Diurnal variations in serum biochemical and haematological measurements

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SUMMARY   Twenty five biochemical and haematological measurements were determined on non-fasting blood and serum samples collected between 9 am and 7 pm from a representative group of 7685 British middle-aged men. Most measurements showed significant diurnal variations, but only for bilirubin, phosphate, and triglyceride did time of day account for more than 5% of the between subject variance. Serum bilirubin concentrations showed a pronounced downward trend in the afternoon, the mean value after 6 pm being 30% lower than the mean value in the morning. Mean serum triglyceride and phosphate concentrations increased steadily through the day. Mean concentrations of potassium, haemoglobin, and haematocrit and red cell count were higher in the morning, while urea and creatinine concentrations and white cell count had higher means in the afternoon. Glucose showed a pattern consistent with short term response to meals.

The effects of these diurnal trends on routine use of biochemical tests needs careful consideration, and a greater understanding of their biological mechanisms is required.

For many biochemical and haematological investigations it is recommended that they be carried out with the patient in a fasting state. Indeed, for some variables such as serum lipids and serum glucose, laboratories may decline to carry out measurement when the patient has not fasted. This is often different for inpatients and outpatients, and in routine practice most biochemical and haematological determinations are made on non-fasting blood samples. The interpretation of these findings is usually made without reference to the time of venepuncture.

This paper reports the findings of diurnal variation in 25 standard biochemical and haematological measurements to assess whether these changes are of sufficient magnitude to require wider recognition and possible adjustment when interpreting results. Whereas most previous studies of diurnal variation have used repeated blood sampling on a small number of subjects,[1-5] our data relate to multiple measures carried out on a single venous blood sample obtained from a large representative sample of British middle-aged men, who were screened during day-time hours in a prospective study of risk factors for coronary heart disease.

Subjects and methods

The British Regional Heart Study includes 7735 men aged between 40 and 59 years, who had been randomly selected from the age and sex registers of representative general practices in 24 towns in England, Wales, and Scotland. The criteria for selecting the towns, the general practice, and the subjects, as well as the methods of data collection, have been reported elsewhere.[6]

From each practice's age-sex register some 420 men, aged between 40 and 59 years, stratified into five year age groups of equal size, were selected at random. The list of names was reviewed by the doctors in the practice, who were asked to exclude those whom they thought would not be able to participate because of severe mental or physical disability. The remaining subjects were invited to take part in the study in a letter signed by the general practitioner. Seventy eight per cent of those invited attended for examination by a team of three research nurses over a period of two and a half years.

BIOCHEMISTRY AND HAEMATOLOGY
The men attended the examination centres in each town over a 10 hour period between 8.30 am and 6.30 pm and were not asked to fast beforehand. Each

man's time of arrival was recorded. As venepuncture was the last part of the examination the estimated time of venepuncture was taken as 35 minutes later. Blood samples for biochemical analysis and haematological studies were drawn into five separate evacuated tubes using a Velcro tourniquet on the upper arm. Serum separation tubes were stood for 30 minutes and spun for 10 minutes. All samples were stored vertically at 4°C and despatched overnight to the Wolfson Research Laboratories, Queen Elizabeth Medical Centre, Birmingham where estimations were completed by noon of the following day. Serum was analysed for concentrations of sodium, potassium, urea, creatinine, urate, calcium, albumin, total protein, bilirubin, glucose, total cholesterol, alkaline phosphatase and aspartate transaminase (AspT) using the SMA 12/60 analyser and standard Technicon methods. High density lipoprotein-cholesterol was estimated using Liebermann Burchard and enzymic methods after precipitation with magnesium phosphotungstate. Gamma glutamyl transferase (GGT) and calcium phosphate were also estimated. Serum lead concentrations were measured at the department of chemical pathology and human metabolism in the University of Southampton and serum triglyceride concentrations in the department of chemical pathology at the Royal Free Hospital School of Medicine, London. Haematological estimations included white cell count, red cell count, haemoglobin and haematocrit, and from these were calculated mean cell volume, mean corpuscular haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC).

Blood samples were not obtained from 45 men and the time of venepuncture was unknown for five. Triglyceride concentrations were not determined for men in the first six towns visited. A number of other biochemical or haematological measurements were not carried out on small numbers of subjects because of inadequate samples or technical problems.

LOG TRANSFORMS
Previous analysis of these data[7-9] found highly skew distributions for several measurements. In each case the use of log transforms and geometric means seemed appropriate.

Results

The time of venepuncture for all 7685 men is shown in table 1. The smaller number of samples in the middle of the day corresponds to the three nurses' midday break which varied slightly from day to day. Few blood samples were obtained before 9.30 am and after 7 pm.

To assess the overall extent of diurnal variation for each biochemical and haematological measurement a

Table 1   *Time of venepuncture for 7685 middle-aged men*

	No of men
9–10 am	603
10–11 am	1041
11–12 noon	947
12– 1 pm	847
1– 2 pm	426
2– 3 pm	317
3– 4 pm	735
4– 5 pm	918
5– 6 pm	912
6– 7 pm	863
7– 8 pm	75
After 8 pm	1
Total	7685

one way analysis of variance was based on 10 time intervals (nine one-hour periods from 9 am to 6 pm, plus 6 pm onwards). Table 2 shows the percentage of variance due to hour of day for each measurement. This percentage variance had the largest values for phosphate (11·7%), bilirubin (10·3%), triglyceride (5·0%) and potassium (3·1%). For haematological measurements the percentage variance due to time of day was generally smaller, with only white cell count and haematocrit exceeding 2%.

As this survey was based on a large number of men even a small percentage variance is significant—for

Table 2   *Between-subject variation in 25 biochemical and haematological measurements attributable to time of day (based on 7685 blood samples from adult men)*

| Biochemistry	Percentage of variance due to time of day
Phosphate	11·7
Bilirubin*	10·3
Triglyceride*	5·0
Potassium	3·1
Urea*	2·1
Creatinine*	1·4
Glucose*	0·9
Sodium	0·8
Calcium	0·7
Alkaline phosphatase*	0·6
Total protein	0·5
Urate	0·5
Lead*	0·4
Albumin	0·4
HDL-cholesterol*	0·3
Asp T*	0·2
GGT*	0·2
Cholesterol	0·0
Haematology:	
White cell count*	2·2
Haematocrit	2·1
MCHC	1·9
Haemoglobin	1·7
Red cell count	1·6
Mean cell volume	0·6
MCH	0·1

*Log transform used.
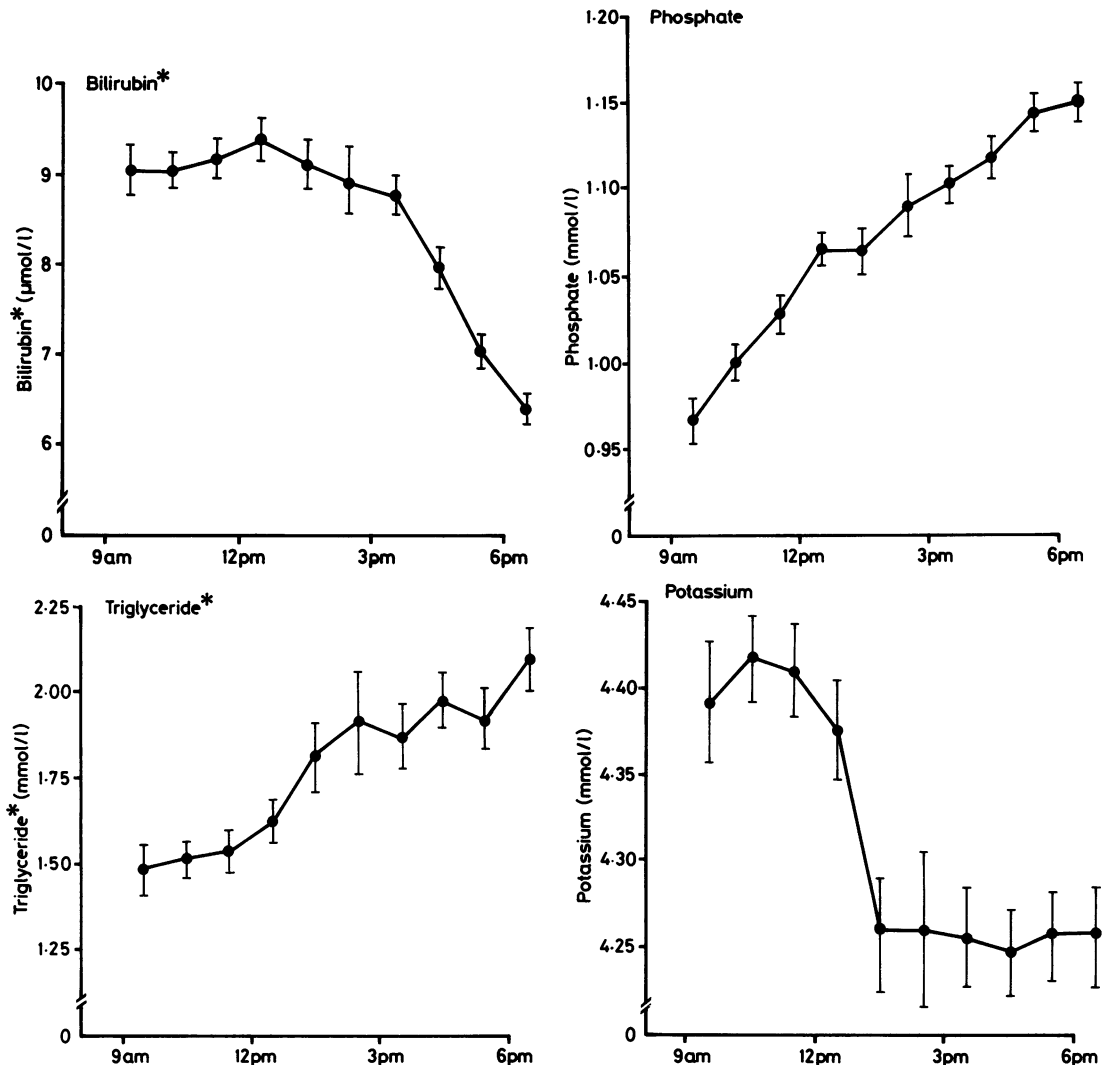
Figure   *Hourly means and 95% confidence limits for 11 biochemical and haematological measurements.*
*Geometric means used for those with skew distributions.

example, a percentage variance of 0·5% has a significance level p < 0·001. Thus there is statistical evidence that diurnal trends exist for most biochemical and haematological measurements. It is the magnitude and shape of such trends, however, that is of practical importance and it seems reasonable to asssume that measurements with a percentage variance of less than 1% have diurnal trends of little clinical relevance.

To describe the diurnal trends of principal interest the figure shows the hourly means and 95% confidence limits for seven biochemical and four haematological measurements. The confidence intervals for 9–10 am,

1–2 pm, and 2–3 pm are slightly wider due to the smaller numbers of subjects screened at those times.

BILIRUBIN
Mean concentrations were reasonably stable in the morning but showed a pronounced downward trend through the afternoon such that the geometric mean after 6 pm was 6·4 μmol/l, 30% lower than the geometric mean obtained between 9 am and 2 pm (9·2 μmol/l).

PHOSPHATE
This showed a consistent upward trend throughout the day; after 6 pm the mean phosphate concentration was 1·15 mmol/l, 19% higher than that between 9 and 10 am (0·97 mmol/l). The mean concentration after

Urea*

Creatinine*
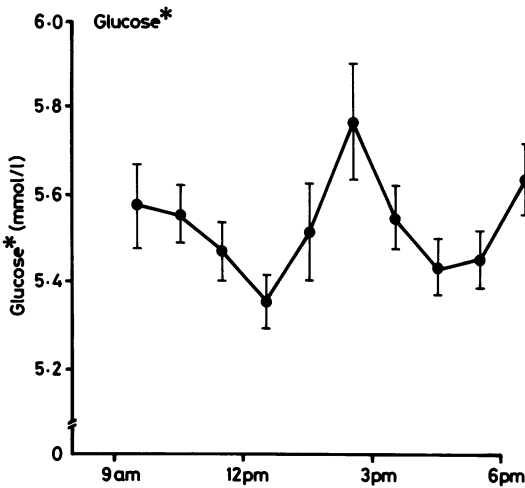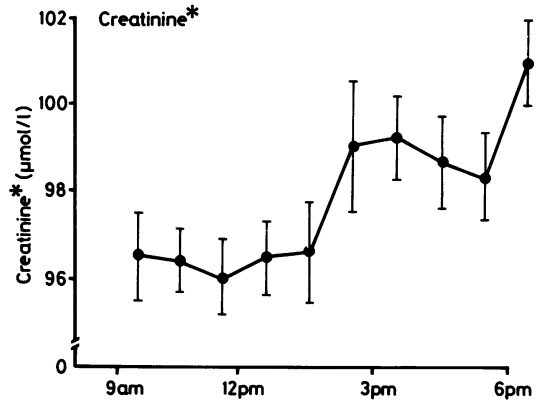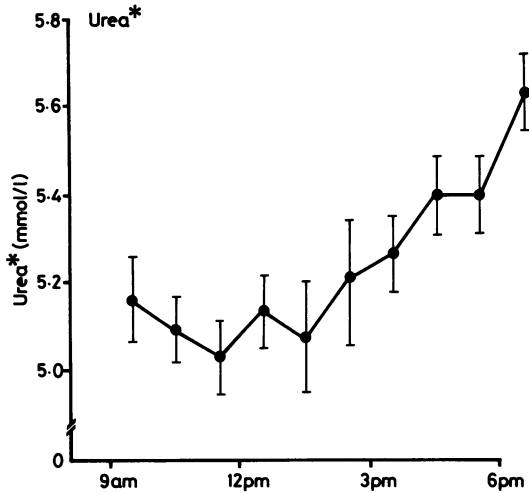
Glucose*

tion throughout the afternoon such that the geometric mean after 6 pm was 5·63 mmol/l compared with 5·10 mmol/l between 9 am and 2 pm.

CREATININE
Mean values were also higher in the afternoon, though the diurnal trend was weaker than for urea.

GLUCOSE
Mean concentrations were highest between 9–10 am, 2–3 pm, and after 6 pm, which probably reflects a short term response to meals.

HAEMATOLOGY
The white cell count showed an increasing trend in mean value up to around 4–5 pm, followed by a slight fall in the late afternoon, while the red cell count, haemoglobin, and haematocrit all showed an opposite trend with highest mean values before 11 am.

The practical relevance of these diurnal trends can be further illustrated by considering how time of

2 pm was 1·13 mmol/l compared with 1·02 mmol/l before 2 pm.

TRIGLYCERIDE
This also rose steadily through the day with a geometric mean of 2·08 mmol/l after 6 pm compared with 1·48 mmol/l between 9 and 10 am. The mean concentration after 2 pm was 1·96 mmol/l compared with 1·57 mmol/l before 2 pm.

POTASSIUM
This showed lower mean concentrations in the afternoon, with a mean value of 4·26 mmol/l after 1 pm compared with 4·40 mmol/l before 1 pm. No such pattern was observed for sodium.

UREA
This showed an increasing trend in mean concentra-

Table 3 *Diurnal variation in prevalence of "high" bilirubin concentrations (> 17 μmol/l)*

Hour of day	No of men	No (%) with serum bilirubin > 17 μmol/l
9 am–	603	36 (6·0)
10 am–	1041	48 (4·6)
11 am–	947	35 (3·7)
12 noon–	847	49 (5·8)
1 pm–	426	22 (5·2)
2 pm–	317	14 (4·4)
3 pm–	735	30 (4·1)
4 pm–	918	33 (3·6)
5 pm–	912	28 (3·1)
6 pm–8.10 pm	939	15 (1·6)
Total	7685	310 (4·0)
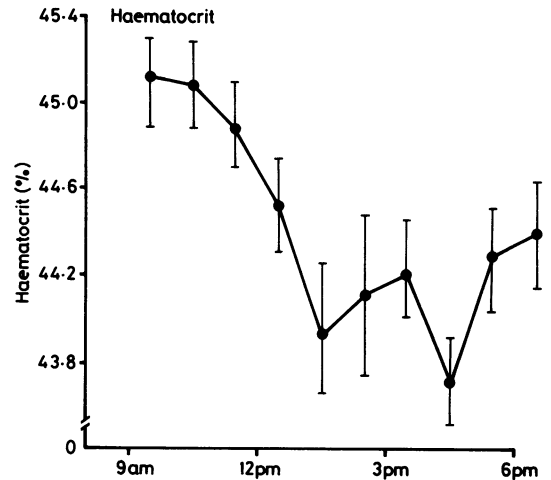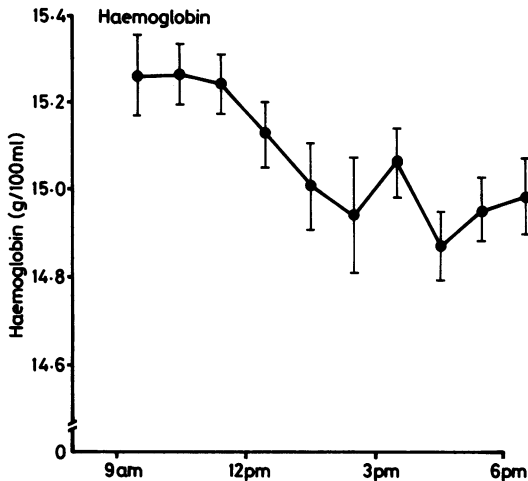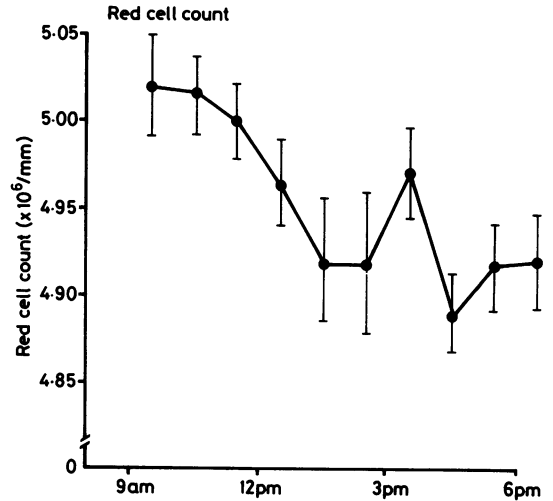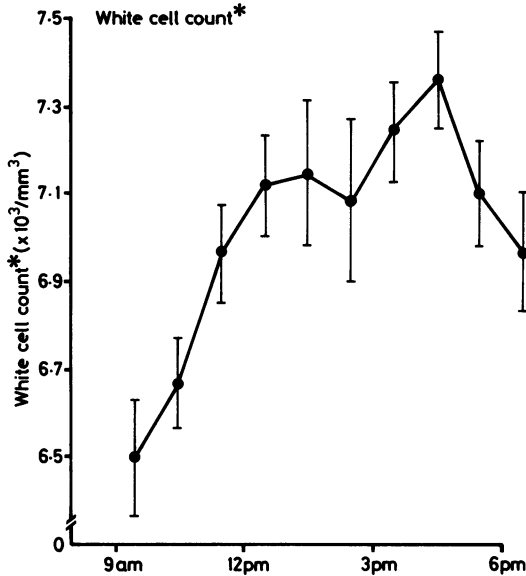
venepuncture affects the chances of men being classified as above some upper reference limit, and serum bilirubin is particularly worthy of attention. Table 3 shows for each hour of day the percentage of men with serum bilirubin of > 17 μmol/l, a commonly used upper reference limit. Between 9 am and 2 pm, 4·9% of men had "raised" serum bilirubin of > 17 μmol/l compared with 1·6% after 6 pm.

Discussion

Within the general field of chronobiology and human circadian rhythms[10][11] there have been numerous investigations into the diurnal variation in routine serum biochemistry and haematology.[1-5] Most of these studies, however, have been on selected small groups of volunteers (usually less than 20 subjects) with repeated blood samples taken at fixed time points over several days. The statistical uncertainty inherent in such limited studies makes it difficult to assess reliably the magnitude of diurnal trends. This report describes an alternative, single venepuncture, large scale epidemiological approach. As subjects were randomly selected from general practice registers and the time of visit should have been unrelated to a subject's condition, an accurate estimation of diurnal trend should be achieved. Two other studies, in blood donors[12] and an industrial workforce,[13] also used this approach to

assess diurnal variation, though in less detail than that described here.

It is important to try and ensure that the diurnal patterns observed in this type of study are genuine and not artefacts attributable to the handling of blood specimens. Each day all samples were delivered by Securicor van overnight to the Wolfson Research Laboratories, so that all laboratory estimations were completed by noon the next day. Such centralised assessment under strict quality control should have eliminated any bias related to sampling time.

The average diurnal patterns may vary from person to person. For instance, differing eating habits would lead to individual variation in the diurnal patterns of triglycerides and glucose. This is an academic point of little practical value as there would be no possibility of correcting a single measurement for an individual diurnal trend, but it does mean that individual trends may be more pronounced than the averages shown in the figure. Studies of repeated sampling over 24 hours in small groups could explore this issue of "randomly phased" diurnal effects in more detail.

BILIRUBIN

We have shown that the mean bilirubin concentration seems to remain stable through the morning and then falls steadily through the afternoon with a particularly steep decline after 3 pm. This finding agrees with a study of 20 volunteers who had mean bilirubin concentrations of 8·5, 8·2, and 7·2 μmol/l at 8.30 am, 12.30 pm, and 4.30 pm, respectively.[1] The relevance of bilirubin's destruction by sunlight should be considered as it has been shown that mean bilirubin concentrations tend to be 1 to 1·4 μmol/l lower in summer,[14] but the diurnal pattern reported here was similar in summer and winter. A more plausible explanation may be the effect of food intake. It has been reported that a two day fast can treble the bilirubin concentration.[15] Overnight fasting might therefore result in an increase in bilirubin concentrations and food intake through the day eventually leads to a fall in level during the afternoon. The relevance of food intake is supported by a study of 11 fasting healthy young men whose mean bilirubin concentrations increased from 11·4 μmol/l at 8 am to 13·2 μmol/l at 2 pm.[2]

The magnitude of the observed decline in serum bilirubin is sufficiently great to suggest that time of day should be taken into account in setting routine upper reference limits for this measurement. As indicated in table 3 the use of a fixed reference limit, such as 17 μmol/l, results in a three-fold reduction in the percentage of "abnormally" high values in the late afternoon compared with those in the morning.

PHOSPHATE

Mean plasma phosphate concentrations show a pro-nounced and continuous rise through the day. This finding has also been reported by several studies of repeated venepunctures in small groups of volunteers.[5 12 16 17] In particular, a 24 hour study of students under waking conditions with identical snacks each hour showed a peak mean concentration at around 8 pm and a trough at 8 am.[16] The reason for such trends is unclear, although cortisol is known to lower plasma phosphate activity. The relation of phosphate activity to carbohydrate metabolism and glucose movement in and out of cells may also be relevant, although the trends found were unrelated to those for serum glucose.

TRIGLYCERIDES

The steady increase throughout the day in mean triglyceride concentration in non-fasting subjects has been shown previously.[18] Food intake seems to be a major contributory cause and type of diet may affect the extent of diurnal trend. For instance, Kuo and Carson showed that diurnal rises in triglycerides seem to be smallest on a diet of rice and fruit.[18] Triglyceride concentrations can increase rapidly in response to a single high fat meal in the morning.[19]

Epidemiological studies of coronary heart disease have usually focused on the measurement of fasting triglycerides to eliminate artefactual differences between subjects caused by differing timings of venepuncture after varying dietary patterns. Fasting concentrations, however, may be an artificial measure, relative to an individual's normal daytime triglyceride pattern. Greater insight might be obtained from non-fasting triglyceride measurements after a subject's usual diet,[20] though some adjustment for the diurnal trend is advisable. There is no evidence of diurnal variation in serum total cholesterol, which supports the view that cholesterol measurements need not be made in the fasting state.

POTASSIUM

Our finding of lower values of mean serum potassium in the afternoon compared with the morning has also been shown in other studies,[1 2] though there seems to be considerable individual variation about this underlying trend. The reasons for this daytime variation in serum potassium are unclear[10] and probably of little clinical importance.

UREA AND CREATININE

Mean values of serum urea and creatinine tended to increase during the day. While these associations are significant, diurnal variation explained only about 1–2% of the between-subject variance in each case. As these findings have not been consistently noted in other studies, they should be interpreted cautiously. It may be, however, that the increase in urea is the result

of the metabolism of dietary protein and that of creatinine the outcome of muscular activity and energy output.

GLUCOSE

The diurnal pattern of mean glucose concentration was predictable and supports the validity of the methods used here to study diurnal variations. The troughs in the late morning (just before lunchtime?) and late afternoon (just before dinnertime?) are consistent with the short term response to carbohydrate intake. It is perhaps surprising that the diurnal trend is not greater: the peak of 5·8 mmol/l between 2 and 3 pm is only 7% higher than the trough of 5·4 mmol/l between 12 noon and 1 pm, and less than 1% of the between-subject variance is attributed to time of day.

RED CELL COUNT, HAEMOGLOBIN, HAEMATOCRIT

The mean red cell count, haemoglobin, and haematocrit all show a weak declining trend through the daytime, as previously reported in a study of both medical students and elderly subjects.[21] Circadian variations in plasma volume may explain these trends.[4]

WHITE CELL COUNT

The increasing mean white cell count through the day has been previously reported, and it was suggested that physical exercise may be a causative factor.[22] Although the mean white cell count is higher in cigarette smokers, the observed diurnal trend was similar in both non-smokers and current smokers.

We have shown that there is a significant diurnal variation in many standard biochemical and haematological tests in a general population of middle-aged men. It has been suggested that such circadian biochemical rhythms should be taken into account by laboratories in setting reference ranges and upper limits of normality for routine use.[23] For most of the biochemical and haematological measurements we studied, the diurnal trends were weak, accounting for 3% or less of the between-subject variance, so that statistical correction for time of day would be a subtle refinement rather than an essential routine. For serum bilirubin, phosphate, and triglycerides, however, the diurnal variation is more substantial. We recommend that the decline in bilirubin concentration through the afternoon and the increasing daytime trends in phosphate and triglycerides values should be taken into account when making clinical interpretations of these three measures.

References

1 Morrison B, Shenkin A, McLelland A, et al. Intra-individual variation in commonly analyzed serum constituents. Clin Chem 1979;25:1799–805.
2 Statland BE, Winkel P, Bokelund H. Factors contributing to intra-individual variation of serum constituents: 1. Within-day variation of serum constituents in healthy subjects Clin Chem 1973;19:1374–9.
3 Winkel P, Statland BE, Bokelund H. The effects of time of venepuncture on variation of serum constituents. Am J Clin Pathol 1975;64:433–47.
4 Young DS. Biological variability. In: Brown SS, Mitchell FL, Young DS, eds. Clinical diagnosis of disease. Amsterdam: Elsevier, 1979:1–113.
5 Markowitz M, Rotkin L, Rosen JF. Circadian rhythms of blood minerals in humans. Science 1981;213:672–4.
6 Shaper AG, Pocock SJ, Walker M, Cohen NM, Wale CJ, Thomson AG. British Regional Heart Study: cardiovascular risk factors in middle aged men in 24 British towns. Br Med J 1981;282:179–86.
7 Shaper AG, Pocock SJ, Ashby D, Walker M, Whitehead TP. Biochemical and haematological response to alcohol consumption Ann Clin Biochem 1985;22:50–61.
8 Ashby D, Pocock SJ, Shaper AG. Ordered polytomous regression: an example relating serum biochemistry and haematology to alcohol consumption. Applied Statistics 1986;35:289–301.
9 Ashby D. A statistical investigation of the relationship of serum biochemistry and haematology to alcohol consumption. (PhD thesis) London University, 1984;7:1–224.
10 Minors DS, Waterhouse JM. Circadian rhythms and the human. Wright PSG: Bristol, 1981.
11 Proceedings of Symposium "Basic and applied chronobiology". Am J Anatomy 1983;168:363–594.
12 McPherson K, Healy MJR, Flynn FV, Piper KAJ, Garcia-Webb P. The effects of age, sex and other factors on blood chemistry in health. Clin Chim Acta 1978;84:373–97.
13 Gidlow DA, Church JF, Clayton BE. Haematological and biochemical parameters in an industrial workforce. Ann Clin Biochem 1983;20:341–8.
14 Broughton PMG, Holder R, Ashby D. Long-term trends in biochemical data obtained from two population surveys. Ann Clin Biochem 1986;23:474–86.
15 Barrett PVD. Hyperbilirubinemia of fasting. JAMA 1971;217:1349.
16 Mills JN. Human circadian rhythms. Physiological Reviews 1966;46:128–71.

17 Markowitz M, Rosen JF, Mizruchi M. Circadian variations in serum zinc concentrations: correlations with blood ionized calcium, serum total calcium and phosphate in humans. *Am J Clin Nutr* 1985;**41**:689·96.

18 Kuo PT, Carson JC. Dietary fats and the diurnal serum triglyceride levels in man. *J Clin Invest* 1959;**38**:1384·93.

19 Thelle DS, Cramp DG, Patel I, Walker M, Marr JW, Shaper AG. Total cholesterol, HDL-cholesterol and triglycerides after a standardized high fat meal *Human Nutr: Clin Nutr* 1982; **36**:469–74.

20 Shaper AG, Pocock SJ, Walker M, Phillips AN, Whitehead TP, McFarlane PW. Risk factors for ischaemic heart disease: the prospective phase of the British Regional Heart Study *J Epidemiol Community Health* 1985;**39**:197–209.

21 Touitou Y, Touitou C, Bogdan A, *et al.* Differences between young and elderly subjects in seasonal and circadian variations of total plasma proteins and blood volume as reflected by hemoglobin, hematocrit and erythrocyte counts. *Clin Chem* 1986;**32**:801–4.

22 Chamberlain AC, Turner FM, Williams EK. The evaluation of white-cell counting in radiation protection. *Br J Radiol* 1952;**25**:169–76.

23 Tedeschi CG. Circadian challenges in quality control. *Hum Pathol* 1973;**4**:281–7.