Plasma Fatty Acids and Lipids in Two Separate, But Genetically Comparable, Icelandic Populations

Gudrún V. Skúladóttir, Sigrún Gudmundsdóttir, Gunnaugur B. Ólafsson, Stefán B. Sigurdsson, Nikulás Sigfusson and Jóhann Axelsson

ABSTRACT: Levels of serum lipids and lipoproteins, and the fatty acid composition of plasma phospholipids, were measured in two genetically comparable, but widely separated, populations. The 1975 mortality rates for ischemic heart disease were significantly higher in one of these populations, the Manitoban residents of pure Icelandic descent, than in the other, a rural population from Northeastern Iceland. Two study populations, Icelanders and Icelandic–Canadians, were drawn from these larger populations. The study populations were matched for age and sex and divided into three age groups, 20–39, 40–59, and 60–69 years. In comparison to the Icelandic–Canadians, the Icelanders exhibited significantly higher levels of total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, but lower triglyceride levels. Their plasma phospholipids contained significantly lower levels of saturated fatty acids (SFA), monounsaturated fatty acids, and n-6 polyunsaturated fatty acids (PUFA) than in the n-3 PUFA levels were three times as high. It was additionally found that fatty acid composition of plasma phospholipids differed among Icelanders of different ages. SFA levels were significantly lower, and n-6 PUFA levels significantly higher, in the 20–39 year group than in the 60–69 year group, possibly due to different dietary fat consumption patterns between generations. No corresponding age-related difference in the fatty acid composition of plasma phospholipids was found in the Icelandic–Canadian study population. As the Icelandic and Icelandic–Canadian groups are assumed to be genetically similar, the biochemical differences between them are evidently due to environmental, probably dietary, differences. The findings indicate that n-3 PUFA may be cardioprotective in the context of an otherwise atherogenic diet. Lipids 30, 649–655 (1995).

By the late 19th century, Icelanders had lived in virtual isolation from the rest of the world for 1,000 years, and the population of Iceland was thus unusually homogeneous. In the period between 1870 and 1914, some 18,000 Icelanders, about 20% of the national population in that period, migrated to North America. Because of meticulous record-keeping, the names, professions, and ages of these emigrants, the farms from which they came, the ships they traveled with, and the places in North America where they settled are known. Many of these Icelanders migrated from Fjöðrásthraéd, a rural district in Northeastern Iceland, to the Interlake District, just north of Winnipeg, Manitoba; and hundreds of individuals currently living there are pure descendants of the Icelandic immigrants from Fjöðrásthraéd. A large number of these purely-descended Icelandic–Canadians have been identified through genealogical records (5,6) and questionnaires. Individuals living in Fjöðrásthraéd were invited to participate in the study if their families had been living in Fjöðrásthraéd for at least three generations (1). The environmental homogeneity within the sample was increased by selecting those who live in the same district as their parents and grandparents. There is every reason to suppose that these two study populations thus identified are highly comparable genetically. This presumed genetic comparability has been confirmed by anthropomorphic studies (1,7). The existence of these two genetically comparable populations living in wide separation from one another and in quite dissimilar surroundings provides an exceptional opportunity for comparative studies aimed at distinguishing environmental from hereditary influences, for example on the prevalence of ischemic heart disease.

In 1975, crude mortality rates for ischemic heart disease were significantly higher in the Icelandic–Canadian communities of Manitoba than in Iceland, for both females and males (1,8). Because the explanation for this difference is evidently not genetic, environmental factors—dietary factors in particular—are indicated. Indeed, the traditional Icelandic diet differs considerably from the normal diet of Icelandic–Canadians. As an effect of diet, it is the lipid profile which is thought to have the most to do with the risk of cardiovascular disease (CVD). The present study investigates serum levels of total chole-
terol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and plasma phospholipid fatty acids of two genetically similar study populations; these profiles exhibit significant differences. We attempt to link the study parameters with CVD risk.

**MATERIALS AND METHODS**

**Subjects.** Two study populations were constructed, one consisting of 119 subjects (60 females and 59 males) drawn from the rural district of Fljótshlídahraed, in Northeastern Iceland, and a second consisting of 119 subjects (59 females and 60 males) drawn from a previously-identified pool of residents of the Interlake District of Manitoba who are pure descendants of immigrants from Fljótshlídahraed (1,2). The Icelandic study population was constructed first; five-year cohorts of persons aged 20–69 years, each cohort containing approximately six females and six males, were selected. The Icelandic–Canadian study population was then constructed by selecting matching cohorts. The two resulting study populations were thus well matched with respect to age and sex, down to the level of the five-year cohorts. For the purpose of analysis in the present study, the two populations were divided into three age groups—20.0–39.9, 40.0–59.9, and 60.0–69.9 years (Table 1).

Overnight fasting blood samples were collected by venipuncture into Vacutainer® tubes (Sarstedt, Nümbrecht, Germany) containing EDTA for fatty acid determinations and no anticoagulant for determinations of lipids and lipoproteins. Plasma or serum was prepared immediately, frozen, and frozen in cold packs to the Heart Preventive Clinic (Reykjavik, Iceland), where it was stored at −22°C until analyzed.

**Analysis of lipids and lipoproteins.** Total serum cholesterol was determined on a Cobas Mira (Roche Diagnostic Systems, Hoffmann-La Roche Ltd., Basel, Switzerland) automatic analyzer by an enzymatic colorimetric method as described by Allain et al. (9) and Trinder (10). Serum triglyceride was determined on the Cobas Mira by an enzymatic colorimetric method as described by Fossati and Principe (11) and McGowan et al. (12). Serum HDL cholesterol was determined on the Cobas Mira according to the method of Burstein et al. (13) and Lopes-Virella et al. (14). The concentration of LDL cholesterol was calculated as follows:

\[
[\text{LDL cholesterol}] = [\text{total cholesterol}] - ([\text{HDL cholesterol}] + [\text{triglyceride}] 	imes 0.37)
\]

Commercial control sera were used for internal quality control, and external quality control was performed by the World Health Organization Lipid Reference Centre in Prague.

**Analysis of phospholipid fatty acids.** Plasma lipids were extracted with chloroform/methanol (2:1, vol/vol) by the method of Sperry and Brand (15) as modified by Nelson (16). The antioxidant butylated hydroxytoluene was added to the extraction medium at 5 mg/100 mL. Phospholipids were separated on thin-layer plates (Adsorbosil H; Alltech, Deerfield, IL) and developed in a solvent system consisting of petroleum ether/diethyl ether/acetic acid (80:20:1, by vol). The phospholipid fatty acids were methylated by the method of Morrison and Smith (17) using 14% boron trichloride/methanol (Sigma

**TABLE 1**

<table>
<thead>
<tr>
<th>Body Weight, Body Mass Index (BMI), Triglycerides, Total Cholesterol, High-Density Lipoprotein (HDL) Cholesterol, and Low-Density Lipoprotein (LDL) Cholesterol in Icelanders and in Icelandic-Canadians</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Icelanders</strong></td>
</tr>
<tr>
<td>20.0–39.9</td>
</tr>
<tr>
<td>n = 42</td>
</tr>
<tr>
<td>(F = 21)</td>
</tr>
<tr>
<td>M = 21</td>
</tr>
</tbody>
</table>

**Body weight (kg):** 74.9 ± 2.6 77.2 ± 2.3 80.0 ± 3.6 75.4 ± 2.4 81.4 ± 2.1 81.6 ± 3.2

**BMI (kg/m²):** 25.3 ± 0.7 26.7 ± 0.6 28.5 ± 0.9 25.6 ± 0.7 27.5 ± 0.5 28.8 ± 1.3

**Triglycerides (mM):** 1.09 ± 0.14 1.33 ± 0.13 1.13 ± 0.09 1.29 ± 0.08 1.70 ± 0.12 1.46 ± 0.11

**Total cholesterol (mM):** 5.4 ± 0.10 6.2 ± 0.10 6.9 ± 0.20 5.0 ± 0.20 5.4 ± 0.10 5.9 ± 0.20

**HDL cholesterol (mM):** 1.3 ± 0.04 1.3 ± 0.05 1.4 ± 0.08 1.04 ± 0.03 5.0 ± 0.03 1.01 ± 0.06

**LDL cholesterol (mM):** 3.7 ± 0.10 4.5 ± 0.10 5.1 ± 0.20 3.4 ± 0.10 3.8 ± 0.10 4.3 ± 0.20

**Notes:**

*Values are mean ± SEM of n determinations, where n represents the number individuals in each age group. Sex distribution among the age groups is shown in parentheses (F = female, M = male).

*Significance calculated by two-way analysis of variance, NS = not significant; G = group of all Icelanders (n = 119) or group of all Icelandic-Canadians (n = 119), A = age.

*Represents age period in years, and mean age is given in parentheses.

**P** < 0.05, compared to the youngest and middle age groups, Scheffe post-hoc test.

**P** < 0.01; **P** < 0.02, compared to the youngest age groups, Scheffe post-hoc test.

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Chemical Co., St. Louis, MO). The methyl esters were analyzed using a gas chromatograph (Hewlett-Packard 5890A; Hewlett-Packard, Palo Alto, CA) equipped with a capillary column, 25 m × 0.2 mm i.d., coated with a 0.25 μm film of 5% diphenyl- and 95% dimethyl polysiloxane. The gas chromatograph was programmed to have an initial oven temperature of 160°C rising in three increments to 235°C. The carrier gas was hydrogen at a flow rate of 1 mL/min. The fatty acid methyl ester peaks were identified and calibrated against those of commercial standards (Sigma Chemical Co.; Applied Science Division, Milton Roy Company, State College, PA).

All measurements in the comparative studies of Icelanders and Icelandic–Canadians have been carried out by the same personnel, using the same techniques and equipment, and the chemical analyses have been performed in the same laboratory.

Statistical analysis. The influence of environment (Fljótshlíðar vs. Interlake District) and age on serum lipids, lipoproteins, and fatty acid composition of plasma phospholipids in all subjects studied was examined by two-way analysis of variance using SYSTAT (18) general linear model procedures. To determine the significant difference in level of lipids, lipoproteins, and phospholipid fatty acids between the age groups within each population, one-way analysis of variance was used, followed by application of Scheffe post-hoc test. Pearson's correlation coefficient was used to measure the correlation between age and the percentage of fatty acids in plasma phospholipids. Differences were considered significant at \( P < 0.05 \).

RESULTS

The study parameters for persons of different ages in the two populations are shown in the tables and the figure. There were no significant differences in body weight and body mass index between the two study populations (Table 1). The body mass index of the oldest age group of Icelanders was significantly higher than that of the youngest age group. The Icelandic study population exhibited significantly lower triglyceride levels and significantly higher levels of total cholesterol, LDL cholesterol, and HDL cholesterol than the Icelandic–Canadian study population (Table 1).

Age-related differences between total cholesterol and LDL cholesterol levels were found among the Icelanders. Within this population the oldest age group exhibited significantly higher total cholesterol and LDL cholesterol levels than the youngest and middle groups. The middle group contained significantly higher levels of total cholesterol and LDL cholesterol than the youngest group. No significant age-related differences in lipid levels were found among the Icelandic–Canadians.

Sex-related differences between lipid levels were found in both study populations (data not shown). Triglyceride levels were significantly higher in males than in females; 1.41 ± 0.15% vs. 1.00 ± 0.06% \((P = 0.046)\), in the Icelanders, and 1.74 ± 0.11% vs. 1.26 ± 0.06% \((P = 0.009)\), in the Icelandic–Canadians. Within the Icelandic study population, total cholesterol levels were significantly lower in males than in females, 5.9 ± 0.1% vs. 6.3 ± 0.2% \((P = 0.039)\). HDL cholesterol levels were also significantly lower in males than in females: 1.17 ± 0.04% vs. 1.43 ± 0.04% \((P < 0.005)\) in Icelanders, and 0.90 ± 0.03% vs. 1.04 ± 0.04% \((P = 0.004)\) in Icelandic–Canadians.

The two study populations differed markedly in the fatty acid composition of plasma phospholipids (Table 2). Seven out of the eleven fatty acids analyzed differed significantly between the two populations. The levels of 20:5n-3 and 22:6n-3 were three times higher in Icelanders than in Icelandic–Canadians, in all age groups \((2.20 ± 0.09\) and \(4.30 ± 0.11%\) vs. \(0.72 ± 0.03\) and \(1.38 ± 0.06%\), respectively). There were no significant differences in the n-6 polyunsaturated fatty acids (PUFAs), 20:3n-6 and 20:4n-6, and in the saturated fatty acids, 14:0 and 18:0. The interaction between gender and age was significant in six fatty acids (Table 2).

Age-related differences between fatty acid levels in plasma phospholipids were found within both study populations. In the Icelandic study population, the level of 14:0 was significantly lower, and the levels of 18:2n-6 and 20:4n-6 were significantly higher in the youngest age group than in the middle and oldest age groups. The plasma phospholipids of the youngest age group of Icelandic–Canadians contained significantly lower levels of 20:5n-3 than the middle and oldest age groups.

In both study populations, significant sex-related differences between fatty acid levels in plasma phospholipids were found only in saturated fatty acids (SFAs) (data not shown). SFA levels were significantly higher in females than in males, 46.6 ± 0.37% vs. 43.3 ± 0.47% \((P = 0.002)\) in Icelanders and 49.0 ± 0.33% vs. 46.9 ± 0.36% \((P < 0.005)\) in Icelandic–Canadians.

Figure 1 shows the relationship between age and levels of SFA (A), n-6 PUFA (B) and n-3 PUFA (C) in plasma phospholipids of Icelanders and Icelandic–Canadians of both sexes. In Icelanders, SFA levels were positively correlated \((r = 0.528, P < 0.0005)\), and n-6 PUFA levels correlated negatively \((r = -0.573, P < 0.0005)\) with age. A small positive correlation between n-3 PUFA levels and age in Icelanders was observed \((r = 0.273, NS)\). No relationship was found between age and levels of SFA \((r = 0.042, NS)\), n-6 PUFA \((r = -0.051, NS)\), and n-3 PUFA \((r = 0.231, NS)\) in the plasma phospholipids of Icelandic–Canadians.

DISCUSSION

Mortality from coronary heart disease is relatively low among Icelanders, even though they exhibit a lipid profile which is normally considered atherogenic. It is recognized that many chronic forms of CVD have long development periods, and total serum cholesterol level has been thought to play a central role in development of atherosclerosis. Hypercholesterolemia in childhood is one of the factors that may affect the development of CHD with aging (19,20). Icelandic
youngsters, ages 6–19, exhibit one of the highest levels of total serum cholesterol yet reported, 5.31 ± 0.86 m[M (mean ± SD)] (4). However, mortality from CVD is relatively low among Icelanders, which indicates that mean total cholesterol levels fail to reflect cardiovascular risk in a population and are a poor predictor of individual risk (4).

In the face of this conundrum, it is worth noting that Icelanders hold the European record in fish consumption (73 g/day/person), and the use of cod liver oil as a daily food supplement is widespread (21). Consumption of fish and fish oil rich in the n-3 PUFAs, 20:5n-3 and 22:6n-3, has been shown to have a beneficial effect on plasma lipid profiles, thereby reducing the risk of CVD (22–27). The effects of fish oil consumption on the levels of total serum cholesterol and LDL cholesterol are less clear (28,29). Some studies have indicated that consumption of fish containing moderate amounts of n-3 PUFAs may cause a deleterious rise in LDL cholesterol levels in normotriglyceridemic males, whereas other studies have found no change (30,31). The relatively high levels of LDL cholesterol exhibited by the Icelandic study population lend support to the argument that the consumption of fish and fish oil increases LDL cholesterol (Table 1).

Low levels of serum triglycerides in humans have been observed to correlate with consumption of fish and fish oil (29). The high Icelandic consumption of these foods may thus explain why serum triglyceride levels were significantly lower in the Icelandic study population than in the Icelandic–Canadian population (Table 1).

It has been maintained that relatively high plasma phospholipid levels of 20:5n-3 and 22:6n-3 are strictly related to diet (32–34). The relatively high levels of plasma phospholipid n-3 PUFA observed in the Icelandic study population may thus be taken to reflect the dietary intake of these two n-3 PUFA found in fish and fish oil, whereas the 30% lower levels of n-3 PUFA observed in the Icelandic–Canadians evidently reflect low dietary levels of n-3 PUFA in the ordinary Canadian diet (Table 2).

Mean levels of plasma phospholipid 20:4n-6, the precursor of the thrombogenic and vasospastic thromboxane A₂, have been shown to be twice as high in Canadians and in Americans (32,33) as in Nordic populations (35–37). It has been suggested that relatively low levels of plasma phospholipid 20:4n-6 may be genetically controlled and independent of diet (33). In the present study, no significant difference was observed in plasma phospholipid 20:4n-6 levels between the two genetically similar study populations, which is consistent with this suggestion (Table 2).

It is generally supposed that the prevalence of CVD correlates positively with the proportion of dietary energy supplied by saturated fatty acids and negatively with the proportion of

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FIG. 1. Relationship between age and percentage of saturated fatty acids (A), n-6 polyunsaturated fatty acids (PUFAs) (B), and n-3 PUFAs (C) of plasma phospholipids in Icelanders (left) and Icelandic-Canadians (right). Each point represents an individual subject. Saturated, sum % (14:0 + 16:0 + 18:0); n-6 PUFAs, sum % (18:2 + 20:3 + 20:4) n-6; n-3 PUFA, sum % (20:5 + 22:5 + 22:6) n-3. NS, not significant.

dietary n-6 PUFAs (38). In Iceland, fat contributes 41% of total dietary energy, with the contribution of saturated fat amounting to 20% (21). However, the contribution of total dietary fat energy in Canada is 38% and saturated fat is estimated to 13% (39). Until the late 1970s, the traditional Icelandic diet consisted of local products, i.e., fish, lamb, and dairy products rich in cholesterol and saturated fat (40). Grains, fruits, and vegetables, which were generally imported and expensive, contributed less to the Icelandic diet. In the Icelandic study population, plasma phospholipid SFA levels were found to increase with age, while in the Icelandic–Canadian population, no age-related difference was observed (Table 2 and Fig. 1). Canadian–Icelanders of all ages exhibited SFA levels of plasma phospholipids similar to those observed in Icelanders 60 years of age and older. Since the late 1970s, a public health campaign in Iceland has advocated the reduction of...
fatty meats and dairy fats in the diet and the increased consumption of vegetable oils and grains. The lower levels of SFA and n-3 PUFA, and higher levels of n-6 PUFA observed in plasma phospholipids of younger Icelanders (Table 2 and Fig. 1) probably reflect the effects of this campaign (4). Older persons in Fljótsdalshérað who tend to eat the traditional Icelandic high-fat diet and consume cod liver oil exhibit higher levels of SFA and n-3 PUFA and lower levels of n-6 PUFA in plasma phospholipids than do younger Icelanders (Table 2 and Fig. 1). The study indicates that the dietary habits of Icelanders--Canadians are rather stable, as no age-related differences in SFA, n-6 PUFA, or n-3 PUFA of plasma phospholipids were observed (Table 2 and Fig. 1). That such age-related differences in plasma phospholipid fatty acids in the Icelanders are due to changes in dietary patterns rather than effects of aging as such is supported by the work of Holman et al. (32).

Of interest in this connection is that lamb, which is a major element in the traditional Icelandic high-fat diet, contains more n-3 PUFA than has been generally realized. Due to short and often hard summers in Iceland, it is customary to supplement the ewes' diet with fish meal, herring, or fish oil during breeding season and prior to the lambing season in the spring. The maternal dietary n-3 PUFA are incorporated into the lean muscle phospholipids of the lamb meat, which in lambs from Fljótsdalshérað contains 7% of n-3 PUFA (unpublished data from our laboratory).

Serum triglyceride level, now recognized as an independent risk factor for CVD (41), is increasing rapidly in Icelanders (42). A 10-year follow-up study (1980–1990) on a subgroup of the population in Fljótsdalshérað (ages 20–60 years) revealed that the cardiovascular risk attributable to triglyceride levels and atherogenic indices has increased markedly (43), evidently reflecting changes in dietary fat intake.

It is generally agreed that n-3 PUFA play an essential role in human growth and development throughout life and are a necessary part of the diet (44). They may be instrumental in preventing the development of CVD (45). The present study supports the idea that n-3 PUFA, such as Icelanders obtain through their consumption of fish, fish oil, and Iceland lamb, can afford protection from atherosclerotic disease, even in the face of a diet that in other respects may be counted as atherogenic (46).

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