Iron nutrition and anaemia in a malaria-endemic environment: haematological investigation of the Gidra-speaking population in lowland Papua New Guinea

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Blood examination was conducted for the four Gidra-speaking village groups in Papua New Guinea, who were characterized by high Fe intake and high malaria prevalence with marked inter-village differences. The northern riverine villagers, whose Fe intake was higher than the other three village groups, did not suffer from Fe-deficiency anaemia in their malaria-endemic environment; nor did the inland villagers, with their second highest Fe intake and their malaria-free environment, suffer from Fe-deficiency anaemia. However, several individuals of the southern riverine village suffered from anaemia in a malaria-endemic environment, although their Fe intake was almost the same as the inland villagers'. A considerable proportion of the coastal villagers were anaemic, reflecting the lowest Fe intake and the highest malaria prevalence. An inter-village comparison of the relationships between haemoglobin levels and transferrin saturation revealed that the southern riverine villagers needed smaller amounts of circulating Fe for erythropoiesis than the northern riverine and inland villagers, reflecting the long-term human-environment conditions such as the density of malaria vectors and the people's dietary habits. Fe supplementation was not judged effective against hypoferraemia and/or anaemia in such a population. As the incidence of malaria had no significant long-lasting effect on Fe stores or circulating Fe concentration, but did have an effect on anaemia, the hypothesis that malaria causes a transfer of Fe from the blood to parenchymal tissues as a defence against infectious diseases was not supported.

Iron: Anaemia: Malaria

Anaemia, which affects approximately 30% of the world's population (Kent, 1992), is an important health problem because it may increase maternal morbidity and decrease physical work capacity owing to reduction in O_2 delivery to the tissues (World Health Organization (WHO), 1975). Diagnosis of anaemia is based solely on the haemoglobin level being lower than the criteria according to the sex and age of a person and the altitude of his or her residence (WHO, 1972, 1975).

Nowadays, Fe supplementation is widely used to treat and prevent any type of anaemia because the commonest cause of anaemia is Fe deficiency and because discrimination between Fe-deficiency anaemia and other types of anaemia is difficult and costly (WHO, 1975). However, Fe supplementation sometimes has two kinds of harmful side-effects: one

is parenchymal tissue injury caused by Fe overload (Pippard, 1990); the other is so-called refeeding-malaria, in which Fe supplementation makes malaria worsen and accelerates anaemia (Murray *et al.* 1975). Malaria, the annual incidence of which is estimated at over 200 million in the tropics, is a serious public health problem and the major cause of haemolytic anaemia. Thus, refeeding-malaria, a quite ironical phenomenon, has aroused interest in health scientists all over the world.

Kent (1992) states that hypoferraemia (low Fe level in serum) is more often attributed to chronic diseases and inflammations than to shortage of dietary Fe. She and her colleagues insist that hypoferraemia, or even anaemia, plays a protective role against infectious diseases as an Fe-withholding defence system (Wadsworth, 1992; Weinberg, 1992). This system of higher Fe retention in tissue than in serum is considered 'adaptive', though it is difficult to confirm it directly because numerous natural and iatrogenic factors can compromise this system. In the present paper this phenomenon is hereafter called 'hypoferraemic adaptation.'

The hypoferraemic adaptation hypothesis can be examined based on the interrelationship between malaria prevalence and Fe nutritional status in a population whose Fe intake level is sufficiently high, whose malaria prevalence differs according to residential area, and whose prevalences of other severe diseases are low over the area. If the hypothesis is true, the extent of a shift of Fe from serum to liver should differ, depending on malaria prevalence, in such a population. The Gidra-speaking population in lowland Papua New Guinea, which has been investigated by the authors for over 25 years, is characterized by high Fe intake levels in all villages (Hongo *et al.* 1989*b*) and large inter-village differences in malaria prevalence (Nakazawa *et al.* 1994), making it suitable for testing the hypoferraemic adaptation hypothesis.

The present paper aims to clarify the Fe metabolic mechanisms in the Gidra and to contribute to answering the question of whether Fe supplementation is beneficial to malaria-endemic areas.

MATERIALS AND METHODS

Subjects

The Gidra-speaking people, whose *de facto* population was slightly over 2000 in 1989, inhabit thirteen villages scattered in their 4000 km^2 territory between the Fly River to the north and the Torres Strait to the south, in Papua New Guinea. The villages are ecologically grouped into four: northern riverine villages along the Binaturi (*n* 2), inland villages set apart from any river (*n* 6), southern riverine villages (*n* 4), and a coastal village (*n* 1). Four villages, each representing one of the four village groups, were selected in our long-standing intensive human ecology study (e.g. Ohtsuka & Suzuki, 1990), and thus are the subjects of the present study.

The Gidra villagers have maintained traditional village life without marked sanitary improvements and medical care. They have basically subsisted on local foods through exploitation of sago, horticulture, hunting, fishing, and gathering, although the cash economy has been introduced to different extents, depending on accessibility to Daru, the capital town of the Western Province. As a result, their dietary habits, for instance, have gradually changed. The coastal villagers consume large amounts of purchased foods such as rice, flour, and tinned fish, whereas other villagers consume almost exclusively local products such as sago flour, garden crops, game meat, and aquatic animals.

A 2-week food consumption survey in these four villages in 1981 revealed three findings relevant to this study. First, the mean energy intake per day per adult male ranged from 12·5 MJ in the southern riverine village to 14·9 MJ in the northern riverine village, and the

mean protein intake ranged from 54·3 g in the northern riverine village to 73·3 g in the coastal village; this result indicated sufficient supply of energy and protein (Ohtsuka *et al.* 1985). Second, animal foods accounted for 47% of protein intake on average (Ohtsuka *et al.* 1985). Third, the Fe intake per day per adult male was 100 mg in the northern riverine villagers, 60 mg in the inland and southern riverine villagers, and 30 mg in the coastal villagers (Hongo *et al.* 1989*b*). These amounts of Fe are much higher than 8 mg, the recommended value for adult males whose foods are recognized at an intermediate level of bioavailability of Fe, and are higher than 15 mg, the recommended value at a low level of bioavailability of Fe (DeMaeyer, 1989); because of the high (47%) contribution of animal foods to protein intake, the intermediate bioavailability of Fe is appropriate to the present study.

Major dietary sources of Fe in the Gidra consisted of sago flour, garden crops and land animals. There were relatively small inter-village differences in contributions of garden crops and land animals to Fe intake, from 19 to 23 mg and from 6 to 9 mg respectively. Sago flour consumption caused inter-village differences in Fe intake level, from 4 mg in the coastal village to 39 mg in the northern riverine village. In addition, the northern riverine villagers consumed 31·3 mg Fe from cycad (*Cycas circinalis*) and lotus (*Nelumbo nucifera*) seeds, which contain 360 and 100 mg Fe/kg respectively (Hongo *et al.* 1989*a*), and the southern riverine villagers consumed 13·5 mg from shellfish (Hongo *et al.* 1989*b*).

In 1989, blood samples were collected by five or six research team members, including a trained local health officer belonging to the Health Department of Western Province, after obtaining informed consent from the villagers. The subjects, who were apparently healthy adult males and females (pregnant females were excluded) aged from approximately 15 to over 50 years, numbered 264 in the four villages; they were more than 80% of the total adult population. For the convenience of the participants, the blood sampling was conducted all day long; thus there was no restriction on eating on the day before sampling. This sampling condition induced undesirable biases in the blood indices reflecting circadian rhythm and/or timing of dietary intake, but they are judged not to have significant influence on inter-village comparisons, the major analytical focus of this study.

The results of examination of antibody titre levels of two *Plasmodium* species have already been reported (Nakazawa *et al.* 1994). The most important result relevant to the present paper was that all coastal villagers and more than 90% of northern and southern riverine villagers had antibodies against *Plasmodium falciparum* with titres of 1:64 or higher, while only 30% of the inland villagers had such antibody levels; in the present paper, an individual with a titre of 1:64 or higher, which indicates repetitive infection and/or recent (within several months) infection, is treated as malaria-positive.

Analytical methods

About 10 ml blood was extracted with a disposable syringe from the antecubital vein, and treated without anti-coagulant. Haemoglobin was measured on a small portion of the whole blood with the cyanmethaemoglobin method (Haemoglobin-Test *Wako*, Wako Pure Chemical Industries, Ltd, Osaka, Japan) within 10 s after sampling. The remaining part was centrifuged at 3000 rev/min (1360 *g*) for 30 min within 5 h after sampling. Our preparatory examination of twelve blood samples from healthy Japanese indicated that 5 h was a short enough time to avoid serum Fe increases induced by haemolysis.

The serum samples were stored in a portable freezer just after centrifugation. Then they were transported by air to Japan within 1 month after sampling, and stored at −20° until measurement. Serum Fe was measured using the bathophenanthroline method (Fe-Test *Wako*, Wako Pure Chemical Industries, Ltd) and the accuracy of measurements was confirmed on each occasion, using the reference serum (Ortho Liquid Reference Serum I

and II for Chemistry, Ortho Diagnostic Systems, Raritan, NY, USA); the measured values of the reference serum ranged within ±5% of the certified value. Serum transferrin concentration was measured by a simple radial immunodiffusion method, using the kit for human serum (NOR partigen plate for transferrin, Hoechst Japan, Tokyo, Japan). The accuracy of the quantification was confirmed with control serum in the kit; all measurements were within the certified ranges, and the CV was 6·3%. Total Fe-binding capacity (TIBC) was estimated according to the following formula (Guindi *et al.* 1988):

$$\text{TIBC (mmol/l)} = 1{\cdot}4 \times \frac{\text{serum transferrin (mg/l)}}{76\,500},$$

where the figure 76 500 is the mean molecular weight of apotransferrin.

It is reasonable to judge that transferrin saturation, which was estimated according to the formula below, indicates the whole circulating Fe amount since protein nutritional status was sufficient among the Gidra:

$$\text{transferrin saturation (\%)} = \frac{\text{serum Fe (mg/l)}/55{\cdot}85}{\text{TIBC (mmol/l)}} \times 100.$$

Serum ferritin, the best indicator of Fe stores and essential for diagnosis of Fe deficiency because of its high specificity (Cook *et al.* 1974; Milman *et al.* 1983; Wickramasingle *et al.* 1985; Goh & Hariharan, 1986; Kumar *et al.* 1989), was measured with the recently developed enzyme-linked immunosorbent assay (ELISA) using the kit for human serum (IR-1400 'MITSUI', Kainos Laboratories, Inc., Tokyo, Japan). All samples were measured in duplicate and were re-measured in cases where the intra-duplication difference exceeded 10%. The accuracy of the determination was confirmed with control serum in the kit; all measurements of the control sera were within the certified ranges, and the CV was 5·9%.

Fe deficiency and Fe overload were defined as follows (Expert Scientific Working Group, 1985). The samples of the former category satisfied the criterion for serum ferritin level and at least one of the two criteria for transferrin saturation and haemoglobin levels, as follows:

(1) serum ferritin < 12 μg/l;
(2) transferrin saturation < 16%;
(3) haemoglobin < 130 g/l for males, haemoglobin < 120 g/l for females.

The samples of the latter category had more than 70% transferrin saturation.

Of the 264 samples, four were not analysed for serum ferritin because of extremely small amounts, and fifty-eight were not analysed for transferrin saturation and/or haemoglobin for such reasons as small amounts and occurrence of haemagglutination. Therefore, the remaining 206 samples were analysed in the present study.

Statistical methods

Three indices for Fe nutritional status (haemoglobin, transferrin saturation, and serum ferritin) were statistically analysed by village and sex. First, the normality of distribution was examined by the Kolmogorov–Smirnov test for each index. For indices with normal distribution, arithmetic means were compared among villages by Tukey's honestly significant difference test. For other indices, logarithmically transformed means were compared by the same test.

An inter-village comparison of haemoglobin concentration adjusted for transferrin saturation was conducted, using the analysis of covariance (ANACOVA) to examine the functional differences of erythropoiesis; to test the differences in slope, the interaction between covariates and group was included in the model, and it was excluded to test the difference in elevations.

Table 1. *Descriptive statistics of haemoglobin concentration (g/l) for four villages in Papua New Guinea by sex*

(Mean values and standard deviations)

						Deficiency	
	n	Mean	SD	Skewness	Normality*	*n*	%
Males							
N. riverine	15	158·9[a]	9·6	−0·83	0·07	0	0·0
Inland	17	158·1[a]	8·5	−0·52	> 0·15	0	0·0
S. riverine	40	145·1[b]	9·3	0·73	> 0·15	1	2·5
Coastal	21	135·6[b]	19·4	−0·86	> 0·15	7	33·3
Total	93	147·6	14·9	−1·06	< 0·01	8	8·6
Females							
N. riverine	26	146·2[a]	10·7	−0·60	> 0·15	0	0·0
Inland	20	146·7[a]	7·6	−1·00	> 0·15	0	0·0
S. riverine	35	132·5[b]	11·0	−0·42	> 0·15	4	11·4
Coastal	32	128·8[b]	15·6	−1·37	< 0·01	6	18·8
Total	113	137·1	14·2	−1·00	< 0·01	10	8·8

[a, b] Mean values within a sex category with unlike superscript letters were significantly different by Tukey's HSD test ($P < 0·05$).

* Probability for the null hypothesis: 'sampled from a normal distribution population'.

Haemoglobin concentration adjusted for transferrin saturation was compared between malaria-positive and -negative groups, using ANACOVA; the judgement of malaria-positive or -negative was based on our previous report (Nakazawa *et al.* 1994). In addition, to examine the effect of malaria infection on the relationship between circulating Fe and Fe stores, transferrin saturation adjusted for serum ferritin was compared between the two groups using ANACOVA.

All of these statistical analyses were conducted using the Statistical Analysis Systems program (SAS Institute, 1982*a*, *b*), and the statistical significance levels were set at 0·05 in all tests.

RESULTS

The distribution patterns of haemoglobin concentration for most village and/or sex groups showed negative skewness, but none of the distributions differed from normal distribution except for females in the coastal village (Table 1). The northern and inland villagers of both sexes showed higher haemoglobin levels than the riverine and coastal villagers. There were no, or few, anaemic individuals in the northern riverine and inland villages, whereas 33·3% of males and 18·8% of females were anaemic in the coastal village; the proportion of anaemic individuals in the southern riverine village was in between these values.

Transferrin saturation did not fit the normal distribution for males of the coastal village and females of the inland, southern riverine, and coastal villages (Table 2), owing to higher outliers (one each in the four groups) who probably suffered from malaria and intravenous haemolysis. Data pooled for all villages showed positive skewness that differed significantly from normal distribution ($P < 0·01$) for either sex, probably due to inequality in sample size and different mean levels among villages. The highest mean transferrin saturation level was found in the inland village for both sexes, although the inter-village difference was not significant.

Table 2. *Descriptive statistics of transferrin saturation (%) for four villages in Papua New Guinea by sex*

(Mean values and standard deviations)

						Deficiency		Overload	
	n	Mean	SD	Skewness	Normality*	*n*	%	*n*	%
Males									
N. riverine	15	39·1	17·4	0·53	> 0·15	1	6·7	1	6·7
Inland	17	40·1	12·5	0·26	> 0·15	0	0·0	0	0·0
S. riverine	40	29·2	10·6	0·70	> 0·15	2	5·0	0	0·0
Coastal	21	34·7	24·3	3·14	< 0·01	2	9·5	1	4·8
Total	93	33·9	16·4	2·40	< 0·01	5	5·4	2	2·2
Females									
N. riverine	26	28·4	8·7	0·24	> 0·15	2	7·7	0	0·0
Inland	20	29·6	12·1	1·09	0·03	1	5·0	0	0·0
S. riverine	35	25·4	11·8	1·03	0·03	6	17·1	0	0·0
Coastal	32	23·2	12·6	1·36	< 0·01	10	31·3	0	0·0
Total	113	26·2	11·6	0·91	< 0·01	19	16·8	0	0·0

* Probability for the null hypotheses: 'sampled from a normal distribution population'.

Table 3. *Descriptive statistics of serum ferritin (μg/l) for four villages in Papua New Guinea*

(Mean values and standard deviations)

							Deficiency	
	n	Mean	SD	GM	Skewness	Normality*	*n*	%
Males								
N. riverine	15	154·5	103·5	118·8[a]	0·97	0·07	0	0·0
Inland	17	80·2	46·9	68·0[a, b]	0·98	0·08	0	0·0
S. riverine	40	55·2	33·4	48·0[b]	2·10	< 0·01	0	0·0
Coastal	21	128·6	97·5	91·1[a]	0·84	0·03	1	4·8
Total	93	92·3	78·2	68·4	1·78	< 0·01	1	1·1
Females								
N. riverine	26	99·7	62·3	81·7[a]	1·07	0·03	0	0·0
Inland	20	67·7	44·8	56·3[a, b]	1·30	< 0·01	0	0·0
S. riverine	35	34·4	21·4	28·7[c]	1·81	< 0·01	3	8·6
Coastal	32	51·5	39·7	39·0[b, c]	2·10	< 0·01	3	9·4
Total	113	60·1	48·8	44·9	1·82	< 0·01	6	5·3

GM, geometric mean.

[a, b, c] Mean values not sharing a common superscript letter were significantly different, $P < 0·05$ (Tukey's HSD test with logarithmic transformation).

* Probability against the null hypothesis: 'sampled from a normal distribution population'.

Only two individuals were judged to have Fe overload: one in the northern riverine village and the other in the coastal village. The latter individual, a male with over 100% transferrin saturation, which theoretically occurs in the case of intravenous haemolysis, was suffering from severe malaria infection at the time of sampling. It is reasonable to judge that

Table 4. *Number of iron-deficient individuals in four villages in Papua New Guinea by village and sex*

Village	Males			Females		
	Normal	Deficient	%	Normal	Deficient	%
N. riverine	15	0	0·0	26	0	0·0
Inland	17	0	0·0	20	0	0·0
S. riverine	40	0	0·0	33	2	5·7
Coastal	20	1	4·8	29	3	9·4
Total	92	1	1·1	108	5	4·4

Table 5. *Comparison of haemoglobin concentrations (g/l) among four villages in Papua New Guinea with transferrin saturation (%) as a covariate*

Village	*n*	Unadjusted mean	Adjusted mean	SEM*
Males				
N. riverine	15	158·9[a]	159·4[a]	3·16
Inland	17	158·1[a]	158·8[a]	3·00
S. riverine	40	145·1[b]	144·5[b]	1·96
Coastal	21	135·6[b]	135·7[c]	2·66
Comparison of slopes: F 5·39 ($P < 0·01$)				
Comparison of elevations: F 16·6 ($P < 0·01$)				
R^2 0·36				
Females				
N. riverine	26	146·2[a]	146·0[a]	2·37
Inland	20	146·7[a]	146·5[a]	2·71
S. riverine	35	132·5[b]	132·5[b]	2·03
Coastal	32	128·8[b]	129·0[b]	2·14
Comparison of slopes: F 0·75 ($P = 0·52$)				
Comparison of elevations: F 14·7 ($P < 0·01$)				
R^2 0·31				

[a, b, c] Mean values with unlike superscript letters were significantly different, $P < 0·05$ (Tukey's HSD test).
* Standard error of the adjusted mean estimates.

his transferrin saturation value did not reflect Fe overload derived from high Fe intake, as will be discussed later. It is noteworthy that males of all villages and females of the northern riverine and inland villages had relatively small proportions of transferrin saturation deficiency (less than 10% in any group), in contrast to females in the southern riverine and coastal villages, who showed proportions of 17·1 and 31·3% respectively.

Serum ferritin levels showed positive skewness in all sex and/or village groups and both sex groups of all villages combined, although those for males of the northern riverine and inland villages did not differ from normal distribution ($P = 0·07$ and $P = 0·08$ respectively). Thus, they were logarithmically transformed for statistical analyses (Table 3), and the log-transformed data satisfied normal distribution. Comparison between sexes revealed that serum ferritin level was higher in males in any village. Inter-village comparison demonstrated that the serum ferritin level was highest in the northern riverine village and

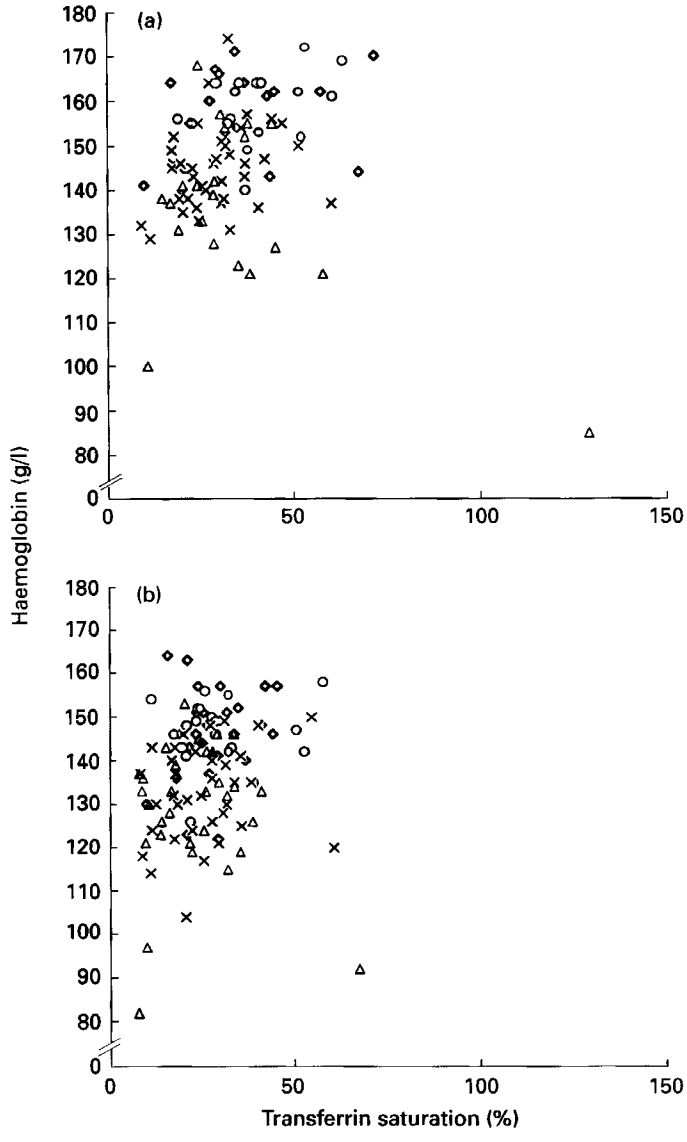

Fig. 1. Relationship between haemoglobin concentration (g/l) and transferrin saturation (%) in (a) male and (b) female subjects from different villages in Papua New Guinea. (◇), N. riverine; (○), inland; (X), S. riverine; (△), coastal.

lowest in the southern riverine village; those in the coastal and inland villages were in between. These inter-village differences accorded with the prevalence of low serum ferritin, i.e., 4·8% in males of the coastal village, 8·6% in females of the southern riverine village, and 9·4% in females of the coastal village.

Fe-deficient individuals numbered six, one male in the coastal village, three females in the coastal village, and two females in the southern riverine village (Table 4). There were possibilities of false negative for the subjects who were classified in the normal category, particularly in the coastal village, as discussed later.

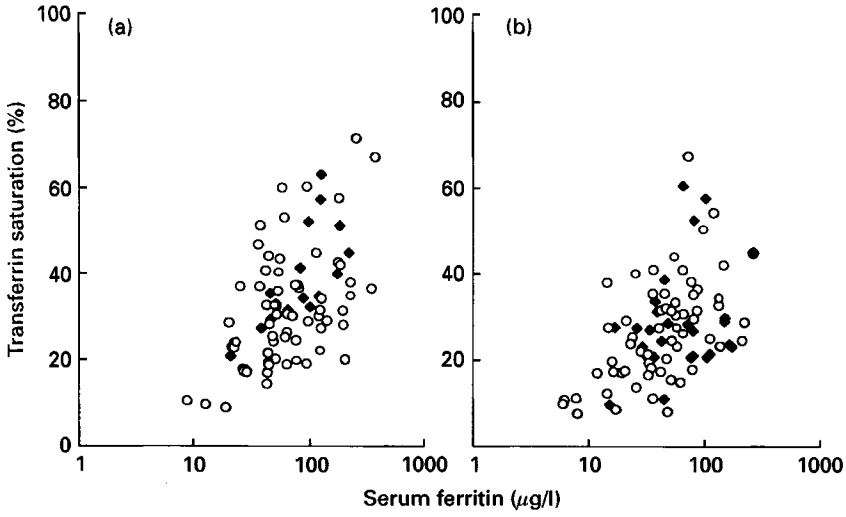

Fig. 2. Relationship between transferrin saturation (%) and serum ferritin concentration (μg/l) in (a) male and (b) female subjects in Papua New Guinea. (○), malaria positive; (◆), malaria negative.

Table 6. *Comparison of transferrin saturation* (%) *between malaria-positives and -negatives with logarithmically transformed serum ferritin* (*μg/l*) *as a covariate*

Malaria infection	*n*	Unadjusted mean	Adjusted mean	SEM*
Males				
Malaria positive	67	31·9[a]	32·3[a]	1·4
Malaria negative	17	38·1[b]	36·5[a]	2·7
Comparison of slopes: F 1·65 ($P = 0·20$)				
Comparison of elevations: F 2·00 ($P = 0·16$)				
R^2 0·31				
Females				
Malaria positive	63	27·2[a]	28·0[a]	1·4
Malaria negative	27	28·1[a]	26·3[a]	2·1
Comparison of slopes: F 0·92 ($P = 0·34$)				
Comparison of elevations: F 0·50 ($P = 0·48$)				
R^2 0·22				

[a, b] Mean values with unlike superscript letters were significantly different, $P < 0·05$.
* Standard error of the adjusted mean estimates.

Levels of haemoglobin and transferrin saturation were significantly correlated in three of the villages but not in the coastal village. The inter-village difference in mean haemoglobin level remained significant after adjustment for transferrin saturation, according to ANACOVA (Table 5). While the adjusted mean haemoglobin levels differed little from the unadjusted values, the slopes of regression from haemoglobin to transferrin saturation differed significantly among the villages for males; males of the southern riverine village had a steeper slope than those of the northern riverine and inland villages. The same tendency was found in females but this was not significant (Fig. 1).

The relationship between serum ferritin and transferrin saturation is shown in Fig. 2,

where the subjects are categorized by malaria infection status. Since a significant positive correlation was similarly found in either the positive or negative groups, and since the adjusted means of transferrin saturation did not differ significantly between the two groups, it is judged that malaria had no significant effect on the Fe stores or on circulating Fe levels (Table 6).

DISCUSSION

Inter-village differences

Occurrence of Fe deficiency in the Gidra was very low in comparison with the 20–30% values reported in tropical countries (Goh & Hariharan, 1986; Hercberg *et al.* 1987). In particular, the northern riverine villages, who maintained the most traditional lifestyle and consumed the largest amounts of Fe, had no Fe-deficient individuals. It is also noteworthy that there was no Fe-deficient individual in the inland village, where malaria prevalence was the lowest. The inland villagers had significantly higher haemoglobin levels than the southern riverine villagers despite the fact that these two groups had the same Fe intake level. It is thus judged that either excess Fe intake or malaria scarcity may prevent Fe deficiency and anaemia. Since the southern riverine villagers had lower Fe intakes and suffered higher malaria prevalence than the northern riverine villagers, there were some anaemic and Fe-deficient people in this village.

The coastal villagers had the lowest Fe intake and the highest malaria prevalence, which accorded with the highest prevalence of anaemia and Fe deficiency in this village group. However, the mean serum ferritin level of the coastal villagers was the second highest, next to the northern riverine villagers. This occurred perhaps because in this village there were several hyperferritinaemic individuals who might suffer from malaria infection and hepatic damage. The common assumption that serum ferritin represents Fe stores when there is a constant release rate of hepatic cells including ferritin from liver is not applicable to individuals with hepatic damage. There is a high possibility that false negative judgements of Fe deficiency occur for people suffering from malaria due to the increased release rate of ferritin into serum, taking into account the clinical findings that high serum ferritin levels were found in malaria patients (Adelekan & Thurnham, 1990). Since indicators of infection and/or inflammation such as C-reactive protein were not measured, it cannot be concretely judged in the present study whether the high serum ferritin levels in some hypoferraemic individuals were caused by malaria or not. Nonetheless, neither glutamate oxaloacetate transaminase (*EC* 2.6.1.1; GOT) nor glutamate pyruvate transaminase (*EC* 2.6.1.2; GPT), which are raised in the case of hepatic damage, were elevated in the coastal village (M. Nakazawa, R. Ohtsuka, T. Kawabe, T. Hongo, T. Suzuki, T. Inaoka, T. Akimichi and M. Alpers, unpublished results). In addition, there were seven males and eleven females in the coastal village who were anaemic or hypoferraemic but whose serum ferritin was not low. Of these, only three females had elevated serum transferrin levels (higher than 3·5 g/l), relatively low serum ferritin levels (lower than 20 μg/l) and low serum Fe levels (lower than 0·5 mg/l); these characteristics manifestly indicate pre-latent Fe deficiency (Wick *et al.* 1991). Thus, it is plausible that there were a few false negative cases in this village.

One Fe-overload individual in the coastal village was judged to have malaria-induced hyperferraemia, in recognition of the fact that malaria causes intravenous haemolysis and that free haemoglobin in serum is subsequently catalysed into peptides and ionic Fe beyond the Fe-binding capacity of serum transferrin molecules; free haemoglobin itself does not alter the serum Fe level, but it apparently does elevate the serum Fe level when catalysed into ionic Fe.

Fe-deficiency in four coastal villagers (Table 4) can be explained by the acute effect of intravenous haemolysis, which maintains the Fe concentration in gastrointestinal mucosal cells at a relatively high level to prevent non-haem Fe absorption (Edwards *et al.* 1989). Since the bioavailability of Fe taken by the Gidra villagers seems to be intermediate as mentioned above, even their intake of more Fe than was recommended was not able to supply sufficient Fe to the tissues.

Mechanisms of iron metabolism

The marked inter-village difference in the interrelationship between haemoglobin and transferrin saturation suggests a difference in the influence of Fe on erythropoiesis. Compared with males of the northern riverine and inland villages, males of the southern riverine village needed smaller amounts of circulating Fe for erythropoiesis, and this may reflect their adaptation to a lower intake than the northern riverine village and higher malaria prevalence than the inland village. The coastal villagers had the lowest intake and highest malaria prevalence, but their haemoglobin and transferrin saturation were not significantly correlated. This result is attributable to the individuals (triangular marks) plotted at the lower right corner in Fig. 1, who suffered from intravenous haemolysis caused by malarial attack. Thus, the lack of significant correlation is considered to come from the mixture of those haemolysed individuals with other individuals who showed a steeper relationship between haemoglobin and transferrin saturation.

Considering that there was no significant effect (or only a short-term effect if any) of malaria experience on the interrelations between transferrin saturation and serum ferritin, it is suggested that endemic malaria does not alter Fe metabolism. The bulk of Fe released from haemoglobin, which is supplied from haemolysed erythrocytes induced by malaria infection, is directly bound to serum transferrin. Transferrin-bound Fe is, in portion, used for erythropoiesis at bone marrow and the remainder is gradually lost through perspiration, urine and faeces; according to Wick *et al.* (1991), about 1 mg Fe is lost, per day, through dead cells of the intestinal mucosa. It is reasonable to judge that malaria does not trigger a shift of Fe from circulating blood into parenchymal tissues as a defence system, although the short-term effects of malaria infection on Fe metabolism are not clarified. Thus, the hypoferraemic adaptation hypothesis of Kent & Dunn (1993) was not supported by the present study.

Haemoglobin levels differed significantly between the malaria-positives and -negatives for both sexes ($P < 0{\cdot}01$): 145·8 (SD 13·7) g/l for malaria-positive males (n 67) and 158·5 (SD 7·1) g/l for negative males (n 17); 134·9 (SD 16·0) g/l for positive females (n 63) and 143·7 (SD 10·5) g/l for negative females (n 27). Considering the significant correlation between haemoglobin and transferrin saturation, the difference between the two groups suggests that malaria infection reduces haemoglobin level, irrespective of Fe stores and Fe intake, as pointed out by Woodruff *et al.* (1979) and Abdalla *et al.* (1980). Further investigations are needed particularly because there is a possibility that the recovery of haemoglobin level after clearance of parasites takes relatively longer periods than the recovery of Fe metabolism.

Oppenheimer *et al.* (1984, 1986) reported that malaria infection is exacerbated by Fe supplementation. If Fe taken from either food or supplementation functions identically, the present results demonstrate the opposite answer: excess Fe intake prevents Fe-deficiency and anaemia. The inconsistency can be explained as follows: people with poor nutritional status, as were the subjects of the study of Oppenheimer *et al.* (1986), tend to develop anaemia in accordance with exacerbation of malaria because Fe supplementation provides

Fe for the parasite; whereas people with good nutritional status like the Gidra do not, because growth of the parasites in the human body has not been restricted by the host's Fe nutrition. Nonetheless, another explanation is possible. Fe supplementation in malaria-endemic areas causes immediate hyperferraemia and raises susceptibility to malarial infection, resulting in the increase of malaria morbidity. In contrast, people whose Fe intake from food has been high may have enhanced protective function against malaria. We consider that the latter explanation is more plausible because in the relationship between malaria and Fe nutrition the inter-village difference was prominent whereas the inter-individual difference was not.

The reason the northern riverine villagers suffered from malaria to a similar extent as the southern riverine villagers but did not suffer from anaemia remains unknown. Comparison of haemoglobin levels between the malaria-positives and -negatives in this village group proved that the latter were not significantly higher than the former ($P > 0{\cdot}15$): 158·8 (SD 10·7) g/l for malaria-positive males (n 12) and 159·3 (SD 4·6) g/l for negative males (n 3); 148·2 (SD 6·8) g/l for positive females (n 18) and 141·5 (SD 16·1) g/l for negative females (n 8). This implies that malaria has no prominent effect on the haemoglobin level. A possible reason is the villagers' significant consumption of cycad seeds, which contain α-amino-β-methylaminopropionic acid of acute neurological toxicity (Garruto *et al.* 1988) and may cause a hyperoxidative condition in the blood vessels. Considering that the consumption of bitter cassava has a protective effect against malaria because of cyanide, which makes erythrocytes fragile (Katz, 1987), it is not impossible that cycad seed consumption has similar protective effects against malaria infection. This possibility should be further examined.

CONCLUSION

An inter-village comparison of haemoglobin levels adjusted for transferrin saturation revealed varying status of erythropoiesis from village to village. In particular, the southern riverine villagers needed smaller amounts of circulating Fe for erythropoiesis than the northern riverine and inland villagers. This inter-village difference should be considered as a result of long-term adaptation, reflecting such local human-environment conditions as the density of malaria vectors and the people's dietary habits. It is thus concluded that Fe supplementation, which affects Fe nutritional status in the short term, is not effective unless erythropoietic status and the people's health status and dietary habits are fully elucidated. Since the Gidra villagers' serum ferritin levels were not related to haemoglobin levels, and since their haemoglobin levels differed significantly between malaria-positive and -negative groups, it is concluded that malaria infection does not affect Fe nutritional status, i.e. Fe stores and circulating Fe, but directly causes anaemia through haemolysis. Thus, the hypoferraemic adaptation hypothesis was not supported by this study. Based on the above two conclusions, the mechanism of the relationship between Fe nutriture, malaria and anaemia is illustrated schematically in Fig. 3.

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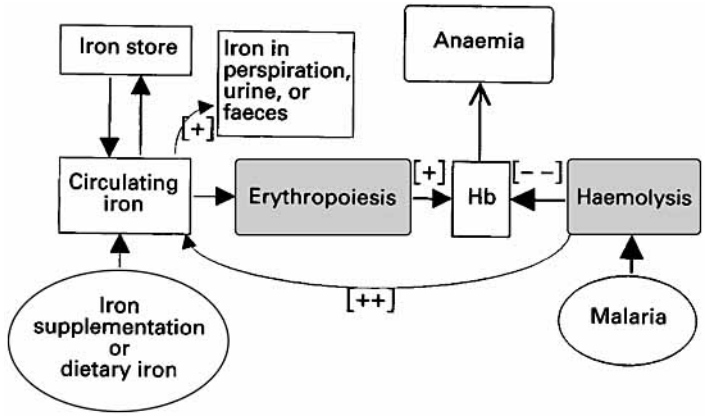

Fig. 3. Schematic diagram showing how malarial infection might trigger anaemia. Hb, haemoglobin; [+], increase; [+ +], large increase; [− −], decrease.

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