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Changes in high density lipoprotein subfractions during alimentary lipaemia

M. Mattock, A. Salter, T. Omer and H. Keen

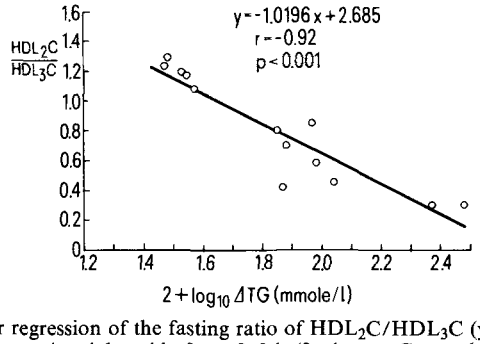
Unit for Metabolic Medicine, Guy's Hospital Medical School, London Bridge SE1 9RT (England), 8 December 1980

Summary. Significant changes in high density lipoprotein subfractions accompanied alimentary lipaemia in normal subjects. Results emphasized the importance of using fasting subjects in HDL studies but did not support an in vivo transformation of HDL_3 to HDL_2.

Low concentrations of plasma high density lipoprotein cholesterol (HDL-C) are associated with increased incidence of coronary heart disease (CHD)[1,2]. Two major subclasses of HDL, HDL_2 and HDL_3 can be isolated in the ultracentrifuge at density (d) intervals of 1.063–1.125 and 1.125–1.21 g/ml respectively[3]. Additional fractions of HDL_2, HDL_{2b} (d 1.063–1.100) and HDL_{2a} (d 1.100–1.125) have also been described[4].

The lipolysis of triglyceride-rich lipoproteins is associated with rapid transfer of lipids and peptides to HDL[5,6]. Patch et al.[7] showed that in vitro lipolysis of very low density lipoprotein (VLDL) triglyceride by lipoprotein lipase is associated with transfer of VLDL components to HDL_3 with subsequent conversion of HDL_3 to HDL_2.

We have sought in vivo evidence for this precursor-product relationship by examining the lipid content of HDL subfractions during the lipolysis accompanying alimentary lipaemia in normal subjects.



Linear regression of the fasting ratio of HDL_2C/HDL_3C (y) on (x) the change in triglyceride from 0–3 h ($2+\log_{10}$ TG mmole/l). The regression equation $y=mx+c$ is given and r is the correlation coefficient.

Methods. 13 normal subjects (6 males, 7 females, age range 22–50 years) from whom informed consent had been obtained, attended the Metabolic Ward after a 12–14 h overnight fast. With the subject resting supine, blood was drawn from an indwelling venous cannula into EDTA tubes with avoidance of venous stasis. Samples were taken fasting and then at intervals to 6 h, following the consumption of a saturated fat-rich meal (1.1 g of fat as double cream and 0.75 g of carbohydrate as canned fruit per kg b.wt). The meal was well tolerated by all subjects.

Duplicate 4-ml plasma samples were adjusted to d = 1.063 background density by addition of 2 ml NaBr solution (d = 1.177) in cellulose nitrate tubes and centrifuged at 115,000 × g 20 h in an MSE.50 preparative ultracentrifuge with No. 2582 angle-head rotor (MSE, West Sussex, Great Britain). The top fraction, containing triglyceride-rich and low density lipoproteins, was removed by tube slicing and the bottom fraction (HDL d > 1.063) quantitatively recovered. The duplicate HDL fractions were then adjusted to d = 1.100 (tube 1) and d = 1.125 (tube 2) by addition of solid NaBr and overlaid with solutions of equivalent densi-

Table 1. Serum triglycerides and cholesterol (mmole/l, mean ± SD) following the fat-rich meal

Time	0 h (fasting)	3 h	6 h
Triglycerides[a]	0.76 ± 0.60	1.75 ± 1.53***	1.09 ± 1.12**
Cholesterol	4.70 ± 1.02	4.89 ± 1.18*	5.03 ± 1.13**

[a]Triglycerides were $\log_{10}$ transformed for statistical analysis. Significantly above fasting levels by paired t-test, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Table 2. Cholesterol and phospholipid content of HDL subfractions (mean ± SD) during alimentary lipaemia

Time	Cholesterol (mmole/l) 0 h	3 h	6 h	Phospholipid (mg/dl) 0 h	3 h	6 h
HDL (total)	1.34 ± 0.31	1.35 ± 0.32	1.38 ± 0.38	126.8 ± 16.4	143.6 ± 19.2***	145.2 ± 21.8***
HDL_2	0.57 ± 0.27	0.65 ± 0.28**	0.66 ± 0.26**	47.2 ± 19.5	58.1 ± 23.6***	58.5 ± 20.8***
HDL_{2b}	0.24 ± 0.16	0.29 ± 0.20**	0.29 ± 0.16***	19.8 ± 11.6	25.3 ± 15.5**	24.8 ± 14.6**
HDL_{2a}[a]	0.38 ± 0.14	0.41 ± 0.10	0.39 ± 0.14	23.6 ± 8.5	30.7 ± 9.6	34.0 ± 10.2**
HDL_3	0.71 ± 0.10	0.63 ± 0.10***	0.65 ± 0.12*	82.0 ± 11.0	92.6 ± 19.2	84.9 ± 19.9

[a]Calculated. Significantly different from fasting level by paired t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ties. After centrifugation at 115,000 × g 40 h the top and bottom fractions were quantitatively recovered. Tube 1 yields HDL_{2b} (d 1.063–1.100) and $HDL_{2a} + HDL_3$ ($d > 1.100$) and tube 2 $HDL_{2a} + HDL_{2b}$ (d 1.063–1.125) and HDL_3 ($d > 1.125$) respectively. HDL_{2a} content was calculated by difference.

Plasma triglyceride and cholesterol and HDL subfraction cholesterol were assayed by Technicon AAII enzymatic methods and HDL phospholipid by a method based on that of Bartlett[8]. Mean recovery of cholesterol in HDL subfractions as a percentage of total HDL cholesterol was 95 ± 5% while mean phospholipid recovery was 101 ± 6%. Overall between-batch precision was 2.5% at a mean HDL cholesterol of 0.85 mmole/l.

Results. Subjects showed the expected alimentary lipaemia, with the highest mean plasma triglyceride (TG) levels 3 h after fat and a small but significant rise in plasma cholesterol (table 1). Inter-individual variation was considerable, with the percentage TG rise over basal levels being significantly greater in men than women at 3 h (178% vs 86%, $p < 0.01$).

The marked changes in mean HDL subfraction cholesterol (C) and phospholipid (PL) that accompanied this in vivo lipolysis are shown in table 2. HDL_{2b} data was log transformed before statistical analysis since the frequency distribution was positively skewed. Although there was no net change in mean total HDL-C there were significant reciprocal changes in the subfractions, with a rise in HDL_2C (chiefly due to HDL_{2b}) and a decline in HDL_3C. In contrast, total HDL-PL rose markedly, with a significant elevation of HDL_2PL. However, the rise in HDL_3PL did not achieve statistical significance. In the fasting state women had a significantly higher total HDL-C than men (1.48 ± 0.30 vs 1.19 ± 0.22, $p < 0.05$), explained by a raised HDL_2C in women (0.73 ± 0.24 vs 0.39 ± 0.14, $p < 0.01$) while HDL_3C levels were similar in both sexes (paralleled by similar sex differences in HDL-PL). However, there was no apparent sex difference in the HDL response to oral fat in this group.

The dependence of these changes on TG-rich lipoprotein metabolism was shown when, 1 week later, 1 subject was re-studied after an isocaloric fat-free meal. No change greater than 2% occurred in any HDL fraction during the following 6 h and plasma TG remained unchanged. The HDL subfraction distribution at a given HDL-C level may be expressed as the ratio $HDL_2C:HDL_3C$[9]. The figure shows that in the fasting state, this ratio is strongly negatively correlated with the peak TG response to dietary fat (expressed as $2 + \log 10\, \Delta$ TG 0–3 h to normalize the skewed distribution and avoid negative numbers).

Discussion. This study shows that the alimentary lipaemia that follows a single saturated fat-rich meal is associated with marked changes in HDL subfraction lipid content. A fat load of this size produces a preponderance of large chylomicrons (with some VLDL) when presented to the small intestinal mucosa[10]. Following hydrolysis of chylomicron TG by capillary endothelial liprotein lipase (LPL), bilayers and vesicles composed of redundant polar surface components may be formed. Such particles may be converted into spherical HDL by interaction with the circulating HDL pool and by lecithin:cholesterol acyltransferase action[11]. The increase in HDL-PL and HDL-PL:C ratio in our data are compatible with such a transfer and confirm earlier observations[6]. Although an influx of free cholesterol into the HDL fraction is likely[11], no significant change in total HDL-C was noted. This may be due to transfer of cholesteryl ester from HDL to other liproproteins[12]. During lipolysis a rise in HDL_2C was evident while HDL_3C declined. Since parallel changes in PL were not seen, these findings do not provide in vivo evidence for the transformation of HDL_3 to HDL_2 observed during in vitro lipolysis of VLDL[7].

Patch and Gotto[9] found that the plasma TG response to oral fat was pronounced in 2 normal men with a low $HDL_2:HDL_3$ ratio, while the response was minimal in 2 other men with a particularly high ratio. A similar inverse relationship (fig.) is present in our larger group of men and women of widely varying HDL subfraction distribution when TG response is expressed as an incremental change from basal levels. We interpret this relationship to indicate that the rate of LPL catalyzed TG-rich lipoprotein catabolism may be an important determinant of HDL subfraction distribution. The increased $HDL_2:HDL_3$ ratio in women may reflect their markedly higher TG removal capacity compared with men[13]. In addition, recent evidence suggests that HDL_2 levels may be modified by hepatic lipase action[14].

The minimal changes in total HDL-C found after meal intake have encouraged the use of non-fasting plasma by some workers[15,16]. With the recent advent of simple precipitation methods for separation of HDL_2 and HDL_3[17], of possible wide application, it is important to recognize that fasting samples should be used in order to avoid meal induced effects on HDL subfractions.

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Effective population number estimates of laboratory populations of *Drosophila melanogaster*

J.M. Malpica and D.A. Briscoe

Departamento de Genética, I.N.I.A. Apartado No.8111, Madrid (Spain), and School of Biological Sciences, Macquarie University North Ryde, N.S.W. 2113 (Australia), 12 December 1980

Summary. A sizeable fraction of the current information in population genetics comes from experiments with population cages of *Drosophila melanogaster* in which a high census (circa 5000 adults) is kept in order to obviate the influence of drift. We have estimated the effective population number in such cages by lethal complementation and our estimates are lower than the census by an order of magnitude.

The estimation of the effective population number *(Ne)* by lethal complementation has a well established theoretical basis[2–4], has been tested experimentally[5], and is of common use in population genetics[5–7]. The expression for the estimate is

$$\hat{N}e = \frac{1 - Ig}{4(Ig\ U - u)} \qquad \text{(Nei}^2\text{)},$$

where U and u are the per chromosome and per locus mutation rates and Ig is the allelic rate of lethal genes, which can be estimated by

$$\hat{Ig} = -\log(1 - Ic\ Q^2)/\{\log(1 - Q)\}^2$$

where Q is the observed frequency of lethal chromosomes in the population and Ic is the proportion of inviable, random heterozygous combinations of such chromosomes.

The estimation was performed in populations kept for different times under cage conditions that maintain a steady census of about 5000 adults[8] (table). The populations were founded with wild caught individuals. Foundation stocks were over 500 inseminated females. Kaduna is known to be free of inversions, as seems to happen with populations kept under laboratory conditions[19]. Riudevella, Amherst and Stellenbosch samples were checked for inversions at the 3rd chromosome, the frequency of inversion-carrying 3rd chromosomes was below 6% in all of them. Values of Ic and Q were obtained for the 3rd chromosome and the usual values of $U = 0.005$ and $u = 10^{-5}$ calculated for the 2nd chromosome were used[9–11], as the available data indicate that the lethal structure for the 2nd and 3rd chromosomes is very similar[12–15] (J.M. Vassallo and J.M.M., unpublished results). The effective population numbers for the investigated populations were in the range 190–1235 (table).

Alternative values for u proposed in the literature[6] would yield lower estimates for the effective population number. The estimation method presupposes that the elimination of lethals occurs mainly as heterozygous combinations. The above population numbers are close to the limit with respect to such a condition[4,16], but if the observed frequencies of lethal chromosomes in our populations were to be explained in terms of elimination of lethals as homozygous combinations, the population numbers would be even lower (range 50–380, estimated as in Wright[17]).

Possible mechanisms for this difference between census and effective population size can be found[18]. Although estimations of the effective population number in line with those reported here have occasionally been used[5,15], the interpretation of a considerable number of experiments in which the number of adults has been used as an estimate of the effective population number should be revised, especially those in which this parameter is used a) to reject drift as an explanation for changes in gene frequencies, b) to predict variability, or c) to calculate expectations of associations among loci.

Estimates of effective population numbers

Populations	Riudevella	Amherst	Stellenbosch	Canberra	Pacific	Kaduna
Months under cage conditions	8	24	36	156	204	276
$Q^a \pm$ SE	0.13 ± 0.04	0.21 ± 0.04	0.24 ± 0.04	0.10 ± 0.03	0.09 ± 0.03	0.13 ± 0.03
$I_c{}^b \pm$ SE	0.05 ± 0.03	0.15 ± 0.04	0.14 ± 0.06	0.13 ± 0.04	0.04 ± 0.03	0.24 ± 0.09
$\hat{N}_e{}^c$	1,123	383	427	395	1,252	190

[a] Q, frequency of lethal 3rd chromosomes. A TM3, Sb, Ser[20] balancer chromosome was used. Males direct drawn from the cage were individually mated to TM3, Sb, Sr/Pr females, only one line from each of the successful matings was subsequently used.
[b] I_c, proportion of inviable, random heterozygous combinations of lethal chromosomes.
[c] $\hat{N}_e{}^c$ estimate of effective population number, for estimation method see text.