Monthly and seasonal variation in plasma lipids in healthy Australian men: a longitudinal study in Melbourne

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A prospective study of seasonal variability of lipids in 36 healthy men, aged 40–45 years, over 14 months in Melbourne showed significant monthly and seasonal variation by paired t-test. Measurement of cholesterol and triglycerides was carried out by standard, automated enzymic assays calibrated with CDC certified materials. High Density Lipoprotein Cholesterol (HDLC) was measured after Polyethylene Glycol 6000 precipitation of Apo B containing lipoproteins. Intra-individual variation ranged wisely; Cholesterol: mean Coefficient of Variation (CV) 6.9%, range 4.1 to 14.0, HDLC: mean CV 9.1%, range 5.3 to 15.6 Low Density Lipoprotein Cholesterol (LDLC): mean CV 10.2%, range 5.2 to 18.4. The seasonal effect showed the most favourable lipid/lipoprotein profile, ie lowest total and LDLC, highest HDLC to occur in the antipodean summer (Nov/Dec) and the least favourable profile in winter (Jul/Aug), with highest (total) Cholesterol and lowest HDLC. This is best observed as the LDLC/HDLC ratio which peaks in July (3.6), with the trough in December (2.7). This pattern is consistent with seasonal effects described previously in the northern hemisphere, except that the months are reversed. Weight did not alter significantly during the period of the study. Seasonal and individual variation in lipids and lipoproteins should be taken into account in the clinical management of lipid disorders.

Introduction

Variation in serum lipids[1-4] requires recognition and measurement in clinical practice, if risk for macrovascular disease is to be adequately defined[5] and the effects of management reliably documented[6]. A number of factors may lead to changes in plasma total cholesterol, apart from food intake and these include: changes in weight and alcohol intake[8]; coffee intake[9]; physical activity[10]; and mental stress[11-13]. Several cross-sectional[14-16] and longitudinal studies[17-19] also indicate that there is biologically significant seasonal variation in plasma total cholesterol, but not all studies support this view[20,21]. As with other determinants of the serum total cholesterol, explanation for its change may reside in any of the lipoprotein subfractions, Very Low Density Lipoprotein (VLDL), Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL)[3]. Seasonal variation has also been observed for a number of other analytes, some of which like glucose, insulin, and lipoprotein lipase may have a bearing on lipoprotein fluctuations[22-24].

There is no reported longitudinal study of seasonal fluctuations in lipoproteins in the southern hemisphere. If the winter were found to be a time associated with higher LDLC (Low Density Lipoprotein Cholesterol) and lower HDLC (High Density Lipoprotein Cholesterol) concentration than in summer, in both northern and southern hemispheres, it would add support to a hypothesis that seasons were a determinant of lipoprotein status. Explanations for such variation by change in behaviour or basic biological rhythms dependent upon length of day, temperature or other variables would still require further research[25-27].

Methods

Subjects

Thirty-six apparently healthy male volunteers accepted and completed this study, which was approved by Prince Henry's Hospital Ethics Committee in accordance with guidelines of the National Health and Medical Research Council of Australia. Four subjects who began the study did not complete it because they had moved from Melbourne or had changed personal circumstances. Informed consent was obtained.

Table 1. Subject characteristics at entry.

	Mean±SEM	Range	
Age (years)	41.6±0.28	39–45	n=40
BMI ($kg.m^{-2}$)	25.3±0.35	18.5–29.7	n=40
BP Systolic (mm Hg)	120.6±2.5	100–158	n=39
BP Diastolic (mm Hg)	79.4±1.4	68–100	n=40
Mean Corposcular Volume	85.4±0.6	79–94	n=37
Gamma Glutamyl Transferase	28.5±3.8	4–135	n=39

Subjects were Australian-born Caucasians and their entry characteristics are shown in Table 1. They had no known health problem and were not on dietary treatment or regular medication. Any intercurrent illness or treatment was documented, but did not lead to exclusion from

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the study. No attempt was made to discriminate between those who did or did not have regular physical activity, but those who took part in irregular intensive or highly competitive winter or summer sports were not included. Those with a body mass index (BMI or Weight/ Height^2) of >30 kg.m^{-2} or those who were known hypertensives were not included. Men who admitted to regular consumption of excessive alcohol (more than eight standard drinks a day on average) or who had a Mean Corpuscular Volume (MCV) greater than 95 or Gamma Glutamyl Transferase (GGT) activity greater than 80 U/L – which could mean excessive alcohol consumption – were not included; two individuals with elevated GGI values were excepted as there was no doubt that alcohol was not used excessively.

Subjects were excluded if, at the first visit, plasma cholesterol exceeded 8 mmol/L or plasma triglycerides exceeded 3 mmol/L. The then current National Heart Foundation of Australia recommendations were that plasma cholesterol be less than 6.5 mmol/L and triglycerides less than 2.0 mmol/L. However, the purpose of this study was to consider the fluctuations which took place over an extended period of time and which may move in and out of the acceptable range. At the end of the study all lipid data were made available to participants and, at their request, to their several medical practitioners.

Monthly blood samples were taken for 14 months from April to May of the following year, inclusive. Sampling was from Tuesday to Thursday, on the same day of the week in each month as far as possible. The same week in the month was used, although a variation of ± one week was allowed to accommodate for vacations or intercurrent illness. Men attended in the morning between 8 and 9.30 am after an overnight fast (water only to drink) from 9 pm the night before. Cigarette smoking was not permitted for one hour prior to sampling. A questionnaire about lifestyle and health in the preceding month was completed on each occasion, and subjects' weight recorded.

Statistical analysis

Significance of difference from baseline, or from any other reference point (peak value), was assessed by paired t-test.

Laboratory methods

Lipid assays were performed by standard enzymic methods for cholesterol (Cholesterol Esterase/Cholesterol Oxidase PAP – Boehringer Monotest High Performance Cat. No. 237574) and Triglycerides as total glycerol (Lipase/Glycerol Kinase/Glycerol Phosphate Oxidase/ PAP – Human Diagnostics Cat. No. H500G). All assays were carried out as routine analytical procedures using an Abbott ABA-100 Bichromatic Analyzer and were standardized and quality controlled with human serum based materials with Centres for Disease Controls (USA) ascribed reference values. (Australian Lipid Standardisation Programme Calibrators and Control.)

The Co-efficient of Variation (CV) for serum cholesterol was 2.4% for a low quality control (4.8 mmol/L) and 2.3% for a high quality control (9.1 mmol/L), while CV for serum triglycerides was 5.1% for the low control (1.3 mmol/L) and 4.8% for the 'high' control (2.1 mmol/L).

HDL was isolated by the method of Allen et al.[28] using Polyethylene Glycol 6000 precipitation and its cholesterol content assayed using a similar principle as for total cholesterol, modified to increase assay sensitivity. Quality control data indicated an overall CV for HDLC of 5.0% at a mean concentration of 1.22 mmol/L.

Results

The most significant plasma lipid relationship with month was that the lowest LDLC/HDLC ratio was found in December (summer) and the highest in July (winter) (Table 2). These reflected trends in total cholesterol in winter and HDLC in summer.

Plasma triglycerides were highest in March (Autumn) and lowest in November (Spring) (Table 2).

The intra-individual variances over 14 months ranged widely (see Table 4). Individual values for the lowest and highest CV, as well as the mean CV for the group are shown for each lipid parameter.

The concept of a 'Δ%' is used to measure the extent of variation from the mean value for each analyte, expressed as a percentage, for individuals with the lowest and highest observed variability, as well as the mean (variability) for the group. The actual values are shown in parentheses.

Discussion

From our studies, there is considerable variation in plasma lipid status over 14 months in apparently healthy men in Australia. This reinforces the case for several observations, even over a year, to define lipid status and to assess the effects of intervention[6,29,30].

The finding that total cholesterol peaked in the winter in a southern hemisphere study is consistent with observations in the northern hemisphere[1,7]. The assessment of the LDLC/HDLC ratio indicated that this also peaked in winter and was lowest in summer.

Lipid values in April and May of the successive years were not identical and indicate that non-seasonal variation in an individual needs also to be taken into account.

Table 2. Peak and trough serum lipids according to month and season.

Analyte	Peak	Month	Season	Trough	Month	Season	*P*
Cholesterol	5.93	August	Winter	5.51	Nov	Spring	<0.05
Triglyceride	1.45	March	Autumn	1.15	Nov	Spring	<0.05
HDLC*	1.42	Dec	Summer	1.21	May	Autumn	<0.05
LDLC†	4.10	May	Autumn	3.61	Dec	Summer	NS
LDLC/HDLC Ratio	3.57	July	Winter	2.73	Dec	Summer	<0.01

*HDLC = High Density Lipoprotein Cholesterol.
†LDLC = Low Density Lipoprotein Cholesterol.

Table 3. Variation in weight with seasons*.

	Winter Jun–Aug	Spring Sept–Nov	Summer Dec–Feb	Autumn Mar–May
Mean (kg)	80.0	79.5	79.0	79.2
SEM	1.5	1.6	1.6	1.6

*No significant changes observed (NS = $P>0.05$).

Table 4. Intra-individual variation for plasma lipids (n=36).

Analyte	Lowest individual CV%	Lowest individual Δ%*	Group mean CV%	Group mean Δ%*	Highest individual CV%	Highest individual Δ%*
Cholesterol	4.1 (5.1–5.8)†	13	6.9	24	14.0 (4.3–7.1)	47
HDLC	5.3 (1.17–1.41)	18	9.1	31	15.6 (0.64–1.16)	59
LDLC	5.2 (3.8–4.6)	18	10.2	36	18.4 (2.3–4.2)	65
LDLC/HDLC Ratio	5.9 (3.3–4.0)	18	14.2	49	25.5 (1.2–3.3)	114

*'Δ%' is the difference between the lowest and highest value, expressed as a percentage of the mean value.
†Figures in parenthesis are the range of an individual's actual values observed for each analyte.

In this study, two of the most important candidates for variation in serum lipids, weight and alcohol intake, have been minimized at entry by exclusion of the obese and excessive consumers of alcohol. Weight was also monitored throughout (Table 3). We were not able to take account of stress.

The occupations of our subjects ranged from clerical to executive and professional with no uniform pattern of work load. However, in Australia, the financial year runs from 1 July to 30 June (winter) and the principal holiday season is summer, notably January. Alcohol intake rises in December towards Christmas (25 Dec) and New Year (1 Jan): 45% of the cohort reported an increase in alcohol consumption in December relative to previous months. It may be that these seasonal activities were contributing to the seasonal variation in plasma lipids.

One reason for interest in an individual's serum lipid variance is that, where it is greater, coronary risk may be greater[9]. In this case, prospective studies of men with differing variances observed in this study, in relation to macrovascular disease outcomes may be worthwhile.

At the very least, the clinical management of serum lipid disorders should take account of the month and season of observation.

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健康澳洲男人血漿脂類的季節和每月的變動：墨爾本一個縱向的研究

摘要

作者在墨爾本選用 36 位 40—45 歲的健康男人爲對象，觀察 14 個月血脂的季節變異性，用配對 t 檢驗，發現有季節和每月的明顯變動。膽固醇和甘油三酯用標准自動酶法測定，並用美國疾病控制中心 (CDC) 可靠的試劑校准。把含有脂蛋白的 APOB 用聚乙烯甘醇 6000 沉澱後測定高密度脂蛋白膽固醇 (HDLC)。個體間差異範圍很大：膽固醇平均變異系數 (CV) 爲 6.9%，範圍在4.1—14.0；高密度脂蛋白膽固醇平均 CV 9.1%，範圍 5.3—15.6；低密度脂蛋白膽固醇平均 CV 10.2%，範圍 5.2—18.4。受季節影響最大的脂類／脂蛋白比值是在澳大利亞的夏季 (11月／12月)，此時總膽固醇和低密度脂蛋白膽固醇最低，而高密度脂蛋白膽固醇含量最高。影響最少的是在澳大利亞的冬季 (7月／8月)，此時總膽固醇含量最高，而高密度脂蛋白膽固醇含量最低。LDLC／HDLC 比值在 7月是高峰，而在 12月的比值在低谷。在北半球除月份不同外，這種恆定的季節的影響已有報導，但體重並未改變研究期間的顯著性。最後作者認爲，血漿脂類和脂蛋白季節和個體的變動，在脂類疾病的臨床處理中應加以重視。