Original Article

Homocysteine concentrations lowered following dietary intervention in an Aboriginal community

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Low circulating folate concentrations lead to elevations of plasma homocysteine. Even mild elevations of plasma homocysteine are associated with significantly increased risk of cardiovascular disease (CVD). Available evidence suggests that poor nutrition contributes to excessive premature CVD mortality in Australian Aboriginal people. The aim of the present study was to examine the effect of a nutrition intervention program conducted in an Aboriginal community on plasma homocysteine concentrations in a community-based cohort. From 1989, a health and nutrition project was developed, implemented and evaluated with the people of a remote Aboriginal community. Plasma homocysteine concentrations were measured in a community-based cohort of 14 men and 21 women screened at baseline, 6 months and 12 months. From baseline to 6 months there was a fall in mean plasma homocysteine of over 2 □mol/L ($P = 0.006$) but no further change thereafter ($P = 0.433$). These changes were associated with a significant increase in red cell folate concentration from baseline to 6 months ($P < 0.001$) and a further increase from 6 to 12 months ($P < 0.001$). In multiple regression analysis, change in homocysteine concentration from baseline to 6 months was predicted by change in red cell folate ($P = 0.002$) and baseline homocysteine ($P < 0.001$) concentrations, but not by age, gender or baseline red cell folate concentration. We conclude that modest improvements in dietary quality among populations with poor nutrition (and limited disposable income) can lead to reductions in CVD risk.

Key Words: homocysteine, community-based intervention, nutrition, Aboriginal people.

Introduction

A low intake of fresh vegetables and fruit may contribute to elevated cardiovascular disease (CVD) risk at least partly through its association with poor folate status. Low circulating folate concentrations lead to elevations of plasma homocysteine by inhibiting the reconversion of homocysteine to methionine. Even mild elevations of plasma homocysteine are associated with significantly increased risk of CVD.[1]

We have previously documented dietary patterns associated with high CVD risk among Aboriginal people: high intakes of saturated fat and low intakes of fresh vegetables and fruit.[2] These observations are consistent with the high CVD mortality among Indigenous Australians.[3] From 1989, a health and nutrition project was developed, implemented and evaluated with the people of a remote Aboriginal community. The project was initiated by community members following premature deaths from CVD of several young people. The intervention resulted in decreases in dietary sugar and saturated fat, increases in fruit and vegetables in the food supply, and corresponding improvements in biochemical indices (cholesterol, folate, vitamins C and B_6) among community members.[4] The aim of the present study was to examine the effect of this intervention on plasma homocysteine concentrations in a community-based cohort.

Subjects and methods

Intervention processes, demography and trends in other risk factors have been described previously.[4] As part of evaluation methodology, fasting blood samples were collected and kept

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cold until centrifugation (within 1 hour). Plasma was subsequently stored frozen at –20 to -45°C. In a longitudinal cohort attending screening at baseline, 6 month and 12 month time points (n = 35) plasma homocysteine concentrations were measured using an automated fluorescence polarisation immunoassay (coefficient of variation: <5%).[5] Red cell folate, pyridoxal and vitamin B_{12} were measured by automated microbiological methods at the department of Haematology, Royal Perth Hospital (reference ranges: red cell folate, 115-600 ng/mL; pyridoxal, 22-54 nmol/L for women and 25-66 nmol/L for men; vitamin B_{12}, 60-850 µg/L). Exact mid-P confidence intervals (CI) around proportions were calculated (PEPI version 3.01; USD Inc., Stone Mountain GA, USA) and adjusted using finite sampling factor correction ($\sqrt{[(N-n)/(N-1)]}$, where N is population size and n is sample size).[6] Changes in plasma homocysteine and red cell folate over time were tested by repeated measures ANOVA. Predictors of change in plasma homocysteine from baseline to 6 months were determined using stepwise linear regression analysis with age, gender, baseline red cell folate concentration, baseline homocysteine concentration and change in red cell folate from baseline to 6 months as independent variables. Statistical analyses were performed using SPSS 10 (SPSS Inc., Chicago IL, USA). Statistical significance was taken as $P < 0.05$.

The study was conducted with the approval of the community and the Joint Institutional Ethics Committee of the Royal Darwin Hospital and the Menzies School of Health Research.

Table 1. Characteristics of the longitudinal cohort, with comparisons to the cross-sectional survey samples. Data for age refer to baseline time point. Continuous data are mean (95% confidence interval), categorical data are prevalence (95% confidence interval)

	Longitudinal cohort (N= 35)	Total† (N= 68, 51, 47)
Age (years)	39 (34 - 44)	38 (35 - 41)
Male gender (%)		
Baseline	40 (29 - 52)	49 (47 - 51)
6 months	-	47 (41 - 53)
12 months	-	47 (40 - 54)
Red cell folate (ng/mL)		
Baseline	82 (74 - 91)	82 (76 - 88)
6 months	140 (123 - 156)	135 (123 - 147)
12 months	208 (172 - 245)	191 (172 - 210)
Serum folate (ng/mL)		
Baseline	2.3 (1.8 - 2.7)	2.1 (1.7 - 2.5)
6 months	3.1 (2.6 - 3.6)	2.8 (2.4 - 3.2)
12 months	3.1 (2.6 - 3.7)	2.8 (2.4 - 3.2)
Vitamin B_{12} (ng/L)		
Baseline	725 (684 – 766)	755 (752 - 758)
6 months	626 (569 – 683)	650 (646 - 654)
12 months	744 (705 – 783)	743 (739 - 747)
Pyridoxal (nmol/L)		
Baseline	42 (38 – 46)	43 (39 - 46)
6 months	47 (41 – 53)	43 (37 - 49)
12 months	66 (58 – 73)	66 (57 - 74)

† from reference 4.

Results

Homocysteine data were available for a cohort of 14 men and 21 women screened at baseline, 6 months and 12 months. This cohort was indistinguishable from the cross-sectional survey samples (the latter representing 94%, 76% and 68% of the resident population respectively)[4] with respect to age, gender and red cell and serum folate levels, and serum pyridoxal and vitamin B_{12} levels (Table 1). The mean (s.d.) age was 39 (14) yr and age ranged from 17 to 72 yr. At baseline, all but 3 subjects were folate deficient (red cell folate <115 ng/mL), none were vitamin B_{12} deficient (<60 µg/L), and one person was pyridoxal deficient (<22 nmol/L). No participants were currently prescribed metformin at the time of the study. Two subjects had minor elevations of serum creatinine: a 64 year-old woman (110 µmol/L) and a 36 year-old man (130 µmol/L). Neither of these subjects showed a decrease in plasma homocysteine.

There was a significant increase in red cell folate concentrations over the course of the intervention ($P < 0.001$) in this cohort, with significant increases from baseline to 6 months ($P < 0.001$) and from 6 months to 12 months ($P < 0.001$). Vitamin B_{12} varied significantly over the course of intervention ($P = 0.015$) due to a decrease at the 6 month time point (all subjects remained Vitamin B_{12} replete throughout; Table 1). Pyridoxal varied significantly over the course of intervention ($P< 0.001$) due to a significant increase between 6 and 12 months (Table 1).

Figure 1A shows the average change in red cell folate concentrations relative to baseline. There was a significant change in mean plasma homocysteine over the course of the intervention ($P = 0.005$; Fig. 1B): from baseline to 6 months there was a fall of over 2 µmol/L ($P = 0.006$), but no further change thereafter ($P = 0.433$). The result remained significant when that individual with a fall of over 10 µmol/L homocysteine was excluded from the analysis (data not shown).

Stepwise multiple regression analysis was used to examine predictors of change in homocysteine from baseline to 6 months as described above. Change in homocysteine concentration was independent of age ($P = 0.338$), gender ($P = 0.124$) and baseline red cell folate ($P = 0.928$). Change in red cell folate concentration ($\beta = -0.343$, $P = 0.002$) was a significant predictor of the change in plasma homocysteine identified by this model, with a fall of 0.32 µmol/L for each 10 ng/mL increase in red cell folate concentration (Fig. 2). Baseline homocysteine concentration was also a significant predictor of change in homocysteine ($\beta = -0.709$, $P < 0.001$): the average decrease was greater by 0.6 µmol/L for each 1 µmol/L increment in baseline homocysteine. R-square for this regression model was 0.65. Change in homocysteine was not related to changes in vitamin B_{12} ($P = 0.878$) or pyridoxal ($P = 0.785$).

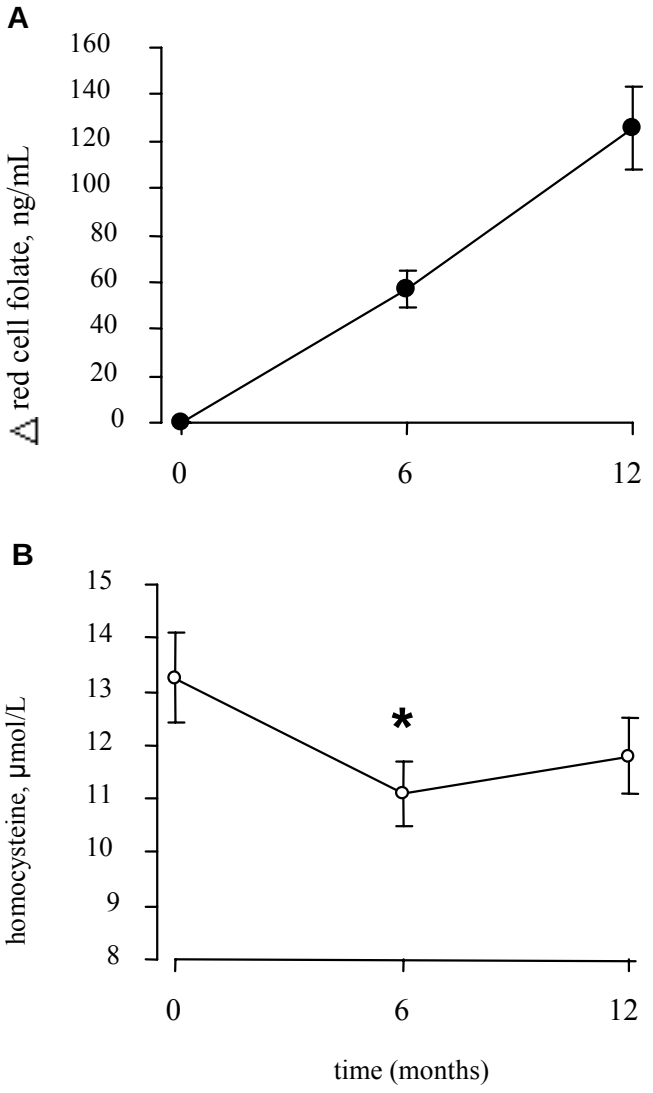

Figure 1. (A) Change in red cell folate concentrations relative to baseline in the longitudinal cohort (n = 35); (B) Plasma homocysteine concentrations, 0-12 months in the longitudinal cohort. Asterisk (*) indicates significant difference from previous time point. Data are mean ± S.E.M.

Discussion

This study has demonstrated major improvements in plasma homocysteine following a community-based dietary intervention. From a high population mean homocysteine at baseline, after 6 months the mean concentration approached that seen in populations at far lower risk of CVD.[7] A difference in average plasma homocysteine of similar magnitude to the change observed here (around 2 µmol/L) was associated with up to a 50% lower risk of CVD in some studies.[8,9] Elevated plasma homocysteine has been previously reported in urban Aboriginal people.[10] That study also reported the prevalence of the thermolabile variant of the methyltetrahydrofolate reductase gene, which is associated with hyperhomocysteinaemia, to be similar to that seen in European populations. Thus hyperhomocysteinaemia among Aboriginal people appears to be largely of dietary origin. In the present study, average intake of fruit and vegetables doubled over the period described here to reach approximately 180g/person/day, a figure approaching that for the wider Australian population (240g/person/day, which was nevertheless substantially less than recommended).[11] These improvements remained apparent three years after the initial intervention period.[12]

A limitation of the present study is a lack of information on renal function, as renal impairment tends to elevate plasma homocysteine. Most participants had normal serum creatinine at baseline and we have assumed that renal function did not alter significantly over the period studied. This might be considered a conservative assumption, given that any renal disease present would likely progress, tending to increase plasma homocysteine and thus biasing our results towards the null.

The present results are encouraging as they indicate that modest improvements in dietary quality among populations with poor nutrition can lead to major reductions in CVD risk, and that this can be achieved with realistic dietary change and in the absence of supplementation.

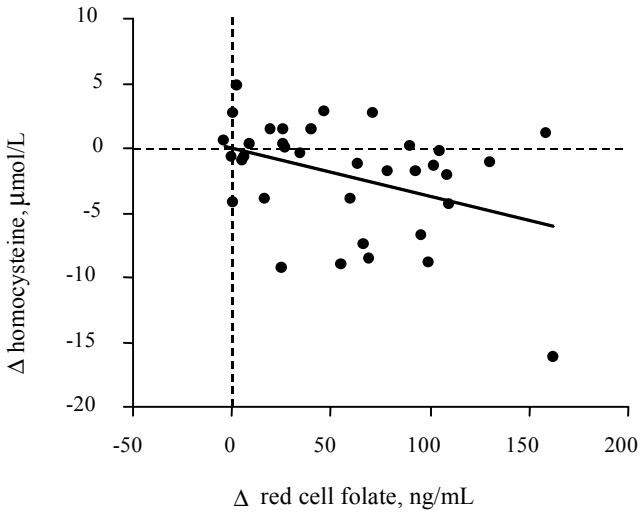

Figure 2. Relationship between change in red cell folate and change in plasma homocysteine concentrations, baseline to 6 months

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