Nutrient Intake Comparisons Between Framingham and Rural and Urban Puriscal, Costa Rica

Associations With Lipoproteins, Apolipoproteins, and Low Density Lipoprotein Particle Size

Hannia Campos, Walter C. Willett, Rose Marie Peterson, Xinia Siles, Stephen M. Bailey, Peter W.F. Wilson, Barbara M. Posner, José M. Ordovas, and Ernst J. Schaefer

To assess cross-cultural relations between dietary intake and plasma lipoproteins, we randomly selected 222 men and 243 women from the urban and rural areas of Puriscal, Costa Rica; related their dietary composition (assessed by a food-frequency questionnaire), fitness level, and body fat to plasma lipids, apolipoproteins, and low density lipoprotein (LDL) particle size; and compared these data with those from a subsample of 280 adults from the Framingham Offspring Study. Total cholesterol and LDL cholesterol levels were significantly (p<0.0001) higher in Framingham (207 and 137 mg/dl, respectively) than in Puriscal (184 and 114 mg/dl, respectively) residents. Elevated triglyceride and apolipoprotein (apo) B levels (25% and 16% higher), low HDL cholesterol and apo A-I levels (12% and 29% lower), and smaller LDL particles (17%) were more frequent in Puriscal than in Framingham residents. Urban Puriscal residents had a significantly lower fitness level; increased body fat, total cholesterol, and triglyceride levels; decreased HDL cholesterol in men; and higher apo B levels in women compared with rural Puriscal residents. Body fat, animal fat, and saturated fat intakes were significantly correlated with total cholesterol, LDL cholesterol, and apo B levels in both men and women in Puriscal. Intakes of protein and animal fat were higher among urban (10.7% and 14.1%, respectively) compared with rural (8.9% and 9.9%, respectively) Puriscal residents and in Framingham (16.0% and 20.8%, respectively) compared with Puriscal residents. No significant differences were found in dietary cholesterol. Saturated fat (largely from palm oil in Puriscal) intakes were significantly different among the three groups: rural Puriscal, 10.7% of calories; urban Puriscal, 11.6%; and Framingham residents, 12.9%. These data indicate that the more atherogenic plasma lipid profile among urban compared with Puriscal residents was largely explained by increased adiposity, decreased fitness level, and higher saturated fatty acid intake. Puriscal residents consumed less animal fat and more carbohydrate than did Framingham residents, and these differences were associated with a 21% lower LDL cholesterol level, a 12% lower HDL cholesterol level, a 29% lower apo A-I level, a 25% higher triglyceride level, a 16% higher apo B level, and a 17% smaller LDL particle size. Some of these cross-cultural differences may be due to differences in ethnic background and physical activity as well. (Arteriosclerosis and Thrombosis 1991;11:1089–1099)
Coronary artery disease (CAD) due to atherosclerosis is the major cause of death in most developed societies, whereas communicable diseases have been responsible for most morbidity and mortality in less industrialized nations. However, in recent years CAD is becoming a major cause of mortality in developing countries, especially in the large urban centers. Age-standardized death rates due to CAD increased in Costa Rica from 125.7 per 100,000 in 1983 to 176.3 per 100,000 in 1986. The current rate now approaches that reported for the United States; CAD mortality rates in the United States have decreased from 229.8 per 100,000 in 1983 to 200.8 per 100,000 in 1986.

Several cross-cultural studies support the concept originally based on animal studies that diets high in cholesterol and saturated fat induce atherosclerosis. Other studies indicated that Japanese living in California had increased CAD, along with higher plasma cholesterol levels and significantly higher dietary cholesterol and saturated fat intakes than Japanese living in Japan. In metabolic ward studies, differences in cholesterol and fatty acid composition intake affect plasma concentrations of low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol. Prospective data have shown that lowering cholesterol levels with diet or diet and drug therapy will reduce CAD risk. Moreover, angiographic regression of coronary and femoral artery atherosclerosis has been observed in some patients with hyperlipidemia after treatment with diet and lipid-lowering drugs; most recently in patients who were randomly assigned to multiple lifestyle changes compared with those patients given usual care.

However, most cross-sectional studies within free-living populations have failed to show associations between diet and lipoproteins. The lack of diet-lipoprotein associations reported in those studies may have been due to intranidividual variability in diet and serum cholesterol and poor assessment of individual usual food intake, as well as analytic considerations and interpretation of epidemiological dietary data. Nevertheless, relatively strong correlations between dietary cholesterol and saturated fat intake and plasma total cholesterol concentrations have been observed within populations with low cholesterol and total fat intake, possibly due to a nonlinear relation with blood cholesterol level.

Apolipoprotein (apo) A-I is a major protein constituent of HDL, while apo B is a major constituent of LDL. These apolipoproteins may serve as better predictors of the presence of CAD risk than LDL cholesterol or LDL cholesterol alone in industrialized countries whose populations are basically consuming a Western diet, although no data on these associations are available in the context of low-fat diets. It has been reported in short-term feeding studies that diets with a low polyunsaturated to saturated fatty acid ratio are associated in women aged 20–65 years. One hundred thirty such households were randomly selected from the 3,415 identified houses in the rural area, and 130 households were also randomly selected from the 918

Study Populations
The study sample in Costa Rica was randomly selected from the canton (county) of Puriscal. The Puriscal region extends from the middle of Costa Rica to the Pacific coast area and comprises about 800 km². There are approximately 26,000 inhabitants in approximately 150 very sparsely distributed localities, with fewer than 500 people in each locality. Santiago, although not a true urban center, is the canton's capital and is the only district in Puriscal with a population of 8,000 people. (Santiago is referred to as urban by the Center of Censuses and Statistics in Costa Rica, and their definition and maps were used in this study.) This region is in rapid transition; approximately 70% of the homes in the rural area and 100% in Santiago have access to electricity and a piped water supply. The rural population subsists mainly on agricultural products and the sale of food crops, while professional careers, retail business, and white-collar jobs predominate in Santiago. The population is predominantly mestizo (mixture of Spanish and Indian ethnic groups), with a small proportion of mulattoes (mixture of black and Spanish ethnic groups). Modern anthropological research suggests that in Costa Rica a tripartite racial mixing accompanied by a European way of life took place before the 1800s. Since that time a comparatively homogeneous Hispanic-American society has formed.

Stratified random sampling was performed in the rural and urban areas to obtain a similar number of participants in each group. Eligible households were defined as those having one man and one nonpregnant woman aged 20–65 years. One hundred thirty such households were randomly selected from the 3,415 identified houses in the rural area, and 130 households were also randomly selected from the 918
identified houses in the urban area. Participation rates were 85% for men and 93% for women in rural and urban areas combined. Subjects with triglyceride levels greater than 400 mg/dl (n = 11), glucose levels greater than 140 mg/dl (n = 9), positive histories of heart disease (n = 4), taking medications known to affect lipids (n = 26), taking oral contraceptives (n = 41), or missing plasma samples (n = 1) were not included in this analysis. The final sample size was 202 men and 174 women.

In Framingham, Mass., the subjects were a subsample from the Framingham Offspring Study Cycle 3 (1983–1987) randomly selected as previously described. The final study population for plasma parameters consisted of 136 men and 142 women. Within this group there were a total of 76 men and 85 women for whom complete nutrient intake data were available.

Data Collection

Data for the study subjects in Costa Rica were collected from January through September 1988. Trained fieldworkers visited participants in their households for recruitment, where subjects signed a consent form, and subsequent appointments were made for data collection. A health history and general characteristics questionnaire was completed for each subject. The questionnaire included socioeconomic and demographic characteristics, as well as a brief medical history, hypertension status, smoking habits, levels of fitness, family history of heart disease, and diabetes status. The design and methodology for the Framingham Offspring Study have been described elsewhere.

Dietary assessment. In Framingham a self-administered semiquantitative food-frequency questionnaire (FFQ) was used to obtain dietary information. The validity and reproducibility of this questionnaire have been previously reported. A similar semiquantitative FFQ, with modifications to account for population differences in the consumption of several foods, was used for the Costa Rican subjects. Dietary data were collected by two standardized nutritionists during an interview, at which time the frequency of consumption of each food item in the modified FFQ was asked. A pretest of this questionnaire was first performed in Costa Rica in 1985 among a sample of 68 subjects from the metropolitan area. The nutrient estimates as a percentage of total energy intake that we obtained with the semiquantitative FFQ were very similar to those obtained by a 24-hour dietary recall in the Metropolitan Nutrition Survey (MNS) in Costa Rica during 1987, including fat (FFQ = 31%, MNS = 31%), protein (FFQ = 14%, MNS = 12%), and carbohydrate (FFQ = 56%, MNS = 58%). Nutrient intake calculations for each subject in both the Framingham and the Costa Rican populations were performed as previously described. Population comparisons in this study were performed by use of nutrient composition rather than absolute nutrient amounts because the use of this questionnaire may overestimate caloric intake when used cross-culturally and composition is most relevant to potential dietary change by individuals.

Anthropometric measurements. All anthropometric measurements were taken by three trained fieldworkers acquainted with standardized methods, with subjects wearing light clothing but not shoes. All measurements were performed in duplicate, and the average was used for analyses. Waist (smallest horizontal trunk circumference) and hip (largest horizontal circumference around the hip and buttocks) girths were measured with nonstretching fiberglass or metal tapes. Skinfold thicknesses measured included triceps (posterior upper arm, midway between the elbow and acromion), subscapular (1 cm below the lower tip of the scapula), umbilical (2 cm to the right of the navel), suprailiac (at the midline and above the iliac crest), and medial calf (interior). All measurements were taken on the right side of the body by use of skinfold calipers (Holtain Ltd, Crymmych, UK). Height and sitting height were measured with a steel anthropometer. To ensure correct readings (to the nearest 0.1 cm) for heights, subjects always stood on a flat surface against a wall. Weight was determined by use of either a Detecto bathroom scale or a Seca Alpha Model 770 digital scale. Biweekly calibration was performed. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²).

Bioelectric impedance assessment (BIA). Bioelectric impedance measurements of Costa Rican participants were performed during the morning before any strenuous activity and after a 12-hour fast with a bioelectric impedance analyzer (model 101, BIA Systems, Detroit, Mich.) following the procedures previously described by Lukaski et al. Four gel electrodes were attached to the anterior surfaces of the foot and ankle and to the posterior surfaces of the arm and wrist. Subjects were supine on a flat surface with the arms relaxed at the sides, not touching the body, and with the thighs separated. Resistance and reactance were read directly from the instrument. Resistance (R), reactance (X), and impedance [(R²+X²)½] values were measured directly on a subsample of 50 men and 44 women. To estimate total body fat in this subsample we used the equation described by Khaled et al, where total body fat = impedance x weight/height². Lean body mass was then calculated as weight minus total body fat and percentage of fat as total body fat divided by weight. Total body fat for the whole population was calculated by deriving an equation by use of a Max R stepwise multiple regression procedure, with total body fat as the dependent variable and several anthropometric measurements available for the whole population as the independent variables. The significant (p < 0.05) anthropometric measurements were used in the following equations:

For men

\[ \text{Body fat (kg)} = 21.86 - (\text{weight} \times 0.0982) \]
Lipoprotein Analysis

Plasma cholesterol, triglyceride, and HDL cholesterol levels were measured with an Abbott Diagnostics ABA-200 bichromatic analyzer and Abbott A-Gent enzymatic reagents as previously described. Total cholesterol, triglyceride, and HDL cholesterol assays were standardized through the Centers for Disease Control Lipid Standardization Program. LDL cholesterol levels were estimated for all subjects by the Friedewald equation.  

\[ \text{LDL cholesterol} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol} + 2 \times \text{triglycerides} + 3} \times 100 \]

Fitness score. To determine the level of fitness in Costa Rican subjects, a modified Harvard step test was performed on a portable wooden 40-cm step. Subjects were instructed to step up and down while following the beats of a metronome. Women were asked to maintain a rhythm of 76 beats/min, whereas men were asked to follow a rhythm of 96 beats/min. Subjects were requested to perform the test for 3 minutes, but when this was not possible the time during which the exercise was performed was recorded. Pulse rates were taken immediately after the test and at 1 and 3 minutes after the test. Fitness score (FS) was calculated as seconds during exercise divided by (pulse + 2 + 3) times 100.

Blood samples. Blood samples were drawn into tubes containing 0.1% EDTA after a 12–14-hour fast. Blood tubes were then immediately stored at 4°C. Within 36 hours they were centrifuged at 2,500 rpm for 20 minutes at 4°C to isolate the plasma. HDL supernates were obtained after precipitation of apo B–containing particles with dextran sulfate-Mg²⁺ by the method of Warnick et al. All plasma aliquots were then stored at −70°C until they were transported by air, on dry ice, within 24 hours to the USDA Human Nutrition Research Center on Aging at Tufts University in Boston, where all the plasma samples were evaluated.

Lipoprotein Analysis

Plasma cholesterol, triglyceride, and HDL cholesterol levels were measured with an Abbott Diagnostics ABA-200 bichromatic analyzer and Abbott A-Gent enzymatic reagents as previously described. Total cholesterol, triglyceride, and HDL cholesterol assays were standardized through the Centers for Disease Control Lipid Standardization Program. LDL cholesterol levels were estimated for all subjects with triglyceride levels less than 400 mg/dl by the Friedewald equation. This method employs a double-sandwich technique in which the microtiter plates (Nunc Immunoplate I, Roskilde, Denmark) were coated with affinity-purified polyclonal antibody for apo B. The same method was used for apo A-I determinations except that affinity-purified apo A-I antibody and plasma dilutions of 1:60,000 were used. Standards used for these assays were calibrated with purified protein standards assayed by amino acid analysis.

Gradient gel electrophoresis on 4.9×82×82-mm 2–16% nondenaturing polyacrylamide gels (PAA 2–16%, Pharmacia, Piscataway, N.J.) was performed with frozen plasma specimens to separate LDL subfractions as previously described. Scanning was performed on an LKB Ultrascan XL laser densitometer (LKB Instruments Inc., Paramus, N.J.) interfaced with an AT&T personal computer (LKB) and a Canon PJ-108A printer by use of the LKB gssx software for peak integration. Seven LDL bands (LDL 1–7) have been identified and classified according to particle density, as determined by sequential ultracentrifugation, or by gradient gel electrophoresis. LDL Is are the largest LDL particles and are found in the density range of 1.019–1.033 g/ml, whereas LDL 7s are the smallest particles and have a buoyant density range of 1.050–1.063 g/ml. Most subjects' LDLs have one major band and one or two minor bands. To account for the minor bands in our analysis we assigned each subject a weighted LDL particle score for which the percent area under each band present was multiplied by the band number. For example, a subject with a predominant LDL band 3 (0.75) and a minor LDL band 4 (0.25) would have an LDL particle score of 3.25[(3×0.75) + (4×0.25)]. A larger LDL particle score corresponds to a smaller LDL particle size because the largest LDL particles are LDL 1 (score=1.00) and the smallest LDL particles are LDL 7 (score=7.00).

Statistical Analysis

Differences between Framingham and the rural and urban Costa Rican men and women for all parameters measured were evaluated by use of sas software (SAs Institute Inc., Cary, N.C.), general linear model procedures for analysis of variance, the Student-Newman-Keuls option for multiple comparisons, and the least-square-means option for age-adjusted comparisons. Values are presented as mean±SD. Pearson correlation coefficients were first used to evaluate associations among nutrient intake, FS, body habitus, plasma lipids, lipoproteins, apolipoproteins, and LDL particle score. Partial correlations were then calculated between nutrient intake and plasma parameters in the combined rural/urban area while controlling for age, FS, total body fat, and caloric intake by use of SPSS software. Controlling for smoking status did not affect the associations between dietary variables and plasma parameters, so this variable was not included in this analysis. The stepwise regression procedure available in sas was used to identify
indirect associations between dietary variables and plasma parameters. Because the distribution of several variables (triglycerides; HDL cholesterol; apo A-I; triglycerides, subscapular, and calf skinfolds; and total carbohydrate, sucrose, animal fat, and cholesterol intakes) skewed, logarithmic transformations were performed for several analyses.

Results

Subjects' General Characteristics

Age and general characteristics for men and women in rural and urban Puriscal, Costa Rica, and in Framingham are shown in Table 1. Framingham men and women were older (mean age, 49 years) than those in rural and urban Puriscal (mean age, 40 years), whereas no age differences were found between rural and urban Puriscal. Framingham men had significantly higher \( p<0.01 \) BMIs than did urban and rural men. In contrast, Framingham women had significantly lower BMIs than did rural and urban women from Puriscal \( p<0.0001 \), with

### Table 1. Age and General Characteristics for Men and Women in Framingham and Rural and Urban Puriscal, Costa Rica Residents

<table>
<thead>
<tr>
<th>Variable</th>
<th>Rural Puriscal (n=103)</th>
<th>Urban Puriscal (n=99)</th>
<th>Framingham (n=138)</th>
<th>Rural Puriscal (n=88)</th>
<th>Urban Puriscal (n=86)</th>
<th>Framingham (n=142)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>41±13†</td>
<td>39±12‡</td>
<td>51±10</td>
<td>40±13†</td>
<td>41±11†</td>
<td>47±9</td>
</tr>
<tr>
<td>BMI (wt/h²)</td>
<td>23.8±3.1‡</td>
<td>25.1±3.7§</td>
<td>26.4±3.7</td>
<td>25.4±4.5§</td>
<td>26.1±4.0†</td>
<td>24.0±3.90</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>11.7±1.1†</td>
<td>12.6±1.4</td>
<td>NA</td>
<td>14.7±1.8§</td>
<td>15.4±1.6</td>
<td>NA</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>52.7±11.2‡</td>
<td>55.9±9.8</td>
<td>NA</td>
<td>43.7±9.5</td>
<td>44.8±9.3</td>
<td>NA</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>18.6</td>
<td>18.7</td>
<td>NA</td>
<td>25.5</td>
<td>26.1</td>
<td>NA</td>
</tr>
<tr>
<td>Fitness score*</td>
<td>60±11§</td>
<td>53±10</td>
<td>NA</td>
<td>48±13†</td>
<td>36±15</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are mean±SD.

BMI, body mass index; NA, data not available.

*Fitness score=time during step test(s)/(pulse 1+2+3) x 100.

Different from Framingham: \( fp<0.0001 \), \( g p<0.01 \). Different from urban Puriscal: \( ||p<0.05 \), \( +p<0.01 \), \( §p<0.0005 \).

Dietary and Lipoprotein Comparisons Between Rural and Urban Puriscal

The age-adjusted average daily nutrient intake for men and women in rural and urban Puriscal is shown in Table 2. Expressed in relation to energy intake (percentage of calories), the average intake of protein, total fat, monounsaturated fatty acids, and

### Table 2. Average Daily Nutrient Intake in Framingham and Rural and Urban Puriscal, Costa Rica Residents

<table>
<thead>
<tr>
<th>Nutrient intake (% of calories)</th>
<th>Rural Puriscal (n=103)</th>
<th>Urban Puriscal (n=99)</th>
<th>Framingham (n=76)</th>
<th>Rural Puriscal (n=88)</th>
<th>Urban Puriscal (n=86)</th>
<th>Framingham (n=85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>9.1±2.7†</td>
<td>10.3±2.6†</td>
<td>15.4±3.0</td>
<td>8.7±3.0§</td>
<td>11.2±3.1†</td>
<td>16.7±2.9</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>64.9±9.4†</td>
<td>59.8±9.6†</td>
<td>47.0±9.4</td>
<td>66.4±10.4§</td>
<td>60.7±9.1†</td>
<td>48.0±8.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12.5±5.1</td>
<td>13.8±6.1</td>
<td>14.0±9.9</td>
<td>12.1±5.1†</td>
<td>14.5±5.4</td>
<td>13.5±8.4</td>
</tr>
<tr>
<td>Total fat</td>
<td>26.4±6.0†</td>
<td>28.9±5.9†</td>
<td>34.5±6.7</td>
<td>26.5±6.6†</td>
<td>29.5±5.8†</td>
<td>34.8±6.3</td>
</tr>
<tr>
<td>Sat FA</td>
<td>10.5±3.0†</td>
<td>11.4±3.1†</td>
<td>12.9±3.4</td>
<td>10.9±3.2†</td>
<td>11.7±3.0§</td>
<td>12.9±2.9</td>
</tr>
<tr>
<td>Mono FA</td>
<td>9.2±2.5†</td>
<td>10.0±2.3†</td>
<td>13.3±3.1</td>
<td>9.4±2.0†</td>
<td>10.4±2.4†</td>
<td>13.1±2.9</td>
</tr>
<tr>
<td>Poly FA</td>
<td>3.1±0.9†</td>
<td>3.3±0.8‡</td>
<td>5.9±1.8</td>
<td>3.1±0.9†</td>
<td>3.5±1.0</td>
<td>6.3±2.0</td>
</tr>
<tr>
<td>Animal fat</td>
<td>10.1±4.8†</td>
<td>13.0±4.9</td>
<td>21.3±7.4</td>
<td>9.8±5.4§</td>
<td>15.2±5.9†</td>
<td>20.3±6.5</td>
</tr>
<tr>
<td>Vegetable fat</td>
<td>16.3±5.5†</td>
<td>15.9±4.7</td>
<td>13.0±4.7</td>
<td>16.7±5.3§</td>
<td>14.0±4.9</td>
<td>14.5±5.4</td>
</tr>
<tr>
<td>Cholesterol*</td>
<td>0.13±0.07</td>
<td>0.13±0.07</td>
<td>0.14±0.07</td>
<td>0.10±0.06‡</td>
<td>0.13±0.06</td>
<td>0.14±0.06</td>
</tr>
<tr>
<td>Dietary fiber*</td>
<td>9.1±3.2</td>
<td>8.4±3.3</td>
<td>8.4±3.9</td>
<td>9.1±3.2‡</td>
<td>7.6±3.2†</td>
<td>10.1±3.3</td>
</tr>
<tr>
<td>Alcohol*</td>
<td>1.5±0.6</td>
<td>2.9±6.3†</td>
<td>6.9±6.5</td>
<td>0.00±2.8‡</td>
<td>0.5±2.8†</td>
<td>3.4±2.9</td>
</tr>
</tbody>
</table>

Nutrient intake is given relative to energy intake (percent) as mean±SD except where nutrient intake is given as g/1,000 kcal (*). Percent calories was calculated with alcohol intake as a source of calories. When alcohol intake was not included, percent calories from fat=36% for men and women in Framingham and 29.4% for urban Puriscal men. Age was adjusted to 43 years.

Sat, saturated; FA, fatty acids; Mono, monounsaturated; Poly, polyunsaturated.

Different from Framingham: \( fp<0.0001 \), \( g p<0.005 \). Different from urban Puriscal: \( ||p<0.05 \), \( +p<0.01 \), \( §p<0.0005 \).
animal fat were significantly higher and carbohydrate intake was significantly lower in the urban than in the rural residents of Puriscal. Decreased vegetable fat and dietary fiber and elevated sucrose intakes were found in urban women compared with rural women, and an elevated saturated fatty acid intake was found in urban men compared with rural men. No significant rural-urban differences (g/1,000 kcal) were found for cholesterol intake in men. The calorie-adjusted cholesterol intake means were 456 mg/day in urban Puriscal men and 399 mg/day in Framingham men. Framingham women had significantly lower vegetable fat intake than did urban Puriscal men, and no significant differences were found in sucrose, dietary fiber, or cholesterol (g/1,000 kcal) consumption. The calorie-adjusted means for cholesterol intake were 456 mg/day in urban Puriscal men and 399 mg/day in Framingham men. Framingham women had higher dietary fiber intake than did urban Puriscal women. No significant differences were detected in vegetable fat, sucrose, or cholesterol intake (g/1,000 kcal) between urban Puriscal and Framingham women. The calorie-adjusted cholesterol intakes were 359 mg/day in urban Puriscal men and 399 mg/day in Framingham men. Framingham women had higher dietary fiber intake than did urban Puriscal women. No significant differences were detected in vegetable fat, sucrose, or cholesterol intake (g/1,000 kcal) between urban Puriscal and Framingham women. The calorie-adjusted cholesterol intakes were 359 mg/day in urban Puriscal men and 399 mg/day in Framingham men.

**Table 3. Plasma Lipids and Lipoproteins in Framingham and Rural and Urban Puriscal, Costa Rica Residents**

<table>
<thead>
<tr>
<th>Plasma parameter (mg/dl)</th>
<th>Rural Puriscal (n=103)</th>
<th>Urban Puriscal (n=99)</th>
<th>Framingham (n=138)</th>
<th>Rural vs. urban</th>
<th>Urban vs. Framingham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>130±72</td>
<td>167±71</td>
<td>126±73</td>
<td>0.003</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>175±35</td>
<td>186±38</td>
<td>208±36</td>
<td>0.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>106±31</td>
<td>114±35</td>
<td>139±36</td>
<td>0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>41±8</td>
<td>38±8</td>
<td>44±9</td>
<td>0.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>111±31</td>
<td>105±30</td>
<td>138±23</td>
<td>0.07</td>
<td>0.0001</td>
</tr>
<tr>
<td>Apo B</td>
<td>110±30</td>
<td>118±31</td>
<td>97±35</td>
<td>0.06</td>
<td>0.0001</td>
</tr>
<tr>
<td>LDL particle score*</td>
<td>3.96±1.10</td>
<td>4.00±1.10</td>
<td>3.61±1.00</td>
<td>0.8</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Dietary and Lipoprotein Comparisons Between Urban Puriscal and Framingham**

Table 2 shows that Framingham residents had significantly higher protein; total fat; saturated, monounsaturated, and polyunsaturated fatty acids; animal fat; and alcohol intakes and significantly lower carbohydrate intake relative to energy intake (percentage of calories) than did urban Puriscal residents. Framingham men had significantly lower vegetable fat intake than did urban Puriscal men, and no significant differences were found in sucrose, dietary fiber, or cholesterol (g/1,000 kcal) consumption. The calorie-adjusted means for cholesterol intake were 456 mg/day in urban Puriscal men and 399 mg/day in Framingham men. Framingham women had higher dietary fiber intake than did urban Puriscal women. No significant differences were detected in vegetable fat, sucrose, or cholesterol intake (g/1,000 kcal) between urban Puriscal and Framingham women. The calorie-adjusted cholesterol intakes were 359 mg/day in urban Puriscal women compared with 326 mg/day in Framingham women. It should be noted that when dietary intake in Framingham residents (n=3,600) was assessed with a 24-hour dietary recall, the percentage of calories from fat was higher by 3%. These differences may be due to systematic underreporting of fat among Framingham residents when an FFQ is used (B.M. Posner et al, personal communication).

Age-adjusted comparisons for lipoproteins are shown in Table 3. Framingham residents had signif-
Rural and Urban Puriscal

Table 4. Partial Correlation Coefficients for Daily Nutrient Intake and Plasma LDL Parameters Among the Combined Rural and Urban Residents of Puriscal, Costa Rica

<table>
<thead>
<tr>
<th>Nutrient (g)</th>
<th>Triglyceride</th>
<th>Chol</th>
<th>LDL</th>
<th>Apo B</th>
<th>LDL score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Protein</td>
<td>-0.14*</td>
<td>0.03</td>
<td>0.09</td>
<td>0.17*</td>
<td>0.13*</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-0.03</td>
<td>0.12</td>
<td>-0.13*</td>
<td>-0.16*</td>
<td>-0.09</td>
</tr>
<tr>
<td>Total fat</td>
<td>-0.11</td>
<td>0.06</td>
<td>0.09</td>
<td>0.16*</td>
<td>0.13*</td>
</tr>
<tr>
<td>Sat FA</td>
<td>-0.07</td>
<td>0.09</td>
<td>0.13*</td>
<td>0.18±</td>
<td>0.17±</td>
</tr>
<tr>
<td>Mono FA</td>
<td>-0.12*</td>
<td>0.04</td>
<td>0.08</td>
<td>0.15*</td>
<td>0.11</td>
</tr>
<tr>
<td>Poly FA</td>
<td>-0.17±</td>
<td>0.00</td>
<td>-0.05</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Animal fat</td>
<td>0.00</td>
<td>-0.04</td>
<td>0.15*</td>
<td>0.17*</td>
<td>0.13*</td>
</tr>
<tr>
<td>Vegetable fat</td>
<td>-0.14*</td>
<td>0.05</td>
<td>-0.06</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>-0.02</td>
<td>-0.13</td>
<td>0.08</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>-0.21†</td>
<td>0.02</td>
<td>-0.06</td>
<td>-0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0.24†</td>
<td>-0.04</td>
<td>0.00</td>
<td>0.10</td>
<td>-0.14*</td>
</tr>
</tbody>
</table>

All correlations were adjusted for caloric intake, age, total body fat, and fitness score. M, male (n=202); F, female (n=174); Chol, cholesterol; LDL, low density lipoprotein; Apo, apolipoprotein; Sat, saturated; FA, fatty acids; Mono, monounsaturated; Poly, polyunsaturated.

See Table 3, footnote for explanation of LDL score.

*tp<0.05, †tp<0.01, ‡tp<0.005.

ificantly higher total, LDL, and HDL cholesterol and apo A-I concentrations and significantly lower triglyceride and apo B levels than did urban Puriscal residents. In addition, Framingham residents had significantly larger LDL particles than did urban Puriscal residents.

Dietary Intake and Plasma Lipid Correlations in Rural and Urban Puriscal

When adjustments for caloric intake, age, total body fat, BMI, and FS were made, differences in lipids and lipoproteins between the rural and urban areas were not statistically significant, so we combined the rural and urban residents and adjusted for the previously mentioned variables. Partial correlation coefficients for daily nutrient intakes and plasma lipids and lipoproteins among the combined rural and urban residents are shown in Table 4. In Puriscal women, increased carbohydrate intake was significantly associated particularly with smaller LDL particles. Increased protein, animal fat, and saturated fatty acid intake and decreased carbohydrate intake were significantly associated with elevated total and LDL cholesterol and apo B levels in Puriscal women. Similar associations with total and LDL cholesterol, apo B levels, and LDL particles were found in Puriscal men, but the correlation coefficients were less striking. In addition, in Puriscal men increased triglyceride levels were significantly associated with increased alcohol and decreased dietary fiber consumption. Decreased consumption of protein, monounsaturated and polyunsaturated fatty acids, and vegetable fat were also significantly associated with elevated triglyceride levels in Puriscal men. No significant associations were found between dietary intake and either HDL cholesterol or apo A-I level (data not shown).

Table 5 shows the calorie-adjusted standardized β-coefficients of the stepwise regression models for independent associations between dietary intake and plasma parameters in men and women from rural and urban Puriscal. In Puriscal men total body fat was significantly correlated with all the plasma parameters; positive correlations were found with triglyceride, total cholesterol, LDL cholesterol, and apo B levels, and inverse correlations were found with HDL cholesterol and apo A-I concentrations. Increased body fat in men was also associated with smaller LDL particles. However, in Puriscal women total body fat was only significantly associated with increased triglycerides and apo B levels, smaller LDL particles, and decreased HDL cholesterol levels. Total body fat was also inversely correlated with FS, particularly in Puriscal men, indicating that increased body fat is probably due to a more sedentary lifestyle (data not shown).

In Puriscal men age was significantly and positively correlated with total cholesterol, LDL cholesterol, and apo B levels. In Puriscal women age was significantly and positively associated with all plasma parameters except for LDL particle score. In addition to age and total body fat, FS also entered the model for HDL cholesterol and apo A-I in Puriscal men. Significant negative associations were found with both parameters. The FS in women was not significantly associated with any plasma parameter.

Of the dietary variables, saturated fat was the preferred predictor for total cholesterol, LDL cholesterol, and apo B levels in Puriscal men and for total cholesterol and triglyceride levels in Puriscal women. Triglyceride levels were associated with different dietary variables in Puriscal men and women. In Puriscal men significant positive associations for triglycerides were found with alcohol intake, and negative associations with polyunsaturated fat and dietary fiber intake were noted. In Puriscal women...

<table>
<thead>
<tr>
<th>Group</th>
<th>TG (mg/dl)</th>
<th>Chol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Apo A-I (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>LDL score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>−0.029</td>
<td>0.014</td>
<td>0.018</td>
<td>0.016</td>
<td>−0.014</td>
<td>−0.003</td>
<td>0.007</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>0.43*</td>
<td>0.34*</td>
<td>0.28*</td>
<td>−0.42*</td>
<td>−0.24†</td>
<td>0.44*</td>
<td>0.23†</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>...</td>
<td>0.21†</td>
<td>0.18†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Fitness score§</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>−0.15‡</td>
<td>−0.24†</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>0.005</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Saturated FA (g)</td>
<td>...</td>
<td>0.02†</td>
<td>0.01‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Poly FA (g)</td>
<td>−0.03</td>
<td>−0.04</td>
<td>...</td>
<td>...</td>
<td>−0.04‡</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Dietary fiber (g)</td>
<td>−0.01</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Alcohol (g)</td>
<td>0.01†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Area‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>−0.18†</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Total $R^2$</td>
<td>0.27</td>
<td>0.20</td>
<td>0.14</td>
<td>0.15</td>
<td>0.10</td>
<td>0.28</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Women

<table>
<thead>
<tr>
<th>Group</th>
<th>TG (mg/dl)</th>
<th>Chol (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>Apo A-I (mg/dl)</th>
<th>Apo B (mg/dl)</th>
<th>LDL score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>−2.483</td>
<td>0.023</td>
<td>0.027</td>
<td>2.461</td>
<td>0.038</td>
<td>−1.83</td>
<td>−0.036</td>
</tr>
<tr>
<td>Total body fat (kg)</td>
<td>0.16*</td>
<td>...</td>
<td>...</td>
<td>−0.16†</td>
<td>...</td>
<td>0.12†</td>
<td>0.26†</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>0.46*</td>
<td>0.57*</td>
<td>0.41*</td>
<td>0.22†</td>
<td>0.31*</td>
<td>0.31*</td>
<td>...</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0.91‡</td>
<td>...</td>
<td>−0.87‡</td>
<td>−0.92‡</td>
<td>...</td>
<td>1.53†</td>
<td>...</td>
</tr>
<tr>
<td>Saturated FA (g)</td>
<td>0.02†</td>
<td>0.01‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Cholesterol (g)</td>
<td>−0.19</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Total $R^2$</td>
<td>0.34</td>
<td>0.28</td>
<td>0.17</td>
<td>0.11</td>
<td>0.09</td>
<td>0.20</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Standardized β-coefficients are given for the variables that met the 0.10 level of significance for entry into the model. Daily nutrient intake data were calorie adjusted. Fitness score=seconds/(pulse 1 + 2 + 3) x 100. Area=rural/urban, where a negative association means lower levels in the rural area. See Table 3 footnote for explanation of LDL score.

TG, triglycerides; Chol, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; Apo, apolipoprotein; Poly, polyunsaturated; FA, fatty acids (men, n=202; women, n=174).

Discussion

Diets high in saturated fat are also more commonly found in industrialized countries than in less developed areas.11 Our data are consistent with these findings. Compared with the Framingham population, Puriscal residents had a significantly lower dietary intake (percentage of calories) of total fat (saturated, monounsaturated, and polyunsaturated fatty acids) and animal fat and a significantly higher carbohydrate intake. Moreover, our data also indicate that urban Puriscal residents consumed 14% of the calories from animal fat compared with 10% observed in the rural residents; these numbers, however, are low if compared with the 20.5% of calories from animal fat observed in the Framingham population. The dietary differences observed are not as striking if saturated fatty acids and cholesterol intake are compared. Saturated fatty acids contributed 11% of the calories in the rural area, 12% in the urban area, and 13% in Framingham, whereas cholesterol intake was not significantly different between rural and urban Puriscal and Framingham residents. The similarity of dietary cholesterol in Puriscal and Framingham may reflect trends toward decreased egg and meat consumption in the United States, whereas the inverse has been observed in Costa Rica. It should be noted that the source of the saturated fat in both populations was very different. In Puriscal, most of the saturated fatty acids were of vegetable origin.

It has been shown in metabolic ward studies that the amount and type of dietary fat affect plasma concentrations of LDL cholesterol and HDL cholesterol,15-17 but most cross-sectional studies have failed to find significant associations with plasma chole-
terol within free-living populations. On the other hand, significant correlations between dietary saturated fat and plasma cholesterol have been found within a population with low mean total cholesterol levels. In our study, we found significant independent correlations between saturated fat and animal fat intake and total cholesterol, LDL cholesterol, and apo B levels within the Puriscal residents.

In recent years, decreased infant mortality rates and increased life expectancy in developing countries, particularly in the urban areas, have been associated with increased rates of degenerative diseases such as CAD. It has been estimated that 77% of the population in Latin America and 43% overall in less developed countries will be living in urban centers and will be affected by health problems similar to those now seen mostly in industrialized countries. Elevated plasma cholesterol levels and a higher prevalence of atherosclerotic lesions have been found more frequently in industrialized countries than in rural areas of less developed nations. In our study, men and women from Puriscal had lower total cholesterol, LDL cholesterol, HDL cholesterol, and apo A-I and higher triglyceride and apo B levels than did men and women from the Framingham Offspring Study. It has also been reported that triglyceride and plasma cholesterol levels are frequently higher in the urban than in the rural residents of Brazil and Puerto Rico. Urban men from Puriscal had significantly higher triglyceride and total cholesterol levels and significantly lower HDL cholesterol levels than did men from the rural area. In addition, urban Puriscal women had significantly higher apo B levels than did women from rural Puriscal. High triglyceride and low HDL cholesterol levels have been found in the Pima Indians and in Mexican-Americans. The same high triglyceride and low HDL cholesterol pattern observed in Puriscal could be due to the mestizo ethnic background of the Costa Ricans. However, it is well established that polyunsaturated fatty acid intake decreases and carbohydrate intake increases triglyceride levels. Thus, a low consumption of polyunsaturated fatty acids along with a high consumption of carbohydrate in Puriscal residents could also explain the higher triglyceride levels in Puriscal compared with Framingham residents. These data also suggest that the high-carbohydrate diet in Puriscal was accompanied by higher levels of total apo B despite the lower total and LDL cholesterol levels. This could be a possible disadvantage of a high-carbohydrate diet. It should be pointed out that because we measured total apo B, a significant portion of apo B is in the triglyceride-rich particle, and there is controversy as to whether these particles are associated with CAD after controlling for HDL levels. An increase in very low density lipoprotein apo B levels with high-carbohydrate diets has been reported. High-carbohydrate diets may decrease the mechanisms that convert very low density lipoprotein to LDL, leading to decreased LDL cholesterol levels but not total apo B.

Small LDL particles have been associated with high triglyceride and low HDL cholesterol levels and with the presence of CAD. Puriscal residents had significantly smaller LDL particles than did Framingham residents. Thus, population differences in apo B and LDL cholesterol levels may be due to differences in LDL composition and plasma particle number. Consistent with previous findings in the Framingham population, small LDL particles in Puriscal residents were associated with higher triglyceride and apo B concentrations and with lower HDL cholesterol levels. Moreover, small LDL particles in Puriscal residents were associated with increased body fat and carbohydrate intake and decreased protein intake. Consequently, our data suggest that small LDL particles are more prevalent in a population that is currently at a lower risk of CAD than that of the United States but that has higher triglyceride and apo B levels and lower HDL and LDL cholesterol levels. Such a finding in these Costa Rican adults is enigmatic. It is reasonable to hypothesize that small LDLs enhance atherogenicity when associated with greater levels of LDL cholesterol but not necessarily when the former are accompanied by greater apo B levels, increased carbohydrate intake, and a less sedentary lifestyle.

Increased adiposity has been associated with increased cardiovascular disease and death rates from all causes. An increased prevalence of obesity has been associated with changes from rural to urban lifestyles. Our data indicate that urban-rural differences in total cholesterol and LDL cholesterol were largely explained by body fat, age, and dietary variables within a free-living population in Costa Rica. The striking rural-urban differences observed in body fat and fitness level in men and women from Puriscal indicate that the trends toward a more atherogenic diet in the urban area were accompanied by a more sedentary lifestyle. Nutrition intervention studies indicate that the effects of weight reduction and exercise on plasma cholesterol levels are related to the composition of the diet. Increased adiposity and saturated fat intake, combined with decreased fitness level, were strong independent predictors of a more atherogenic plasma lipid profile in Puriscal residents. A recent report by Ornish et al indicated that comprehensive lifestyle changes were necessary to induce regression of severe coronary atherosclerosis. Our population-based study indicates that the more atherogenic lipid profile in urban Puriscal residents was associated with a cluster of significant lifestyle differences. In addition, given the similarity in dietary cholesterol between Puriscal and Framingham residents despite the significant differences found in saturated fatty acids, not only diet but also factors such as adiposity and physical activity should be emphasized in cross-cultural studies.

Our data indicate that men and women in urban Puriscal have increased body fat, are more sedentary,
and have a tendency toward a more atherogenic diet and plasma lipid profile than do men and women from rural Puriscal. Body fat, fitness level, and saturated fatty acid intake, as assessed by an FFQ, were important determinants of total cholesterol, LDL cholesterol, and HDL cholesterol within residents of Puriscal, Costa Rica. Puriscal residents have a less atherogenic lifestyle and plasma lipid profile than do men and women from Framingham. Ethnic background may also explain some of these cross-cultural differences.

Acknowledgments

We want to thank Leonardo Mata and Marcela Vives at el Instituto de Investigaciones en Salud en San José y en Santiago, Puriscal, Costa Rica, for their support and assistance.

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Key Words • dietary intake • plasma lipoproteins • triglycerides • cholesterol • apolipoproteins • low density lipoprotein particle size • physical activity • population comparisons

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