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SERUM VITAMIN LEVELS AND HUMAN NUTRITION

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Summary

The development of automated techniques for measuring vitamin levels in blood made it possible to examine samples from 2965 volunteers taking part in the Busselton population survey. Red cell folate levels were reduced in 3.1% of those studied and comprised 41 males and 51 females. Eight males and seven females had a reduced level of serum vitamin B12 but with two exceptions the reduction was mild (100-150 ng/l). Five volunteers had an increased vitamin B12 level. Red cell thiamine levels were measured in 1977 samples using a direct automated microbiological assay. Sixty four (3.2%) were found to have a reduced level of this vitamin. Pyridoxal-5-phosphate (vitamin B6) levels were measured in 210 women and 10.7% were below the lower limit of the reference range.

With the exception of pyridoxal which requires further study the blood vitamin levels measured in the adult members of the Busselton population appeared to be adequate.

I. INTRODUCTION

The assessment of the nutritional status of a particular population or group of individuals is not difficult when a large number fall at the lower end of the nutritional scale. That is gross under-nutrition is easily observed. However, the more subtle forms of sub-clinical malnutrition are often difficult to identify even in small groups of people and may present insuperable problems when the numbers run into many thousands.

In dealing with borderline undernutrition a clinical examination may not be helpful and it is necessary to look at other indices which may reflect the level of nutrition.

The measurement of serum vitamin levels may provide a useful means of estimating the nutritional standing of a population. Vitamin B12 is present only in meat, and dairy products. Folic acid is present in green vegetables and pyridoxal is found in wheat and potatoes. If the serum level of these three vitamins is found to be in the normal range, then it may be assumed that nutrition is adequate. A normal blood level must reflect on adequate intake of a particular vitamin. A reduced blood level may be associated with malnutrition or an increased demand. Unfortunately there are a number of complicating factors, for example in protein malnutrition the serum vitamin B12 level is often raised above the upper limit of the normal range. The reason for this is thought to be the inability of the liver to store the vitamin. Under normal conditions the liver is the major storage organ for vitamin B12. On the other hand among members of the Seventh Day Adventist Church 12% have been found to have a sub optimal level of vitamin B12 this is because some of them eschew meat but in other respects maintain an adequate standard of nutrition.

Chronic alcoholics may have a raised serum vitamin B12 level and

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women taking oral contraceptive agents have a reduced level of both folate and vitamin B12 compared with those not taking the pill. However, the levels in these women seldom fall below the lower limits of the reference range (Davis and Smith 1974). The measurement of folate can provide much useful information. For example folate levels within the red blood cell reflect tissue concentration of the vitamin at the time the red cell was produced, while the measurement of serum folate concentrations relate to the intake during the past two weeks. The use of blood vitamin assays in isolated groups such as some Aboriginal communities has in the past provided valuable information on nutrional status.

The conventional methods for measuring these vitamins are tedious and time consuming and are impractical for large scale studies. In recent years a number of automated microbiological assay systems have been developed and these are able to process very large numbers of samples (Davis et al 1970, Davis et al 1973). This facility has made possible the measurement of serum vitamin B12 and serum and red cell folate levels on 2965 samples collected during the Busselton survey. In addition a smaller number of serum pyridoxal (vitamin B6) assays were run as a pilot study. In a more recent survey of the same population group red cell thiamine levels were determined on 1977 samples.

II. RESULTS AND DISCUSSION

The group studied comprised 1518 males and 1447 females, their ages ranged from 20 to over 80 years. Fifty women were pregnant and a further eight thought that they might be. For the purposes of this study the eight were included with the non pregnant group. One hundred and sixty six women admitted to taking oral contraceptive agents.

Blood was collected from volunteers throughout the day over a two week period. No account was taken of so called diurnal variation or the effect of recent meals. It had previously been found that food had only a minor effect on serum vitamin levels table 1.

TABLE 1. Effect of food on serum folate levels.

Subjects	Age Yrs	Fasting	1 hour post Breakfast	1 hour post Lunch	1 hour post Dinner
Male	24	4.9 ug/l	5.6	4.1	4.4
Female	22	7.9	10.3	9.3	6.9
Female	19	5.0	6.5	6.8	7.3
Male	21	5.8	6.1	6.5	6.6

The results of red cell and serum folate levels in both males and females are shown in table 2.

The normal range was derived from measurements made on 100 healthy people and is similar to the range used by most workers in the field. However, there are some difficulties when looking at large population groups particularly when it is likely that the majority of the volunteers will be in good health. Table 2 shows that the use of the mean plus or minus 2 standard deviations as a reference range would bring the lower limit of the range to a point where the level is associated with a marked folate deficiency. The use of the 95 percentile would also predetermine the number of volunteers who were going to be placed in the subnormal group. For this reason we have used our long established reference range to determine which measurements fall into the low range.

TABLE 2. Serum and red cell folate levels in the Busselton population.

	No.	Mean µg/l	Range µg/l	SD	No. below the lower limit of reference range
Serum folate					
Males	1518	5.30	1-17	2.39	56
Females	1447	5.06	0.7-18.5	2.44	59
Red cell folate					
Males	1518	308	54-954	130	41
Females	1447	289	26-1368	135	51

Reference range for both sexes: serum folate 2.7-18.5 µg/l, (levels below 2.5 were counted as low). Red cell folate 115-600 µg/l.

With respect to folate the most important group were those who had both a low serum and red cell folate and there were 17 of these (nine males and eight females). Eight of these were people between the ages of 40 and 60 years. A total of 88 males and 102 females had either a low serum or red cell folate. The means of the folate results appear to be satisfactory but this is difficult to evidence because surveys of this size have not previously been undertaken. Certainly the mean red cell folate appears to be adequate.

There were 166 women taking oral contraceptive agents and their results were compared with the female participants in the study not taking these agents. The results are shown in table 3.

TABLE 3. Mean results of women taking oral contraceptives compared with those of women who were not taking these agents.

	No.	Serum folate (μg/l)	Red cell folate (μg/l)	Serum vitamin B12 (ng/l)
Women taking oral contraceptives	166	4.6	260.9	310.0
Women not using oral contraceptives	901	5.1	292.9	363.4
Significance		$p<0.001$	$p<0.001$	$p<0.001$

There was a significant difference between the mean serum and red cell folate levels and also a significant difference between the mean serum vitamin B12 levels. Eight of the women taking oral contraceptives had a serum folate level below the lower limit of the reference range but only one had both a low serum and red cell level. Decreased vitamin B12 levels have previously been reported in women taking oral contraceptives (Wertalk et al 1972). None of the women in this series had a level below the lower limit of the reference range.

The fall in the mean serum folate level in women taking oral contraceptive agents is very similar to that seen in pregnancy. Davis et al (1969) measured the serum folate concentration in 100 pregnant women and found a mean value of 4.7 μg/l compared with a mean of 4.6 μg/l in the present series.

Recently a microbiological assay for serum and red cell thiamine has been designed using *Lactobacillus fermenti* as the test organism. A direct assay of the vitamin could be expected to provide more substantive evidence than those tests based on the level of activity of a coenzyme such a transketolase in erythrocytes. During the 1978 Busselton Survey red cell thiamine was measured in samples from 1977 volunteers, (987 females and 990 males). There was no significant difference in the results on the basis of sex nor was there any significant difference related to age (tables 4 and 5).

TABLE 4. Busselton survey red cell thiamine. Females

Age (Yrs)	No.	Mean	SD	Range	95 percentile
10-19	95	72.2	11.67	46-110	54-94
20-29	194	72.9	13.9	33-188	52-102
30-39	158	71.0	12.6	52-173	54-110
40-49	189	71.0	13.4	42-135	50-105
50-59	137	75.0	14.0	50-160	54-105
60-69	154	73.6	14.3	35-158	54-105
70-79	46	73.8	15.0	42-140	54-100
80+	14	68.4	11.0	50-84	50-84

Units are in µg/l.

TABLE 5. Busselton survey red cell thiamine. Males.

Age (Yrs)	No.	Mean	SD	Range	95 percentile
10-19	105	73.0	11.0	34-104	54-94
20-29	150	70.6	21.2	40-215	44-94
30-39	141	70.1	12.3	44-118	50-96
40-49	159	69.7	12.0	44-150	50-100
50-59	177	69.2	13.4	44-106	46-100
60-69	187	70.2	14.1	36-185	44-100
70-79	48	69.1	12.0	46-100	48-94
80+	23	71.7	13.4	48-92	48-92

Units are in µg/l.

A total of 64 volunteers (51 males and 13 females) were found to have a reduced level of the vitamin, that is less than 50 µg/l of red cell thiamine pyrophosphate. A minimum level of 50 µg/l was based on the 95 percentile. This is satisfactory for the establishment of a normal range but could be questioned when used to judge the adequacy of the vitamin nutrition within a population. An alternative approach is to use the mean plus or minus 2 standard deviations. This reduced the lower limit of the reference range to 44 µg/l and reduced the number of volunteers with thiamine levels below this lower limit to 26, 20 males and six females. The use of a range based on the mean plus or minus 2 standard deviations brought the lower limit very close to that found in alcoholics. Levels in eight alcoholics ranged from 30 to 50 with seven below 45, mean 39 µg/l. This problem is likely to be due to a skewed distribution of results. Although a number of surveys have been done on thiamine nutrition, measurements have been based on transketolase activity. No large survey embracing nearly 2000 people has previously been attempted. This raised the problem of assessing the adequacy of the thiamine levels found in this population. As an interim measure it was decided that 50 µg/l would be used as the lower limit of the reference range. This was based on the 95 percentile and the levels found in alcoholics. On this basis 3.2% of the group studied in Busselton were thiamine deficient, this is similar to the number who showed evidence of folate deficiency on the basis of red cell folate levels (3.1%). On the basis of smaller studies using the measurement of transketolase other workers have claimed a much higher incidence of thiamine deficiency. Wood and Penington (1974) found 19% of apparently normal people were thiamine deficient. The direct measurement of thiamine levels needs to be used in further population studies before its value can be properly assessed.

The reference range for serum vitamin B12 is 160 to 850 ng/l. One thousand five hundred and eighteen Busselton males had a mean of 365 with an SD of 132 and a range between 90 and 1200 ng/l. For females the mean was 363, SD 144, range 55-1450 ng/l. Eight males and seven females had a vitamin B12 level below 160 ng/l. One female had a concentration of 55 ng/l which is within the range usually associated with pernicious anaemia. One male had a level of 90 ng/l the remainder ranged from 100 to 150 ng/l. Three males and two females had levels above the upper limit of the reference range table 6.

TABLE 6. Distribution of vitamin B12 levels in the Busselton population.

	No.	Mean	SD	Range
		Males		
All	1518	365 ng/l	132	90-1200
Reduced levels	8	121	19	90-150
Raised levels	3	1270	61	1200-1210
		Females		
All	1447	357 ng/l	142	55-1450
Reduced levels	7	118	33	55-145
Raised levels	3	1213	203	1090-1450

Only 0.53% of males and 0.48% of females showed a reduced level of vitamin B12. Possible reasons for the raised level of the vitamin found in six of the volunteers include poor nutrition, alcoholism, liver disease and neoplasia. One hundred and sixty six women taking oral contraceptive agents had significantly lower vitamin B12 levels when compared with women not taking these agents ($p<0.001$) but none in this group fell below the lower limit of the normal range.

For technical reasons it was only possibly to measure serum pyridoxal levels in 210 women. The measurement of this vitamin was done primarily to assess the effect of oral contraceptives. Several workers have claimed that the level of this vitamin is reduced in users of these agents (Adams et al 1973, Baumblatt 1977). Two groups of 105 women matched for age were used in this study one group were taking oral contraceptive agents while the other was not (table 7).

TABLE 7. Comparison of mean serum pyridoxal-5-phosphate levels in 105 women taking oral contraceptive agents and age matched controls.

Age (Yrs)	No.	Taking oral Contraceptives Mean nmol/l	Controls Mean nmol/l	Significance
20-29	46	48.5	51.8	NS
30-39	28	43.2	36.5	NS
40-49	31	36.3	35.1	NS

Among women taking oral contraceptives 9.4% had a level below the lower limit of the reference range compared with 11.3% among the controls. The reference range was constructed from results obtained on samples from 371 apparently healthy volunteers. There was no significant difference in the pyridoxal levels of the two groups and this refutes the claim that oral contraceptives can reduce serum vitamin B6 levels. There is however, little doubt that in some women oestrogenic steroids reduce the affinity for pyridoxal phosphate of two B6 dependent enzymes (kynureninase and kynurenine transaminase).

The methods used in this survey have been used previously to examine underprivileged communities and on these occasions up to 50% of results have been found to be below the lower level of the reference range. In this survey only a modest incidence of vitamin deficiency was apparent. A surprising feature was the result of the thiamine assays which suggested that in Busselton at least thiamine malnutrition was not as serious a problem as has been reported elsewhere in Australia (Wood and Penington 1974). Although it was only possible to examine a few samples for pyridoxal a surprising number 10.7% were below the lower limit of the reference range. This suggests that the intake of this vitamin is borderline and further study is required particularly with respect to vitamin B6/protein intake relationship.

Folate nutrition appeared to be generally adequate with 3.1% of the population having a mildly reduced red cell concentration.

Vitamin B12 nutrition was good. Only 0.4% had reduced levels and this would be compatible with either poor nutrition or vegetarianism in the small number of people involved.

With the exception of pyridoxal-5-phosphate, the vitamin nutrition of the Busselton adult population of the basis of blood vitamin levels appears to be adequate.

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