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Comparative Determinations of Low-Density-Lipoprotein-Cholesterol

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Two different methods for low-density-lipoprotein-cholesterol (LDL-C) determination were comparatively used. The heparine-sodium citrate (pH 5.12) precipitation method gave similar LDL-C results to the ones given by the Friedewald *et al.* formula (3.2 *vs* 3.3 mmol/l) in 187 men. Values obtained using both methods show a very high and significant correlation ($r > 0.9$; $p < 0.001$). However, LDL-C values obtained with the precipitation method were 15 % higher in hypertriglyceridemics (triglycerides (Tg) $\geq$ 2.3 mmol/l). A paired-comparison between data obtained by both methods indicates a clear serum Tg-values influence, because LDL-C values obtained by the precipitation method were significantly more frequently higher ($p < 0.05$ or $p < 0.01$) than LDL-C values obtained using the Friedewald's formula in hypertriglyceridemic men (Tg $\geq$ 1.7 mmol/l or Tg $\geq$ 2.3 mmol/l respectively). When a 3.9 mmol/l LDL-C level break was chosen, Friedewald's formula gave 13 % false hypercholesterolemics. The influence of Tg was again significant in men with both, hypercholesterolemia and hypertriglyceridemia, while LDL-C values obtained by the precipitation method were significantly more frequently higher ($p < 0.01$).

Key Words: Friedewald's formula, Lipoproteins, LDL-cholesterol, Precipitation method, Triglycerides.

Nowadays, the determination of cholesterol transported by low-density-lipoproteins (LDL-C) is frequently made by the formula proposed by FRIEDEWALD *et al.* (10), according to which

$$\text{LDL-C} = \text{TC} - (\text{HDL-C}) - \text{Tg}/5$$

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Abbreviations: TC = Total cholesterol; HDL-C = Cholesterol transported by high density lipoproteins; Tg = Triglycerides; VLDL-C = Cholesterol transported by very low density lipoproteins.

This formula has been widely used for the study of hyperlipemic groups as well as normo or hypolipemic (6, 7, 10), but it has been questioned because of the influence that the relation VLDL-C/Tg may have on the values of LDL-C (2, 19).

It must be noted that in most European countries the Friedewald formula would be based on molar units and would therefore be LDL − C = TC − (HDL-C) − Tg/2.3 (personal communication).

On the other hand there are some studies that correlate the values obtained indirectly using the above mentioned equation with the ones obtained using methods which directly measure the concentrations of LDL-C (3, 4, 16).

In this work a comparative study between the values of LDL-C obtained using a sodium-citrate (pH = 5.12) precipitation method and the ones obtained indirectly using Friedewald's formula is made. We also analyze the correlation between both methods and the influence that the values of triglycerides may have in the determination of LDL-C, specially in hypercholesterolemic people.

Materials and Methods

Subjects. — Serum samples from 187 people were randomly chosen from between 20 and 50 year-old people who were taking part in a study of risk factors for coronary heart disease (CHD) made by the Spanish Army (*Grupo de Estudios Cardiovasculares de las Fuerzas Armadas*). The blood withdrawal was made after a 10-12 h fasting from the antecubital fossa and following the WHO rules. Blood samples were centrifugued at 700 × *g* for 30 min and serum samples were stored at 4 °C until analyzed, being all the samples tested within a lap of time of 48 h.

Apparatus. Reagents and Chemicals. — Tube electrophoresis was developed using a Bio-Rad's Model 155 tube gel electrophoresis cell. Rocket immunoelectrophoresis was performed with a model 1415 horizontal electrophoresis cell from Bio-Rad. Tube gel or horizontal electrophoresis cells were connected to a highly regulated constant current-constant voltage model 500/200 power supply from Bio-Rad. Spectrophotometric measurements were done using a PU8620 UV/VIS/NIR spectrophotometer from Philips.

Diethylbarbituric acid was obtained from Merck AG (Darmstadt, Germany). Controls, standards, enzymes and other reagents needed for lipid determinations were purchased from Boehringer Mannheim, (Barcelona, Spain). Controls, standards, antiserum needed for apoprotein (apo) determinations were provided by Behring (Madrid, Spain). All other reagents and chemicals used were analytical grade.

Laboratory procedures. — Cholesterol as TC, HDL-C and LDL-C were determined using the enzymatic-colorimetric method of RÖSCHLAU *et al.* (17). LDL-C was also measured using FRIEDEWALD's formula (10).

Tg were determined following the enzymatic-colorimetric method of BUCCOLO and DAVID (5).

HDL-C was measured after precipitation of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) with dextran sulfate and magnesium chloride (9). LDL precipitation was made with heparine (100,000 IU/I) and sodium citrate (64 mmol/l) at the isoelectric point (pH = 5.12) using WIELAND and SEIDEL method (18). In this way VLDL and high density lipoproteins (HDL) could be found in the supernatant.

After studying several serum samples to which different volumes of precipitating reagent were added (700, 800, 900, 950 and 1,000 µl, respectively), precipitation of LDL was made adding 800 µl of pre-

cipitating reagent to 100 µl of serum, instead of the 1,000 µl of reagent proposed by the original technique. Then the samples were settled for 15 min and centrifugued at 1000 × g. After this, the supernatants were analyzed by polyacrilamide gel electrophoresis (15) and determinations of apo A1 and apo B by immunoelectrophoresis (13) were also performed in order to prove the precipitation of LDL and the lack of co-precipitation of VLDL and/or HDL. According to the results of apo B and electrophoresis and to the lack of variation in apo A1 concentration, a neat precipitation of LDL could only be obtained with 800 µl of precipitating reagent.

The quality control of the different parameters was made according to the Lipid Research Clinics Program specifications (14). The interassay variation coefficients were 3.5 % for TC, 3.9 % for Tg, 7.0 % for HDL-C and 7.5 % for LDL-C.

Statistical analysis. — Due to the influence of age in TC and LDL-C levels (6, 11, 12) and the heterogeneous distribution in the population studied (102 people between 20 and 29 years old, 66 between 30 and 39, and 22 between 40 and 49), the most probable kind of theoretic distribution was studied in order to apply the most adequate statistical tests.

The values of LDL-C obtained using both methods were adjusted to 3 kinds of theoretic distributions: Normal, Gamma and Normal-logarithmic (N-Log). The χ^2 test (8) was used to verify how the reliability of the parameters adjust to the different theoretic distributions. This test was done for each kind of distribution, varying the number of equally-probable intervals and choosing the one with higher reliability grade. The χ^2 test shows that the LDL-C values found with both methods in the same population fit better with a N-Log distribution than with a Normal or Gamma distribution.

Since the samples did not follow a Normal distribution, the differences between the values obtained with the two methods were studied using the Mann-Whitney «U» non-parametric test (8). The statistical comparison of the paired values obtained by both methods was made with the Wilcoxon «T» non-parametric test (8). The dependence grade between methods was tested with the Pearson product-moment correlation coefficient (8).

Results and Discussion

The LDL-C values determined by the FRIEDEWALD *et al.* formula (10) or by the precipitation method are similar (table I). From the 187 individuals analyzed with both methods, the values were higher in 103 and 84 cases, respectively. The statistical study with the Wilcoxon test does not significant differences.

All this information supports the fact that FRIEDEWALD's equation can be accepted as a useful way for LDL-C determination in clinical practice and epidemiological studies (6, 7, 10, 12).

Taking into consideration the objections pointed by WILSON *et al.* (19) who said that when seric Tg levels were above 200 mg/dl the VLDL/Tg relation (1/5) lost its lineality, we distributed 187 individuals into three groups (one with Tg levels between 150 and 199 mg/dl (1.7-2.3 mmol/l), another with Tg levels above 150 mg/dl ($\geq$ 1.7 mmol/l), and a third one with this level above 200 mg/dl ($\geq$ 2.3 mmol/l) in order to see the influence that the Tg levels may have in the determination of LDL-C values using Friedewald's formula.

Table I shows that in those individuals with Tg values above 1.7 mmol/l LDL-C values obtained with the precipitation were 8.2 % lower than the ones obtained with Friedewald's equation, while in individuals with Tg values between 1.7 and 2.3 mmol/l LDL-C concentrations were 7.4 % higher. Finally, in individuals with

Table I. *LDL-cholesterol (LDL-C) concentrations (mg/dl) obtained by the precipitation method (Method A) and by the Friedewald's formula (Method B), in the whole male population, in men with hypertriglyceridemia and in men with hypercholesterolemia.*

Data are mean values ± standard deviations. In parenthesis mmol/l. n = number of determinations. Tg = serum triglycerides. Data in the same row were non-significantly different (U Mann-Whitney test).

	n	LDL-C (Method A)	LDL-C (Method B)
Total male population	187	122.9 (3.2) ± 46.8 (1.2)	126.8 (3.3) ± 46.9 (1.2)
Hypertriglyceridemic men			
Tg = 150-199 mg/dl or 1.7-2.3 mmol/l	19	133.5 (3.5) ± 59.0 (1.5)	145.5 (3.8) ± 60.6 (1.6)
Tg ≥ 200 mg/dl or ≥ 2.3 mmol/l	12	165.7 (4.3) ± 7.0 (0.2)	143.8 (3.7) ± 7.0 (0.2)
Tg ≥ 150 mg/dl or ≥ 1.7 mmol/l	31	156.8 (4.1) ± 63.4 (1.6)	142.6 (3.7) ± 61.2 (1.6)
Hypercholesterolemic men			
LDL-C ≥ 150 mg/dl or ≥ 3.9 mmol/l			
All of them	41	185.3 (4.8) ± 44.3 (1.1)	184.3 (4.8) ± 49.4 (1.3)
Tg < 150 mg/dl (< 1.7 mmol/l)	32	178.0 (4.5) ± 26.6 (0.7)	184.3 (4.8) ± 37.5 (1.0)
Tg ≥ 150 mg/dl (≥ 1.7 mmol/l)	9	208.6 (5.4) ± 80.8 (2.1)	184.5 (4.8) ± 82.3 (2.1)

Tg values above 2.3 mmol/l LDL-C levels were 15 % higher with the precipitation method than with the formula.

The detailed analysis of LDL-C values obtained with both methods in hypertriglyceridemic men indicates that a higher number of individuals showed higher LDL-C values using the precipitation method and this situation involves significant differences ($p < 0.05$) between the methods (table II).

The correlation between LDL-C values obtained with both methods in all 187 individuals was highly significant ($r = 0.902$; $p < 0.001$). FRIEDEWALD *et al.* (10) found a correlation of 0.98 between LDL-C values obtained using their formula and the ones obtained with ultracentrifugation. ASSMANN *et al.* (3) also found a very high correlation between the LDL-C results obtained using precipitation with polivinylsulfate and the ones obtained with Friedewald's formula.

The correlation between LDL-C values obtained with both methods was $r = 0.963$ ($p < 0.001$) for hypertriglyceridemic individuals (Tg ≥ 1.7 mmol/l), and remained significant ($p < 0.001$) in high levels ($r = 0.958$) for Tg levels ≥ 2.3 mmol/l.

FRIEDEWALD *et al.* (10) found different correlations between LDL-C values obtained with ultracentrifugation and with their formula due to the seric Tg levels. In this way, in patients with hyperlipemia of type I, correlation was 0.99, while in those with hyperlipemia of type IV it was 0.85. Correlation rose to 0.94 in these patients when Tg levels were below 400 mg/dl.

WILSON *et al.* (19) correlated LDL-C values obtained with Friedewald's equation and those obtained by ultracentrifugation and in this way proved that in individuals who did not suffer hyperlipoproteinemias of types I, III, or IV and those Tg values were below 400 mg/dl, the linearity loss of the VLDL-C/Tg relation occurred for Tg values of 200 mg/dl and over, and this could mean an error of 7-10 % in the LDL-C determination with Friedewald's formula. All these considerations support the data obtained in the present study.

Since the LDL-C determination may have a prognostic importance for individuals suffering hypercholesterolemia, it was deemed of interest to study the influence of the analytical method used in in-

Table II. *Influence of serum triglyceride (Tg) levels in the LDL-cholesterol (LDL-C) values for hypertriglyceridemic men, and for hypercholesterolemic men obtained by the precipitation method (Method A) and by the Friedewald formula (Method B).*

	n	Cases in which LDL-C (Method A) > LDL-C (Method B)	p*
Hypertriglyceridemic men			
Tg = 150-199 mg/dl or 1.7-2.3 mmol/l	19	14	< 0.05
Tg ≥ 200 mg/dl or ≥ 2.3 mmol/l	12	9	< 0.01
Tg ≥ 150 mg/dl or ≥ 1.7 mmol/l	31	23	< 0.01
Hypercolesterolemic men (LDL-C ≥ 150 mg/dl or ≥ 3.9 mmol/l)			
All of them	41	22	N.S.
Tg = 150-199 mg/dl or 1.7-2.3 mmol/l	32	18	N.S.
Tg ≥ 200 mg/dl or ≥ 2.3 mmol/l	9	8	< 0.01

n = number of individuals
p* = Wilcoxon's non-parametric test
N.S. = non-significant differences

dividuals in whom a future risk for coronary heart disease could be considered, according to their serum LDL-C levels (LDL-C ≥ 3.9 mmol/l) (1, 10). Forty seven cases appeared to be above such level with at least one of the two methods. Six of them were considered false hypercholesterolemic as their LDL-C values were below 3.9 mmol/l when the precipitation method was used. The LDL-C mean value of the 41 true hypercholesterolemic individuals was similar using either of the methods, and no relevant differences were found (table I).

Due to the influence that Tg has on LDL-C determinations, such influence was studied in individuals who were hypercholesterolemic (TC ≥ 3.9 mmol/l) as well as hypertriglyceridemic (Tg ≥ 1.7 mmol/l). The LDL-C mean value in these 9 cases turned out to be 13.11 % higher with the precipitation method (table I). Although the statistical comparison was not significant, the analysis of the paired values shows that the precipitation method gave significantly higher values more often ($p < 0.01$) than the formula (table II).

This influence disappears in the remaining 32 hypercholesterolemics whose LDL-C mean values using both methods were almost equal (tables I and II).

In conclusion, LDL-C determination using the precipitation method can be generally considered comparable to Friedewald's equation, but as Tg values increase Friedewald's formula yields up to 15 % lower LDL-C values than the precipitation method. The paired analysis of the values shows a statistically significant effect of the Tg. It must also be taken into account that when the formula is used in epidemiological studies in order to select hypercholesterolemic individuals, it presents other inconveniences as it shows a high percentage of false hypercholesterolemic cases, and gives lower LDL-C values in hypercholesterolemic population with Tg levels ≥ 1.7 mmol/l.

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Resumen

Se comparan dos métodos utilizados para la determinación del colesterol transportado por lipoproteínas séricas de baja densidad (LDL-C). El método de precipitación que utiliza heparina-citrato sódico (pH 5,12) da en 187 hombres resultados similares de LDL-C a los obtenidos con la fórmula de Friedewald *et al.* (3,2 *vs* 3,3 mmol/l). La correlación obtenida entre ambos métodos es elevada y significativa ($r > 0,9$; $p < 0.001$). Sin embargo, los valores de LDL-C obtenidos con el método de precipitación son un 15 % más elevados en individuos hipertrigliceridémicos (triglicéridos (Tg) $\geq$ 2,3 mmol/l). Una comparación pareada de los datos obtenidos por los dos métodos señala una clara influencia de los niveles séricos de Tg ya que en individuos hipertrigliceridémicos (Tg $\geq$ 1,7 mmol/l o Tg $\geq$ 2,3 mmol/l) los resultados de LDL-C obtenidos por el método de precipitación son más elevados en un número mayor de casos ($p < 0.05$ o $p < 0.01$, respectivamente) que con la fórmula. Cuando se selecciona el valor $\geq$ 3,9 mmol/l de LDL-C como índice de hipercolesterolemia, se obtiene un 13 % de falsos hipercolesterolémicos al emplear la fórmula de Friedewald. En individuos con hipercolesterolemia e hipertrigliceridemia, de nuevo el método de precipitación da más veces niveles más elevados ($p < 0.01$) de LDL-C que la fórmula.

Palabras clave: Fórmula de Friedewald, Lipoproteínas, LDL-colesterol, Método de precipitación, Triglicéridos.

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