SAS statistical software (SAS Institute Inc.). Genotypic associations on basic characteristics were tested by linear models (SAS-PROC GLM) adjusted for age, center, and paternal history of MI. The area under curve (AUC) of GTT and FTT was calculated by the trapezoidal rule. Heterogeneity between cases and controls and between European regions was tested by introducing a corresponding interaction term in the general linear model. The time of peak values were compared using the Kruskal–Wallis rank-sum test. The distribution of alleles between cases and controls was tested using  $\chi^2$  test for each region and also for the whole study population. The power of this study was over 0.90 to detect a 0.2 mmol/l difference in fasting serum triacylglycerol or glucose between the Ala/Ala or Thr/Thr homozygous individuals and a 0.1 mmol/l difference between Ala/Ala homozygotes and Ala/Thr heterozygotes. The theoretical power to infer genotype associations from studies comparing matched offspring has been discussed in a previous study [33].

## 3. Results

### 3.1. Distribution of genotypes

The allele and genotype frequencies of the FABP2 polymorphism in four regions, Baltic, United Kingdom, middle and southern Europe, are shown in Table 1. There were no significant differences in allele frequencies between cases and controls in any region or in all regions together. The allele frequencies did not differ significantly between European regions. The mean frequency of the alanine encoding allele was 0.729.

### 3.2. Clinical and biochemical characteristics according to genotypes

Some basic characteristics of the study subjects, divided in groups according to their genotype, are given in Table 2. The mean age of the students was 23 years, BMI 23.3, total cholesterol 4.4 mmol/l, HDL-C 1.2 mmol/l and glucose 5.2 mmol/l. There were no significant differences between the three genotypic groups in any of the parameters measured.

### 3.3. Postprandial response to glucose and fat load

The curves representing the mean glucose and insulin levels following the GTT and triacylglycerol levels following the FTT are shown in Figs. 1–3. The results of GTT and FTT are summarized in Table 3. Because there were no significant interactions between genotype and case/control status or region when results of GTT or FTT were analyzed, only pooled results are shown in Figs. 1–3 and in Tables 2 and 3. There were no significant differences between groups of individuals having different genotypes, in response to GTT or FTT.

## 4. Discussion

Based on the widespread distribution of FABPs in mammalian cells and on their high expression level in specific cell types, it is evident that FABPs most probably play a critical role in intracellular physiology. FABP2 is mainly expressed in epithelial cells of small intestine, in areas where fatty acid absorption is known to be most effective. Also FABP1 is expressed at high levels in these cells. Because the FABP1 and FABP2 are not expressed coordinatively during developmental stages and because they have different tissue distribution, they probably have different roles within the cells [19]. Intestinal epithelium has been shown to preferentially oxidize fatty acids derived from plasma and esterify fatty acids derived from the intestinal lumen [34]. This led to a hypothesis that FABP2 transports long

chain fatty acids across cytosol for triacylglycerol formation in smooth endoplasmic reticulum (apical pathway) and FABP1 supplies fatty acids for oxidation and phospholipid synthesis (basolateral pathway) [35].

However, based on structural studies by NMR, it has been suggested that FABP2 exclusively binds and transports fatty acids whereas FABP1 binds and transports variety of ligands including bile salts, monoacylglyc-

Table 1  
Genotype and allele frequency distribution for the Ala54Thr polymorphism of the FABP2 gene in cases and controls in four regions, Baltic, United Kingdom, middle and southern Europe

Study group	Genotype			Allele		<i>P</i> (Alleles) ( $\chi^2$ test)
	Ala/Ala	Ala/Thr	Thr/Thr	Ala	Thr	
<i>Baltic countries</i>						
Number of cases	33	34	4			
Frequency	0.465	0.479	0.056	0.704	0.296	
Number of controls	38	26	11			
Frequency	0.507	0.347	0.147	0.680	0.320	0.65
<i>United Kingdom</i>						
Number of cases	34	30	3			
Frequency	0.508	0.448	0.045	0.731	0.269	
Number of controls	43	22	4			
Frequency	0.623	0.319	0.058	0.783	0.217	0.32
<i>Middle Europe</i>						
Number of cases	51	49	9			
Frequency	0.468	0.450	0.083	0.693	0.307	
Number of controls	52	44	6			
Frequency	0.510	0.431	0.059	0.726	0.275	0.46
<i>Southern Europe</i>						
Number of cases	48	33	2			
Frequency	0.578	0.398	0.024	0.777	0.223	
Number of controls	51	33	6			
Frequency	0.567	0.367	0.067	0.750	0.250	0.55
<i>All regions</i>						
Number of cases	166	146	18			
Frequency	0.503	0.442	0.055	0.724	0.276	
Number of controls	184	125	27			
Frequency	0.548	0.372	0.080	0.734	0.266	0.73
<i>All participants</i>						
Number (total = 666)	350	271	45			
Frequency	0.526	0.407	0.068	0.729	0.271	

Table 2  
Selected characteristics of subjects according to FABP2 genotype (mean  $\pm$  S.E.M. in the fasting state)

Characteristic	Genotype			<i>P</i> <sup>a</sup>
	Ala/Ala ( <i>N</i> = 350)	Ala/Thr ( <i>N</i> = 271)	Thr/Thr ( <i>N</i> = 45)	
Age, years	22.7 $\pm$ 0.1	22.8 $\pm$ 0.1	23.5 $\pm$ 0.3	0.09
BMI, kg/m <sup>2</sup>	23.3 $\pm$ 0.2	23.3 $\pm$ 0.2	23.4 $\pm$ 0.4	0.95
Waist/hip	0.85 $\pm$ 0.00	0.85 $\pm$ 0.00	0.84 $\pm$ 0.01	0.51
Glucose mmol/l	5.20 $\pm$ 0.02	5.17 $\pm$ 0.03	5.20 $\pm$ 0.06	0.69
Insulin mU/l	11.75 $\pm$ 0.27	11.28 $\pm$ 0.30	10.85 $\pm$ 0.70	0.31
Triacylglycerol mmol/l	1.01 $\pm$ 0.03	0.98 $\pm$ 0.03	1.07 $\pm$ 0.07	0.27
Cholesterol, mmol/l	4.42 $\pm$ 0.05	4.40 $\pm$ 0.06	4.36 $\pm$ 0.13	0.86
HDL-C, mmol/l	1.18 $\pm$ 0.015	1.19 $\pm$ 0.017	1.13 $\pm$ 0.037	0.28
LDL-C, mmol/l	2.81 $\pm$ 0.047	2.78 $\pm$ 0.05	2.79 $\pm$ 0.19	0.89
Apo-B, mg/dL	72.0 $\pm$ 1.0	70.6 $\pm$ 1.1	71.2 $\pm$ 2.7	0.61
Apo-AI, mg/dL	99.6 $\pm$ 1.0	100.8 $\pm$ 1.1	98.8 $\pm$ 2.7	0.58

<sup>a</sup> General linear model (SAS-PROC GLM), adjusted for age, center and paternal history of MI.

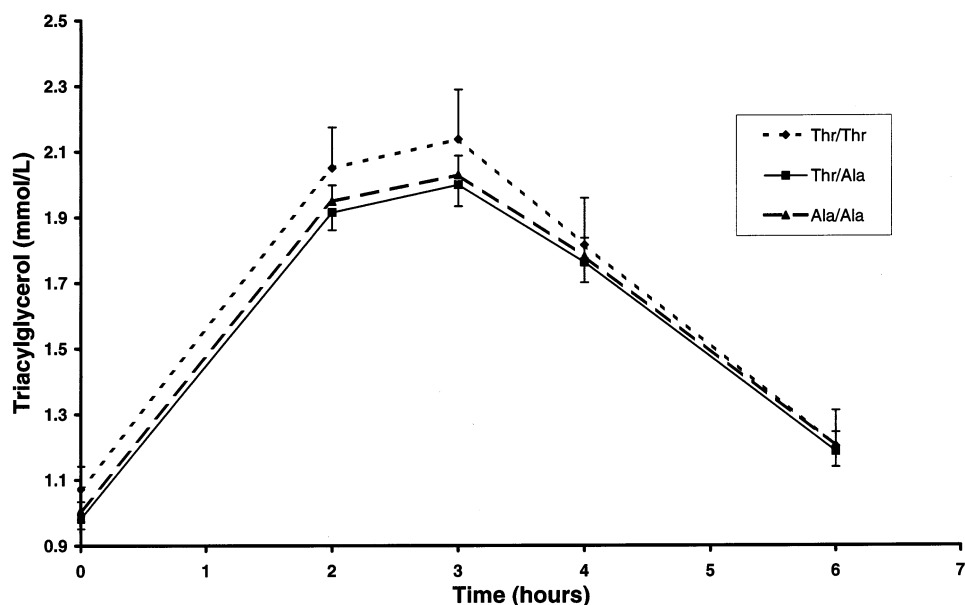


Fig. 1. Postprandial mean triacylglycerol concentrations ( $\pm$  S.E.M.) in male individuals with different Ala54Thr genotypes of the FABP2 gene. Cases and controls are pooled (no interaction with genotype). The values shown are adjusted for age, center and paternal history of MI.

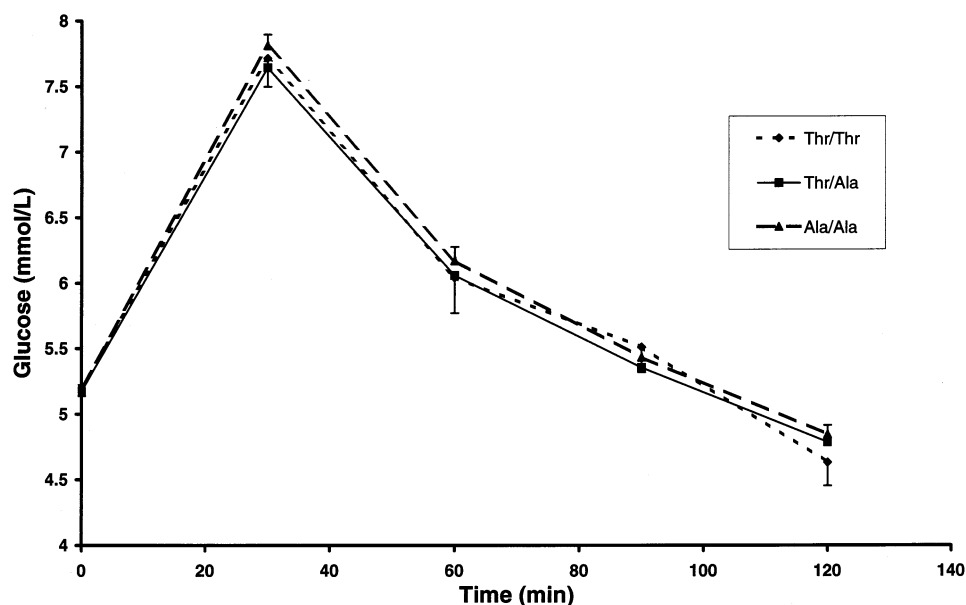


Fig. 2. Postprandial mean serum glucose concentrations ( $\pm$  S.E.M.) in male individuals with different Ala54Thr genotypes of the FABP2 gene during an oral glucose tolerance test. S.E.M. is indicated with a bar only for Thr/Thr and Ala/Ala homozygotes. Cases and controls are pooled (no interaction with genotype). The values shown are adjusted for age, center and paternal history of MI.

erols and fatty acyl-CoA esters. According to that view, both proteins operate on both basolateral and apical pathways [36]. Other functions suggested for FABPs include regulation of mitosis, cell growth, cell differentiation, fatty acid mediated signal transduction, gene expression, and protection of cells against the toxic effects of fatty acids [37].

There are still many open questions concerning the Ala54Thr polymorphism. The findings that the threonine encoding allele is associated with increased

fasting insulin concentration and higher insulin response in glucose tolerance test in Pimas [23] has not been repeated in other populations [26,38]. The Ala54Thr polymorphism was not associated with type 2 diabetes in Pimas [23], with obesity in Finns [38] or with familial combined hyperlipidemia (FCHL) in Finnish FCHL families [26]. It has been suggested that the Ala54Thr polymorphism might associate with the risk for atherosclerosis because it causes a compositional change in LDL particles [26], an altered post-

prandial lipemia [27] or a diabetes-related phenotype [24]. In screens for polymorphisms in FABP2 gene the Ala54Thr has been the only polymorphism identified to change an amino acid in the mature FABP2 protein thus far [23,26,39]. The functional role of the Ala54Thr polymorphism is supported by the findings that this codon may be important for the conformational adjustment between apo- and holoproteins [40], the threonine-containing protein has greater affinity for oleate (C18:1) and arachidonate than the protein hav-

ing alanine at that codon [26] and by the fact that the Ala54Thr polymorphism of FABP2 can alter the transfer of fatty acids across Caco-2 cells [41]. A counter argument on the importance of FABP2 in regulating intestinal fatty acid absorption is that the rate-limiting step of fatty acid transport in intestinal epithelial cells is believed to be between the movement of triacylglycerol from the endoplasmic reticulum to Golgi [42].

In the present study there were no specific associations of the Ala54Thr polymorphism on any of the

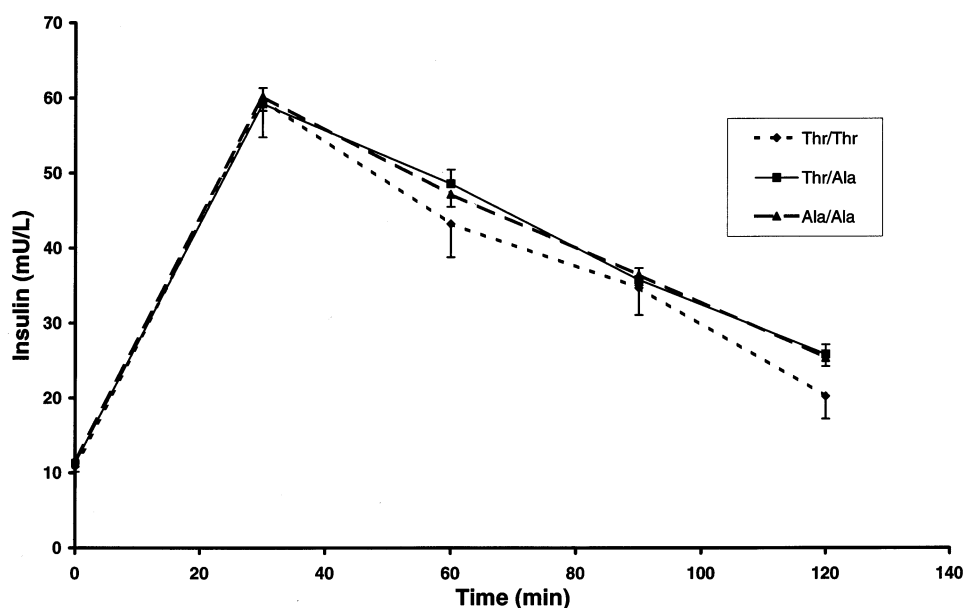


Fig. 3. Postprandial mean serum insulin concentrations ( $\pm$  S.E.M.) in male individuals with different Ala54Thr genotypes of the FABP2 gene during an oral glucose tolerance test. Cases and controls are pooled (no interaction with genotype). The values shown are adjusted for age, center and paternal history of MI.

Table 3  
Glucose and fat tolerance test results of the study subjects according to FABP2 genotype (mean  $\pm$  S.E.M.)

Test group <sup>a</sup>	Peak <sup>b</sup>	Time at peak <sup>c</sup> (h)	Area under curve <sup>b</sup>
<i>Glucose in GTT (mmol/l)</i>			
Ala/Ala	2.73 $\pm$ 0.08	0.56 $\pm$ 0.01	1.82 $\pm$ 0.11
Ala/Thr	2.61 $\pm$ 0.10	0.57 $\pm$ 0.02	1.67 $\pm$ 0.13
Thr/Thr	2.67 $\pm$ 0.21	0.56 $\pm$ 0.04	1.69 $\pm$ 0.29
P-value	0.59	1.00	0.60
<i>Insulin in GTT (mU/l)</i>			
Ala/Ala	55.51 $\pm$ 1.94	0.75 $\pm$ 0.02	57.66 $\pm$ 1.95
Ala/Thr	55.86 $\pm$ 2.17	0.76 $\pm$ 0.02	58.51 $\pm$ 2.19
Thr/Thr	55.27 $\pm$ 5.11	0.73 $\pm$ 0.06	54.89 $\pm$ 5.15
P-Value	0.89	0.83	0.76
<i>Triacylglycerol in FTT (mmol/l)</i>			
Ala/Ala	1.20 $\pm$ 0.04	2.87 $\pm$ 0.05	3.79 $\pm$ 0.14
Ala/Thr	1.19 $\pm$ 0.04	2.90 $\pm$ 0.06	3.79 $\pm$ 0.15
Thr/Thr	1.22 $\pm$ 0.10	2.79 $\pm$ 0.13	3.78 $\pm$ 0.35
P-value	0.78	0.74	0.60

<sup>a</sup> Thr/Thr  $N = 45$ , Thr/Ala  $N = 271$ , and Ala/Ala  $N = 350$ .

<sup>b</sup> P-values for peak and AUC are based on a general linear model (SAS-PROC GLM) adjusted for fasting values. Peak values represent change from baseline. AUC is calculated by trapezoidal rule. AUC units are relative (mmol h/l or mU h/l).

<sup>c</sup> P-value for time at peak is based on Kruskal–Wallis rank-sum test.

studied lipid or glucose related parameters. The results were negative both in fasting and in postprandial state. The European populations did not differ in this regard. The allele frequencies did not depend on the paternal history of MI. The reasons for the obvious discrepancy between these results and earlier positive results are unknown. Different results between association studies conducted in different populations could be explained by linkage disequilibrium to another locus in one population but not in the other. Because Pima Indians and Mexican-Americans are genetically related populations, there is the possibility that the locus associated with insulin levels may be specific for them [22]. FABP2 gene contains also an intragenic ATT-trinucleotide polymorphism [43]; the allele 1 of that polymorphism was associated with increased insulin sensitivity and allele 3 with insulin resistance in Pima Indians [21]. In a European case-control study between type II diabetic and non-diabetic individuals from Finland, the UK, and Wales, there was no association between this trinucleotide polymorphism and glucose intolerance [44]. It is also possible to get a true negative result in an association study even if there is a true positive result in a linkage study, even within one population. To test whether this region contains a gene causing an increased risk of type 2 diabetes in Europeans, a linkage study using family material should be done.

Whether or not Ala54Thr polymorphism is functional, environmental factors and gene to gene interactions may affect study results. One potential environmental variable is diet. Increased absorption of polyunsaturated  $\omega$ -3 fatty acids might even improve insulin sensitivity whereas increased absorption of saturated long chain fatty acids would reduce it [26]. Ala54Thr polymorphism might affect the absorption efficiency of long-chain fatty acids and change the serum lipid composition. However, that has not been detected in previous studies [38]. It has been suggested that the associations of genetic factors with physiological variables are more easily seen in young individuals with relative absence of confounding factors [24]. Our study population consisted of healthy young students. Many of the previous studies, reporting significant association of FABP2 on glucose and lipid metabolism, have been focused on populations with obesity, diabetes or hyperlipidemia. Maybe FABP2 polymorphism Ala54Thr is only associated with serum glucose and lipid levels when there is a clustering of other pathological factors. An example of such a modification can be found from a study in which the Ala54Thr polymorphism was genotyped in familial combined hyperlipidemia patients and in their family members. In the study by Pihlajamäki et al. [26] the patients having Thr54 allele had 20% higher LDL-C than Ala54Ala homozygous patients, non-affected family members having Thr54 allele had 2.7% lower LDL-C than those

having Ala54Ala genotype. In conclusion, there are several unanswered questions concerning Ala54Thr polymorphism of FABP2 that have to be studied and verified in carefully planned study settings.

### Acknowledgements

This study was supported by the European Community Concerted Action MRH4 COMAC Epidemiology, the Research Foundation of Orion Corporation and by the Finnish Foundation for Cardiovascular Research. The authors want to thank Liisa Ikävalko for her valuable help in laboratory.

### Appendix A

EARS II Project Leader:

D.St.J. O'Reilly, UK

EARS II Project Management Group:

F. Cambien, France

G. De Backer, Belgium

D. St. J. O'Reilly, UK

M. Rosseneu, Belgium

J. Shepherd, UK

L. Tiret, France

The EARS II Group Collaborating Centers and their Associated Investigators:

Austria:

H. J. Menzel, Institute for Medical Biology and Genetics, University of Innsbruck, laboratory.

Belgium:

G. De Backer, S. De Henauw, Department of Public Health, University of Ghent, recruitment center.

Belgium:

M. Rosseneu, Laboratorium voor Lipoproteïne Chemie/Vakgroep Biochemie, University of Ghent, laboratory.

Denmark:

O. Faergeman, C. Gerdes, I. C. Klausen, Medical Department I, Aarhus Amtssygehus, Aarhus, recruitment center.

Estonia:

M. Saava, K. Aasvee, Department of Nutrition and Metabolism, Estonian Institute of Cardiology, Tallinn, recruitment center.

Finland:

C. Ehnholm\*, R. Elovainio\*\*, J. Peräsalo, \* National Public Health Institute; \*\* The Finnish Student Health Service, Helsinki, recruitment center.

Finland:

Y.A. Kesäniemi\*, M.J. Savolainen\*, P. Palomaa\*\*, \* Department of Internal Medicine and

Biocenter, Oulu, \*\* The Finnish Student Health Service, University of Oulu, recruitment center and laboratory.

France:

L. Tiret, V. Nicaud, R. Rakotovoao, INSERM U258, Hôpital Broussais, Paris, EARS data center.

France:

S. Visvikis, Centre de Médecine Préventive, Nancy, laboratory.

France:

J. C. Fruchart, J. Dallongeville, Service de Recherche sur les Lipoprotéines et l'Athérosclérose (SERLIA), INSERM U325, Institut Pasteur, Lille, laboratory.

Germany:

U. Beisiegel, C. Dingler, Medizinische Klinik Universitäts-Krankenhaus Eppendorf, Hamburg, recruitment center and laboratory.

Greece

G. Tsitouris, N. Papageorgakis, G. Kolovou, Department of Cardiology, Evangelismos Hospital, Athens, recruitment center.

Italy:

E. Farinero, Institute of Internal Medicine and Metabolic Disease, University of Naples, recruitment center.

The Netherlands:

L. M. Havekes, IVVO-TNO Health Research, Gaubius Institute, Leiden, laboratory.

Portugal:

M. J. Halpern, J. Canena, Instituto Superior de Ciencias da Saude, Lisbon, recruitment center.

Spain:

L. Masana, J. Ribalta, A. Jammoul, A. LaVille, Unitat Recerca Lipids, University Rovira i Virgili, Reus, recruitment center and laboratory.

Switzerland:

F. Gutzwiller, B. Martin, Institute of Social and Preventive Medicine, University of Zurich, recruitment center and laboratory.

United Kingdom:

D.St.J. O'Reilly, M. Murphy, Institute of Biochemistry, Royal Infirmary, Glasgow, recruitment center and laboratory.

United Kingdom:

S.E. Humphries, P.J. Talmud, V. Gudnason, R.M. Fisher, University College London School of Medicine, London, laboratory.

United Kingdom:

D. Stansbie, A.P. Day, M. Edgar, Department of Chemical Pathology, Royal Infirmary, Bristol, recruitment center and laboratory.

United Kingdom:

F. Kee \*, A. Evans \*\*, \* Northern Health and Social Services Board, \*\* Department of Epi-

demology and Public Health, the Queen's University of Belfast, Belfast, recruitment center.

## References

- [1] Schleicher CH, Cordoba OL, Santome JA, Dell'Angelica EC. Molecular evolution of the multigene family of intracellular lipid-binding proteins. *Biochem Mol Biol Int* 1995;36:1117.
- [2] Glatz JF, Luiken JJ, van Nieuwenhoven FA, Van der Vusse GJ. Molecular mechanism of cellular uptake and intracellular translocation of fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 1997;57:3.
- [3] Glatz JF, van Nieuwenhoven FA, Luiken JJ, Schaap FG, van der Vusse GJ. Role of membrane-associated and cytoplasmic fatty acid-binding proteins in cellular fatty acid metabolism. *Prostaglandins Leukot Essent Fatty Acids* 1997;57:373.
- [4] Burnett DA, Lysenko N, Manning JA, Ockner RK. Utilization of long chain fatty acids by rat liver: studies of the role of fatty acid binding protein. *Gastroenterology* 1979;77:241.
- [5] Thompson ABR, Schoeller C, Keelan M, Smith L, Cladinin MT. Lipid absorption: passing through the unstirred layers, brush-border membrane, and beyond. *Can J Physiol Pharmacol* 1993;71:523.
- [6] Davidson NO. Cellular and molecular mechanisms of small intestinal lipid transport. In: Johnson LR, editor. *Physiology of the Gastrointestinal Tract*, 3rd ed. New York: Raven, 1994:1909–34.
- [7] Schroeder F, Jolly CA, Cho TH, Frolov A. Fatty acid binding protein isoforms: structure and function. *Chem Phys Lipids* 1998;92:1.
- [8] Ockner RK. A binding protein for fatty acids in cytosol of intestinal mucosa, liver, and other tissues. *J Clin Invest* 1972;51:70 Abstract.
- [9] Ockner RK, Manning JA. Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J Clin Invest* 1974;54:326.
- [10] Ockner RK, Manning JA, Kane JP. Fatty acid binding protein. Isolation from rat liver, characterization, and immunochemical quantification. *J Biol Chem* 1982;257:7872.
- [11] Offner GD, Brecher P, Sawlilich WB, Costello CE, Troxler RF. Characterization and amino acid sequence of a fatty acid-binding protein from human heart. *Biochem J* 1988;252:191.
- [12] Peeters RA, Veerkamp JH, Geurts van Kessel A, Kanda T, Ono T. Cloning of the cDNA encoding human skeletal-muscle fatty-acid-binding protein, its peptide sequence and chromosomal localization. *Biochem J* 1991;276:203.
- [13] Baxa CA, Sha RS, Buel MK, Smith AJ, Matarese V, Chinander LL, Boundy KL, Bernlohr A. Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry* 1989;28:8683.
- [14] Madsen P, Rasmussen HH, Leffers H, Honore B, Celis JE. Molecular cloning and expression of a novel keratinocyte protein (psoriasis-associated fatty acid-binding protein [PA-FABP]) that is highly up-regulated in psoriatic skin and that shares similarity to fatty acid-binding proteins. *J Invest Dermatol* 1992;99:299.
- [15] Oelkers P, Dawson PA. Cloning and chromosomal localization of the human ileal lipid-binding protein. *Biochim Biophys Acta* 1995;1257:199.
- [16] Bennett E, Stenvers KL, Lund PK, Popko B. Cloning and characterization of a cDNA encoding a novel fatty acid binding protein from rat brain. *J Neurochem* 1994;63:1616.
- [17] Shimizu F, Watanabe TK, Shinomiya H, Nakamura Y, Fujiwara T. Isolation and expression of a cDNA for human brain

- fatty acid-binding protein (B-FABP). *Biochim Biophys Acta* 1997;1354:24.
- [18] Godbout R, Bisgrove DA, Shkolny D, Day RS. Correlation of B-FABP and GFAP expression in malignant glioma. *Oncogene* 1998;16:1955.
- [19] Gordon JI, Elshourbagy N, Lowe JB, Liao WS, Alpers DH, Taylor JM. Tissue specific expression and developmental regulation of two genes coding for rat fatty acid binding proteins. *J Biol Chem* 1985;260:1995.
- [20] Sparkes RS, Mohandas T, Heinzmann C, Gordon JI, Klisak I, Zollman S, Sweetser DA, Ragnathan L, Winokur S, Lusis AJ. Human fatty acid binding protein assignments intestinal to 4q28–4q31 and liver to 2p11. *Cytogenet Cell Genet* 1987;46:697 Abstract.
- [21] Prochazka M, Lillioja S, Tait JF, Knowler WC, Mott DM, Spraul M, Bennett PH, Bogardus C. Linkage of chromosomal markers on 4q with a putative gene determining maximal insulin action in Pima Indians. *Diabetes* 1993;42:514.
- [22] Mitchell BD, Kammerer CM, O'Connell P, Harrison CR, Manire M, Shipman P, Moyer MP, Stern MP, Frazier ML. Evidence for linkage of postchallenge insulin levels with intestinal fatty acid-binding protein (FABP2) in Mexican-Americans. *Diabetes* 1995;44:1046.
- [23] Baier LJ, Sacchettini JC, Knowler WC, et al. An amino acid substitution in the human intestinal fatty acid binding protein is associated with increased fatty acid binding, increased fat oxidation, and insulin resistance. *J Clin Invest* 1995;95:1281.
- [24] Hegele RA, Harris SB, Hanley AJG, Sadikian S, Connelly PW, Zinman B. Genetic variation of intestinal fatty acid-binding protein associated with variation in body mass in aboriginal Canadians. *J Clin Endocr Metab* 1996;81:4334.
- [25] Hegele RA, Connelly PW, Hanley AJG, Sun F, Harris SB, Zinman B. Common genomic variants associated with variation in plasma lipoproteins in young aboriginal Canadians. *Arterioscler Thromb Vasc Biol* 1997;17:1060.
- [26] Pihlajamaki J, Rissanen J, Heikkinen S, Karjalainen L, Laakso M. Codon 54 polymorphism of the human intestinal fatty acid binding protein 2 gene is associated with dyslipidemias but not with insulin resistance in patients with familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 1997;17:1039.
- [27] Ågren JJ, Valve R, Vidgren H, Laakso M, Uusitupa M. Postprandial lipemic response is modified by the polymorphism at codon 54 of the fatty acid-binding protein 2 gene. *Arterioscler Thromb Vasc Biol* 1998;18:1606.
- [28] The EARS Group. The European Atherosclerosis research study (EARS): design and objectives. *Int J Epidemiol* 1994;23:465.
- [29] EARS, Rosseneu M, Fruchart JC, Bard JM, Nicaud V, Vinaimont N, Cambien F, De Backer G. Plasma apolipoprotein concentrations in young adults with a parental history of premature coronary heart disease and in control subjects. The EARS study. *Circulation* 1994;89:1967.
- [30] EARS, Humphries SE, Nicaud V, Margalef J, Tiret L, Talmud P. Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides. *Arterioscler Thromb Vasc Biol* 1998;18:526.
- [31] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
- [32] Syvänen AC, Sajantila A, Lukka M. Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. *Am J Hum Genet* 1993;52:46.
- [33] Tiret L, Nicaud V, Ehnholm C, Havekes L, Menzel HJ, Ducimetière P, Cambien F. Inference of the strength of genotype-disease association from studies comparing offspring with and without parental history of disease. *Ann Hum Genet* 1993;57:141.
- [34] Gangl A, Ockner RK. Intestinal metabolism of plasma free fatty acids. Intracellular compartmentation and mechanisms of control. *J Clin Invest* 1975;55:803.
- [35] Bass NM, Manning JA, Ockner RK, Gordon JI, Seetharam S, Alpers DH. Regulation of the biosynthesis of two distinct fatty acid-binding proteins in rat liver and intestine. Influences of sex difference and of clofibrate. *J Biol Chem* 1985;260:1432.
- [36] Cistola DP, Sacchettini JC, Banaszak LJ, Walsh MT, Gordon JI. Fatty acid interactions with rat intestinal and liver fatty acid-binding proteins expressed in *Escherichia coli*. A comparative <sup>13</sup>C NMR study. *J Biol Chem* 1989;264:2700.
- [37] Glatz JF, Borchers T, Spener F, van der Vusse GJ. Fatty acids in cell signalling: modulation by lipid binding proteins. *Prostaglandins Leukot Essent Fatty Acids* 1995;52:121.
- [38] Sipilainen R, Uusitupa M, Heikkinen S, Rissanen A, Laakso M. Variants in the human intestinal fatty acid binding protein 2 gene in obese subjects. *J Clin Endocr Metab* 1997;82:2629.
- [39] Saarinen L, Pulkkinen A, Kareinen A, Heikkinen S, Lehto S, Laakso M. Variants of the fatty acid-binding protein 2 gene are not associated with coronary heart disease in nondiabetic subjects and in patients with NIDDM. *Diabetes Care* 1998;21:849.
- [40] Sacchettini JC, Gordon JI. Rat intestinal fatty acid binding protein. *J Biol Chem* 1993;268:18399.
- [41] Baier LJ, Bogardus C, Sacchettini JC. A polymorphism in the human intestinal fatty acid binding protein alters fatty acid transport across Caco-2 cells. *J Biol Chem* 1996;271:10892.
- [42] Mansbach CM, Nevin P. Intracellular movement of triacylglycerols in the intestine. *J Lipid Res* 1998;39:963.
- [43] Polymeropoulos MH, Rath DS, Xiao H, Merrill CR. Trinucleotide repeat polymorphism at the human intestinal fatty acid binding protein gene (FABP2). *Nucleic Acids Res* 1990;18:7198.
- [44] Humphreys P, McCarthy M, Tuomilehto J, et al. Chromosome 4q locus associated with insulin resistance in Pima Indians. Studies in three European NIDDM populations. *Diabetes* 1994;43:800.