

The prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin

Abstract

The aim of this study is to determine the prevalence of vitamin B12 deficiency among Irish patients with type 2 diabetes mellitus on metformin therapy. We also seek to determine if the dose and duration of metformin use is associated with vitamin B12 deficiency and if the vitamin B12 deficiency is associated with clinical complications. The incidence of type 2 diabetes mellitus in the Irish population is increasing, and it is therefore important to minimise morbidity. Vitamin B12 deficiency is an easily preventable side effect of metformin therapy. It is essential to determine the prevalence of this condition in order to prevent the occurrence of complications, such as peripheral neuropathy and megaloblastic anaemia. Data was collected through the examination of participants' hospital charts and the use of the electronic database (Patient Information Profile Expander) in Beaumont Hospital, Dublin. This study finds a high prevalence of vitamin B12 deficiency in patients with type 2 diabetes mellitus on metformin therapy. An inverse relationship exists between vitamin B12 levels and the dose and duration of metformin use. Therefore, we suggest that the measurement of vitamin B12 should become an essential part of the annual review in all patients with type 2 diabetes mellitus on metformin therapy.

Keywords: Vitamin B12 deficiency, metformin, type 2 diabetes mellitus.

Royal College of Surgeons in Ireland Student Medical Journal. 2011;4(1):16-20.

Introduction

Type 2 diabetes mellitus (T2DM) affects 4.8% of the Irish population, and this figure is expected to increase by 37% over the next decade.¹ The epidemic of T2DM is driven primarily by a sedentary lifestyle, increasing obesity, lack of physical exercise, increased life expectancy, and earlier diagnosis to a certain extent. Metformin, a biguanide, is the first-line oral hypoglycaemic agent used in the treatment of the overweight or obese patient with T2DM.

Metformin acts on several tissues via activation of the adenosine monophosphate-activated protein kinase (AMPK) system to reduce serum glucose. The primary effect of metformin is to suppress hepatic gluconeogenesis.² Side effects of metformin therapy are gastrointestinal upset and, rarely, lactic acidosis. Another clinically relevant side effect of metformin therapy is vitamin B12 deficiency. This side effect is well described, frequently forgotten and not routinely screened for. In 2009, Pflipsen *et al.* showed that 22% of patients with T2DM had a vitamin B12 deficiency, and those on metformin had reduced serum vitamin B12 levels with an increased risk of vitamin B12 deficiency.³ Moreover, Ting *et al.* showed that the dose and duration of metformin use is directly correlated to a decreased

serum level of vitamin B12.⁴ The mechanism by which metformin therapy causes vitamin B12 deficiency is not clear, but it is thought to be due to either alterations in small bowel motility, which stimulate small bowel bacterial overgrowth and subsequent vitamin B12 deficiency, or by directly decreasing vitamin B12 absorption.^{2,5} The absorption of the vitamin B12-intrinsic factor complex by cells of the terminal ileum is calcium dependent, and metformin alters intracellular handling of calcium, thereby reducing absorption.^{2,5} The latter theory is supported by the fact that the administration of calcium reverses metformin-induced vitamin B12 deficiency.⁵

It is important to recognise the clinical consequences of vitamin B12 deficiency. Vitamin B12 is required for DNA synthesis, cellular repair and the normal production of red blood cells. Thus, vitamin B12 deficiency is a known cause of megaloblastic anaemia, characterised by enlarged red blood cells and distinctive changes in neutrophils.⁶ Prolonged vitamin B12 deficiency may also result in neuropathy, ranging from paraesthesia and decreased peripheral sensation to altered mental status, subacute combined degeneration of the spinal cord and dementia.⁶ A proposed mechanism

Omar Marar¹,

Sera Senturk¹,

Amar Agha²,

Chris Thompson²,

Diarmuid Smith²

¹RCSI medical students

²Department of

Endocrinology/RCSI Medical

School, Beaumont Hospital,

Dublin

for these neurological effects is the disturbance of myelin synthesis due to impaired production of methionine.⁷ Therefore, it is worthwhile to consider the prevalence of vitamin B12 deficiency among the growing T2DM population.

The prevalence of vitamin B12 deficiency in patients with T2DM on metformin therapy in Ireland is unknown and the measurement of serum vitamin B12 in T2DM patients on metformin is not part of the standard annual review examination. The objective of this study is to determine the prevalence of vitamin B12 deficiency among Irish patients with T2DM on metformin therapy. This would inform the need for routine annual vitamin B12 measurements in T2DM patients on metformin. We also want to determine whether dose and duration of metformin therapy is associated with vitamin B12 deficiency, and whether vitamin B12 deficiency in patients on metformin is associated with clinical complications such as anaemia or peripheral neuropathy.

Methods

This is a cross-sectional study to examine the prevalence of vitamin B12 deficiency in patients with T2DM on metformin attending the Beaumont Hospital diabetes service.

I. Participant selection

Inclusion criteria for study group: a diagnosis of T2DM and a prescription history of metformin for ≥ 18 months.

Inclusion criteria for control group: a diagnosis of T2DM and no history of metformin use in the past five years.

Exclusion criteria: a diagnosis of pernicious anaemia, vitamin B12 supplementation, malabsorption (coeliac disease, inflammatory bowel disease, gastrointestinal surgery), malnutrition (pure vegans, anorexia nervosa), iron deficiency anaemia, history of thyroid disease and thyroxine treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison's, primary ovarian failure, hypoparathyroidism).

II. Demographic data

The following data was recorded for each patient: age, sex, weight, height, body mass index (BMI), years with diabetes, total daily dose of and years on metformin, haemoglobin (Hb), mean corpuscular volume (MCV), glycosylated haemoglobin (HbA1c), vitamin B12, folate, thyroid-stimulating hormone (TSH), free thyroxine (T4), urine albumin-creatinine ratio (ACR), serum creatinine, and the presence or absence of peripheral neuropathy measured using a 10g monofilament. All data collected was acquired by the examination of participants' hospital charts and their diabetes folders stored in the Diabetes Day Centre, and identification of patients at the weekly diabetes outpatient clinics and through the use of the hospital's electronic database, Patient Information Profile Expander (PIPE). Number of years with diabetes was calculated based on the date of diagnosis as indicated in the medical chart. For those charts that did not mention a particular date of diagnosis, the date of the earliest documentation of a diagnosis of diabetes was used. If the date that metformin was commenced was not available in the medical records, the number of years on metformin was calculated based on the date of the earliest medical record documenting

the use of metformin. The dose of metformin was based on the most recent medical record documenting the dose.

Patients with a vitamin B12 measurement in the three months preceding study initiation were included in the study. Serum vitamin B12 was measured using an immunoassay (Beckman Coulter – UniceL Dxl 800 Access Immunoassay System) with a co-efficient of variation of approximately 10% or less. Low vitamin B12 measurements have low reproducibility. As such, all vitamin B12 readings below 140ng/L are repeated, and the average of the two readings is reported. The severity of vitamin B12 deficiency was split into three groups: mild vitamin B12 deficiency, defined as a vitamin B12 level of 160-180ng/L; moderate deficiency (120-160ng/L); and, severe deficiency (<120ng/L). Anaemia is defined by the World Health Organisation (WHO) guidelines as a Hb value of <12g/dl for females and <13g/dl for males.⁸ A macrocytic anaemia was defined as the presence of a low Hb in conjunction with an MCV of >96fl.

Patients with T2DM attending the diabetes service are reviewed annually to assess their metabolic control and to screen for diabetes-related complications. We are interested in determining whether low vitamin B12 levels are associated with complications such as peripheral neuropathy, and thus refer to data from annual reviews.

III. Aims

The aims of the study were as follows:

1. Determine the background prevalence of vitamin B12 deficiency in patients with T2DM not on metformin and with no history of or treatment for autoimmune disease(s).
2. Determine the prevalence of vitamin B12 deficiency in patients with T2DM on metformin and if the presence of vitamin B12 deficiency correlates with dose or duration of metformin therapy.
3. Determine if metformin-induced vitamin B12 deficiency is associated with anaemia as measured by Hb and MCV, or peripheral neuropathy as measured by the absence of sensation to 10g monofilament.

IV. Statistics

Data was analysed using student's t-test (Microsoft Excel 2007) and Fisher's test (GraphPad 2007).

Results

Two hundred and sixteen patients with T2DM on metformin therapy (137 males, 79 females) were identified, and a control group of 70 patients with T2DM not on metformin therapy (40 males, 30 females) was included in the study. Groups were similar with respect to age, sex, duration of diabetes, weight, BMI and HbA1c (**Table 1**). Seventy-one (33%) patients on metformin had a vitamin B12 deficiency compared to only five (7.5%) in the control group ($p < 0.00001$) (**Table 2**). Among patients with vitamin B12 deficiency on metformin therapy, there was no difference in relation to age, sex, duration of diabetes, BMI or diabetes control as measured by HbA1c (**Table 3**). The dose of metformin inversely affected serum vitamin B12 levels (**Table 3**). We compared the vitamin B12 levels among patients taking less than 1,000mg of metformin per day to patients taking doses over

1,000mg per day, and the difference was found to be statistically significant ($p < 0.008$). This correlation also existed when comparing the average dose of metformin among B12-deficient patients (~2,024mg/day \pm 597.43) to the average dose among the normal patients (~1,793mg/day \pm 578.68) (Figure 1). Duration of metformin therapy was also found to influence vitamin B12 levels. The metformin group and the vitamin B12-deficient group was divided into those who were on metformin therapy for over five years and those who had taken it for less than five years. In the metformin group, those who were on it for less than five years had an average vitamin B12 of 261.5ng/L \pm 132.6, compared to an average vitamin B12 of 227.5ng/L \pm 110.8 in those patients taking metformin for more than five years ($p < 0.043$). With the vitamin B12-deficient patients in the metformin group, no statistical significance was found when comparing those on metformin for less than five years to those on it for over five years ($p < 0.13$) (Figure 2). In the metformin group, 17 patients had a mild vitamin B12 deficiency, 33 had a moderate deficiency and 21 had a severe deficiency. Out of the five deficient patients in the control group, two patients were found to be mildly vitamin B12-deficient, two were moderately deficient and one was severely deficient (Figure 3). Among all patients on metformin therapy, 27% (58/216) were found to be anaemic. In the vitamin B12-deficient metformin group, 31% (22/71) were anaemic. None of the patients had a macrocytic anaemia and only two patients had a macrocytosis. Some 29% (5/17) of the mildly, 18% (6/33) of the moderately and 52% (11/21) of the severely vitamin B12-deficient patients were found to be anaemic. In the group with normal vitamin B12 levels, 24% (35/145) of the patients were anaemic, and none were found to have macrocytic anaemia or macrocytosis. Within the control group,

Table 1: Demographics.

Demographics	Metformin group	Control group	P-value
Sex			
Males (%)	137 (63%)	39 (56%)	
Females (%)	79 (37%)	31 (44%)	
Age (years)			
Average	64 \pm 10.6	66 \pm 10	0.09
Years with diabetes			
Average	8.45 \pm 6.07	8 \pm 6.59	0.83
Weight (kg)			
Average	90.99 \pm 17.60	87.10 \pm 17.66	0.15
BMI (kg/m²)			
Average	33.37 \pm 6.17	32.10 \pm 6.51	0.42
HbA1c (%)			
Average	7.27 \pm 1.29	7.13 \pm 1.32	0.44

BMI: body mass index; HbA1c: haemoglobin A1c.

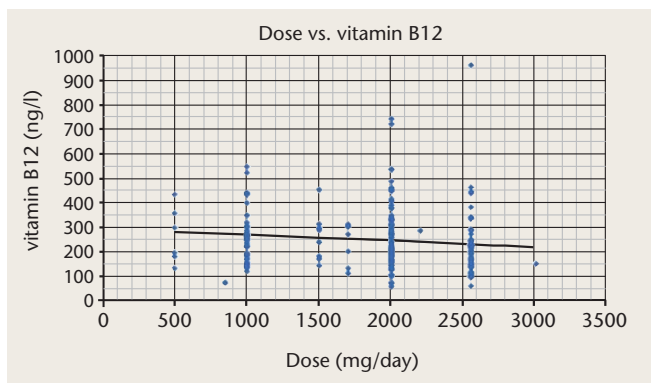


FIGURE 1: The inverse relationship (r -value = -0.12) between the dose of metformin (mg/day) and vitamin B12 levels (ng/L).

Table 2: Metformin group vs. control group.

Demographics	Metformin group	Control group	P-value
Weight (kg)			
Average	90.99 \pm 17.60	87.10 \pm 17.66	0.15
Range	50-141.8	50.90-140	
B12 (ng/L)			
Average	247.98 \pm 125.23	323.23 \pm 112.21	0.00001
Range	60-961	81-658	
Hb (g/dL)			
Average	13.30 \pm 1.51	13.38 \pm 1.47	0.7
Range	9.8-16.7	9.1-16.8	
MCV (fl)			
Average	86.13 \pm 4.99	86.76 \pm 4.59	0.34
Range	70.9-103.5	69.4-95.7	
HbA1c (%)			
Average	7.27 \pm 1.29	7.13 \pm 1.32	0.44
Range	5-13.3	5.2-11.1	
Creatinine (umol/L)			
Average	79.76 \pm 21.41	88.89 \pm 38.08	0.07
Range	45-159	13-245	
TSH (mIU/L)			
Average	2.01 \pm 1.21	1.84 \pm 0.95	0.22
Range	0.27-9.37	0.19-4.92	
Neuropathy			
Absent	151 (94%)	43 (96%)	
Present	10 (6%)	2 (4%)	

Hb: haemoglobin; MCV: mean corpuscular volume; HbA1c: haemoglobin A1c; TSH: thyroid-stimulating hormone.

Table 3: Metformin group – vitamin B12-deficient vs. normal.

Metformin group			
Demographics	Deficient (≤180ng/L)	Normal (>180ng/L)	P-value
Number of patients	71 (33% of total)	145 (67% of total)	
Number of males	49 (69%)	88 (61%)	
Number of females	22 (31%)	57 (39%)	
Age (years)			
Average	64 ± 9.85	63 ± 10.96	0.46
Range	41-90	36-91	
Weight (kg)			
Average	94.47 ± 17.66	89.35 ± 17.40	0.067
Range	61.2-141.8	50-133.4	
BMI (kg/m²)			
Average	33.61 ± 6.61	33.26 ± 6.05	0.84
Range	27.5-48.6	22.3-51.4	
B12 (ng/L)			
Average	136.51 ± 29.56	302.57 ± 117.74	<0.00001
Range	60-179	180-961	
Hb (g/dL)			
Average	13.05 ± 1.50	13.43 ± 1.50	0.085
Range	9.8-16.3	9.9-16.7	
MCV (fl)			
Average	85.47 ± 5.08	86.46 ± 4.94	0.18
Range	72.1-98.5	70.9-103.5	
HbA1c (%)			
Average	7.25 ± 1.13	7.28 ± 1.36	0.87
Range	5.3-11.8	5-13.3	
Creatinine (umol/L)			
Average	79.39 ± 23.62	79.94 ± 20.38	0.88
Range	45-159	48-158	
TSH (mIU/L)			
Average	2.02 ± 1.42	2.01 ± 1.10	0.98
Range	0.46-9.37	0.27-5.76	
Dose (mg/day)			
Average	2023.94 ± 597.43	1793.45 ± 578.68	0.008
Range	500-3000	500-2550	
Years on metformin			
Average	5.59 ± 2.72	5.40 ± 3.39	0.66
Range	2-14	2-24	
Years with diabetes			
Average	9.45 ± 6.98	8.12 ± 5.66	0.28
Range	2-42	2-34	
Neuropathy			
Absent	49 (92.5%)	102 (96%)	0.73
Present	4 (7.5%)	6 (4%)	

BMI: body mass index; Hb: haemoglobin; MCV: mean corpuscular volume; HbA1c: haemoglobin A1c; TSH: thyroid-stimulating hormone.

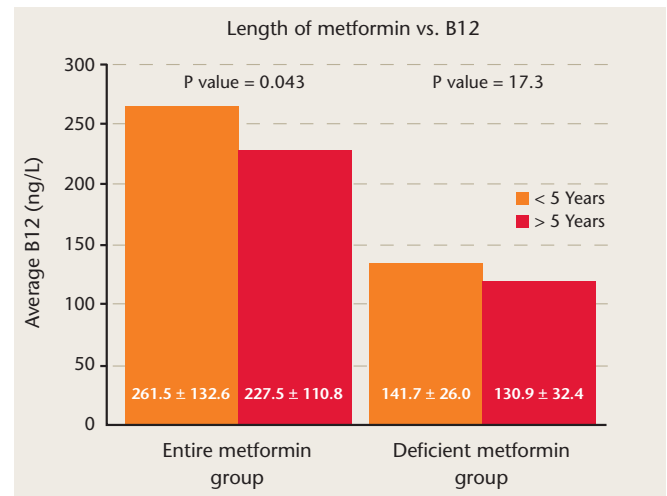


FIGURE 2: This relationship suggests that the duration of metformin therapy may influence vitamin B12 levels in the entire metformin group and the vitamin B12-deficient metformin subgroup.

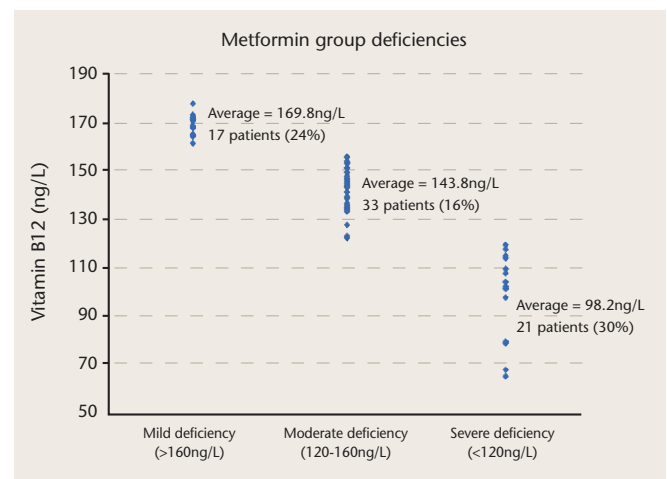


FIGURE 3: The number of patients in the vitamin B12-deficient metformin sub-divisions with mild (>160ng/L), moderate (120-160ng/L) and severe (<120ng/L) vitamin B12 deficiency.

14% (10/70) were anaemic. No patients in the control group had a macrocytosis. Peripheral neuropathy was not recorded in all patients. In the vitamin B12-deficient patients on metformin, four out of 53 (7.5%) recorded values indicating neuropathy compared to six out of 108 (4%) recorded values for the patients on metformin with normal vitamin B12 levels. Among the vitamin B12-deficient controls (five patients), three neuropathy values were recorded, one of which was positive (33%). Within the normal controls, 42 neuropathy values were recorded, one of which was positive (2%).

Discussion

In this cross-sectional study, 27% (76/286) of patients with T2DM in the study group had a vitamin B12 deficiency. In those patients on metformin therapy, it was found that vitamin B12 deficiency existed in one out of three patients, compared to one out of 14 patients in the

control group. Both of these figures are higher than in previously reported studies.^{3,4}

Pflipsen *et al.* found that 22% (44/203) of their patients with T2DM had a vitamin B12 deficiency.³ The researchers obtained informed consent from patients that met the inclusion criteria and collected data by having the participants complete a survey with questions about demographics, medication and supplement use, and exclusion criteria.³ Although the demographics and exclusion criteria used by Pflipsen *et al.* are very similar to those used in this study, the method of data collection differs; Pflipsen *et al.* only examined participant medical records through charts and the hospital's database, and identified patients at the weekly diabetes outpatient clinics. Pflipsen *et al.* had an advantage by using surveys, which allowed for the immediate and up-to-date delivery of information. Another part of the survey required the patient to indicate their use of metformin, insulin (any form), other hypoglycaemic medications, acid blockers (H₂ blockers and/or proton pump inhibitors), herbal supplements, multivitamins, and B-complex vitamins;³ this study only documented patients taking metformin and excluded those on vitamin B12 supplements. Additionally, Pflipsen *et al.* defined vitamin B12 deficiency as serum vitamin B12 levels <100pg/mL or serum vitamin B12 levels of 100-350pg/mL with elevation of serum methylmalonic acid >243nmol/L or homocysteine >11.9nmol/L.³ In this study, deficiency parameters consisted of three subdivisions of vitamin B12 levels into mild (160-180ng/L), moderate (120-160ng/L) and severe (<120ng/L). Therefore, it is evident that the methods and parameters used by Pflipsen *et al.* produced a more refined and representative T2DM study population in comparison to this study with regard to the level of vitamin B12 deficiency overall.

Likewise, Ting *et al.* showed an increased risk of vitamin B12 deficiency associated with duration of metformin and dose, and found these parameters to be high risk factors for developing B12 deficiency.⁴ In this study, the data showed a statistically significant inverse relationship between length of metformin use and vitamin B12 levels when comparing patients on metformin for five years or less to those taking it for more than five years. Thus, there is an increased risk of developing vitamin B12 deficiency in patients with T2DM on metformin therapy for over five years. There was a statistically significant correlation between the dose of metformin and vitamin B12 deficiency – the higher the dose, the lower the average vitamin B12 level. This is similar to the

finding by Ting *et al.*, in which there was a two-fold increase in risk of developing vitamin B12 deficiency with each 1g/day dose increment.⁴ Despite the significantly increased prevalence of vitamin B12 deficiency among the metformin group when compared to the controls, it was not associated with neuropathy or macrocytic anaemia. The majority of patients, both in the metformin and control groups, did not have neuropathy. However, the recorded values for neuropathy were too few to draw any solid conclusions regarding the risk of neuropathy among those with vitamin B12 deficiency. Possible explanations for the lack of association with neuropathy or macrocytic anaemia in our study may be the small study population, or that these side effects require longer periods of deficiency in order to manifest.

Anaemia was found to be prevalent in all groups of patients: 31% in the vitamin B12-deficient metformin group, 24% in the metformin group with normal serum vitamin B12 levels and 14% in the control group. The high incidence of anaemia in patients with T2DM is a concern and may reflect the association of T2DM with shortened red cell lifespan or possibly mild renal impairment. However, since there was no statistically significant correlation between the average Hb or MCV and vitamin B12 levels in the groups, we cannot conclude that vitamin B12 is responsible. The weaknesses of the study are a lack of sufficient data, as illustrated by the incomplete peripheral neuropathy data, and dependency on diabetic charts without full examination of each patient's medical records, since many of the diabetic files had incomplete data. However, the data on serum vitamin B12 levels and metformin therapy, including dose and duration of therapy, were available for all patients. All of the serum vitamin B12 levels were performed within three months of the start of the study and so reflect the current risk of vitamin B12 deficiency in patients with T2DM on metformin attending Beaumont Hospital's diabetes service. We have thus demonstrated a high prevalence of vitamin B12 deficiency in patients with T2DM treated with metformin. As such, we suggest that the measurement of vitamin B12 should become an essential part of the annual review in all patients with T2DM on metformin therapy.

Acknowledgements

We would like to thank the staff of the Diabetes Day Centre in Beaumont Hospital for their tremendous help during this study.

References

1. Diabetes Federation of Ireland [homepage on the internet]. Tablets for type 2 diabetes. Updated 2009. Cited February 14, 2010. Available from: http://www.diabetes.ie/WebSite/Content/educational_articles/2009_Type2medication.aspx.
2. Diamanti-Kandarakis E, Christakou CD, Kandaraki E, Economou FN. Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol*. 2009;162(2):193-212.
3. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Topolski R. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross sectional study. *J Am Board Fam Med*. 2009;22(5):528-34.
4. Ting RZ, Szeto CC, Chan MH, Ma KK, Chow KM. Risk factors of vitamin B12 deficiency in patients receiving metformin. *Arch Internal Med*. 2006;166:1975-9.
5. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. *Age and Ageing*. 2006;35(2):200-1.
6. American Association for Clinical Chemistry [homepage on the internet]. Vitamin B12 and folate deficiency. Updated 2010. Cited February 21, 2010. Available from: <http://www.labtestsonline.org/understanding/conditions/vitaminb12-4.html>.
7. Toosi TD, Shahi F, Afshari A, Roushan N, Kermanshahi M. Neuropathy caused by B12 deficiency in a patient with ileal tuberculosis: A case report. *J Med Case Reports*. 2008;2:90.
8. World Health Organisation. Indicators and Strategies for Iron Deficiency and Anemia Programmes. Report of the WHO/UNICEF/UNU Consultation. Geneva, Switzerland, December 6-10, 1993. WHO, 1994.