Relationship between Smoking Status and Serum Lipids in a Hyperlipidemic Population and Analysis of Possible Confounding Factors

E. Bruckert, N. Jacob, L. Lamaire, J. Truffert, F. Percheron, J. L. de Gennes

The aim of our study was to estimate the potential relationship between smoking behavior and other coronary heart disease risk factors in 250 hyperlipidemic patients. We present data obtained through self-reporting of the number of cigarettes smoked per day, measurements of three tobacco markers, and data on dietary habits and lipid variables. We measured cotinine (by HPLC) and thiocyanate and used a recent colorimetric assay for the indirect evaluation of the nicotine metabolites in a single urine specimen. Mean values of nicotine metabolites, expressed as cotinine equivalents, were 6.7, 39.9, and 79.4 \( \mu \text{mol/L} \), respectively, for nonsmokers, light smokers (7.7 cigarettes per day), and heavy smokers (25.8 cigarettes per day). We found that light smokers have higher concentrations of cotinine and nicotine metabolites in proportion to the number of cigarettes smoked per day than do heavy smokers. Thus, the simple colorimetric assay can accurately evaluate smoking status. Hyperlipidemia and smoking are linked by an intricate network of multiple relations. The concentration of high-density lipoprotein (HDL) cholesterol is lower in heavy smokers, and the concentrations of triglycerides and cholesterol are higher. The 0.11 mmol/L difference in HDL cholesterol between light and heavy smokers is close to the results of previous papers; however, when gender, dietary habits (including alcohol intake), and data on body mass index are included in a multiple regression analysis, there is no longer an association between HDL cholesterol concentrations and smoking status. Therefore, these different dietary habits may be confounding factors that partly explain the pattern of lipid variables.

Additional Keyphrases: nicotine · cotinine · thiocyanate · high-density lipoprotein · cholesterol

Smoking has been identified as a major risk factor for cardiovascular disease (1). Two major issues remain unresolved: the reliability of self-reporting of smoking status, and the mechanisms by which tobacco intake interacts with potential confounding factors, such as the presence of other unhealthy habits. Tobacco biochemical markers have been demonstrated to provide a reliable verification of patients' smoking status, with cotinine being the analyte of choice for quantifying nicotine intake during tobacco consumption (2–4) and exposure to environmental tobacco smoke (4, 5). In recent years, several procedures (5–11) have been widely used to detect and quantify this marker in blood, urine, and saliva but, in general, these require the use of expensive laboratory instruments. Recently, a colorimetric assay that detects various nicotine metabolites in urine has been shown to be highly correlated to results of a cotinine radioimmunoassay (12, 13). Unfortunately, cotinine is eliminated from the body within a few days (14). Thiocyanate, on the other hand, has a long half-life in plasma (15) and is mainly excreted in the urine; for these reasons, the determination of this product of the detoxification of hydrogen cyanide in mainstream cigarette smoke may be of interest (2, 3, 15–17).

To estimate the impact of smoking behavior on a hyperlipidemic population, we present in this study data obtained through the self-reporting of smoking status and contrast these with the determination of cotinine by high-performance liquid chromatography (HPLC) and with the colorimetric assays of thiocyanate and nicotine metabolites in a single urine specimen. We also include data on the dietary habits of 250 patients in relation to their smoking status.

Materials and Methods

Patients

Between May and June 1989, all patients referred to our outpatient clinic (Hôpital Pitié-Salpêtrière, Service d'Endocrinologie-Métabolisme, Paris) systematically underwent a blood sampling for the evaluation of lipid variables and a urine test for the evaluation of the tobacco markers. In this clinic, all the patients are referred for the evaluation of a dyslipidemia (mainly hypercholesterolemia). The first 58 nonsmokers referred constituted the control population.

Between September 1989 and June 1990, only the patients who were exsmokers and smokers at the time of their admission to the clinic underwent such an evaluation. Exsmokers were patients who had smoked regularly before the study, regardless of when they stopped. However, the patients who stopped within a month before the sampling were not included in the study.

All patients were questioned about their medical history and previous treatments. Routine medical examination included measurements of weight, height, and diastolic and systolic blood pressure, taken in a supine position after a 10-min rest. Body mass index (BMI) was calculated as weight divided by height squared. Smoking status was evaluated as cigarettes per day and pack-years (average number of packs per day multiplied by the number of years of smoking).

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Received September 16, 1991; accepted February 12, 1992.

Dietary habits were assessed by a registered dietitian who obtained a four-day food record for each patient (three weekdays and one day of the weekend), in which all foods and beverages consumed were recorded. For alcoholic beverages, the amount of absolute alcohol was calculated on the basis of the number and the type of drinks taken by each subject and then expressed in grams of ethanol per day. Mean calories; the calories constituted by fats, proteins, and carbohydrates; cholesterol intake; and the ratio of polyunsaturated to saturated fats were calculated by using INSERM’s standard text for determining the nutritional values of foods consumed by each patient (18).

Patients who had modified their smoking status during the three weeks before the study, who had major bulimia, or who had been treated with nicotinic acid were excluded. Nibbling (defined as the consumption of food during the interprandial phase more than three times a week) was recorded for sweet and salty foods separately.

Urine Specimens

Patients provided a sample of urine (untimed collection before 1200). After determination of the refractive index, the urine specimens were stored at -20 °C without a preservative. All the samples were analyzed for cotinine, thiocyanate, and metabolites of nicotine within a week after collection.

Lipid Analyses

Venous blood samples were taken between 0730 and 0930 after a 12-h overnight fast. Lipid analysis was performed within 3 h of blood sampling. Concentrations of total cholesterol and triglyceride in serum were determined by enzymatic methods (Biotrol, Paris, France, and bioMérieux, Marcy-l’Étoile, France, respectively), and high-density lipoprotein cholesterol (HDL-C) by an enzymatic procedure after precipitation with phosphotungstic acid/magnesium chloride, a method selected by the French Society of Clinical Biology (19). Serum concentrations of lipoprotein(a) (Lp(a)) were determined by an immunonephelometric assay with polyclonal antibodies from ImmunoFrance (Orly, France) and a nephelometer (Behringwerke AG, Marburg, FRG) as previously described (20).

Quantification of Urinary Cotinine by HPLC

We quantified urinary cotinine by HPLC with the following chromatographic equipment: a 150 x 4.6 (i.d.) mm Altex Ultrasphere-IP C18 column (5-μm particle size; Beckman Instruments Inc., Brea, CA); a Model 114 M chromatographic pump (Beckman Instruments); a pulse dampener (Touzart & Matignon, Vitry-sur-Seine, France); a Model 7125 injector (Rheodyne, Cotati, CA); an ultraviolet detector (Du Pont Instruments, Wilmington, DE); and an Enica 10 integrator (Delia Instruments, Suresnes, France).

Reagents. (−)Nicotine and (−)cotinine were purchased from Sigma Chemical Co., St. Louis, MO; 2-phenylimidazole was supplied by Aldrich Chemical Co., Milwaukee, WI. Methanol, methylene chloride and acetonitrile were from Merck, Darmstadt, FRG. Hep-tane sulfonic acid, sodium salt, was provided by Eastman Kodak Co., Rochester, NY. Stock solutions of nicotine (3.1 mmol/L), cotinine (2.85 mmol/L), and the internal standard solution (2-phenylimidazole, 3.74 mmol/L) in methanol were prepared monthly and stored at 4 °C. Before analysis, working standard solutions were prepared by diluting 10-fold in water.

Sample preparation. We mixed 5.0 mL of urine, 50 μL of 175 μmol/L internal standard solution, 10 mL of methylene chloride, and 1 mL of 3 mmol/L NaOH with shaking for 10 min on a rotary mixer. The aqueous and organic phases were separated by centrifugation (10 min, 3000 x g). The aqueous layer was removed and discarded. The organic phase was washed with 1 mL of 3 mmol/L NaOH. The samples were vigorously shaken, then centrifuged (5 min, 3000 x g). We gently evaporated 5 mL of the organic phase to dryness under reduced pressure at 45 °C. The sample residues could be stored for at least two days at 4 °C before being analyzed.

HPLC conditions. The mobile phase was a mixture of 100 mL of acetonitrile and 900 mL of pH 4.6 buffer containing, per liter, 0.05 mol of sodium dihydrogen phosphate, 0.8 mmol of sodium heptane sulfonate, and 0.1 mmol of EDTA. The mixture was degassed before use by filtration on a HAWP 0.45-μm pore-size filter (Millipore Corp., Milford, MA) under reduced pressure. The chromatographic flow rate was 1 mL/min. For analysis, we dissolved the sample residues in 250 μL of mobile phase and injected 50 μL of this. Cotinine and cotinine were detected at 254 nm.

Colorimetric Assays of Thiocyanate and Nicotine Metabolites

The analytical methods for thiocyanate (21, 22) and nicotine metabolites (12, 13) were based on the König reaction. Reactions were performed directly with a small urine sample in disposable polystyrene cuvettes. We measured the optical absorbance at 510 nm with a Compact Clinical Analyzer (OLLI C; Koné, Espoo, Finland). Results for the thiocyanate assay varied linearly with concentrations up to 30 μmol/L in the sample. The interassay reproducibility (n = 30) was 5.7% and 5% for the 5 and 12 μmol/L thiocyanate standards, respectively. For nicotine metabolites, the assay was slightly modified: we used a 50 mmol/L solution of 2-thiobarbituric acid and let the color develop for 25 min at room temperature. We expressed the concentration of nicotine metabolites in urine as “μmol/L cotinine equivalents,” calibrating the assay with a working standard solution of (−)-cotinine. Results for this assay varied linearly with concentrations up to 200 μmol/L cotinine equivalents in urine. The intersay reproducibility (n = 30) was 8% and 7% for the 25 and 85 μmol/L cotinine standards, respectively. Blood and bile pigments were found to interfere.
Statistical Analysis

The mean values and standard deviations were calculated for all continuous variables. A multiple-regression analysis of HDL-C was undertaken to assess the role of different confounding factors (partial Y- and P-values were determined). The Pearson correlation coefficient was calculated for each tobacco marker in relation to the number of cigarettes smoked per day. The comparisons between the categories were performed by ANOVA. The statistical analysis was carried out with a Macintosh SE computer, with the use of Statview (Abacus) and Excel (Microsoft) software (Apple Computer, Inc., Cupertino, CA).

Results

There were 250 patients in our population (160 men and 90 women). Patients were divided into four groups according to their self-evaluation of daily cigarette consumption (Table 1). The first group consisted of 58 nonsmokers; 49 exsmokers formed the second group. The third group comprised the 75 light smokers (7.7 ± 4.4 cigarettes per day), and the fourth group was 68 heavy smokers (25.8 ± 8.9 cigarettes per day).

Tobacco Markers

Results for the assays of tobacco markers are reported in Table 1. Smokers displayed a wide range of cotinine values (0.3 to 35 μmol/L) determined by HPLC, the mean value (SD) being 6.3 (6.1) μmol/L for light smokers and 12.4 (11.6) for heavy smokers. The correlation between cotinine concentrations and the number of cigarettes per day, was _r_ = 0.388 ( _P_ <0.01). Through the alkaline extraction step, nicotine and cotinine were recovered from urine at 87% and 81%, respectively. The internal standard solution had a slightly lower analytical recovery rate (75%).

A chromatogram for analysis of a standard is shown in Figure 1. All peaks are baseline-resolved. The retention times were 3.4 min for nicotine, 4.6 min for cotinine, and 8.4 min for the internal standard. The calibration curve is linear up to 5 μg per 50-μL injection; quantification was by the peak-height method. For daily routine assays, we used a two-point calibration curve, created with duplicate injections of the aqueous standard. The day-to-day ( _n_ = 20) coefficients of variation for 2.85 and 5.7 μmol/L cotinine standards obtained through the extraction step were 6.5% and 10%, respectively. Cotinine as little as 2.5 ng per 50-μL injection was detected, corresponding to a concentration of 0.3

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Table 1. Clinical and Biological Characteristics in Patients Grouped According to Smoking Status

<table>
<thead>
<tr>
<th>Mean (SD)</th>
<th>Non-smokers (n = 58)</th>
<th>Ex-smokers (n = 49)</th>
<th>Light smokers (n = 75)</th>
<th>Heavy smokers (n = 68)</th>
<th><em>p</em>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>52.16 (14.55)</td>
<td>52.86 (10.47)</td>
<td>46.16^b (13.11)</td>
<td>43.44^b (10.41)</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>24.07 (3.04)</td>
<td>25.59 (3.86)</td>
<td>24.24 (3.8)</td>
<td>24.11 (3.3)</td>
<td>0.10</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>132.00 (15.30)</td>
<td>134.49 (21.55)</td>
<td>130.36 (15.2)</td>
<td>128.65 (17.0)</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>83.00 (8.20)</td>
<td>84.60 (11.07)</td>
<td>84.29 (9.68)</td>
<td>82.36 (11.45)</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.20 (1.47)</td>
<td>6.30 (1.31)</td>
<td>6.43 (1.34)</td>
<td>6.85 (1.52)</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.33 (1.42)</td>
<td>1.63 (1.92)</td>
<td>1.81^a (2.21)</td>
<td>2.34^a (2.99)</td>
<td>0.006</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.35 (0.36)</td>
<td>1.30 (0.35)</td>
<td>1.27^a (0.41)</td>
<td>1.24^a (0.41)</td>
<td>0.09</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>2.4 (2.3)</td>
<td>1.8 (1.4)</td>
<td>2.0 (2.0)</td>
<td>2.2 (1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>0</td>
<td>0</td>
<td>7.7 (4.4)</td>
<td>25.8 (8.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Pack-year</td>
<td>0</td>
<td>19.7 (14.4)</td>
<td>18.3 (17.3)</td>
<td>28.6 (16.9)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Urine refractive index</td>
<td>1.022 (0.012)</td>
<td>1.024 (0.006)</td>
<td>1.023 (0.006)</td>
<td>1.025 (0.006)</td>
<td>0.071</td>
</tr>
<tr>
<td>Tobacco markers, μmol/L</td>
<td>6.7 (6.4)</td>
<td>13.8 (27.2)</td>
<td>39.9^a (34.1)</td>
<td>79.4^a (51.5)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Nicotine metabolites</td>
<td>72.1 (28.9)</td>
<td>84.0 (41.7)</td>
<td>107.4^b (65.3)</td>
<td>164.4^b (98.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cotinine</td>
<td>6.3 (6.1)</td>
<td>12.4 (11.6)</td>
<td>10.3 (11.3)</td>
<td>16.4 (11.6)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure.

*a* By ANOVA; NS, not significant.

*b* Significantly different from non-smokers group ( _P_ <0.05).
\( \mu \text{mol/L} \) in urine. Through the alkaline extraction step, caffeine was extracted from the urine with a mean recovery rate of 90\% and appears on the chromatogram with a retention time of 5.9 min. Because even a large caffeine peak does not interfere with the peaks for cotinine or the internal standard, it was not necessary to add an acidic extraction step to remove caffeine from urine samples (23). Conversely, unknown peaks are sometimes eluted near the nicotine peak, making a correct evaluation difficult. In those cases, we did not routinely quantify nicotine. Finally, with the one-step extraction procedure, we used a routine analysis time of 20 min to ensure that unknown peaks with long retention time were excluded from the next assay.

In nonsmokers, exsmokers, light smokers, and heavy smokers, the mean (SD) thiocyanate values were, respectively, 72 (28), 84 (41), 107 (65), and 164 (86) \( \mu \text{mol/L} \). In the nonsmokers, thiocyanate in urine was mostly <120 \( \mu \text{mol/L} \). Only 8 of 107 samples from non- and exsmokers had thiocyanate between 120 and 150 \( \mu \text{mol/L} \), whereas 43 of the 68 heavy smokers had values from 120 to 400 \( \mu \text{mol/L} \). The correlation coefficient between thiocyanate and the number of cigarettes smoked per day was 0.488 (P <0.01). There is a great deal of overlap between the values for the nonsmokers and light smokers, but the difference was significant at 95\% for both these groups and for the light and heavy smokers (ANOVA P-value = 0.0001).

For nicotine metabolites, the mean (SD) was 6.8 (6.4) \( \mu \text{mol/L} \) cotinine equivalents for nonsmokers, 13.8 (27.2) for exsmokers, 39.9 (34.1) for light smokers, and 79.4 (51.5) for heavy smokers. Correlation with the number of cigarettes per day was \( r = 0.578 \) (P <0.01). The correlation between the colorimetric assay and cotinine HPLC determination was \( r = 0.7 \) (P <0.01). In the light and heavy smokers, the mean for nicotine metabolites was sixfold greater than the mean for cotinine.

Figure 2 shows the relationship between the concentrations of the tobacco markers and the number of cigarettes per day. The patients were divided into six groups according to increasing numbers of cigarettes smoked per day.

**Dietary Habits and Risk Factors Assessment**

Clinical and biological variables are indicated in Table 1. The concentrations of cholesterol and triglycerides in smokers are higher than in nonsmokers; conversely, the concentrations of HDL-C are lower. The difference in cholesterol values is not significant. Lp(a) concentrations are not significantly different between the four groups.

The result of the calculation of the four-day dietary record is indicated in Table 2. Smokers have a higher calorie intake despite having the same BMI as the other groups. The amount of fats and carbohydrates is higher in the smoker group, although the ratio of polyunsaturated to saturated fats is not significantly different from the other groups. The intake of alcohol is higher in the smoker group than in the group of patients who never smoked. Interestingly, the exsmokers also had a significantly higher intake of alcohol. The number of patients with nibbling habits for sweetened and salted foods are 19 and 9, respectively, in the nonsmokers, 8 and 4 in the exsmokers, and 28 and 21 in the smokers. We did not find any statistical difference in the nibbling behavior; however, there tend to be fewer nibblers of sweet foods (P = 0.067) among the smokers and exsmokers (20 pieces of data missing).

The BMI at age 20 years and the maximal BMI were recorded for 183 patients. Because some patients could not provide the relevant information, there are several pieces of missing data. However, exsmokers tended to have a higher maximum BMI (26.98, SD 4.24) than those of nonsmokers (25.48, SD 3.61), light smokers (24.67, SD 3.65), and heavy smokers (25.76, SD 3.31) (ANOVA P = 0.033). The BMI at age 20 was not significantly different between the four groups of patients: 21.3 (3.01), 22.87 (3.20), 21.87 (3.30), and 22.28 (3.16) for the non-, ex-, light, and heavy smokers, respectively.

**Discussion**

In this study we have expressed the results for tobacco markers as concentrations (\( \mu \text{mol/L} \)), whereas others have reported the results for urinary tobacco markers as ratios with creatinine excretion (3, 4). Thompson et al. (24) demonstrated that the adjustment of urinary cotinine to creatinine improves the relationship between urinary and serum cotinine in smokers. Nevertheless, it has been accepted that, as a measure of passive exposure tobacco smoke, cotinine in human body fluids should be expressed as mass per volume (11). Because urinary creatinine excretion is related to muscle in human subjects, it is at least theoretically relatively stable for a given healthy individual (25). However, the value can be influenced by diet or physical activity and urine flow (26). Therefore, we favored the simple and inexpensive method of determining the refractive index of the urine specimens to take into account possible variations in urine concentration between the four groups. We found no significant differences between the groups.

Thiocyanate has already been extensively studied in
plasma and saliva of smokers and nonsmokers (2, 17, 22, 23, 27, 28). Only recent studies take into account
urinary thiocyanate determination to test smoke intake (2, 3) or to monitor smoking cessation (16). Our results for
nonsmokers and smokers are comparable with those obtained by Jarvis et al. (2), using a similar assay
method; however, they reported an average cigarette consumption of 13.2 cigarettes per day. The correlation
found here with the number of cigarettes smoked per day for thiocyanate in urine is close to the correlation
for blood specimens or saliva (27, 28). The major advantage of thiocyanate quantification is the slow clearance
of this compound from the body, unlike nicotine-related metabolites (16).

Although cotinine is influenced by diet and industrial pollution (17, 29), our data show that it remains a
reliable indicator of smoking status. Urinary cotinine values by HPLC determination correspond to previously
reported values obtained by HPLC (30), gas chromatography (2, 4), radioimmunoassay (8, 12, 13), and enzyme-linked
immunosorbent assay (5). However, cotinine is no longer considered the major metabolite of nicotine, which probably explains why the cotinine concentration in serum or plasma is only roughly related to daily
cigarette consumption (27, 28, 31). Because the correlation of urinary cotinine with the number of cigarettes
smoked per day is close to that observed with serum or plasma specimens, the present study and other data (14)
suggest that this noninvasively obtained sample is suitable for evaluating smoking status.

For nicotine metabolites, this study complements findings in previously published reports (12, 13, 16).
Although apparently nicotine cannot be considered a tobacco-specific marker (32), the exsmoker population
shares with smokers various characteristics of tobacco markers, which may be explained by a higher exposure
to environmental tobacco smoke or by an attempt to hide a smoking relapse.

The colorimetric assay has been described as giving results closely correlated with those of cotinine
radioimmunoassay \( r = 0.85 \) (12) and 0.91 (13). Cotinine as determined by HPLC and colorimetric determinations
of nicotine metabolites are not so highly correlated. Previous works disagree as to the ratio of the colori-
metric assay to cotinine radioimmunoassay: 8:1 for smoking pregnant women (12) and 3:1 to 4:1 for male smokers
(13). We obtained intermediate results (6:1). Of course, nicotine metabolism varies from individual to individual
(33) and yields many metabolites. At present, neither the amount nor the relative concentration of metabolites in the urine, serum, and saliva of smokers is well defined. The colorimetric assay measures nicotine
metabolites that contain an intact pyridine ring: nicotine, cotinine, trans-3'-hydroxyco
tinine, and pyridylcarbinol (34). trans-3'-Hydroxyco
tinine is the nicotine metabolite detected in the highest concentration in urine of smokers (35, 36), whereas cotinine represents only 15% of urinary nicotine-related metabolites (36). This probably explains the close correlation between nicotine metabolites and the number of cigarettes
smoked per day.

We report here a large overlap in concentrations of cotinine and nicotine metabolites between light and
heavy smokers, despite no apparent overlap in the number of the cigarettes smoked. Although heavy
smokers [25.8 (8.9) cig/day] consumed threefold as many cigarettes as light smokers [7.7 (4.4) cig/day], mean
concentrations of cotinine and nicotine metabolites were increased only twofold in the heavy smokers. Indeed,
smoke intake is largely determined by the individual pattern of puffing and depth of inhalation. As has been
demonstrated, smokers of low-yield cigarettes adjust their patterns and do not consume less nicotine than
smokers of "regular" cigarettes (37, 38). Moreover, smoking induces changes in nicotine disposition: the rate of cotinine disappearance from urine is signifi-
cantly slower in nonsmokers than in smokers (39, 40).

Therefore, the self-reported status of smoking is an imprecise datum, and the determination of tobacco
markers, especially nicotine metabolites, in a single urine sample is a good alternative.

High values for cardiac risk factors tend to cluster and act synergistically (41). These factors are linked by an intri
cate network of multiple relations. This complexity is illustrated here in several ways. Smokers have both a
different lipid pattern and different dietary habits, as also noted in several other publications. For example,
HDL-C is lower and triglyceride concentrations are greater in smokers than in nonsmokers in several stud-
ies (42-44). Conversely, as also reported previously, there is no difference in Lp(a) concentrations between
these groups (45). The same results have been reported
for coffee drinkers, patients with high alcohol consumption, and patients with a more atherogenic diet.

In view of the aggregation of smoking status with several sociodemographic and behavioral variables (46, 47), we evaluated the effects of dietary habits as potential confounding factors. We found calorie intake, alcohol consumption, and total cholesterol intake per day to be quite different in the four groups of patients. The higher average intake of alcohol among smokers and exsmokers has already been described. For instance, Stamford et al. (49) showed that heavy smokers consume 78% more alcohol calories than do nonsmokers. Interestingly, we found the alcohol intake of ex-, light, and heavy smokers to be identical. We therefore assume that a common behavior pattern may lead to alcohol consumption and cigarette smoking.

On the other hand, BMI did not differ significantly in the four groups, although the exsmokers had a higher BMI (P = 0.10), by an amount close to what has been already published (49). The body weight for current smokers was the same, although they consumed on average 300 to 400 calories per day more than nonsmokers. Such findings support the possibility that smokers have a higher metabolic rate than nonsmokers, as previously described. However, this group of patients also had a lower mean age and included more men than other groups; moreover, their alcohol intake might adversely impact the absorption of nutrients (50) or participate in the non-energy-producing microsomal system (51). There was no difference among groups in BMI at age 20. However, there are several pieces of missing data for the maximal BMI and the BMI at age 20. Nonetheless, although such missing data might have introduced a bias, the amount of missing data is similar in each of the different groups (data not shown), and the magnitude of the difference (3 kg) is close to the results recently found in a large epidemiologic study (52).

We have emphasized here the results that relate to the potential relationship between smoking status and HDL-C because prior studies have consistently shown a lower concentration of HDL-C in smokers. However, several separate confounding factors may interfere, including gender, alcohol intake, age, and sedentary habits (53, 54). Dietary habits are only slightly involved in the analysis. Our results show a 0.11 mmol/L decrease in HDL-C between nonsmokers and heavy smokers, findings close to those reported by Freedman et al. (42), 0.11 mmol/L, and Braschotet al. (43), 0.10 mmol/L. They are also consistent with the 5.7% mean decrease in HDL-C reported by Craig et al. (44), who analyzed 22 previous studies comparing HDL-C among smokers and nonsmokers. Craig et al. emphasized that in most studies lipid concentrations were not adjusted for variations in age, sex, weight, or even alcohol consumption and only a few results were adjusted for the amount and partition of calories between lipids, proteins, and carbohydrates. This lack of adjustment of data fails to take into account reports that smokers share different behaviors in regard to their diet. The regression analysis (Table 3), which included factors known to influence HDL-C concentrations, showed a positive association with age, BMI, calories provided by fats, the amount of cholesterol consumed per day, and sex; all of these associations have been previously described. When we included these data in the analysis, the smoking status did not correlate with HDL-C concentrations. This finding needs further examination, but suggests that several confounding factors may have been neglected in previous papers and may thus have led to an overestimation of the influence of smoking status on the HDL-C.

Our results must be interpreted cautiously because the patients were being treated with hypolipidemic drugs. However, the inclusion of this variable did not modify our results. In addition, the percentage of patients being treated by hypolipidemic drugs was the same in all four groups.

In conclusion, smoking status evaluation with three different markers produces results that agree with those in previous publications. Our findings strongly support the idea that smoking status can be accurately evaluated in clinical practice by simple, nonspecific tests, such as colorimetry of untimed morning urine samples. Besides several other confounding factors, dietary habits may strongly interfere with the relation between lipids and smoking status. Consequently, dietary habits should be taken into account when the relationship between smoking status and the concentrations of different lipids are evaluated. The clustering of these behavioral factors is probably important in terms of the medical consequences of smoking and in the management of patients who have several risk factors.

Table 3. Predictive Value* of Variables for HDL-C Concentrations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Partial F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>13.05</td>
<td>0.0004</td>
</tr>
<tr>
<td>Gender</td>
<td>10.57</td>
<td>0.0014</td>
</tr>
<tr>
<td>Alcohol, g/day</td>
<td>0.10</td>
<td>0.74</td>
</tr>
<tr>
<td>Total calories</td>
<td>0.30</td>
<td>0.59</td>
</tr>
<tr>
<td>Fat calories</td>
<td>6.96</td>
<td>0.0009</td>
</tr>
<tr>
<td>Carbohydrate calories</td>
<td>2.72</td>
<td>0.10</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>1.50</td>
<td>0.22</td>
</tr>
<tr>
<td>BMI</td>
<td>6.02</td>
<td>0.015</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.63</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* From the multiple-regression analysis in whole study population.

References

CLINICAL CHEMISTRY, Vol. 36, No. 9, 1992 1703
