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INFLUENCE OF DIETARY INTAKE AND PHYSICAL ACTIVITY ON ANNUAL RHYTHM OF HUMAN BLOOD CHOLESTEROL CONCENTRATIONS

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ABSTRACT

Seasonal variation in the plasma total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) have been repeatedly reported, with contradictory results regarding the pattern of seasonal variation of these parameters. Furthermore, it is still not well established whether the variation is due to changes in the nutrition or changes in physical activity depending on the season. The aim of this study was therefore to determine plasma TC and HDL-C in different groups of healthy participants: 19 vegetarians with a constant diet independent of the season, 14 athletes with almost constant physical activity over the year, and 114 controls in the age groups 20–26 years (mean age 24 + 1.5 years) and 40–48 years (mean age 44.3 + 2.1 years). Over 2 years, blood samples were collected every 2–3 months and were analyzed for plasma TC and HDL-C. At all visits, body mass index (BMI) and waist-to-hip ratio (WHR) were calculated, and nutrition and physical activity profiles were obtained. The seasonal model was calculated using object-oriented software for the analysis of longitudinal data in S (OSWALD); multiple regression analysis was used to determine the influence of

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age, gender, diet, and physical activity on seasonal changes of the lipid parameters. In all groups, we found an annual rhythm of the plasma TC and HDL-C concentrations, which can be mathematically described by a sine curve with a maximum in winter and a minimum in summer. This rhythm was independent of the age, gender, BMI, diet, or physical activity. The observed seasonal differences between the maximum and the minimum were about 5%–10% for TC and about 5%–8% for HDL-C concentration. These differences were greater than the determined circadian (TC 3.5%, HDL-C 4%) and day-to-day changes for TC and HDL-C (coefficient of variation <5% for both). In conclusion, annual rhythm of TC and HDL-C is not primarily induced by seasonal differences in dietary intake or physical activity. Therefore, the annual rhythm in cholesterol levels is most likely determined by endogenous factors or factors directly related to seasonal changes in the environment. (*Chronobiology International, 18(3), 541–557, 2001*)

Key Words: Annual rhythm; Cholesterol; HDL-cholesterol

INTRODUCTION

Seasonal variations in plasma total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels have been the subject of a number of investigations in different countries (1–14). However, there are contradictory results with respect to the seasonality of these variables. In some studies, no seasonal patterns of cholesterol plasma concentration were observed (3,5,10,12,13,15–18), whereas other investigations described a seasonal pattern with the highest levels in winter and the lowest in summer (1,4,7,9,19–22).

Furthermore, it is still controversial whether possible circannual variations in the TC and HDL-C plasma concentrations are primarily due to environmental factors (23), dietary intake (2,15), or physical activity (23,24) or whether these variations are due to intrinsic biological rhythms (25,26). The hypothesis that a circannual rhythm of the plasma cholesterol concentration may be involved is supported by the results from animal studies, which show a seasonal pattern of plasma lipid parameters under constant conditions (26–29).

Therefore, our first aim was to determine the alterations in plasma TC and HDL-C concentrations in a group of healthy probands over 2 years. Since a major source of variability in annual changes of a variable relates to circadian changes (25,30), we further determined circadian changes in TC and HDL-C levels in all seasons for a subgroup.

We questioned whether possible seasonal variations in these parameters are primarily due to seasonal differences in dietary intake, body mass index (BMI), or physical activity. We addressed this question with a group of vegetarians who applied a constant diet over the 2 years to control for the influence of dietary intake on the seasonal variation of TC and HDL-C. To test for the possible influence of physical activity on seasonal changes of TC and HDL-C, a group of

athletes with a constant daily training time for the 2 years was included in the study.

SUBJECTS AND METHODS

Study Design

The present investigation was a prospective and longitudinal study during a period of 2 years. We studied 147 healthy volunteers who gave their informed consent to participate in our study. The following exclusion criteria were defined for all participants:

- history of any metabolic diseases, especially disorders in the lipid metabolism
- pregnancy
- treatment with any medication
- changes of nutrition behavior during the study
- significant trauma
- extreme physical activity prior to the visit
- smoking and frequent alcohol consumption

Group A consisted of 19 vegetarians (9 males and 10 females), mean age 23.9 ± 1.1 years, and was characterized by a constant dietary intake over the 2 years. The vegetarians adhered to strict dietary instructions without seasonal differences. The constant dietary intake was confirmed by 7-day nutrition self-reports.

Group B consisted of 14 athletes (9 males, 5 females), mean age 25.1 ± 0.7 years, with a daily training time of constant duration. The constant daily training time (athletics or swimming) was confirmed by the analysis of the training plans obtained from the trainers.

The control group (group C) consisted of 114 students and their relatives. This group was randomly divided into four subgroups with respect to age and gender. Subgroup C1 was composed of 20–26-year-old males ($n = 30$) and 20–26-year-old females ($n = 41$) (mean age 24.0 ± 1.5 years). Subgroup C2 encompassed 40–48-year-old males ($n = 20$) and 40–48-year-old females ($n = 23$) (mean age 44.3 ± 2.1 years).

Starting 45 ± 10 days after the first day of each season (defined by the calendar), data were obtained from at least four visits (spring, summer, autumn, winter) per year. At all visits, TC and HDL-C concentrations were measured. The BMI defined as weight in kilograms/height^2 in square meters and the waist-to-hip ratio (WHR) were calculated. A questionnaire was answered at all visits, including assessment of special nutrition conditions, smoking habits, treatment with medication, physical activity and, infections.

The evaluation of the nutrition of the subjects was estimated from 7-day nutrition self-reports done in all four seasons. The purpose of the nutrition re-

ports was to evaluate possible seasonal differences in the nutrition behavior in groups B and C and to confirm a constant diet in group A. The participants could choose from over 100 items in different amounts from standardized protocols (31). This protocol was chosen since it could be shown that it minimizes the subjective bias of the answers (31,32). The protocols were analyzed using the program EBIS (Ernährungsanamnese, Beratungs- und Informationssystem) (33).

Subject Synchronization

TC and HDL-C were measured every 60 minutes during the daytime in all seasons in a subgroup of 5 males and 5 females to control for the influence of circadian changes in TC and HDL-C plasma concentrations on annual changes of these parameters (25). The sample time was standardized for all subjects in all seasons (between 07:00 and 08:00). All subjects were identically synchronized all year with regard to sleep-activity cycle, duration of daylight (lights on 07:00 ± 1h, lights off 21:00 ± 1h), eating behavior on the day before the visit (overnight fast starting at 21:00), outside temperature, and for females, the phase of the menstrual cycle.

Sample Collection and Analyses

Capillary blood samples were collected from all subjects (at a standardized sampling time, after sitting for 5 minutes) at each visit. TC and HDL-C were measured by a dry chemistry method, the Reflotron system (Boehringer, Mannheim, Germany). For all samples, the same Reflotron system was used. The blood samples were all taken by the same investigator because it is known that different investigators cause a significant variation coefficient (34). It is not necessary to take fasting blood samples for the cholesterol measurements (35,36). The coefficient of variation for day-to-day changes in TC and HDL-C levels was less than 5% for both parameters.

After capillary blood collection, plasma was isolated by low-speed (5000*g*) centrifugation for 5 minutes. The cholesterol measurement was done with 32 µl plasma using a test strip. The enzymatic reactions (cholesterolesterase and cholesteroloxidase) occur in the dry phase after contact with the plasma on the test strip. The reactions release superoxide, which oxidizes the indicator tetramethylbuxidine by means of peroxidase activity, resulting in a color change into blue. The intensity of the blue indicator, measured at 642 nm, correlates with the cholesterol concentration. The inter- and intraassay coefficients of the method are less than 5% (34,37). The quality control was performed with the control material Precinorm U (Boehringer Mannheim GmbH). A functional check of the Reflotron system was done using Reflotron Check (Boehringer Mannheim GmbH).

HDL-C was measured after precipitation of Apo B–containing lipoproteins with dextransulfat/Mg^{2+}. The reactions were identical to those for the cholesterol measurements. The variation coefficient was less than 5% (38,39). The quality control was performed with the control material Precinorm HDL (Boehringer Mannheim GmbH).

Statistical Analysis

Since it is known that females have higher plasma cholesterol concentration than males, analysis of the lipid parameters was done separately for each gender. After testing for a normal distribution of the data using the Kolmogorov-Smirnov test, data were given with mean and standard deviation. To detect statistically significant differences, the Student *t* test and analysis of variance (ANOVA) were performed to validate time-related changes and intergroup differences in TC and HDL-C levels, BMI, amount of physical activity, and intake of carbohydrate, protein, fat, cholesterol, and the like. Because constant nutrition behavior throughout the 2-year period (group A) and a constant amount of physical activity (group B) was confirmed for the corresponding groups, a bimodal split (i.e., yes/no response for physical activity constant or constant nutrition behavior) could be used for the evaluation of the influence of these parameters on the annual rhythm. For each variable, we fit a seasonal model to the 3-month data from each subject separately. Data sets were object oriented, and for all tests, including multiple regression analysis, the entire matrix was studied. We included terms to account for a linear trend in the dependent variable so that the occurrence of a maximal value (acrophase) was estimated after adjusting for the possibility of changes in the linear trend. We then tested whether there was a significant tendency among the subjects for the acrophase to occur at the same time of the year with the equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 \cos(\alpha t) + \beta_4 \sin(\alpha t) \quad (1)$$

where y is the measurement of the variables, x_1 is the variable for gender (0 = male, 1 = female), x_2 is the variable for the influence of constant physical activity or constant dietary intake (0 = yes, 1 = no), $\alpha = 2\pi/12$, t ranges from 1 (January) to 12 (December), and β_0–β_4 are the coefficients of the regression model estimated by ordinary least-squares regression. The cosine characteristics refer to the average of the two annual cycles and were calculated from this equation in the multiple regression model.

For each subject and each dependent variable, we derived the month (season) in which the maximum occurred. We estimated the phase Φ for each subject regardless of the goodness of fit of the regression line or overall significance of specific coefficients as described in Ref. 12. To test for statistically significant differences in the phase or acrophase between the groups, the object-oriented software for the analysis of longitudinal data in S (OSWALD) of the Statistical

Package for Social Sciences (SPSS, release 7.5, Seattle, WA) was used (40,41). For the evaluation of the influence of age, gender, nutrition behavior, and physical activity, multiple regression analysis was performed; therefore, the bimodal split could be used for the evaluation of the influence of these parameters on the annual rhythm, which was tested with multiple regression analyses.

RESULTS

Total Cholesterol

Females had significantly higher plasma TC levels than males in all groups (A, B, C1, C2) ($P < .05$) (Fig. 1). In the control group (group C), the older subgroup (group C2) had significantly higher TC plasma concentrations compared to the younger subgroup (group C1) for each gender ($P < .05$) (Fig. 1). Groups A and B were age matched with group C1. The mesor average of the plasma TC concentrations in the different groups, calculated from the rhythm analysis, are shown in Fig. 1.

In all groups, we found a strong seasonal effect, with 5%–10% higher TC concentrations in winter compared to the summer. To explore evidence for seasonality of the TC plasma concentrations, we fit a seasonal model (sine curves) to the data for each proband separately and calculated seasonal models for all groups from the individual models. The rhythm analysis showed a significant sinusoidal rhythm for the seasonal variation of the TC concentration ($P < .01$), with a maximum in winter and a minimum in the summer in males (Fig. 2) and females (Fig. 3) of all groups. The significant rhythm was also evident in the groups with an almost constant dietary intake (group A) and a constant physical

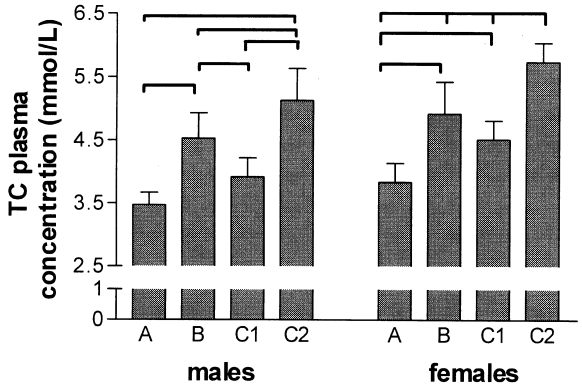

Figure 1. Mesor total cholesterol concentration ($\beta_0 \pm +$ SEM) for males and females obtained from the regression equation $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 \cos(\alpha t) + \beta_4 \sin(\alpha t)$. Brackets indicate significant differences ($P < .05$). In addition, females had significantly higher TC levels than males within each group ($P < .05$).

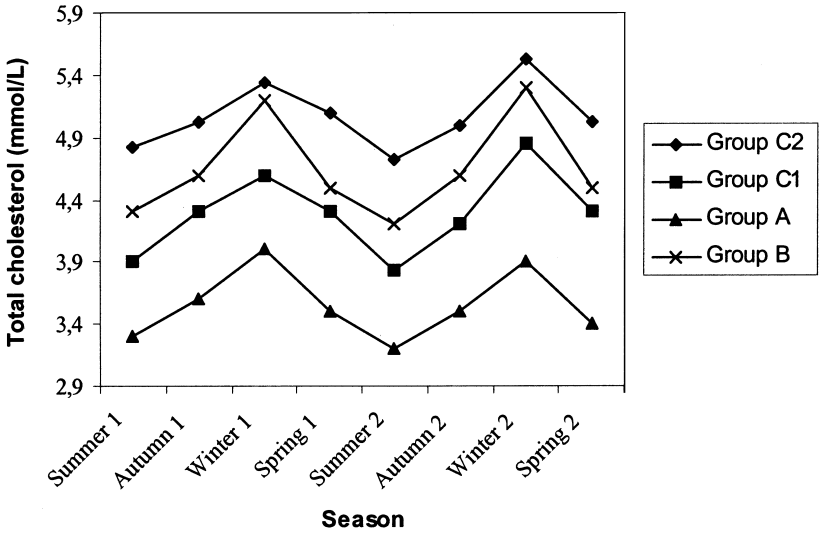

Figure 2. Seasonal pattern of total cholesterol concentration for males of all groups. The annual rhythm and the intergroup differences are statistically significant between all groups ($P < .05$).

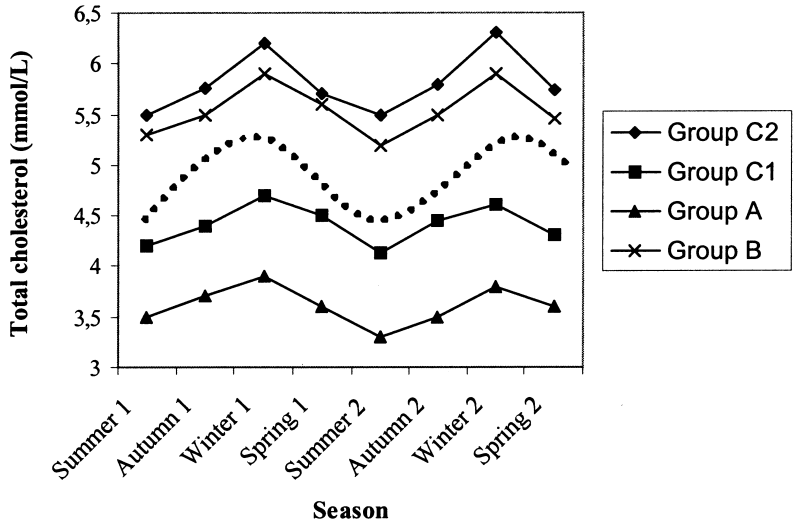

Figure 3. Seasonal variation and rhythm analysis of total cholesterol concentration for females of all groups. (• • • = sinus curve of the regression model obtained from object-oriented software for the analysis of longitudinal data in S (OSWALD).

activity (group B) throughout the year. To evaluate the influence of age, gender, dietary intake (group A), and physical activity (group B) on the TC plasma concentration in this model, multiple regression analysis using Eq. 1 was performed. The results of the regression analysis for the different models are shown in Table 1.

High-Density Lipoprotein Cholesterol

In analogy to the TC levels, the plasma HDL-C concentrations were significantly higher in females of all groups compared to the males (Figs. 4 and 5) ($P < .05$).

The annual rhythm was parallel to that observed for TC. The changes in HDL-C levels between winter and summer were 5%–8% (i.e., lower than the change in TC levels). However, the seasonal changes were larger than the interassay coefficient of variation (CV = 5%) for the HDL-C measurement. We found an annual rhythm of the HDL-C plasma concentrations, which is characterized by a significant sine curve ($P < .05$). The maximum was detected in winter and the minimum in summer (Figs. 4 and 5). The rhythm was apparent in all groups. The multiple regression analysis showed that, after normalization for the influence of changes in dietary intake (group A) and in physical activity (group B), the rhythm was still significant ($P < .05$).

TC/HDL-C Quotient

A quotient TC/HDL-C greater than 5 is associated with an increased risk for arteriosclerosis and coronary artery disease (42). Only in males of group C2

Table 1. Parameters of the Regression Analysis for the Seasonality of Total Cholesterol Concentration

Regression Parameter (Covariates for)	Influence of Diet, Group A vs. C1	Influence of Sport, Group B vs. C1	Influence of Age, Group C1 vs. C2
β_0 (mesor)	3.8 ($P < .001$)	4.43 ($P < .001$)	5.46 ($P < .001$)
β_1 (gender)	–0.41 ($P < .001$)	–0.41 ($P < .001$)	–0.41 ($P < .001$)
β_2 (age)	—	—	–1.11 ($P < .01$)
β_2 (diet)	–0.93 ($P < .001$)	—	—
β_2 (physical activity)	—	0.73 ($P < .001$)	—
β_3 (cos for the season)	0.09 ($P < .001$)	0.13 ($P < .001$)	0.13 ($P < .001$)
β_4 (sin for the season)	0.02 ($P < .05$)	0.03 ($P < .05$)	0.03 ($P < .05$)

The mesor is the mean of the calculated sinus curve, the covariates (β_{1-4}) determine the regression equation for the age (comparison group C1 vs. C2), diet (comparison group A vs. C1), and physical activity (comparison group B vs. C1) influence. Significance level in parentheses.

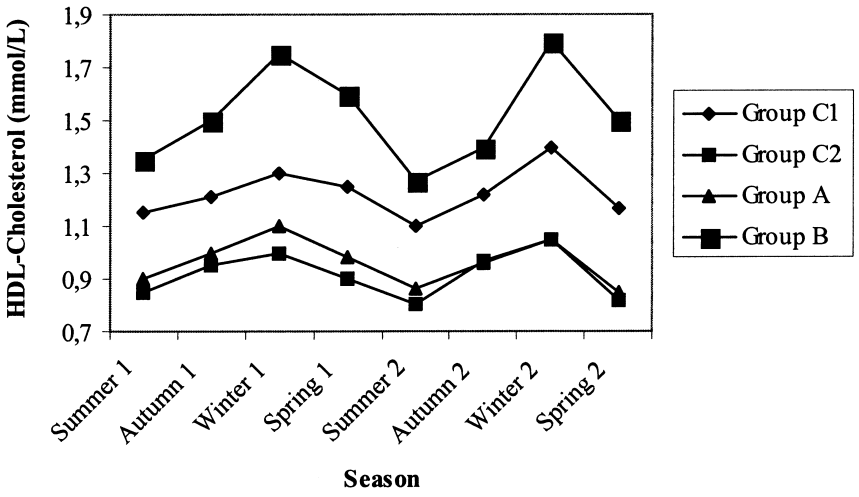

Figure 4. Seasonal variation of HDL cholesterol concentration for males of all groups.

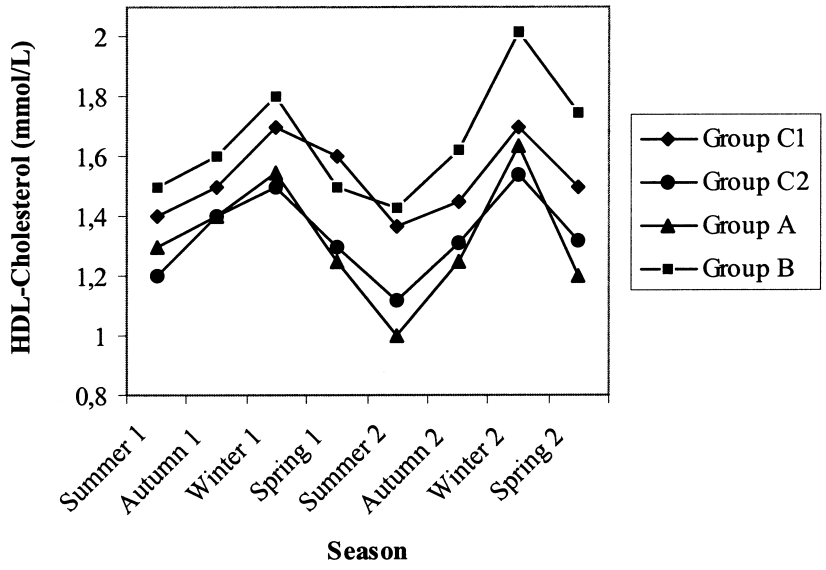

Figure 5. Seasonal variation of HDL cholesterol concentration for females of all groups.

was a higher TC/HDL-C quotient detected (TC/HDL-C = 5.6). As further evidence that TC and HDL-C plasma concentrations have the same seasonal pattern, with a maximum in winter and a minimum in summer, no variation of the quotient was found.

Circadian Changes in Total and High-Density Lipoprotein Cholesterol Levels

In 10 participants (5 males and 5 females of group C), TC and HDL-C levels were measured every hour between 06:00 and 22:00 in all seasons. There were no significant changes in TC and HDL-C plasma concentrations ($P < .01$). The variation coefficient for the intraday differences in TC levels was 3.5%, and for HDL-C levels, it was 4%. Since annual changes in TC (5%–10%) and HDL-C (5%–8%) were larger than the intraday coefficient of variation for both variables, once-a-day sampling can be regarded as adequate.

Body Mass Index, Waist-to-Hip Ratio

The mean BMI in the different groups is shown in Table 2. There were moderate variations in the BMI, with higher values in winter compared to summer. However, these differences in BMI were not significant. Since there was no correlation between the BMI and the TC or HDL-C levels ($r = 0.45$), a significant influence of the body weight on the seasonal variation of these parameters was not detected.

The mean WHR for the younger females of groups A, B, and C1 was 0.79 ± 0.07, and for the older females (group C2), it was 0.84 ± 0.09. The mean WHR for the 20–26-year-old males (groups A, B, C1) was 0.87 ± 0.1, and for the 40–48-year-old males (group C2), it was 0.96 ± 0.13. The differences between the younger and the older study participants were not significant. As expected, females had a significantly lower WHR than males in both age groups ($P < .05$).

Table 2. Body Mass Index (Mean ± SEM) for the Different Groups

Group	Males	Females
A (constant diet)	22.2 ± 0.6	20.6 ± 0.3
B (constant physical activity)	23.1 ± 0.7	21.3 ± 0.4
C1 (young control group)	23.3 ± 0.8	21.4 ± 0.5
C2 (older control group)	25.1 ± 1.1	24.6 ± 0.9

Evaluation of the Seven-Day Nutrition Reports

Nutrition behavior with respect to total energy intake and the total and relative intake of carbohydrates, protein, fat, cholesterol, polyunsaturated fatty acids, and alcohol was evaluated for all participants using standardized 7-day nutrition self-reports at all seasonal visits. Participants could choose from over 300 given food examples, which was shown to be a suitable method for the assessment of nutrition behavior (31). As supposed by the strict dietary instructions of the vegetarians in group A, the constant dietary intake was confirmed for both genders by the analysis of the nutrition protocols. No significant differences in the main components of nutrition and in total energy consumption were detected. The diet contained an average of 9200 kJ for the females and 10,400 kJ for the males. Carbohydrates provided about 52%, proteins about 13%, and fat about 30% of the total energy for both genders of group A. A constant diet throughout the year also applied to the nutrition behavior of groups B and C1, whereas the dietary intake of the older participants (group C2) was characterized by a 25% higher total energy intake in autumn and winter. The higher dietary intake was due to an increase of total fat and carbohydrate intake in this group.

Evaluation of the Training Time Protocols

Physical activity indication was obtained by an individual questionnaire from all participants. To correct for seasonal differences in the physical activity in groups A, C1, and C2, a group of competitive athletes with a constant daily training time over the 2-year study period was investigated. We confirmed the constant daily training time and intensity by written reports obtained from the trainers. All athletes were on the level of competitive athletes fulfilling the standards for international competition such as European championships in athletics or swimming. Throughout the year, the constant weekly training time was 20h ± 2h for the male athletes and 17.5h ± 1.3h for the female athletes; this contained 65% of submaximum and 15% of maximum training for both genders and without differences between the seasons. We did not measure the subjective energy expenditure in these athletes, but training time and intensity can be regarded as a valuable estimate of the individual energy output of competitive athletes (43).

DISCUSSION

The results of previous studies that showed no seasonal variation in plasma TC or HDL-C levels (3,5,10,12,13,15–18,44,45) are in part restricted by a small number of participants (16,45) or by a short time period covered or a retrospective study design (44,45). In contrast, studies without these restrictions clearly showed an annual rhythm of cholesterol levels (1,2,6–8). Robinson et al. (1)

showed, in more than 200,000 men and women from the United Kingdom and Japan over 7 years, a significant seasonal pattern, with a peak in winter and lowest levels in summer. This pattern is in accordance with the results from a number of studies (4,7,19,22,46), whereas Kristal-Boneh et al. (2) and Harlap et al. (8) found maximum cholesterol levels in spring and a minimum in summer.

Our results provide further evidence of annual changes in plasma cholesterol levels, with highest levels in winter and lowest in summer for a healthy population in central Europe. In contrast to other studies (1,20), the study design was longitudinal and prospective; all measurements and visits were done by the same investigator; and subjects were synchronized regarding the sampling time, the duration of daylight, the sleep-activity cycle, the outside temperature, and for females, the phase of the menstrual cycle. Patients with hypercholesterolemia or any other metabolic disorders were excluded, and the annual rhythm was confirmed by use of a 2-year period. Moreover, the statistical analysis of the data was object oriented and focused on the detection of longitudinal changes in the variables TC, HDL-C, BMI, nutrition behavior, and physical activity. The statistical analysis of our data is adequate for the detection of longitudinal changes (12). The differences between the minimum in summer and the maximum in winter (TC 5%–10%, HDL-C 5%–8%) were higher than the previously described differences between the maximum and minimum values of about 3%–5% variation (1).

In our study, the summer-winter differences in TC and HDL-C levels were higher than the interassay coefficient of variation for the method (5%) and higher than the proposed variation due to interday differences (30). A major source of variability in annual changes of a variable relates to circadian changes (25). Therefore, we determined circadian changes in the TC and HDL-C levels in a subgroup of study participants. The variation coefficient resulting from circadian changes of TC (3.5%) and HDL-C (4%) levels was smaller than the summer-winter differences for these parameters. Therefore, once-a-day sampling can be regarded as an adequate method. Seasonal changes of TC and HDL-C were larger in the group of older participants and in female patients. This result is in accordance with the results of Kristal-Boneh et al. (2) and may indicate the age and gender differences in cholesterol levels. However, the pattern and the phase of the annual rhythm of the lipid parameters was not affected by age and gender differences.

Seasonal differences in the diet, physical activity, body weight (7,21), ethnic background or environmental factors like temperature (1,2), duration of sunlight exposure, or geographic differences may affect the cholesterol levels (47). Therefore, our subjects were synchronized with regard to the sampling time, the sleep-activity cycle, the sunlight exposure, the environmental temperature, and others, and for females, the phase of the menstrual cycle. However, seasonal changes have been described in persons of all ethnic groups and from countries as diverse as France (48), Holland (44), Israel (2,8,49), Norway (17), Finland (9), and the United Kingdom (1,4,19,50); different climates of the United States

(7,24), Japan (1), and Germany (20). Since these studies describe the annual rhythm in TC and HDL-C levels independently from differences in the subjects' synchronization (duration of daylight, outside temperature, sampling time, etc.), the annual rhythm of cholesterol levels could be induced by endogenous factors in addition to environmental factors.

In accordance with several studies (1,4,15,48), annual rhythm of TC and HDL-C concentrations was not related to seasonal changes in body weight. This result is a further suggestion for the hypothesis that intrinsic factors probably influence the cholesterol rhythm throughout the year.

With some exceptions (12,48), the previous studies of seasonal changes in TC and HDL-C were performed on persons with normal nutrition behavior and physical activity. Buxtorf et al. (48) found, for 34 nuns with a constant diet and an almost constant physical activity, an annual pattern of cholesterol levels that suggested that dietary factors and physical activity are not the predominant factors leading to the annual rhythm. A further aim of our study, therefore, was to evaluate the impact of seasonal differences in diet and physical activity on the annual rhythm of TC and HDL-C under constant conditions for the dietary intake and the amount and intensity of physical activity. We confirmed that the seasonal changes in dietary intake (group A) or in the physical activity (group B) of the participants were minimal, and no statistically significant differences were detectable with standardized methods. The only German study (20) suggested that seasonal differences in nutrition behavior lead to the described seasonal cholesterol changes, whereas Fuller et al. (15) found no seasonality of the cholesterol levels, although the dietary intake of the probands significantly changed with the seasons. Animal studies showed that defined constantly controlled diets did not affect the annual rhythm of lipid parameters (26,27). Multiple regression analysis of our data showed that the influence of the dietary intake on the annual rhythm in TC and HDL-C levels was not significant. This result suggests that the nutrition behavior was not the predominant determinant of the annual rhythm in our probands.

More physical activity in the spring and summer months compared to the winter was suggested as a main reason for seasonal changes in cholesterol levels (24,51). We compared the influence of physical activity on the seasonal changes of cholesterol levels between a group with an average physical activity (group C1) and a group with constant physical activity (group B). There were no significant differences in the pattern, phase, and maximum of the annual rhythm of cholesterol plasma levels between these groups. In accordance with the results of Buxtorf et al. (48), this result provides further evidence that seasonal changes of TC and HDL-C are not primarily induced by seasonal differences in the extent of physical activity.

In summary, we found an annual rhythm of TC and HDL-C plasma concentration. This rhythm was independent from the age, gender, dietary intake, and physical activity of the participants. We therefore conclude that the seasonal changes of TC and HDL-C levels could be determined by intrinsic factors, like

seasonal changes in endocrine factors or a circannual rhythmicity, in addition to environmental factors.

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