than 20 enzyme results per day. For the analytes studied here, the present cost of reagent per test result ranges from $1.80 to $2.00, compared with $1.00 to $1.50 for the Seralyzer (Ames Division, Miles Laboratories, Elkhart, IN 46515), another physician's office analyzer.

We thank the Eastman Kodak Company for providing the DTSC analyzer and supplies for these studies.

References

1. Ng RH. Selecting instrumentation for physician's office testing. Clinics Lab Med 1986;6:305–15.

2. El-Deriny SE, Ng RH, Statland BE. Evaluation of the Kodak DT-60 analyzer [Technical Brief]. Clin Chem 1986;32:1415.

3. Glick MR, Ryder KW, Jackson SA. Graphic comparisons of interferences in clinical chemistry instrumentation. Clin Chem 1986;32:470–5.

4. Bruns DE, Savory J, Titheradge AC, et al. Evaluation of the IFCC-recommended procedure for serum aspartate aminotransferase as modified or use with the centrifugal analyzer. Clin Chem 1981;27:156–9.

5. Ash KO, Smith A, Ng RH, Statland BE. Selecting instrumentation. Check Sample Clinical Chemistry, American Society of Clinical Pathologists. Chicago: ASCP, 1985;25:1–7.

CLIN. CHEM. 33/10, 1913–1915 (1987)

Importance of Time Interval between Repeated Measurements of Total or High-Density Lipoprotein Cholesterol When Estimating an Individual's Baseline Concentrations

Ernest P. Rotterdam, Martijn B. Katan,[1] and Jan T. Knuiman

We studied intra-individual variation in total and high-density lipoprotein (HDL) cholesterol in healthy volunteers (22 men and 19 women, ages 19 to 62 years) on controlled natural diets. The within-person coefficient of variation (CV) depended on the interval between blood samples, increasing from about 2% to 3% for measurements made 24 h apart to 4% to 5% for measurements made at four-day intervals or longer. We conclude that within-subject fluctuations in total and HDL cholesterol have a time constant of several days. Multiple measurements are generally needed to decide whether an asymptomatic subject exceeds a certain concentration of total or HDL cholesterol; we recommend that such measurements be made at least four days apart.

Additional Keyphrases: *intra-individual variation · diet-related effects · heart disease*

A high concentration of cholesterol in blood is among the most clearly established risk factors for coronary heart disease. To decrease the incidence of coronary heart disease, it is recommended that people with high- and moderate-risk concentrations of cholesterol be treated (*1*). However, the classification of people into high-, moderate-, and low-risk categories is complicated by the high ratio of within- to between-person variance. Within-person coefficients of variation (CVs) for subjects on their usual diets range between 1.5% and 8.0% for total cholesterol and between 4.0% and 9.4% for high-density lipoprotein (HDL) cholesterol (*2, 3*). The extent of the variation is related to the nature of the subjects, the degree of dietary control, the laboratory precision, and the interval between consecutive measurements (*2–4*). To decrease the within-person variance, serial samples should be drawn (*5*). However, if these samples are drawn within a relatively short interval, then the estimations may be interdependent, so that the precision gained may be less than optimum for the number of samples analyzed. Therefore we have studied the relationship between within-person variance and the interval between serial measurements. Our findings may be helpful in planning studies involving estimates of a subject's true mean concentrations of total and HDL cholesterol in serum.

Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands.

[1] Address correspondence to this author.

Received April 20, 1987; accepted July 1, 1987.

Materials and Methods

Study Design

The data were obtained in a series of four controlled experiments on the effect of diet on serum lipids. Subjects received natural mixed diets in which 11–14% of the energy (calories) was provided by protein, 41–45% by fat, 39–45% by carbohydrates, and 1–3% from ethanol; 30 to 43 g of dietary fiber was ingested daily. The fatty acid composition and cholesterol content of the diets varied between experimental periods. Each diet period lasted from two to four weeks. When a diet was begun, the concentrations of lipids in the subjects' blood achieved a new steady state in about 11 days (*6*). Therefore, we used the results for blood lipids only after they had stabilized after the change in diet. These results were the "baseline" values for that diet. Details of the experimental design and particulars of the diets have been published (*6, 7*).

Subjects

The participants in the four experiments were healthy normolipemic volunteers from the general population, living in or near Wageningen, a college town of 30 000 inhabitants in the central part of The Netherlands, 80 km from Amsterdam. Altogether, 22 men and 19 women, ages 19–62 years (mean 32 years), participated in two or more of the different studies. The mean body mass index was 22.3 kg/m^2 (range, 17.7–29.4 kg/m^2). Each individual's body weight remained within 2% of the initial body weight during these studies.

The design and execution of the experiments were thoroughly explained to the subjects and informed consent was obtained. Prior approval was obtained from the Medical-Ethical Committee of the Department.

Blood Sampling and Laboratory Methods

Blood was sampled after an overnight fast, the serum stored at −80 °C, and total and HDL cholesterol concentrations were determined by the Liebermann–Burchard colorimetric assay in a rigidly standardized laboratory as earlier described (*8, 9*). All serum samples obtained from one person during one dietary period were analyzed in the same run. The within-run analytical CV ranged from 0.6 to 1.7% (17 pools) for total cholesterol in serum and from 1.8 to 2.2% (two pools) for HDL cholesterol, as determined with internal control pools over a range of concentrations and in blind duplicate determinations of sera from normolipemic patients.

Statistical Methods

The interdependence of serial cholesterol values for each subject can be studied by calculating the so-called semi-variance (*10, 11*), estimated as $\Sigma(x_t - x_{t+d})^2/2n$, where x is the concentration of total or HDL cholesterol, t is the day of blood sampling, d is the number of days between successive blood samplings, and n is the number of pairs in the summation. If the values obtained at an interval of d days are totally uncorrelated then the semi-variance is identical to the full within-person variance; i.e., the variance of $(x_t - x_{t+d})$ will then equal twice the variance of x. However, when x_t and x_{t+d} are correlated—e.g., when both measurements are obtained during a temporary increase or decrease of the total or HDL cholesterol concentration—then the semi-variance (x, d) is equal to the full variance of x minus the covariance of x_t with x_{t+d}. Commonly, covariance decreases and (consequently) semi-variance increases with increasing d until semi-variance equals the full within-person variance.

After estimating the semi-variance for each person, we calculated the group means and SDs of the semi-variances. We then calculated the corresponding within-person CVs by taking the square root of the semi-variances and dividing the resulting SDs by 1% of the average mean concentration of total (2000 mg/L, 5.20 mmol/L) or HDL cholesterol (560 mg/L, 1.45 mmol/L). By doing this, instead of dividing by the individual concentrations, we ensured that the fluctuations in the within-person CVs represent the fluctuations in the semi-variance values. We then plotted the within-person CVs as a function of time span d (Figure 1). Because the semi-variances were approximately log-normally distributed, we took the logarithm of the within-person semi-variance before calculating group means and SDs. The total number of pairs involved in the calculation of the group means is given in the legend to the figure.

The true within-person variance was estimated as the value to which semi-variance approached after sufficiently long intervals of time.

As an illustration of the use of the within-person CV values, we calculated the number of measurements (k) needed to estimate the individual value for total or HDL cholesterol to within a certain percentage of a person's true (baseline) mean value by using the equation:

$$k = [1.96 \times (\text{within-person CV}/D)]^2$$

where D = acceptable departure of observed mean from the true (baseline) mean, as a percentage of the latter. If k measurements are made, then the chance that their mean will differ from the "true" mean by more than D% is less than 1 in 20.

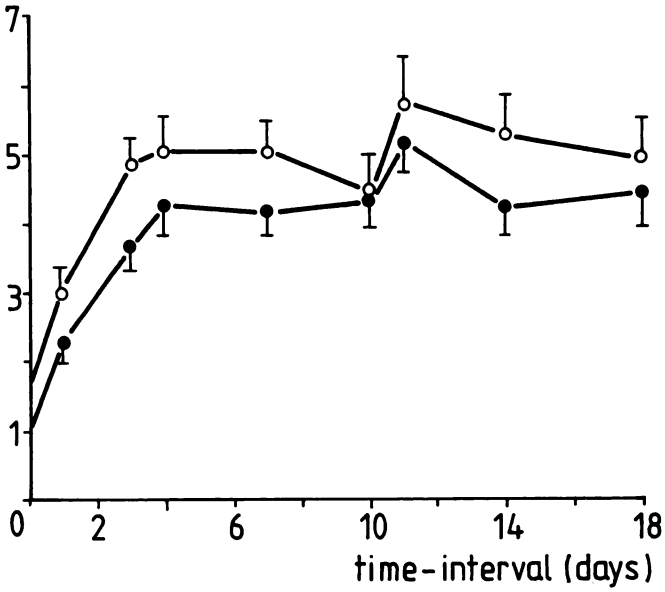

Fig. 1. Within-person CV (%, *ordinate*) for the concentration of total (●) and HDL (○) cholesterol in serum, as a function of the time between repeated blood samplings

The within-person CV was calculated by taking the square root of the semi-variance and dividing the resulting SD by 1% of the average mean concentration of total or HDL cholesterol. The "zero" days interval refers to laboratory variance for duplicate samples. Data are from volunteers from the general population of Wageningen and surroundings, The Netherlands, in 1982–1984. *Bars* indicate 1 SE. The number of measurement pairs used for total cholesterol was 164, 208, 272, 208, 91, 91, 128, and 64 for intervals of 1, 3, 4, 7, 10, 11, 14, and 18 days, respectively. Numbers for HDL cholesterol were similar

Theoretically, the "crude" within-person variance values should be corrected for the within-run analytical variance to find the true within-person variance. However, for practical purposes such as calculating the number of observations that must be made to obtain acceptable precision at a given CV, the analytical variance must also be considered. Therefore we did not correct our data for analytical variance.

Results

The relationship between the mean within-person CV and sampling interval is shown in Figure 1. The CV at a time interval of 0 days represents the CV for duplicates of a single sample divided into two parts after the blood was collected. Thus this is the within-run laboratory CV. The mean within-person CVs for both total and HDL cholesterol increased strongly when the interval increased from zero to four days. A further increase of the interval did not further increase the CV except for a transitional increase at an interval between 10 and 14 days. Further analysis of the data revealed that this transitional increase was related to the day of the week. Blood sampled on Monday had slightly lower concentrations of total and HDL cholesterol than that on the other days of the week. Thus sampling intervals that began or ended on Monday were associated with higher estimates of the CV than intervals that did not begin or end on Monday.

Having established that four days is the minimum interval required, we estimated the full within-person variance from the data on variance for intervals of at least four days and with weighting factors proportional to the number of pairs involved in the calculation of a particular variance (legend to Figure). The mean within-person SD was 85 mg/L (0.22 mmol/L) for total cholesterol and 29 mg/L (0.07 mmol/L) for HDL cholesterol. The laboratory CV averaged

1.1% for total cholesterol and 2.0% for HDL cholesterol. About 7% of the within-person variance (i.e., SD squared) for total cholesterol and 15% of that for HDL cholesterol were attributable to laboratory variance.

The within-person SDs do not, of course, encompass long-term changes in concentrations of total and HDL cholesterol in serum, such as might result from seasonal influences or changes in lifestyle.

Discussion

This study shows that if serial measurements of total and HDL-cholesterol are made at intervals of four days or less, the within-person variance will be underestimated. Therefore, the estimated total and HDL cholesterol concentrations will appear to be more constant than they actually are. Thus, we recommend that serial measurements should be made at least four days apart.

The number of samples needed for the correct estimation of someone's true mean is determined by the amount of imprecision that is still acceptable, and it increases with the square of the within-person CV. With use of the equation given in *Methods*, it can be calculated that at a within-person CV of 6% for total and HDL cholesterol a single determination (i.e., $k = 1$) will yield a value to within $D = 12\%$ of the true mean value on 19 out of every 20 occasions, provided that the laboratory methods used are free from bias. Six samples are required to estimate an individual's value to within 5% of the true mean value. This applies to healthy normolipemic subjects with relatively constant diets and regular lifestyle, and measurements in a strictly standardized laboratory. The within-person CV may well be higher if patients are studied who have various diseases or an irregular lifestyle or dietary habits, or if laboratory precision is poor. In this study analytical variability was minimized by storing specimens so that all cholesterol values for a given subject could be determined in a single run. In a clinical setting the contribution of laboratory variation to within-person variation may be higher than found in this study. If the within-person CV, including laboratory imprecision, increases to 12% then a single sample will yield a value to within 24%, and 16 independent blood samples are needed to determine cholesterol to within 6% of the true mean value. In one of every 20 cases the discrepancy D will still exceed these percentages.

A different way to approach the question of how within-person variability affects the usefulness of a single measurement is to ask how much a second measurement tends to differ from the first one. This difference has a median absolute value of $(0.6745\sqrt{2}) \times$ within-person CV, or $0.954 \times$ within-person CV. Thus for a within-person CV of 6%, in half of the cases a second measurement will yield a value that differs by more than 5.7% from the first value. A difference of more than 10, 20, or 30% will be found in 22, 1.5, and 0.02% of the cases, respectively, on the average. For a within-person CV of 10%, a difference between consecutive measurements of more than 10, 20, or 30% will, on average, be found in 48, 16, and 3.4% of the cases, respectively.

Our data on within-person variance for total and HDL cholesterol are similar to those reported for free-living subjects (*2, 3, 12*). Within-person CVs between 1.5% and 8.0% for total cholesterol and between 4% and 9.4% for HDL cholesterol have been reported. This implies that day-to-day fluctuations in a more or less constant habitual dietary pattern have only a relatively small effect on the concentrations of total and HDL cholesterol.

The causes of within-person fluctuations have not yet been identified. Theoretically, they could be due to spontaneous fluctuations in the metabolism of low-density lipoprotein (LDL), which is the main carrier of cholesterol in serum. Each temporary change in the production or clearance rate of LDL will lead to a temporary change in serum cholesterol concentration. LDL turns over at the rate of about 0.4 pools/day (*13*), so such a change in concentration should be 50% complete in about 1.7 days and 80% complete in about 4.0 days after a change in LDL production or clearance rate. These intervals are similar to the time constants we found for variations in serum cholesterol in this study.

References

1. National Institutes of Health Consensus Development Conference Statement. Lowering blood cholesterol to prevent heart disease. J Am Med Assoc 1985;253:2080–8.
2. Demacker PNM, Schade RWB, Van 't Laar A. Intra-individual variation of serum cholesterol, triglycerides and high density lipoprotein cholesterol in normal humans. Atherosclerosis 1982;45:259–66.
3. Mjøs OD, Rao SN, Bjøru L, et al. A longitudinal study of the biological variability of plasma lipoproteins in healthy young adults. Atherosclerosis 1979;34:75–81.
4. Jacobs DR, Barrett-Connor E. Retest reliability of plasma cholesterol and triglyceride. The Lipid Research Clinics Prevalence Study. Am J Epidemiol 1982;116:878–85.
5. Grundy SM, Bilheimer D, Blackburn H, et al. Rationale of the diet-heart statement of the American Heart Association. Report of Nutrition Committee. Circulation 1982;65:839A–54A.
6. Katan MB, Beynen AC, De Vries JHM, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol 1986;123:221–34.
7. Glatz JFC, Berns MAM, Katan MB. Whole-body cholesterol synthesis and fecal neutral steroid and bile acid excretion in man on diets high or low in linoleic acid. In: Beynen AC, Geelen MJH, Katan MB, Schouten JA, eds. Cholesterol metabolism in health and disease: studies in the Netherlands. Wageningen: Ponsen and Looyen, 1985;120–5.
8. Van der Haar F, Van Gent CM, Schouten FJM, et al. Methods for the estimation of HDL cholesterol, comparison between two laboratories. Clin Chim Acta 1978;88:469–81.
9. Katan MB, Van der Haar F, Kromhout D, et al. Standardization of serum cholesterol assays using serum calibrators and direct addition of Liebermann–Burchard reagent. Clin Chem 1982;28:683–6.
10. Clark I. Practical geostatistics. London: Applied Science Publishers Ltd, 1982.
11. David M. Development in geostatistics. 2. Geostatistical or reserve estimation. Amsterdam: Elsevier, 1977.
12. Hölzel GE. Intra-individual variation of some analytes in serum of patients with insulin-dependent diabetes mellitus. Clin Chem 1987;33:57–61.
13. Grundy SM, Vega GL, Bilheimer DW. Kinetic mechanisms determining variability in low density lipoprotein levels and rise with age. Arteriosclerosis 1985;5:623–30.