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The impact of vegetarianism on some haematological parameters

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Abstract: *Objective*: Subjects adopting a vegetarian diet are liable to vitamin B12 and iron deficiencies. Co-existing vitamin B12 and iron deficiencies may give an equivocal haematological picture, which may, in turn, delay making an early diagnosis. The current work was undertaken to investigate some haematological parameters in relation to vitamin B12 and iron status in vegetarians. *Subjects and methods*: Twenty-nine vegans, 64 lacto- and lacto-ovo-vegetarians, in addition to 20 occasional meat eaters, were enrolled for this study. The total group included 49 males and 64 females aged [mean (SD) = 46(15) yr]. Complete blood count, methylmalonic acid (MMA), homocysteine (HCY), ferritin, and transferrin concentrations and percentage transferrin saturation were assayed, using conventional methods. *Results*: Vegans displayed the highest MMA and HCY levels (median MMA = 708 nmol L^{-1}; HCY = 12.8 μmol L^{-1}). A lower lymphocyte count and a higher mean corpuscular volume (MCV) were found in vegans compared with lacto- or lacto-ovo-vegetarians (median = 1.51×10^9 vs. 1.83×10^9 L^{-1}; 92 vs. 89 fL, respectively). Vitamin B12-deficient subjects in the higher range of transferrin saturation percentage had higher MCV than vitamin B12-deficient subjects in the lower transferrin saturation range (mean MCV = 92 vs. 89 fL). A lower platelet count was found in the highest quartile of MMA (mean = 211×10^9 L^{-1}) and in the highest quartile of HCY (mean = 215×10^9 L^{-1}), compared with the other quartiles. Lower lymphocyte and platelet counts and higher MCV were found in subjects with elevated MMA and HCY, compared to those with normal metabolites. Factors that explained the variations in MCV were red blood cell count, ferritin, transferrin saturation, and methylmalonic acid levels. *Conclusion*: vitamin B12 and iron status were compromised by a vegetarian diet. Variations in mean corpuscular volume were determined by iron and vitamin B12 status. Lower lymphocyte and platelet count were accompanied by metabolic evidence that indicated vitamin B12 deficiency.

Key words: cobalamin; iron; homocysteine; methylmalonic acid; ferritin; transferrin; mean corpuscular volume

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Extensive efforts have been made in recent years to try to promote the health of vegetarians by clarifying the concept of a balanced diet and defining the requirements for some essential micronutrients (1). Follow-up studies supplied evidence in favour of a vegetarian lifestyle (2). However, the beneficial effects of a vegetarian diet may be counterbalanced by the deprivations of micronutrients, mostly available in animal products (3).

Vitamin B12 is among the many nutrients reported to be reduced in vegetarians (4, 5). Vitamin B12, the cofactor for methionine synthase, plays a crucial role in the one-carbon metabolism and in transferring a methyl group from 5-methyltetrahydrofolate to homocysteine (HCY). Vitamin B12 occurs almost exclusively in foods of animal origin (6). Vitamin B12 deficiency might be expected in subjects ingesting predominantly, or solely, plant foods. On the contrary, folate requirements may be satisfactorily met by a plant-rich diet. However, cells chronically depleted of vitamin B12 are expected to display impaired folate utilization, due to impaired generation of tetrahydrofolate (7). This anomaly is important in rapidly proliferating cells such as the enterocytes and the haematopoietic system, in the latter case being expressed as macro-ovalocytic erythrocytes (7, 8).

Iron is another micronutrient reported to be marginal in vegetarians (9). Iron occurs in a plant-origin diet in the non-heme form, which is less bioavailable than the heme form in animal products (10). The effect of the diet on iron haemostasis may vary greatly and may depend on the concomitant availability of other nutrients (10).

A vegetarian diet is therefore common ground for the etiologies of vitamin B12 and iron deficiencies. Concomitant vitamin B12 and iron deficiencies may be presented in the absence of a classic haematological picture (11–13). Additionally, vitamin B12 and iron deficiencies were related to increased susceptibility to infectious diseases, which has suggested a role for these micronutrients in the immune system (14, 15). The present work was undertaken to investigate some haematological aspects resulting from different degrees of animal-food deprivation. We also studied these aspects with simultaneous insights on iron, vitamin B12, and folate status.

Subjects and methods

Subjects included in this study were volunteers recruited at a conference of the German Federation of Vegetarians, or at a summer camp of the Vegan Society of the Netherlands. Subjects were stratified into three groups, according to their habitual dietary pattern. Twenty-nine subjects reported a plant-based diet (vegans), 64 subjects were lacto-or lacto-ovo-vegetarians, and 20 subjects had a reduced intake of meat (once weekly). The last group was called semi-vegetarians. Subjects with acute or chronic diseases were not eligible for this study. The total group included 64 females and 49 males [mean age (SD) = 46 (15) yr]. Blood samples were collected after a 12 h fast. Serum samples were placed directly on ice and centrifuged within 45 min at 4000 r.p.m. for 10 min Several aliquots were prepared and kept at −70 °C for subsequent analyses. All subjects agreed to participate in the study and gave their consent after being informed in detail about the study.

Total serum homocysteine (HCY) and methylmalonic acid (MMA) concentrations were measured by gas chromatography – mass spectrometry (GCMS). Deuterated HCY and MMA were used as internal standards. The between-day coefficient of variation for HCY and MMA assays was 5.3% and 3.2%, respectively. Serum folate and total serum vitamin B12 levels were assayed by chemiluminescence immunoassay (Bayer). Complete blood count was performed, using a haematology analyser (Sysmex XE-2100^{TM}). Serum iron levels were photometrically assayed, and serum ferritin concentrations were measured using immunoturbidimetric assay (Roche Diagnostics). Serum transferrin levels were evaluated, using nephelometric assay (DADE Behring).

Statistics

Data analyses were performed using SPSS for windows (version 9.0). The two-tailed Mann–Whitney test was used for inter-group comparisons. Backward regression analyses were applied, using the row data or the log transformed one (for the skewed parameters). All tests were considered significant whenever $P < 0.05$.

Results

Vegans had the highest median MMA level, the lowest median serum vitamin B12, and the highest median HCY level, compared to the other two groups (Table 1). A higher mean corpuscular volume (MCV) and a lower lymphocyte count were found in vegans compared with lacto- lacto-ovo-vegetarians (Table 1). The prevalence of some abnormal metabolic and haematological markers are presented in Fig. 1. Data from all subjects were pooled and divided into subgroups, according to HCY and MMA levels (Table 2). Compared to subjects who had normal HCY and MMA levels, subjects with elevated HCY and MMA levels had been on a vegetarian diet longer (median 13 vs. 7 yr) and they had a lower lymphocyte count (median 1.60 vs. 1.79×10^9 L^{-1}), a lower platelet count (median 228 vs. 243×10^9 L^{-1}), and higher MCV (median 91 vs. 88 fL) (Table 2). A lower platelet count was found in the upper HCY quartile compared with the other three quartiles (mean 215×10^9 vs. 249×10^9 L^{-1}; $P = 0.019$) (Fig. 2A). A lower platelet count was also found in subjects within the upper quartile of MMA, compared to the other three quartiles (mean 211×10^9 vs. 250×10^9 L^{-1}; $P = 0.006$) (Fig. 2B).

To investigate the influence of concomitant low iron and vitamin B12 status on mean corpuscular volume, subjects were stratified with respect to transferrin-saturation percentage values into two subgroups (below or equal to and greater than the median in the total group) (Fig. 3). Only within the upper range of transferrin saturation was the mean MCV significantly higher in vitamin B12-deficient than non-deficient subjects (mean MCV = 92 vs. 88 fL; $P = 0.003$). Comparing vitamin B12-deficient subjects, higher mean MCV was found in subjects within the higher transferrin-saturation

Table 1. Biochemical and haematological parameters as displayed by the study participants

Parameter	Semi-vegetarians	Lacto-/lacto-ovo-vegetarians	Vegans	P^a	P^b	P^c
Number	20	64	29			
Male/Female	8/12	28/36	13/16			
Serum vitamin B12, pmol L^{-1}	218 (158–302)	192 (135–358)	148 (112–290)	0.105	0.004	0.016
MMA, nmol L^{-1}	206 (147–649)	355 (154–1535)	708 (243–3024)	0.005	0.000	0.001
Homocysteine, μmol L^{-1}	8.7 (6.5–44.9)	10.5 (7.1–23.3)	12.8 (8.1–37.0)	0.159	0.006	0.050
Folate, nmol L^{-1}	32.0 (14.6–52.8)	28.8 (17.1–57.3)	31.8 (20.7–69.5)	0.717	0.508	0.160
Creatinine, μmol L^{-1}	75 (62–88)	71 (53–88)	71 (53–80)	0.062	0.020	0.448
Haemoglobin, g dL^{-1}	13.2 (11.0–15.6)	13.5 (11.9–15.4)	13.9 (12.4–15.6)	0.809	0.193	0.132
Lymphocyte count, ×10^9 L	–1.72 (1.35–2.36)	1.83 (1.26–2.76)	1.51 (1.08–2.37)	0.450	0.090	0.014
MCV, fL	90 (84–95)	89 (82–95)	92 (87–96)	0.347	0.136	0.007
Platelet count, ×10^9 L^{-1}	242 (196–310)	241 (173–318)	228 (143–306)	0.987	0.146	0.120
MPV, fL	10.9 (9.9–11.9)	11.1 (9.9–12.2)	11.4 (10.1–12.9)	0.474	0.132	0.201
Iron, μg dL^{-1}	93 (26–169)	91 (42–162)	80 (51–201)	0.652	0.729	0.486
Transferrin, g L^{-1}	2.3 (1.9–2.9)	2.4 (1.9–2.8)	2.3 (1.9–2.8)	0.682	0.807	0.813
Transferrin saturation,%	29 (7–45)	28 (12–49)	26 (15–59)	0.797	0.745	0.504
Ferritin, μg L^{-1}						
Female	20 (12–184)	30 (12–66)	21 (12–76)	0.457	0.792	0.478
Male	76 (28–163)	36 (17–100)	30 (15–160)	0.059	0.247	0.624

Data are median (10th/90th) percentiles.

[a] Comparing semi with lacto-lacto-ovo-vegetarians.

[b] Comparing vegans to semi-vegetarians.

[c] Comparing vegans to lacto-lactoovo-vegetarians. MCV values were missing in 12 subjects.

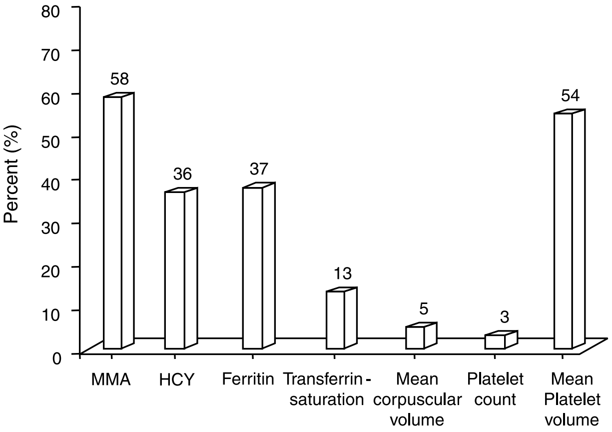

Fig. 1. Prevalence of some abnormal metabolic and haematological markers in the total study population. The following cut-off levels were considered; elevated MMA > 271 nmol L^{-1}; elevated HCY > 12 μmol L^{-1}; low ferritin < 30 and 15 μg L^{-1} for males and females, respectively; low transferrin-saturation percentage < 16%; high mean corpuscular volume > 97 fL; low platelet count < 140 × 10^9 L^{-1}; elevated mean platelet volume > 11.0 fL.

percentage compared to those in the lower transferrin-saturation percentage (mean MCV = 92 vs. 89 fL; $P = 0.036$) (Fig. 3).

Backward multiple regression analyses were performed on the pooled data to find out the determinants of MCV or platelet count (outcome variable) that can be explained by changes in some independent variables (Table 3).

Discussion

Our current study included subjects who had been on several types of alternative diet. Not surprisingly, vitamin B12 status was compromised by the degree of animal food restriction. Eighty-five percent of our subjects had metabolic evidence that indicates vitamin B12 deficiency (elevated MMA) (Fig. 1). Vitamin B12 deficiency was consistently found in vegetarians (4, 5, 16). Concern has grown in recent years about neurological damage, which may occur in vitamin B12-deficient subjects, in the absence of classic haematological disturbances (16, 17). We observed in the current study several haematological liabilities that seemed to be related to the degree of animal food restriction.

Iron-deficient erythropoiesis is a stage that proceeds anaemia. In this stage ferritin levels become decreased, transferrin levels become increased and percentage saturation of transferrin become reduced (18). These sensitive tests are supposed to uncover iron-deficient subjects who lack the classical features of deficiency (18). 37% of our total subjects had low ferritin levels. Out of 64 females aged [mean(SD)] 47(16) yr, low ferritin levels were presented in 28 females. Impaired erythropoiesis in the case of vitamin B12 deficiency may result in an iron overload picture, and normal iron status indices may not exclude iron deficiency (13). Our data supplied evidence that vitamin B12 deficiency-induced macrocytosis can be masked when iron status is in a state of negative balance (Fig. 3) (11, 12). Vitamin B12 deficiency might induce a secondary defect in the enterocytes, which may further cause iron loss before being assimilated (8).

Only 5% of the currently investigated subjects had MCV > 97 fL (Fig. 1). Variations in MCV were accounted for by iron and vitamin B12 status

Table 2. The influence of vitamin B12 deficiency on some haematological parameters

Parameter	Normal MMA/ normal HCY	Elevated MMA/ normal HCY	Elevated MMA/ elevated HCY	P^a	P^b
Number (%)	41 (36)	31 (27)	35 (31)		
Age (yr)	48 (24–66)	47 (17–76)	43 (25–62)	0.695	0.340
Time on alternative diet (yr)	7 (2–22)	10 (3–43)	13 (4–44)	0.199	0.002
Serum vitamin B12 (pmol L^{-1})	235 (158–423)	177 (127–302)	148 (117–230)	0.006	<0.001
MMA (nmol L^{-1})	200 (144–258)	477 (296–1599)	936 (461–2708)	<0.001	<0.001
Homocysteine (μmol L^{-1})	8.4 (6.3–10.7)	9.3 (7.1–11.3)	17.9 (12.7–45.8)	0.009	<0.001
Folate (nmol L^{-1})	32.2 (16.5–60.6)	31.6 (20.5–75.3)	29.5 (18.1–60.0)	0.666	0.191
Creatinine (μmol L^{-1})	71 (55–88)	71 (53–80)	71 (62–88)	0.279	0.799
Lymphocyte count (×10^9 L^{-1})	1.79 (1.35–2.67)	2.04 (1.34–2.77)	1.60 (0.98–2.27)	0.699	0.010
Mean corpuscular volume (fL)	88 (83–95)	90 (85–95)	91 (83–98)	0.149	0.026
Platelet count (×10^9 L^{-1})	243 (193–318)	231 (179–335)	228 (143–293)	0.397	0.024

Data are median (10th–90th) percentiles. Comparing (normal MMA/normal HCY) group; [a]to (elevated MMA/normal HCY) group; [b]to (elevated MMA/elevated HCY) group. Normal HCY <= 12 μmol L^{-1}; normal MMA <= 271 nmol L^{-1}. Only six subjects had an isolated elevation of HCY concentrations.

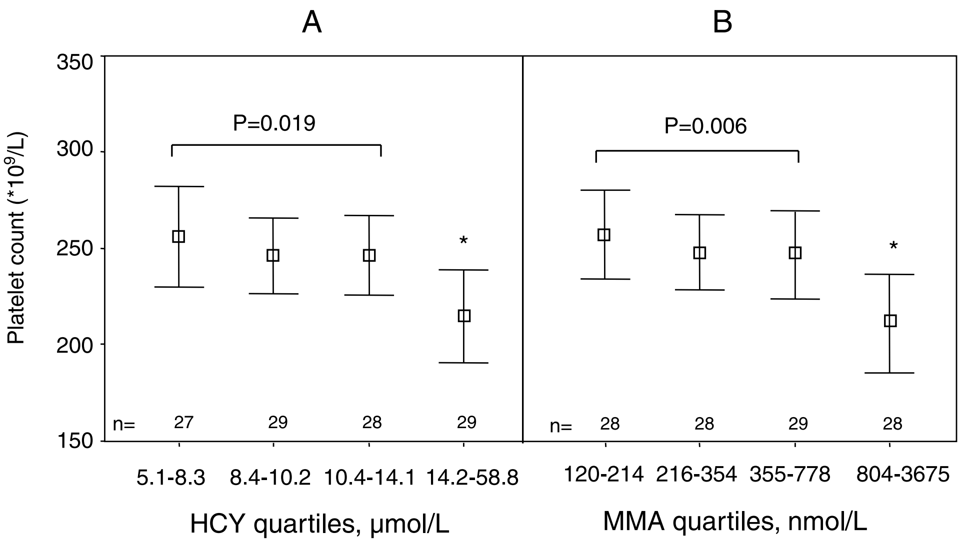

Fig. 2. Mean platelet count (95% confidence intervals) according to quartiles of (A) homocysteine concentrations and (B) methylmalonic acid concentrations. *Significant differences compared with the last group presented.

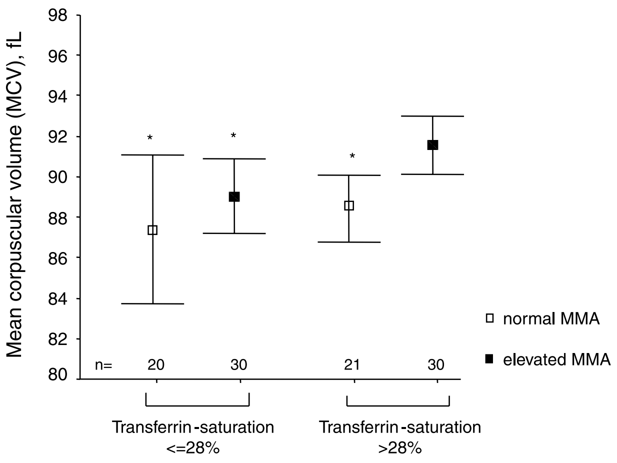

Fig. 3. The effect of cobalamin and iron status on mean corpuscular volume (MCV). Transferrin saturation = 28% is the median of the total collective. Mean MCV and 95% confidence intervals are illustrated in the figure. *Significant differences compared with the last group presented.

Table 3. Stepwise multiple regression analyses for the pooled data to find out the determinants of mean corpuscular volume or platelet count

Independent variables	Beta (95% CI)^a	P
Mean corpuscular volume (outcome variable)		
Red blood cell count	−5.96 (− 8.31/− 3.61)	<0.001
Ferritin	+3.82 (1.17/6.47)	0.005
Methylmalonic acid	+3.87 (1.62/6.12)	0.001
Transferrin-saturation	+0.1 (0.04/0.16)	0.003
Platelet count (outcome variable)		
Ferritin	−43.9 (− 71.76/− 16.2)	0.002
Methylmalonic acid	−31.0 (− 57.5/− 4.6)	0.022
Mean platelet volume	−24.5 (− 35.7/− 13.3)	<0.001

[a] Beta coefficient and 95% confidence intervals are indicated. All the independent variables were continuous.

(MMA), with a dominating effect for iron status (Table 3, Fig. 3). Microcytosis was reported to dominate over macrocytosis when iron deficiency is more severe than vitamin B12 deficiency (8).

Vitamin B12 deficiency was accompanied by increased susceptibility to some infections (14), and the role of vitamin B12 in the production of antibodies has been addressed (19). We found lower lymphocyte and platelet counts in subjects with elevated MMA and HCY levels, compared with those with normal metabolites (Table 2). Thrombocytopenia was found in only 3% of our subjects; however, 54% had elevated mean platelet volume (> 11.0 fL) (Fig. 1). Given that platelets have a six-fold higher content of vitamin B12 than red blood cells (20), the present data suggest a prominent role for vitamin B12 in platelet life cycle. Thrombocytopenia was reported in vitamin B12-deficient subjects (16), and remission of most of the haematological disturbances was achieved after vitamin B12 therapy (21, 22). Contrary to our findings, a higher platelet count was found in vegetarians compared with non-vegetarians (3). Increased platelet aggregation, in addition to shorter bleeding time, could be corrected after supplementation of n-3 fatty acids (3). Other micronutrient deficiencies or unknown factors that may have contributed to the present findings could not be ruled out in our study. Further studies are needed to find out the clinical significance of our findings. Vitamin supplementation trials may help to find out if these observations are reversible.

In conclusion, our findings in subjects who have adopted different types of diet suggest that concomitant iron and vitamin B12 deficiencies may account for the near absence of megaloblastic anaemia. The lower platelet count and the giant platelet form were accompanied by metabolic evidence that indicated vitamin B12 deficiency. The beneficial effects expected from a vegetarian diet should be evaluated in the light of the potential harm which may arise from iron and vitamin B12 deficiencies.

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