Bioquímica Aplicada 2(1985)55-57.

Bioq. Aplic., Vol. II, N.° 1, pp. 55-57, 1985

FLUCTUATIONS OF THE CONCENTRATION OF TOTAL SERUM CHOLESTEROL IN HEALTHY SUBJECTS: SEPARATION OF PLASMA VOLUME EFFECTS FROM OTHER INFLUENCES

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Recebido em 22/11/1984

ABSTRACT — Within-person variations of serum cholesterol concentrations may be caused by changes in the concentration of water in the intravascular space. We have tested this possibility by means of determination of the ratio of the concentration of serum protein to serum cholesterol, which should be constant if no other influences were present.

It was found that as the subjects went from the supine to the standing position, the concentration of serum cholesterol increased, but the ratio of serum protein to cholesterol remained constant. The day-to-day and week-to-week variation in serum cholesterol was not decreased by expressing serum cholesterol on a serum protein basis, indicating that influences other than plasma volume changes are involved.

Serum cholesterol concentrations in clinically healthy subjects show within-person variations (in terms of percent coefficient of variation) of about 10%, 5% and 3,5% from week-to-week[1], day-to-day[2] and hour-to-hour[2], respectively. Part of this variation could be due to changes in the concentration of water in the intravascular space, which cause either dilution or concentration of serum constituents. Such changes should leave the ratio of the concentration of lipoprotein-bound cholesterol to other macromolecular serum components constant ar every time of sampling. This might offer a way to increase the precision of the determination of a person's serum cholesterol concentration without increasing the number of blood samples taken. We have tested serum total protein as an index for changes in serum volume, because earlier studies[2,3] have shown that changes in posture, tourniquet application or exercise caused the concentration of serum cholesterol and total protein to shift in the same direction.

MATERIALS AND METHODS

Six healthy volunteers (aged 20-28 years) participated in this study. Specimens of blood were obtained at 10.30 a.m. on days: 0, 12 and 13. Before blood sampling the subjects had stood upright for 30 min. On day 0, another blood sample was taken after the subjects had layed supine for the next 30 min. Blood samples were collected from an antecubital vein into evacuated tubes without anticoagulant and allowed to clot at room temperature for 1-2 hours. Serum was prepared by low speed centrifugation at room temperature and stored at −20°C.

Total cholesterol was measured according to Röschlau et al.[4] using the kit (CHOD-PAP method) supplied by Boehringer-Mannheim GmbH, F.R.G. Three calibration sera with low, medium and high cholesterol concentrations were used; the cholesterol concentration of these sera was determined by the method of Abell et al.[5].

Total protein was measured in serum according to Henry et al.[6] by the biuret reaction. As calibration standards sera were used of which the protein concentration was determined by Kjeldahl analysis[7].

All samples were analysed in duplicate within one batch. The standard error of the mean of a duplicate analysed in this manner was 1.1% for cholesterol and 0.2% (C.V.) for protein.

RESULTS AND DISCUSSION

Table 1 documents that lying down for 30 min caused a significant decrease in the concentration of serum cholesterol and serum protein in all subjects. These results agree with those reported earlier by e.g. Statland and Winkel[2] and Dixon and Paterson[3]. This effect of posture was not observed when the ratio of serum protein to serum cholesterol was calculated (Table 1). Thus it may be suggested that the decline seen in the concentrations of serum cholesterol and serum protein when the subjects lie down are due exclusively to an increased plasma volume.

As can be appreciated from Table 2, the mean week-to-week variability in the concentration of serum cholesterol was 7%; the mean day-to-day variability was 2%. Table 2 also shows that calculating the ratio of cholesterol to protein does not improve the within-person stability in the concentration of serum cholesterol, when measured over a period of two weeks if posture was kept as constant as possible.

We conclude that a correction procedure for variations in the concentration of serum cholesterol as described here is useful when

Table 1

Serum total cholesterol and protein concentrations after 30 min upright and after 30 min supine

person no.	sex	serum cholesterol (mmol/l) upright	supine	difference (%)	serum protein (g/l) upright	supine	difference (%)	ratio protein/cholesterol (g/mmol) upright	supine	difference (%)
1	female	4.56	4.36	-4.4	71.2	67.7	-4.9	15.6	15.5	-0.6
2	male	4.09	3.80	-7.1	75.9	70.4	-7.2	18.6	18.5	-0.5
3	female	4.87	4.56	-6.4	71.0	67.5	-4.9	14.6	14.8	+1.4
4	male	3.78	3.48	-7.9	77.4	71.7	-7.4	20.5	20.6	+0.5
5	female	4.29	3.93	-8.4	71.5	65.0	-9.1	16.7	16.5	-1.2
6	female	6.99	6.54	-6.4	71.0	66.4	-6.5	10.2	10.2	0.0
mean		4.76	4.45	-6.8	73.0	68.1	-6.7	16.0	16.0	0.7

Table 2

Serum total cholesterol and protein concentrations after 30 min of standing upright at three different days

person no.	serum cholesterol (mmol/l) nov.19	dec.1	dec.2	difference (%) nov.19-dec.1	dec.1-dec.2	serum protein (g/l) nov.19	dec.1	dec.2	difference (%) nov.19-dec.1	dec.1-dec.2	ratio (protein/cholesterol) nov.19	dec.1	dec.2	difference (%) nov.19-dec.1	dec.1-dec.2
1	4.56	5.40	5.49	+18.4	+1.7	71.2	73.7	74.1	+3.5	+0.5	15.6	13.6	13.5	-12.8	-0.7
2	4.09	4.20	4.32	+2.7	+2.9	75.9	75.2	76.4	-0.9	+1.6	18.6	17.9	17.7	-3.8	-1.1
3	4.87	5.02	5.01	+3.1	-0.2	71.0	70.2	73.9	-1.1	+5.3	14.6	14.0	14.8	-4.1	+5.7
4	3.78	3.85	3.71	+1.9	-3.6	77.4	77.4	73.3	0.0	-5.3	20.5	20.1	19.8	-2.0	-1.5
5	4.29	4.53	4.42	+5.6	-2.4	71.5	69.9	71.2	-2.2	+1.9	16.7	15.4	16.1	-7.8	+4.5
6	6.99	6.30	6.41	-9.9	+1.7	71.0	68.6	67.3	-3.4	-1.9	10.2	10.9	10.5	+6.9	-3.7
mean	4.76	4.88	4.89	6.9	2.1	73.0	72.5	72.7	1.9	2.8	16.0	15.3	15.4	6.2	2.9

plasma volume changes are known to be an important source of variation, for instance when changes in posture, exercise or tourniquet application are involved. However, the week-to-week and day-to-day variation are most likely true metabolic variations and thus cannot be corrected for by the determination of the ratio of the concentrations of serum protein and cholesterol.

ACKNOWLEDGEMENTS

We thank the volunteer for their cooperation.

This work was supported by the Netherlands Heart Foundation, grant no. 26.003. MBK is an established investigator of the Netherlands Heart Foundation.

REFERENCES

1 — Keys A. Blood lipids in man — a brief review. *J Am Diet Ass* 1967; 51:508-516.

2 — Statland BE, Winkel P. Variations of cholesterol and total lipid concentrations in sera of healthy young men Differentiating analytic error from biologic variability *Am J Clin Pathol* 1976; 66 935-943

3 — Dixon M, Paterson CR. Posture and the composition of plasma *Clin Chem* 1978; 24 824-826

4 — Röschlau P, Bernst E, Gruber W Enzymatische Bestimmung des Gesamt-Cholesterins im Serum *Z Klin Chem Klin Biochem* 1974; 12 403-407.

5 — Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity *J Biol Chem* 1952; 195 357-366

6 — Henry RJ, Sobel C, Berkman S Interferences with biuret methods for serum proteins Use of Benedicts's qualitative glucose reagent as a biuret reagent *Anal Chem* 1957; 29:1491-1494.

7 — Henry RJ, Cannon DC, Winkelman JW eds *Clinical Chemistry: Principles and Technics* New York Evanston San Francisco London Harper & Row 1974, pp 409-411