

# Cardiovascular Risk Factors in Children. Main Findings of the Four Provinces Study

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Atherosclerosis is a process that begins during the first few decades of life, progresses asymptotically, and generally shows no clinical manifestations until adulthood. The Four Provinces study, which involved 1275 prepubertal children, was an investigation into childhood risk factors for atherosclerosis (e.g., diet, anthropometric variables, lipid levels, and some genetic factors) that may be related to the occurrence of coronary artery disease in adults. In this review, we summarize the study's most important findings. The Four Provinces study showed that several factors associated with the metabolic syndrome (i.e., obesity, and raised glucose and triglyceride levels), which can lead to coronary disease in adults, are already present in schoolchildren. What is more, children from provinces with high coronary disease mortality weighed more and had higher plasma triglyceride and glucose levels. A high percentage of children in all provinces studied had total cholesterol and low-density lipoprotein cholesterol plasma levels above the recommended values. Despite the presence of some positive features in the population as a whole, such as a raised high-density lipoprotein cholesterol level and low prevalence of the apolipoprotein-E  $\epsilon$ 4 allele, which is known to be related to cardiovascular risk, if the metabolic changes described above persist, the risk of cardiovascular disease in Spain can only increase in the future. These metabolic characteristics are associated with a high-fat, low-carbohydrate diet which is far from that currently recommended for children. Correction of this poor diet at an early age would have significant benefits for the prevention of cardiovascular disease.

**Key words:** *Lipids. Obesity. Diet. Genetics. Risk factors. Coronary disease. Children.*

The researchers taking part in the Four Provinces study are listed at the end of the article.

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## Factores de riesgo cardiovascular en la edad infantil. Resultados globales del estudio Cuatro Provincias

La aterosclerosis es un proceso que se inicia en las primeras décadas de la vida y evoluciona de forma asintomática, en general, sin expresión clínica hasta la edad adulta. En el estudio Cuatro Provincias hemos analizado, en 1.275 niños de edad prepuberal, factores de riesgo (dieta, variables antropométricas, concentraciones de lípidos, vitaminas y algunos determinantes genéticos) que pueden estar relacionados con la aparición de la enfermedad coronaria en la edad adulta. En esta revisión resumimos de forma global, las principales aportaciones del estudio. El estudio 4P ha reflejado que los aspectos relacionados con el síndrome metabólico (obesidad, concentraciones elevadas de glucosa y triglicéridos) que conducen a la enfermedad coronaria en el adulto están ya expresados en la edad escolar. En este sentido, los niños de las provincias con una alta mortalidad coronaria pesan más y tienen unas concentraciones más altas de triglicéridos y glucosa. El porcentaje de niños que supera las concentraciones recomendables de colesterol total y colesterol unido a lipoproteínas de baja densidad es elevado en todas las provincias. A pesar de que hay aspectos positivos, como que las concentraciones de colesterol unido a lipoproteínas de alta densidad son también elevadas y que la prevalencia del alelo  $\epsilon$ 4 del gen de la apo-E, claramente relacionada con el riesgo cardiovascular, es baja en el conjunto de la población, si las alteraciones metabólicas descritas persisten, la situación de España respecto al riesgo cardiovascular puede empeorar en el futuro. Estos aspectos metabólicos se asocian con una alimentación rica en grasas y con un bajo consumo de hidratos de carbono, alejada de las recomendaciones actuales para la infancia. Su corrección en edades tempranas tendría enormes beneficios en la prevención de la enfermedad coronaria.

**Palabras clave:** *Lípidos. Obesidad. Dieta. Genética. Factores de riesgo. Enfermedad coronaria. Niños.*

## INTRODUCTION

At present, we know that the pathological processes that lead to the development of atherosclerosis and coronary disease begin in childhood.<sup>1</sup> These progress asymptotically and generally without clinical

**ABBREVIATIONS**

FFA: free fatty acids  
 DHEA-S: dehydroepiandrosterone sulfate  
 HDL-C: high-density lipoprotein cholesterol  
 LDL-C: low-density lipoprotein cholesterol  
 BMI: body mass index

manifestations until adulthood. Intravascular ultrasound imaging studies have detected atherosclerotic lesions in the coronary arteries of 17% of individuals aged less than 20 years,<sup>2</sup> demonstrating the very early onset of this disease. These processes seem to be associated with cardiovascular risk factors in the first decades of life.<sup>3</sup>

The Four Provinces study<sup>4</sup> (4P) was an epidemiological project investigating such risk factors in prepubertal children that may explain the incidence of coronary disease in adults. It was a comparative cross-sectional study which investigated cardiovascular risk factors in 1275 children aged 6-8 years living in 4 Spanish provinces. In 1993,<sup>4</sup> there was a significant difference in adult coronary mortality in these provinces which comprised Cádiz and Murcia, with high mortality, and Madrid and Orense, with low mortality. The children were selected from clusters of schools through random sampling and were stratified by sex and socioeconomic level.<sup>4</sup>

We investigated diet (food and nutrient intake), anthropometric variables (weight, prevalence of obesity, etc), plasma lipid levels, vitamins, and insulin, and genetic factors clearly related to lipid levels, such as the apolipoprotein-E genotype (APOE). Fieldwork and data collection were conducted during 1998-2000 in the same season in each province. We summarize the study's main findings in this review article.

**ANTHROPOMETRIC VARIABLES AND OBESITY**

Analysis of the anthropometric variables showed that obesity was very prevalent among the children included in the study. Child obesity is reaching alarming proportions and is becoming a significant public health problem.<sup>5</sup> Using the cut-off points proposed by Cole et al,<sup>6</sup> the estimated prevalence of obesity for each age and sex was 9.4% in boys and 10.5% in girls.<sup>7</sup> One of the most interesting results of the 4P study was the significant difference in the prevalence of obesity between the provinces studied. The percentage of children who had a Body Mass Index (BMI) above 20.1 was almost double in the high-mortality provinces (15.9% in Cádiz and 14.1% in Murcia) than in the low-mortality provinces

(8% in Madrid and 9.4% in Orense).<sup>8</sup> Associated with this, the average levels of glucose and triglycerides in the children in the high-mortality provinces were statistically higher than those in the children in the low-mortality provinces, which indicates that these children have different metabolic profiles.

When the metabolic changes associated with obesity in prepubertal children of both sexes were analyzed, we found that the obese children had higher plasma triglyceride and lower high-density lipoprotein cholesterol (HDL-C) levels than the nonobese children. No differences were found in plasma glucose and low-density lipoprotein cholesterol (LDL-C) levels.<sup>7</sup> As expected, insulin levels and the homeostasis model assessment index were significantly higher in the obese children of both sexes than in the nonobese children; however, free fatty acid (FFA) levels were lower in the obese children than in the nonobese children, with a statistically significant difference in girls.<sup>7</sup> Our data demonstrate that some of the metabolic features typical of obesity in adults (high triglyceride, insulin and homeostasis model assessment levels, and low HDL-C levels) are present in obese prepubertal children. However, the metabolic features highly characteristic of adult obesity, such as high glucose and FFA levels, are not present in obese prepubertal children. These data indicate that obesity-related metabolic disorders become established in different ways as a function of age and sex, apparently depending on the chronology of sexual maturation. This is supported by reports in the literature that show that the metabolic changes associated with obesity differ according to the sex and age of the children studied.<sup>9,10</sup>

**BIOCHEMICAL VARIABLES**

Table shows the mean values of glucose and lipid levels observed in the children studied. Plasma glucose and ApoA-1 levels were higher in the boys, and triglyceride, LDL-C, and ApoB levels were higher in the girls.<sup>11</sup>

**Glucose and Triglycerides**

Analysis of the biochemical variables showed that there were significant differences between provinces. In line with the lipid theory of atherosclerosis, differences in plasma lipid levels associated with ApoB (total cholesterol and LDL-C) should be expected; however, our data suggest otherwise. Interestingly, we found that plasma glucose, triglyceride and ApoA-1 levels in the high-mortality provinces were clearly higher than those in the low-mortality provinces.<sup>11</sup> Some 21% and 11.7% of the child population in Cádiz and Murcia, respectively, had glucose values >100 mg/dL versus 8.9% and 3.7% in Orense and Madrid, respectively. Similarly, the percentage of children with triglyceride levels >100

### Plasma Lipid and Glucose Levels in Prepubertal Children in the Four Provinces Study\*

	Boys (n=633; 50.4%)	Girls (n=622; 49.6%)	P
Glucose, mg/dL	91.7 (9.7)	89.5 (12.5)	.0001
Cholesterol, mg/dL	181.1 (25.4)	183.7 (28.2)	NS
Triglycerides, mg/dL	70.8 (23.1)	73.7 (24.5)	.029
LDL-C, mg/dL	106.9 (24.5)	110.5 (26.7)	.016
ApoB, mg/dL	68.5 (13.7)	71.2 (14.8)	.001
HDL-C, mg/dL	59.6 (13.4)	58.4 (13.6)	NS
Apo A-1, mg/dL	138.1 (19.1)	135.8 (18.9)	.034

\*HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NS, nonsignificant. Values are expressed as mean (standard deviation).

mg/dL in the high-mortality provinces (13.5% in Cádiz and 13.6% Murcia) was considerably higher than the percentage in the low-mortality provinces (7.7% and 8.5% in Madrid and Orense, respectively). In the high-mortality provinces, we found that, in addition to the aforementioned, weight and BMI values were higher and there was a positive correlation between BMI and glucose and triglyceride levels. High BMI and high glucose and triglyceride levels are the most characteristic clinical features of insulin resistance. It is well-known that insulin resistance is a cardiovascular risk factor<sup>12</sup> and its presence in children could be a clear marker of future coronary mortality. However, we did not find any differences in mean insulin values between provinces, due to the fact that insulin resistance is probably not yet established at this age. We found that the prepubertal girls had higher plasma insulin levels than the boys, which indicates that the girls had begun to be more resistant to this, although other manifestations of insulin resistance were not yet present at this age.<sup>13</sup> We found higher levels of dehydroepiandrosterone sulfate (DHEA-S),<sup>14</sup> the only hormone present in children of this age, which has a clear association with insulin values.

### Total Cholesterol and Low-Density Lipoprotein Cholesterol

Mean total cholesterol levels were 181.1 mg/dL and 183.7 mg/dL in boys and girls, respectively (Table). The percentage of children with cholesterol levels above the 200 mg/dL limit was high in all the provinces<sup>11</sup> and ranged between 19.2% in Madrid and 26.6% in Murcia (Figure 1A). The percentage of children with LDL-C levels >130 mg/dL was 13% in Madrid and 22% in the remaining provinces (Figure 1B). Previous studies<sup>15</sup> performed in Madrid showed that hyperlipidemia was prevalent and this was confirmed in the 4P study.<sup>11</sup> These findings can be generalized to Spain as a whole, as confirmed by

abundant data by region<sup>16-19</sup> and in a metaanalysis of childhood cholesterolemia.<sup>20</sup>

It may seem illogical to recommend a level of 200 mg/dL total cholesterol as optimum in adults given that practically a quarter of school-age children are above this limit. Lack of concern regarding this problem may be based on the results of some studies demonstrating that in 40% of children these high levels do not persist after sexual maturation.<sup>21,22</sup> However, while accepting that some of these children reach standard lipid levels at puberty, we now know that atherosclerotic lesions begin to form early in life<sup>2</sup> and may determine risk in adults.

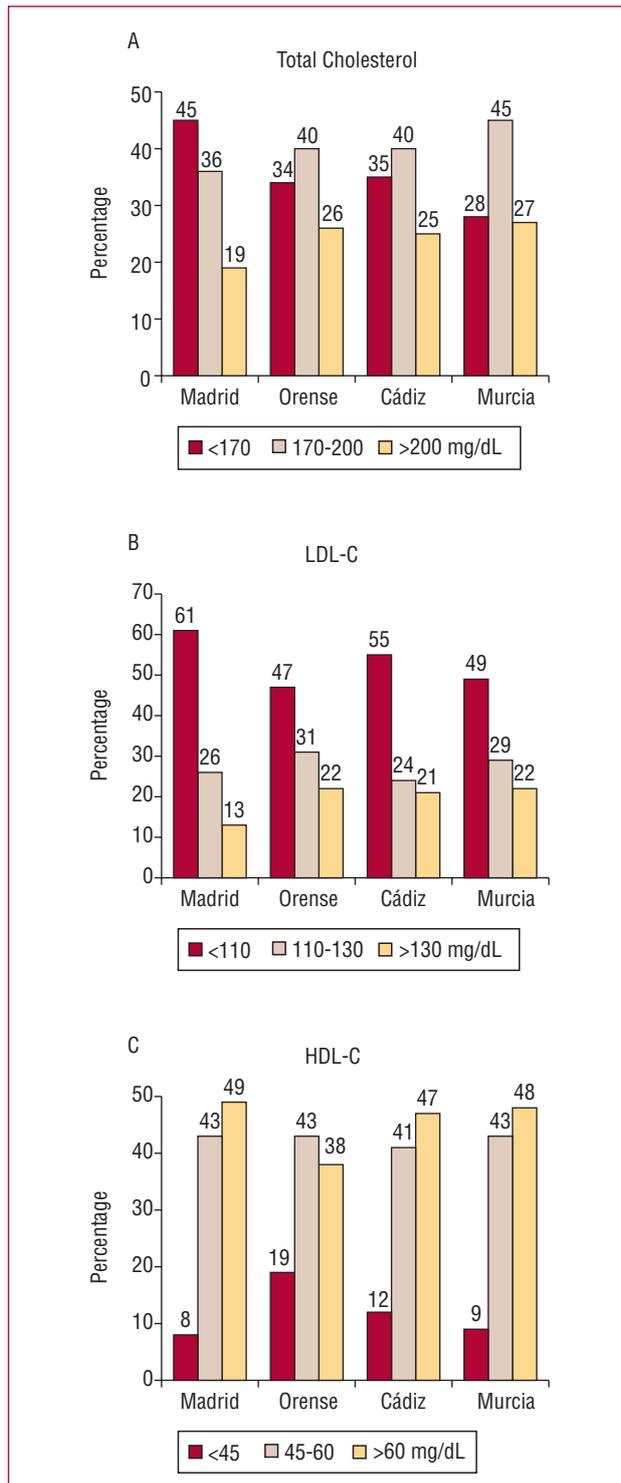
### High-Density Lipoprotein Cholesterol

We also observed high levels of HDL-C, however, together with these high values of total cholesterol and LDL-C. The level of HDL-C was 59 mg/dL in the prepubertal population.<sup>11</sup> Some 38% of the children in Orense and 47%-49% of the children in the remaining provinces had levels higher than 60 mg/dL (Figure 1C). Very high levels of HDL-C have been observed in all the studies involving school-age children conducted in Spain.<sup>15,18,19</sup> This situation may be based on dietary and/or genetic factors. Cholesterol and total fat, both saturated and monounsaturated, increase HDL-C levels. The results of our dietary pattern survey<sup>8</sup> shows that we cannot attribute the cause of these high values only to olive oil consumption, since total and saturated fat consumption is also very high.

An important question is whether these HDL-C levels remain high in the adult population thereby contributing to low coronary mortality in Spain.<sup>23,24</sup> As confirmed in the post-pubertal population, HDL-C levels decrease in boys and are high in girls,<sup>15</sup> and will persist in the latter until menopause when the levels return to equilibrium.

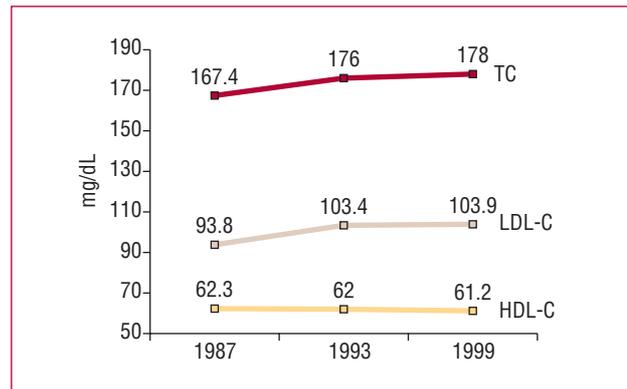
However, while accepting that lipid levels can change in children during puberty and later in maturity, some studies have established an inverse correlation between HDL-C levels in children and the incidence of coronary disease in the countries studied.<sup>25</sup> In this regard, HDL-C levels in Spanish children are higher than in children from other countries with greater coronary mortality.

Three different studies conducted by our group comparing plasma lipid levels in the school-age population in the Community of Madrid (Barrio de Pilar study<sup>26</sup> in 1987, NICAM study<sup>15</sup> in 1993, and children from Madrid in the 4P study<sup>11</sup> in 1999) (Figure 2) showed that in little more than 1 decade cholesterol levels had risen by 15 mg/dL in children of 6-8 years.<sup>27</sup> A decrease of 7 mg/dL<sup>28</sup> has been observed in North American children of the same age. Studies on trends in coronary mortality in the USA showed that the decrease in cholesterol levels in the child and adult population was



**Figure 1.** Percentage of children in each range in relation to total cholesterol, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in each province studied.

associated with a decrease in coronary heart disease mortality in adults.<sup>29</sup> In Spain, total plasma cholesterol and LDL-C levels have gradually increased. However, recent analyses of HDL-C levels have demonstrated some very high stable values, which indicates that the



**Figure 2.** Changes in plasma levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) between 1987 and 1999 in prepubertal children (6 to 8 years) in the Community of Madrid. Taken from Garcés et al.<sup>27</sup>

low coronary mortality in Spain could be related to lipid values in Spanish children.<sup>28</sup>

### Antioxidant Vitamins

We measured the plasma levels of fat-soluble antioxidant vitamins (vitamin E, carotenes, and retinol) in our prepubertal population. Without going into detail, we found that these correlate with plasma lipid levels and have very acceptable values, higher than those in other countries, and are similar to those found in populations of a similar age in Italy.<sup>30</sup>

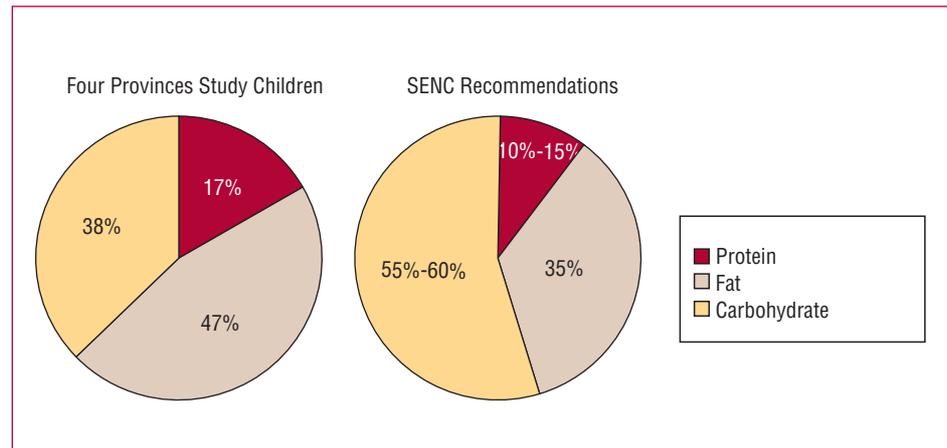
### DIET

We analyzed diet by administering a Food Consumption Frequency Questionnaire.<sup>8</sup> We found that participants had a high-calorie diet, with a high intake of fats (47% of the energy intake) (Figure 3), especially saturated ones (17%).<sup>31</sup> It was also a high-protein (17% of energy intake), low-carbohydrate (38% of energy intake)<sup>31</sup> diet (Figure 3). As can be seen in Figure 3, this diet is far from meeting the current recommendations on macronutrient intake.<sup>31</sup> In contrast, micronutrient intake, in particular antioxidant vitamins and minerals, was not deficient and was more than that recommended in all cases except in the case of vitamin B<sub>6</sub>.<sup>31</sup>

We observed that the mean energy intake was higher in the high-mortality provinces.<sup>8</sup> This diet seems to contribute to the anthropometric and biochemical variables described in the children studied and is consistent with the high prevalence of overweight and obesity.

We assessed the association between saturated fat intake and the children's anthropometric and biochemical variables. Low saturated fat intake is associated with a better lipid profile (more HDL-C and low LDL-C).<sup>32</sup> Furthermore, children with low saturated fat intake,

**Figure 3.** Macronutrient intake as a percentage of dietary energy in children in the 4P study compared to the recommendations of the Spanish Society of Community Nutrition (SENC).



despite consuming more calories, do not weigh more and have a more varied diet. These data suggest the following: a varied diet is associated with greater calorie intake, does not lead to obesity, and has a better biochemical profile.<sup>33</sup> The hypothesis that the child who has a more varied diet, despite consuming more calories, does not gain weight and has a better risk profile, is very attractive.

Regarding nutrients in the diet of Spanish children, the most important source of carbohydrates was bread; that of proteins, whole milk; that of fats, olive oil; and that of saturated fat, fried potatoes.<sup>34</sup> This food pattern lies between the typical Mediterranean diet and that in Anglo-Saxon countries since, although consumption of fruit, vegetables, fish, and olive oil is quite high, so is consumption of meat and milk derivatives, pastry products, snacks, precooked meals, and carbonated drinks.<sup>31</sup> This pattern probably stems from economic development in the Mediterranean region which has led to an influx of high-density energy foods and fast foods that include animal products while replacing cereals. If this food pattern is maintained until adulthood, it could lead to greater coronary risk than is current and contribute in the future to Spain losing its position in Europe regarding its relatively low levels of coronary mortality.

## GENETIC DETERMINANTS

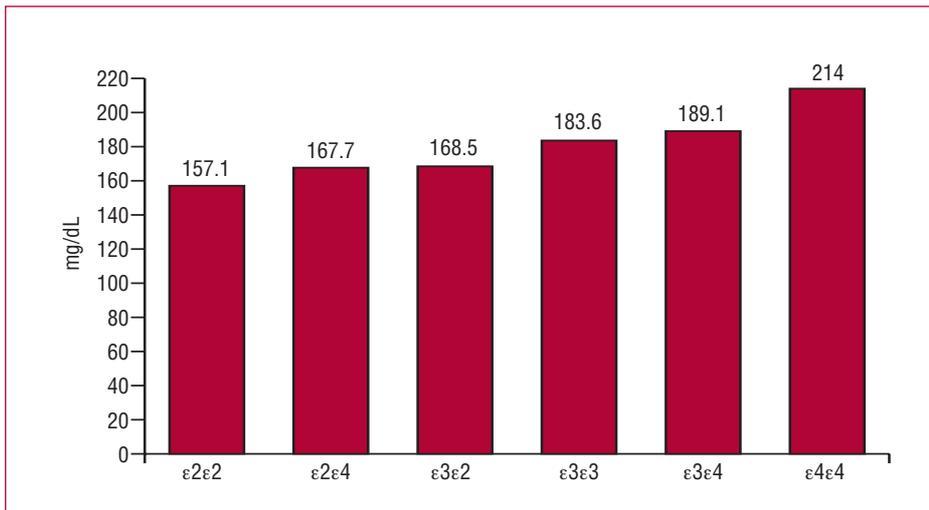
### Apolipoprotein E Genotype

The 4P study also analyzed how APOE gene polymorphism (APOE genotypes) affects lipid variables. Among the genetic alterations associated with lipid metabolism, the APOE genotype has gained particular prominence within the problem of atherosclerosis, given its key role in lipid metabolism, and because it is one of the genetic determinants that has been consistently associated with variations in lipid levels in the population and risk of coronary disease.

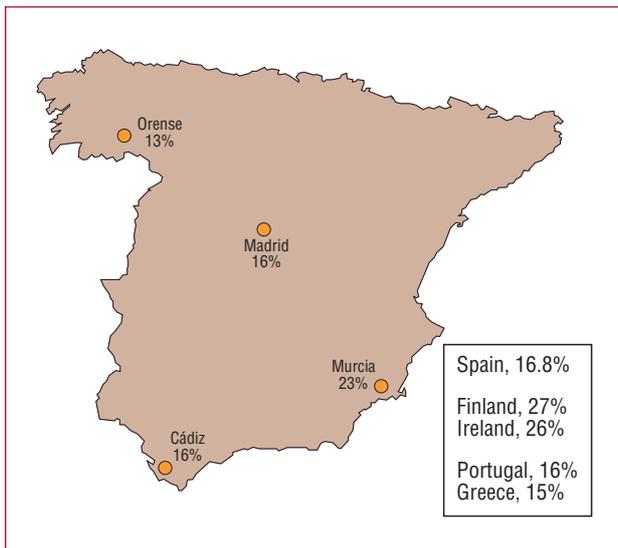
This is a polymorphic gene with 3 codominant alleles ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) that give rise to 6 genotypes:  $\epsilon 2\epsilon 2$ ,  $\epsilon 2\epsilon 3$ ,  $\epsilon 3\epsilon 3$ ,  $\epsilon 3\epsilon 4$ ,  $\epsilon 4\epsilon 4$  and  $\epsilon 2\epsilon 4$ . Several population studies (epidemiological studies<sup>35,36</sup> and case-control studies<sup>37</sup>) have shown that the  $\epsilon 4$  allele, and in particular the  $\epsilon 3\epsilon 4$  genotype, are strongly associated with coronary disease. The relationship between the APOE genotype and atherosclerosis is basically due to its effect on lipid levels,<sup>38</sup> although it can have other effects.

Results from the study population showed that the  $\epsilon 3\epsilon 4$  genotype is associated with significantly higher values of total cholesterol (Figure 4), LDL-C and ApoB.<sup>39</sup> However, we found that these polymorphisms had different effects on these lipid variables depending on sex<sup>39</sup> and birth weight,<sup>40</sup> indicating that these effects were influenced by hormones. In fact, we have shown that DHEA-S levels modify the influence of the APOE genotypes on plasma lipid levels.<sup>41</sup> Based on this data, we can conclude that the lipid levels in the study population are the result of complex interactions between diet, genes, and hormone levels.

When we investigated APOE genotype frequency we found a low prevalence of the  $\epsilon 4$  allele (10.1%) and the  $\epsilon 3\epsilon 4$  genotype (16.8%).<sup>42</sup> This low prevalence makes biological sense in a Mediterranean country with low coronary mortality and is similar to that of other low-mortality Mediterranean countries, as well as being lower than in North European countries, where coronary mortality is greater.<sup>35,36</sup> Interestingly, however, the distribution of genotypes by province varies considerably, with a very similar pattern in Cádiz and Madrid (16% of  $\epsilon 3\epsilon 4$ ) but with very different prevalences in Orense and Murcia (12.9% and 22.5%, respectively) (Figure 5).<sup>42</sup> The lowest frequency of the  $\epsilon 4$  allele and the  $\epsilon 3\epsilon 4$  genotype was found in Galicia (where Orense is located), which is similar to regions with a Mediterranean environment (Figure 5), which fits in with the low coronary mortality in this region. Galicia<sup>43</sup> is geographically isolated, with high emigration and low immigration, and has maintained its linguistic, economic,



**Figure 4.** Plasma levels of total cholesterol in prepubertal children in relation to the APOE genotype.



**Fig. 5.** Frequency of the ε3ε4 genotype by province in the Four Provinces study

in this Mediterranean country, but it has areas where there is high expression of this allele and a high incidence of coronary mortality, such as Murcia, and areas of low prevalence of the allele and low coronary mortality, such as Orense. We can conclude that this genetic determinant is associated with coronary mortality in relatively isolated populations and loses its predictive value in genetically heterogeneous populations.

Our results have practical importance. Despite protective factors in children in the Spanish population, such as high HDL-C levels or the generally low prevalence of the ε3ε4 genotype, which is clearly related to cardiovascular risk, we should control the risk factors obviously related to excess weight (high levels of glucose, triglyceride, and cholesterol, etc), through educational interventions aimed at improving the current diet of children. The prevention of obesity in children plus physical exercise will help to reduce the alterations associated with excess weight, and prevent both the onset of adult obesity and the increase in mortality coronary in Spain. Thus, our findings provide new arguments for initiating coronary prevention programs from childhood onwards.

and cultural identity. Galicia has low genetic diversity. Murcia lies at the opposite extreme,<sup>44</sup> where the ε4 allele and ε3ε4 genotype are highly prevalent, similar to that in countries with high mortality (Figure 5), which again fits with the very high coronary mortality found there. The identical distribution of APOE genotypes in Madrid and Cádiz poses a very interesting problem. Both areas have experienced multiple civilizations throughout history and this population mixture has gradually produced identical genetic frequencies. This explanation is strengthened by observing that 16% of the prevalence of the ε3ε4 genotype is the mean of the total group under study. Spain is not homogeneous regarding the distribution of the APOE genotype. The ε4 allele has low expression

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