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Fasting time and vitamin B_{12} levels in a community-based population

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ABSTRACT

Objectives: Vitamin B_{12}, also known as cobalamin (Cbl), is an essential vitamin that manifests with numerous severe but non-specific symptoms in cases of deficiency. Assessing Cbl status often requires fasting, although this requirement is not standard between institutions. This study evaluated the impact of fasting on Cbl levels in a large community-based cohort in an effort to promote standardization of Cbl testing between sites.
Design and methods: Laboratory data for Cbl, fasting time, patient age and sex were obtained from laboratory information service from Calgary Laboratory Services (CLS) for the period of April 2011 to June 2015. CLS is the sole supplier of laboratory services in the Southern Alberta region in Canada (population, approximately 1.4 million). To investigate potential sex-specific effects of fasting on Cbl levels, males and females were analyzed separately using linear regression models.
Results: A total of 346,957 individual patient results (196,849 females, 146,085 males) were obtained. The mean plasma Cbl level was 386.5 (±195.6) pmol/L and 412.0 (±220.8) pmol/L for males and females, respectively. Linear regression analysis showed fasting had no significant association with Cbl levels in females; however a statistically significant decrease of 0.9 pmol/L/hour fasting ($p < 0.001$) was noted in males.
Conclusions: The broad population variance in Cbl suggests the slight gender-specific differences noted in this study are insignificant. Despite this, fasting has the potential to contribute to higher rates of Cbl deficiency in men. Together, these data suggest fasting should be excluded as a requirement for evaluating plasma Cbl.

1. Introduction

Vitamin B_{12}, also known as cobalamin (Cbl), is a water-soluble essential vitamin required for numerous enzymatic functions in the body [1,2]. Cbl deficiency exhibits a broad spectrum of clinical manifestations including neurological, psychiatric, and hematological symptoms and is especially prevalent in the elderly. Indeed, it is estimated that 5–40% of the elderly population may be affected [3,4]. Cbl deficiency commonly results from chronic dietary deficiency, loss of bioavailability due to reduced gastric motility, malabsorption, or autoimmune activity against carrier proteins [2,5]. Deficiency is readily treated with intramuscular injections or oral supplementation of Cbl, though diagnosis remains especially problematic due to the ambiguity of symptoms Statistical summaries of female and male population data are shown in Tables 1 and 2, respectively.

Laboratory measurement of Cbl often requires a fasting sample; however this requirement is not standard between labs. A lack of published data on the effects of fasting on plasma Cbl concentration has resulted in confusion regarding the pre-analytical impact of fasting on plasma Cbl concentration. Some studies have shown that Cbl absorption through the gut requires up to ≥7 h to appear in circulation, however there are no large scale studies demonstrating the effects of fasting on Cbl concentrations [5,6].

2. Materials and methods

2.1. Patient data

Data for this study was collected from the Laboratory Information System at Calgary Laboratory Services (CLS), the sole supplier of laboratory services in the Southern Alberta region of Canada. Annual workload volumes at CLS approach 30 million tests, serving a population of >1.4 million persons. CLS employs a policy whereby lipid panel testing may be conducted at any time, provided the time since last meal (i.e., fasting time) is recorded. This study utilized the data collected for Cbl levels taken at the same time as lipid panels from patients with associated fasting times. The data for Cbl level, patient age, sex, and fasting time were analyzed from April 2011 to June 2015. Data for patients with undefined sex and ages >100 years were excluded to prevent error in analysis due to inaccurate patient information. This study was a quality improvement initiative and therefore did not require ethics approval.

All testing was performed at CLS using a competitive immunoassay based on electrochemiluminescence (Roche Cobas 8000) using a normal range of 155–700 pmol/L, thus Cbl deficiency is defined as plasma Cbl concentration ≤155 pmol/L. The Roche Cbl assay measures total

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Table 1
Summary statistics of the female population data included in this study. The 2.5th and 95th percentiles correspond to the calculated upper and lower limits of normal in this population. The current definition of Cbl deficiency is 155 pmol/L.

Fasting time (h)	Mean age	Sample size (N)	Mean Cbl (pmol/L)	Standard deviation	2.5th Percentile (pmol/L)	97.5th Percentile (pmol/L)	Deficiency rate (%)
1	45.3	5260	423.2	217.6	146	1175	3.5
2	47.2	2861	424.1	214.0	142	1169	3.0
3	48.5	2299	420.5	219.0	142	1122	4.1
4	48.7	1780	416.3	212.7	145	1103	3.4
5	48.5	1208	421.0	217.6	138	1110	4.0
6	47.4	634	393.3	215.6	139	957	4.5
7	47.4	380	431.9	211.0	113	1337	4.8
8	47.1	1093	415.4	239.8	138	1196	6.1
9	45.5	1876	421.9	205.3	134	1221	4.0
10	46.5	9916	428.9	220.1	141	1190	3.8
11	48.7	16,096	429.8	223.9	138	1161	4.2
12	49.9	50,672	427.5	224.3	140	1174	3.9
13	50.9	42,945	428.8	222.0	142	1170	4.0
14	51.4	29,457	428.3	220.8	141	1145	3.6
15	51.5	16,682	424.0	222.0	142	1136	3.8
16	51.4	8762	418.7	216.6	138	1108	3.8
17[a]	49.0	7732	416.1	214.3	139	1107	4.1
Total	50.4	199,653	426.4	250.8	139	1152	3.9

[a] Cbl result differs significantly from the 11–15 hour fasting interval.

circulating Cbl levels by dissociating it from binding proteins using reducing substances and a basic pH. Cbl is then detected by competitive binding using ruthenium-labeled intrinsic factor and biotin-labeled Cbl and streptavidin coated magnetic beads.

Fasting time was recorded by patient estimation of time since last meal and categorized into 1 h intervals from one to 16 by rounding up to the nearest hour. Patients fasting >16 h were grouped into the 16-h interval. Patient results missing information for fasting time, sex or age and repeat measures on the same patient were excluded. Patient ages were grouped into five year intervals.

Statistical analyses were conducted by univariate analysis in the general linear model with Bonferroni post hoc correction in the statistical package SPSS (IBM), ver 20. Analyses were conducted on each sex separately to account for sex-specific differences in fasting effects.

3. Results

A total of 346,949 Cbl records (199,653 (57.6%) female, 147,296 (42.4%) male) with associated fasting time were retrieved from the Laboratory Information System at CLS. Fig. 1A shows the frequency of Cbl deficiency rates in the population by age group. On average, 4.25% of the population were Cbl deficient, with a slightly higher proportion being found in males (4.62%) than females (3.98%). Mean plasma Cbl values were 386.5 and 412.0 pmol/L in males and females, respectively. Fig. 1B shows the total number of tests within each age group to allow interpretation of deficiency rates as a function of test volume.

Fig. 2 summarizes the effect of gender and fasting time on plasma Cbl levels. A boxplot of the data with the population standard deviation (Fig. 2A) demonstrates the high variability in plasma Cbl levels within the entire test population. Fig. 2B shows the mean ± 95% CI in plasma Cbl in male and female populations. Linear regression analysis showed no statistically significant relationship between plasma Cbl level and fasting time (p = NS) in females. Conversely, linear regression of the male population showed a statistically significant decrease of approximately 0.9 pmol/l/h fasting ($p < 0.001$).

4. Discussion

Cobalamin deficiency (plasma concentration < 155 pmol/L) is associated with megaloblastic anemia and neurological disorders, and is especially prevalent in the elderly. If not diagnosed, Cbl deficiency can have significant adverse effects on the quality of life of the deficient

Table 2
Summary statistics of the male population data included in this study. The 2.5th and 95th percentiles correspond to the calculated upper and lower limits of normal in this population. The current definition of Cbl deficiency is 155 pmol/L.

Fasting time (h)	Mean age	Sample size (N)	Mean Cbl (pmol/L)	Standard deviation	2.5th percentile (pmol/L)	97.5th percentile (pmol/L)	Deficiency rate (%)
1[a]	46.9	1747	403.4	202.2	144	1024	3.0
2[a]	48.4	1687	402.8	202.1	133	1017	3.3
3	48.8	1798	400.9	207.4	142	1078	4.7
4	50	1382	397.4	194.3	140	991	3.5
5	48.2	1197	394.9	201.8	124	991	3.9
6	49.5	784	390.1	195.5	150	1081	4.8
7	50.5	576	388.3	207.3	133	923	3.5
8	48	495	381.3	192.4	133	926	3.2
9	46.7	1288	393.6	204.4	132	1110	4.4
10	47.8	2046	386.8	205.6	130	1030	5.2
11	49.3	9594	387.3	204.3	131	1022	5.2
12	50.8	12,972	387.3	204.0	132	999	5.1
13	51.6	44,344	384.5	195.5	134	977	4.7
14	52.4	26,271	385.7	193.7	136	955	4.6
15	52.6	18,847	384.9	191.8	136	968	4.3
16	52.2	10,236	385.6	192.9	140	930	4.1
17	50.8	12,030	388.0	184.7	143	929	3.6
Total	51.3	147,294	386.5	195.6	136	997	4.5

[a] Cbl result differs significantly from the 11–16 hour fasting intervals.