Understanding the impact of malaria on the interpretation of micronutrient biomarkers

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Statement of own work

I, Fanny Sandalinas, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Accurate estimates of population micronutrient status are needed to guide policies, programmes, and the choice of interventions to prevent and control micronutrient deficiencies. It is widely accepted that some micronutrient biomarker concentrations should be corrected for inflammation status with the use of inflammation marker concentrations, to improve the specificity with which deficiency is indicated. However, there is evidence to suggest that malaria infection could have an inflammation-independent, additive effect on certain biomarkers, particularly ferritin, which complicates the assessment of iron status in malaria-endemic areas. Often, the population groups with greater risk of micronutrient deficiencies also have greater risk of contracting malaria. This research started with a systematic literature review, finding that micronutrient biomarkers are affected by malaria infection, and the intensity of this relationship varied according to the stage of infection. Then, an analysis of cross-sectional data from representative population-based surveys (eight datasets, n=7,886 children) investigated the relationship between malaria, ferritin concentrations, and inflammation. Inflammation-adjusted ferritin concentrations were 44% (95% CI 39, 52; $p<0.001$) greater in malaria-infected children, compared to uninfected children. Age and malaria exposure, the two main determinants of malaria immunity, were shown to modify this association. The potential role of malaria immunity in mediating the relationship between malaria and micronutrient biomarkers was explored further through an analysis of the 2015 Malawi micronutrient survey dataset. This analysis demonstrated a clear pattern of association between low level of immunity and greater changes in ferritin concentrations during malaria infection. The results of this research indicate the need to account for malaria in the assessment of iron status in malaria-endemic areas, particularly in populations with low immunity to malaria. The findings have potential applications for research on the epidemiology and aetiology of micronutrient deficiencies, and in population micronutrient surveillance, particularly in malaria endemic areas.

Preface

This thesis is written in research paper style format. The supplementary material for each paper is presented at the end of the thesis.

Chapter 1 provides an overview of the relationship between micronutrients, inflammation and malaria.
Chapter 2 presents the research gaps the PhD addresses and provides an overview of the PhD aims and objectives.
Chapter 3 reports a literature review and meta-analysis on the relationship between micronutrients and malaria infection. The paper is published in a peer-reviewed journal.
Chapter 4 reports a multi-country secondary data analysis of the relationship between ferritin and malaria infection in pre-school children in representative micronutrient surveys. The paper has been submitted for US CDC clearance prior to submission for publication.
Chapter 5 reports a secondary data analysis of the association between malaria infection and biomarkers in the 2015 national micronutrient survey of Malawi. The paper has been submitted for US CDC clearance prior to submission for publication.
Chapter 6 reports a consultation with stakeholders and presents a technical brief aimed at policy and programme stakeholders, based on the findings of the thesis.
Chapter 7 provides a discussion of the findings from the overall thesis.

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Abbreviations

AGP: α1-acid glycoprotein

APP: Acute phase proteins

BRINDA: Biomarkers reflecting inflammation and nutritional determinants of anaemia

CDC: United States Centers for Disease Control and Prevention

CI: Confidence interval

CRP: C-reactive protein

DHS: Demographic and health survey

G6PD: Glucose-6-phosphate dehydrogenase

HRP2: Histidine-rich protein 2

MAPS: Micronutrient Action Policy Support

PfPR: Plasmodium falciparum parasite rate

PSC: Pre-school children

RDT: Rapid diagnostic test

SAC : School-age children

sTfR: Soluble transferrin receptors

RBP: Retinol binding protein

WHO: World Health Organization

WRA: Women of reproductive age

1 Chapter 1 Background

1.1 Measuring micronutrient status in population

Micronutrients are needed in small amounts for a wide range of functions in human health. Micronutrient deficiencies underlie a large disease burden, especially in low-income countries[1]. Deficiencies in iron and vitamin A are common around the world[2]. In a recent analysis of individual-level biomarker data for micronutrient status from nationally representative, population based surveys, Stevens et al. found the global prevalence of deficiency in at least iron, zinc or vitamin A to be 56% (95% CI: 48–64) among preschool-aged children[3].

1.1.1 Iron status

Iron deficiency is estimated to affect 1.5-2 billion people worldwide[4], with considerable adverse health effects as iron is needed for energy production, oxygen transport and utilization, cellular proliferation, and pathogen destruction[5]. Iron deficiency affects all populations, but the most vulnerable are women and children due to their greater requirements[6]. Nutritional iron deficiency occurs when the diet supplies insufficient bioavailable iron to meet the body's requirements for growth and pregnancy and to replace iron lost from the gastrointestinal tract and skin, in the urine, and through menstruation[4].

There are several biomarkers indicating iron status which may be used in public health contexts, as outlined below.

Serum ferritin

Ferritin is the major form of iron storage. Serum ferritin is a measure of the amount of iron in body stores, although its concentration can be affected by concurrent infection or inflammation. The serum ferritin measurement is widely available and well standardized[5].

Soluble transferrin receptors

Transferrin is the main iron transport protein found in the blood. Cells can regulate their iron uptake by modulating the expression of the transferrin receptor on the cell surface[7]. When there is insufficient iron for the synthesis of haemoglobin, there is an increased concentration of soluble

transferrin receptor (sTfR) in circulating blood[8]. However, sTfR concentrations are also elevated in conditions with increased red cell production and/or turnover[7]. Consequently, sTfR can only be used as an indicator of depleted iron stores when there are no other known causes of abnormal erythropoiesis[7].

Haemoglobin

The measurement of haemoglobin is an important screening tool for the detection of iron deficiency; however there are many potential causes of anaemia, including other nutritional deficiencies, infectious diseases and red blood cell disorders[5].

Hepcidin

Hepcidin is a peptide hormone and has emerged as the master regulator of iron metabolism. Hepcidin controls the absorption and distribution of iron through different mechanisms, and its regulation responds to the need of iron for erythropoiesis, the amount of iron stores and the degree of inflammation[5].

1.1.2 Vitamin A status

Vitamin A deficiency disorders include xerophthalmia and increased risk of death from infectious diseases, especially among preschool children[9]. The most commonly used biomarker to measure vitamin A status is serum/plasma retinol. As the measurement of serum retinol requires expensive laboratory equipment, serum retinol binding protein (RBP), its carrier protein, can be used as a proxy for serum retinol[9].

1.2 Interpreting micronutrient status in the context of inflammation

Accurate interpretation of micronutrient biomarker data is important for the correct evaluation of micronutrient status, whether this status is measured as part of micronutrient surveys or in research settings. These data can be used to estimate the prevalence of micronutrient deficiencies in a population, and to evaluate the effect of an intervention on micronutrient status. Accurate micronutrient biomarker data are therefore needed to inform the needs of programmes and policies, and for robust evidence to guide decisions on public health interventions. However, micronutrient

status and prevalence of micronutrient deficiencies can be incorrectly reported because certain micronutrient biomarkers are affected by inflammation and infections[10].

1.2.1 What is inflammation?

The biochemical changes in a body that are initiated in response to microbial invasion, tissue injury, chronic disease states, immunologic disorders, and psychological stress are termed the inflammatory response[11]. It begins when activated macrophages release a broad spectrum of mediators including cytokines, which then stimulate hepatocytes in the liver to produce acute phase proteins (APPs)[11; 12]. Positive APPs are markedly elevated during states of inflammation[10]. The acute phase response is usually transient and its main purpose is to prevent further damage to the tissues affected and to remove harmful molecules[13]. A common feature is a rapid fall in the blood concentration of several micronutrients, including iron, zinc and retinol[14]. In the absence of overt disease, inflammation is usually mild, but nutritional biomarkers are still altered[14]. Although these depressions are transient and reversible, they have the potential to affect the accurate estimation of micronutrient status if the level of inflammation is high during the sample collection period, or if a large proportion of individuals with inflammation are sampled in a population survey. These problems are particularly important where people are apparently healthy but live in areas where there is a high prevalence of infectious disease[15].

The particular issue of inflammation in childhood

Children are born with very little protection against pathogens and need exposure to pathogens in order to develop their own immune defences[16]. There is ample evidence to show that even apparently healthy children can have elevated inflammatory markers[16].

1.2.2 How serum biomarkers fluctuate during inflammation

The concentration of several biomarkers including serum retinol, ferritin and zinc change by 40% or more in the 48 hours following infection or trauma [14].

Iron biomarkers

Serum iron concentration decreases in a wide variety of stressful situations, commonly by around 50-75%[17]. A reduction in serum iron is believed to be beneficial to the host by depriving invading microorganisms of an element for growth and reproduction, and by limiting free radical production[17]. During this process, iron is neither lost (except in case of blood loss) nor excreted but stored in ferritin. As a consequence, serum ferritin rises. Serum iron can remain low (and serum ferritin high) for several weeks before returning to baseline concentrations[17]. Hepcidin plays a major role in the redistribution of iron during the inflammation process, by reducing iron absorption from the diet, and its release from body stores[17], maintaining serum iron low and serum ferritin high. Although sTfR is not an acute phase protein, a consistent pattern of increased sTfR concentrations has been reported during inflammation[8].

Vitamin A biomarkers

During inflammation, there is a decrease in hepatic synthesis of RBP, which interrupts the release of retinol and RBP from the liver, lowering serum retinol and serum RBP concentrations[9].

Zinc, folate and vitamin B12 biomarkers

Serum zinc is depressed during inflammation, as the acute-phase response can lead to a redistribution of zinc from the plasma or serum to the liver[18]. A recent review by Young et al. reported weak and inconsistent correlations between C-reactive protein (CRP) or α1-acid glycoprotein (AGP) and vitamin B-12 or folate biomarkers[19].

1.2.3 Accounting for inflammation when analysing micronutrient biomarkers

When analysing micronutrient biomarker data, the most commonly measured APPs are CRP, and AGP. CRP is a sensitive marker of systemic inflammation[20]. At the onset of infection, CRP increases rapidly over the first 24–48h[12]. AGP levels rise more slowly in response to inflammation or infection and remain elevated for longer than CRP levels[21]. These proteins are especially useful for detecting and monitoring inflammation in people with no, or non-specific, clinical symptoms[12]. Several approaches have been proposed to account for inflammation when interpreting biomarker values. With the example of ferritin, these approaches include increasing the ferritin concentration cut-off, excluding

individuals with elevated inflammation markers, or correction approaches using the values of these APPs[10].

1.2.3.1 Increasing the concentration cut-off

In the updated guidelines on using ferritin to assess iron status in populations, the World Health Organization (WHO) still recommends to increase the concentration cut-off to define deficiency in individuals with inflammation[22]. This is, however, not the method that is most commonly used by researchers and data analysts, as it comes with several limitations. Increasing the cut-off is unlikely to capture the extent to which ferritin can increase during inflammation, which can be up to 300%[6].

1.2.3.2 Excluding individuals with elevated inflammation markers

While this can appear as the most conservative approach, excluding individuals with elevated inflammation markers comes with several limitations as the sample size could be substantially reduced, and excluding people living with inflammation can make the findings less representative of the wider population. This could therefore be considered as a large, biased exclusion. Nevertheless, some stakeholders still apply this technique, as shown in the Indian Comprehensive Nutrition National Survey[23], in which all participants with a CRP >5 mg/L were excluded for the analysis of biomarker data.

1.2.3.3 Correction approaches

The correction approaches use the relative difference in biomarker concentrations between individuals with and without inflammation.

- Thurnham correction factors

One of these correction approaches was developed by David Thurnham and involves the use of correction factors that are defined according to three stages of infection, defined by the levels of CRP and AGP[24]. As these two APPs respond differently over the time-course of the infection, we can use their respective concentration to characterise three stages of subclinical inflammation, in contrast to the healthy group for which there are no elevated APP[24]. This method is relatively easy to use and does not require advanced statistical training. As expected with the use of a cutoff, this method may

miss some cases of inflammation, where marker concentrations are below the chosen cutoffs. There is moreover another issue related to individuals with very high APP, as the correction is not proportional to the level of inflammation.

This method was originally proposed for application to apparently healthy populations, in which people with sicknesses such as, for example, diarrhoea, fever and respiratory tract infection were excluded[25]. This would exclude individuals with very high APP, and in general the inflammation would be relatively uniform and mild[25]. However, in most micronutrient surveys for example, morbidity data are collected but sick children and adults are usually not excluded, which could question the relevance of this technique in these large population-based surveys.

- BRINDA regression method

The other correction method was developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) group. BRINDA is a multi-agency and multi-country research partnership designed to improve the interpretation of nutrient biomarkers in settings of inflammation and to generate context-specific estimates of risk factors for anaemia[26]. The rationale for BRINDA grew from an expressed need by the global nutrition community to improve assessment of micronutrient status[26], as there are no universally accepted methods for accounting for inflammation in estimating micronutrient status, which can lead to incorrect diagnosis of individuals, as well as over- or underestimation of the prevalence of deficiency in a population[11]. The BRINDA correction method is a regression method, whereby a linear regression is used to adjust the biomarker concentration by the concentrations of CRP and AGP[10]. This technique uses the slopes (regression coefficients) of the two APP and uses the maximum of the lowest decile of each APP concentration to define the reference, that is the group with no inflammation[10]. Several papers published by the BRINDA group outline the recommended inflammation adjustments for the most common biomarkers (Figure 1).

Micronutrient biomarkers	Preschool-age children	Women of reproductive Age	References
Retinol binding protein & serum retinol	AGP + CRP	No adjustment	Larson, Nutrients, 2018 (9) Namaste et al., AJCN, 2020 (8)
Serum ferritin	AGP + CRP	AGP + CRP	Namaste et al., AJCN, 2020 (8)
Soluble transferrin receptor	AGP	AGP	Rohner et al., AJCN, 2017 (10)
Serum zinc*	AGP + CRP	No adjustment	McDonald et al., AJCN, 2020 (11)
Serum and RBC folate	No adjustment	No adjustment	Young et al., AJCN, 2020 (12)
Serum B-12	No adjustment	No adjustment	Young et al., AJCN, 2020 (12)
Vitamin D	No adjustment	No adjustment	Young et al., AJCN, 2022 (13)

Figure 1: Inflammation markers used to adjust micronutrient biomarkers among preschool-age children and women of reproductive age based on the latest publications, from Luo et al[27].

According to a study in children in Indonesia, the regression analysis is preferable to the correction factors approach when adjusting serum ferritin for inflammation, as it accounts for the severity of inflammation and morbidity[28]. In this study, the BRINDA regression analysis resulted in greater corrections than the Thurnham correction factors for all biomarkers studied[25].

1.2.4 Impact of inflammation adjustment on the interpretation of micronutrient status

A recent report by Hess et al. has examined the differences in micronutrient deficiency prevalence estimates according to different adjustment methods[29]. The impact varied widely by context. For example, in Liberia in 2011, the prevalence of iron deficiency among pre-school children (PSC) based on ferritin concentration was 20.4% unadjusted, 29.8% adjusted categorically, and 55.6% adjusted using the BRINDA method[29]. In other contexts where inflammation was not as prevalent, the impact of the adjustment method was less dramatic. It has now become current practise to report biomarker data adjusted for inflammation and these adjustment methods are widely used by researchers when estimating micronutrient status, and in most micronutrient surveys.

1.2.5 Limits to defining inflammation by CRP and AGP only

The definition of inflammation based on elevated CRP and AGP markers is imperfect, as inflammation is a complex process than cannot be captured simply by the elevation of either one of both of these two acute-phase proteins. However, in the absence of other widely available biomarkers of inflammation in population-based surveys, we continue to rely on a definition of inflammation based on these two markers.

1.3 Malaria

1.3.1 Generalities

Infants and young children, as well as women of reproductive age (WRA) are at considerably higher risk of contracting malaria, and developing severe disease, than other demographic groups living in the same endemic region[30]. According to the World Health Organization (WHO), there were 249 million cases of malaria in 2022[31]. Malaria is caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. Most malaria cases and deaths occur in sub-Saharan Africa. The clinical presentation of disease ranges from asymptomatic malaria to uncomplicated, to severe or complicated[32]. Asymptomatic malaria, i.e. the presence of parasitaemia in the absence of fever or other malaria-related symptoms, is very common in malaria-endemic regions, with prevalence exceeding 50% in some areas[33]. Uncomplicated malaria is symptomatic malaria parasitaemia (e.g. with fever, malaise) without signs of severity or evidence of vital organ dysfunction[34]. Severe malaria is multi-syndromic and may manifest as cerebral malaria, severe malaria anaemia, respiratory distress and/or kidney failure.

There are five well-established malaria parasite species that infect humans, namely *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. P. falciparum* accounts for 99.7 % of infections in sub-Saharan Africa[31].

1.3.2 Malaria immunity

Natural acquired immunity is one of the factors that conditions the extent to which malaria infection can develop into severe disease[35]. Naturally acquired immune responses against *P. falciparum* require repeated parasite exposure to attain protection and therefore host immunity is determined mainly by age and exposure[36], which can be defined by the total number of infections experienced by an individual[37]. The transmission intensity in a given area (often measured as number of infectious bites per person per year) varies with a number of factors. Malaria endemicity is the degree of malaria transmission in an area[38] and the parasite prevalence in children aged 2-10 years is a measure commonly used to define levels of endemicity (Figure *2*).

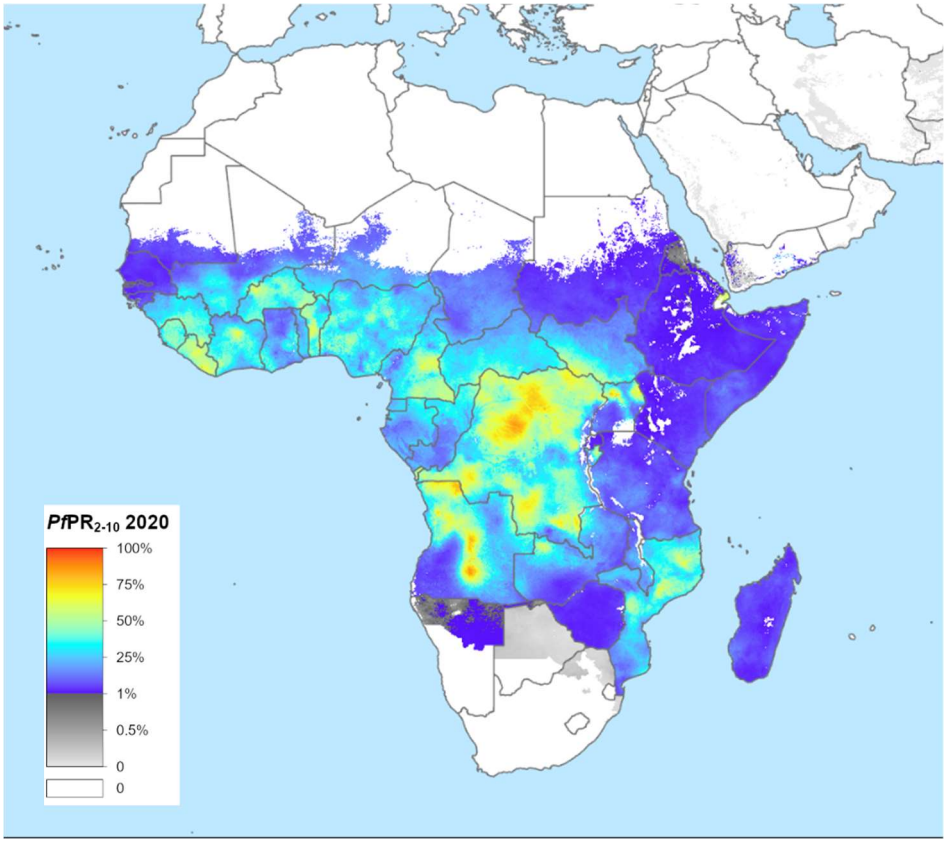

Figure 2: *Plasmodium falciparum* parasite rate in two- to ten-year-olds in sub-Saharan Africa in 2020. Source: Malaria Atlas Project[39]

In settings of higher and more holoendemic malaria transmission (parasite prevalence constantly ≥ 75%), more individuals in a population (especially the adults with longer exposure, and the non-pregnant adults who don't have altered immunity) will have some degree of partial immunity to malaria. In areas of moderate transmission, a child will require more time to develop immunity than a child living in a malaria holoendemic area[40].

1.3.2.1 Environmental factors

Acquired malaria immunity is greatly dependant on the level of transmission of *P.falciparum,* which is influenced by environmental and climatic factors.

Altitude and urbanization

Transmission intensity can vary with altitude because of the associated changes in temperature, which affects the development of *P. falciparum*[41]. A lower malaria transmission intensity in high altitude could therefore result in lower malaria immunity among populations living there, than among those living in surrounding lower-altitude areas. Urbanization causes marked entomological, parasitological and behavioural changes that result in reduced risks of malaria[42], and therefore reduced malaria immunity.

Vector control measures

WHO recommends the use of long-lasting insecticidal nets (LLINs) for the prevention and control of malaria in children and adults living in areas with ongoing malaria transmission[43]. A systematic review reported that LLINs significantly reduce all-cause child mortality by 17% and incidence of *P. falciparum* malaria by 45%[43]. There has been, however, much debate over the long-term effects of control measures which reduce malaria transmission upon the development of immunity to malaria[44]. A minimum amount of exposure to infection is required to develop effective clinical immunity. The reduction in exposure associated with the use of mosquito nets could in theory lead to lower acquired immunity to malaria.

Rainfall, humidity and other climatic conditions

Based on the available data, climate is considered to be the most important factor in limiting transmission and distribution of malaria on a large scale[45]. Temperature determines parasite and

vector development, rainfall provides mosquito breeding sites, and humidity, together with temperature, affects mosquito survival[46]. However, these factors are not covered in this thesis, mostly because no data were available in the datasets included in the analysis. With the rise in global temperatures associated with climate change, it is expected that more areas will become malaria-endemic and these areas will be characterized, at least initially, by absent or very low levels of malaria immunity.

1.3.2.2 Individual factors

In addition to environmental factors, certain individual factors such as age and the presence of hemoglobinopathies can influence individuals' malaria immunity.

Age

As stated earlier, age is one of the largest determinants of malaria immunity. In areas of moderate and high transmission intensity, as characterize much of malaria-endemic Africa, parasitaemia density peaks in children less than five years old and subsequently declines in an age-dependent manner. Clinical immunity (i.e. immunity against the symptoms of malaria) has been defined as a reduction in parasite density and prevalence of disease with age[47].

Hemoglobinopathies and enzymopathies

Hemoglobinopathies such as sickle cell disease and alpha-thalassemia are relatively common in sub-Saharan Africa[48; 49]. It is believed that the genetic defence mechanisms of the human host have evolved to resist malaria infection[50].

- Sickle cell

While the genetic mutation that causes sickle cell disease can lead to early death in individuals who are homozygous for the mutation, in its heterozygous form (sickle cell carrier), it partially protects against severe malaria caused by *P. falciparum* infection[51]. Compared with persons with normal haemoglobin, individuals with sickle cell trait have a 50–90% reduction in parasite density[51]. A number of mechanisms have been proposed, including reduced parasite growth and enhanced removal of parasitized cells through innate or acquired immune processes[52].

- Alpha-thalassemia

The thalassemias are also inherited blood disorders that result from mutations in the globin genes. Alpha-thalassemias are now the most common genetic disorders of human beings[53]. Alpha-thalassemia is considered to be protective in cases of severe malaria but to have no effect on asymptomatic parasitaemia[54].

- Glucose-6-phosphate dehydrogenase (G6PD) deficiency

G6PD plays a key role in the control of oxidative damage in erythrocytes, and G6PD deficiency is the most common known enzymopathy, affecting more than 400 million people worldwide[55]. G6PD-deficient alleles appear to confer a protection against malaria[56]. In a study in Southeast Asians, Louicharoen et al. found no significant effect of the G6PD-deficient alleles on falciparum density, although *P. vivax* density was reduced by 30% to 61%[50].

Vaccination

As of October 2023, WHO recommends the programmatic use of malaria vaccines for the prevention of *P. falciparum* malaria in children living in malaria endemic areas, prioritizing areas of moderate and high transmission[57]. As the vaccination programmes are still new, populations vaccinated against malaria were not included in the datasets considered.

Pregnancy

Pregnancy is a time of increased vulnerability to malaria, as the immune system of women adapts to the pregnancy and becomes more vulnerable to malaria. Due to the scarcity of data, pregnant women were not included in the analyses.

2 Chapter 2 Thesis overview

This chapter presents the rationale and research gap this thesis addresses, in addition to the aim, objectives, and overview of the work found in this thesis.

2.1 Rationale for the thesis

2.1.1 Introduction

The presence of malaria parasites in the blood can produce a chronic or mild acute phase response, even in an asymptomatic individual, resulting in changes of micronutrient biomarker concentrations. The main question of this research is whether the correction for CRP and AGP described in the first chapter fully captures the modification in biomarker concentrations during a malaria infection. The correction methods do not apply to an infection that is not accompanied by an elevation in CRP and AGP. It is also likely that malaria can increase ferritin independently of inflammation, although the specific pathways are not yet fully understood[58].

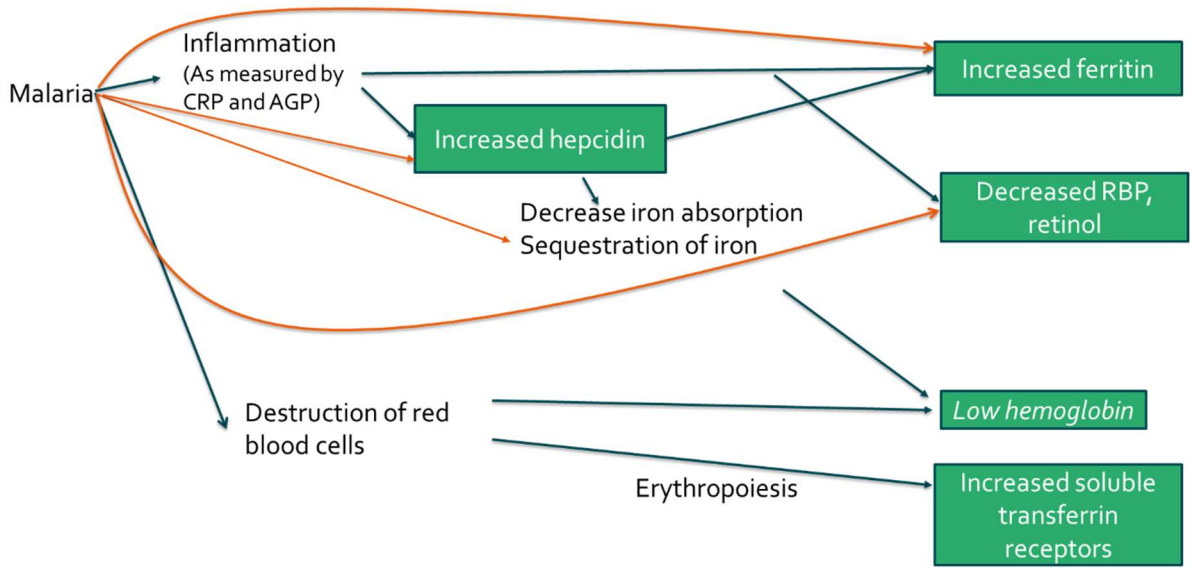

Figure 3: Summarized pathways by which malaria can affect the interpretation of certain micronutrient biomarker concentrations.

RBP: retinol binding protein. CRP: C -reactive protein, AGP: α1-acid glycoprotein.

During malaria infection, even during an asymptomatic infection, iron is sequestrated in macrophages and iron absorption from the gut is decreased, through hepcidin-mediated mechanism[59](Figure 3). Inflammation causes elevated ferritin and reduced retinol. Although it is not an acute phase protein, soluble transferrin receptor concentrations might be increased, if there is any haemolysis of red cells resulting in erythropoiesis[6]. It is unclear whether malaria can affect micronutrient biomarker concentrations independently of inflammation (orange arrows).

2.1.2 Evidence

In 2020, the WHO published an updated guide on the recommended adjustments for inflammation, and also stated that an additional adjustment for malaria was possible[60]. Several recent studies suggested that ferritin values differ by malaria infection status after correcting for inflammation. In a study in Zambian children, Barffour et al. reported ferritin values in children with and without inflammation, and with and without asymptomatic malaria infection[61]. During the high transmission period, children with asymptomatic malaria but no inflammation had ferritin concentrations that were almost two-fold greater than those in children with inflammation but no malaria[58]. Muriuki et al. found that children with asymptomatic malaria had greater ferritin concentrations at every decile of CRP, compared with those without malaria[62]. They found that malaria parasitaemia also increased ferritin levels independently of increased CRP and/or AGP in multivariable analyses[63]. Retinol is also known to be affected by malaria infection[64]. In children aged 6 – 59 months in Ghana, increasing malaria parasite density was significantly associated with decreasing serum retinol, possibly due to increased vitamin A requirements during malarial infection[64]. These reductions have been largely attributed to the inflammatory response[64]. In young children in Liberia, Larson et al. found a significant added effect of malaria infection on RBP concentrations and vitamin A deficiency prevalence estimates even after adjusting for CRP and AGP using the regression approach[65]. Similarly, in Burkina Faso, Wessells et al. found that asymptomatic malaria was associated with plasma ferritin and RBP levels in young children, even after adjusting for CRP and AGP[66].

Some research indicates that malaria might also affect the concentration of sTfR[8; 67] and serum zinc[68]. The mechanisms are largely unknown but could involve the incomplete capture of the acute phase response by CRP and AGP[69], increased erythropoiesis for sTfR[8], or micronutrient redistribution[67]. Other micronutrient biomarkers such as serum folate, red blood cell folate and serum vitamin B12

seem to not be affected by inflammation[19]; however, there have been reports of elevated folate status in children during malaria infection, probably due to the de-novo synthesis of folate by the malaria parasite[70]. It is unknown if elevated folate status, or modified vitamin B12 status are common during malaria in WRA, and if these could affect the estimates of folate and B12 deficiency prevalence, and therefore decisions on the implementation of programmes aimed at controlling these deficiencies.

In 2017, Namaste et al. conducted a multi-country analysis of the effect of malaria on the interpretation of ferritin using the BRINDA datasets in PSC and WRA[71]. They reported that malaria infection was independently associated with ferritin after controlling for CRP and AGP, but they concluded that when CRP and AGP are measured, there appears to be limited utility in measuring malaria status to adjust ferritin concentrations, because malaria adjustments alone or in addition to CRP and AGP adjustments did not change the estimates of iron deficiency prevalence. A recent paper by Luo et al. also concluded that in PSC, when estimating iron deficiency using ferritin and sTfR, the prevalence estimates of micronutrient deficiencies were similar using the BRINDA inflammation adjustment method with or without malaria as a binary variable[27]. Therefore, adding malaria as a binary variable in the BRINDA inflammation adjustment for biomarkers of iron was considered not necessary, although the necessity to consider the stage of infection was mentioned[27].

2.1.3 Gaps in research

In both BRINDA analyses cited above, malaria infection was considered only as a dichotomous variable and the possible variations per malaria species, diagnostic methods, endemicity profile, stage of infection, or age of the participants were not reported, although these factors could modify the association between malaria and micronutrient biomarker concentrations[72]. While the conclusion from the BRINDA papers were valid in the databases considered, it is worth noting that the estimates of micronutrient deficiency prevalence could be affected differently in other settings and when the factors mentioned above are considered. As no stratified analyses were conducted, it is unclear whether some associations between micronutrient biomarker concentrations and malaria may have been masked when reporting associations for the entire population group. In addition, understanding to what extent malaria can affect micronutrient biomarker concentrations is also important to evaluate programmes that intend to improve micronutrient status. It is very common to measure the impact of an intervention on micronutrient status by measuring the changes in micronutrient biomarker

concentration, rather than only assessing the change in the prevalence estimates. In these cases, even small changes in the concentration of micronutrient biomarkers due to malaria need to be considered.

2.1.4 Malaria immunity and biomarkers

In previous studies analysing malaria and micronutrient biomarkers, it has been hypothesised that the relationship between malaria and micronutrient biomarkers could be modified by malaria immunity[69; 73; 74]. Evidence from Zanzibar was reported by Stoltzfus et al. in a study on iron supplementation in young children[74]. Although all children studied were clinically well on the day of assessment, the authors found that 75% of children had malaria parasitaemia. Serum ferritin was higher with greater malaria parasitaemia in children under 30 months old, but there was no association in children over 30 months[74]. The authors speculated that age-dependent immune mechanisms might protect older children. In this study, ferritin was not adjusted for inflammation.

In a study in Burkina Faso, Wessells et al. reported that asymptomatic malaria was associated with inflammation-adjusted ferritin and inflammation-adjusted RBP and questioned the role of immunity as well[69]. The authors suggested as a possibility that in populations with a relatively higher immunity to malaria (e.g., school-age children and nonpregnant adults in holoendemic regions), the relation between asymptomatic malaria status, APP, and indicators of micronutrient status, or the impact of APP and malaria infection on the estimated prevalence of micronutrient deficiencies, would be attenuated[69] .

Other factors associated with malaria immunity as described in the first chapter such as malaria endemicity, hemoglobinopathies or the use of bed nets have, to our knowledge, never been studied in the relationship between malaria and inflammation-adjusted biomarkers.

Identifying whether malaria immunity can modify the relationship between malaria and micronutrient biomarker concentrations, could improve our understanding of the impact of malaria on micronutrient biomarkers interpretation and result in more accurate estimates of micronutrient status in individuals and populations. Previous work by BRINDA explored a malaria adjustment based on malaria as a binary variable. However, if different factors related to malaria immunity affect this relationship, a more refined adjustment accounting for these factors might be more suitable.

2.1.5 Direction of the intended impact

Although there is evidence of bi-directional effects between malaria and biomarkers[5], the assumption explored in this thesis is that, generally, malaria causes disturbances in micronutrient biomarker concentrations, rather than the micronutrient biomarker levels impacting the risk of malaria. Additionally, it is possible that malaria infection leads to sustained changes in micronutrient status, notably by decreasing iron absorption through increased hepcidin[75]. However, the hypothesis explored in this work is that the increase in ferritin observed during a malaria infection does not result from a real change in iron status but is linked to transient disturbances and redistribution of serum iron. These assumptions are guided by a series of observations on ferritin, specifically:

- Experimental study

De Mast et al. followed fluctuations in ferritin concentrations in Dutch volunteers after an experimental infection with *P. Falciparum*[76]. They observed the presence of parasites by blood smear microscopy every eight hours after parasite inoculation and effective treatment was started once the parasites were detectable. Ferritin concentrations increased dramatically as soon as parasites were detectable and it took several weeks for the ferritin levels to go back to pre-infection levels, even when effective treatment was given[76]. The fact that ferritin levels started to rise immediately after infection suggests a causal effect from malaria on ferritin concentrations.

- Prospective studies on malaria and biomarker status.

In longitudinal studies looking at ferritin concentrations and inflammatory markers concentrations after a malaria infection, it was notable that ferritin concentrations were reduced after effective malaria treatment, but remained elevated for about a month after the infection[75; 77; 78]. The response to malaria treatment favours a causal relationship between malaria infection and ferritin increase.

- Direction of change

The direction of change in serum ferritin (elevated ferritin during malaria) contradicts the hypothesis of a change of iron status during malaria. The most plausible impact of malaria on iron status would be to negatively affect the iron status due to reduced dietary iron absorption, and not to improve the iron status.

- Biological mechanisms

As outlined in Figure 3, it is believed that malaria can increase ferritin through an inflammation mechanism and possibly inflammation-independent pathways.

2.2 PhD aims and objectives

The objective of the research is to assess the relationship between micronutrient biomarker concentrations, inflammation and malaria, and to investigate adjustment algorithms to account for inflammation and malaria when estimating micronutrient status. The specific objectives are:

- to assess the relationship between micronutrient biomarker concentrations and clinically defined asymptomatic and uncomplicated malaria in different population groups (systematic review and meta-analysis, chapter 3);
- to assess the relationship between serum ferritin concentrations, inflammation and malaria in population-based surveys (multi-country analysis of individual-level data, chapter 4);
- to assess the role of immunity in the relationship between malaria and micronutrient biomarkers (multi-country analysis and analysis of Malawi micronutrient survey data, chapters 4 and 5).

While the initial focus of my research was on iron and vitamin A, the scope evolved slightly during the PhD, to focus mainly on ferritin while exploring other micronutrients. The main reasons were the availability of survey data, the available literature to support the assumptions and the relevance of survey findings.

2.3 Methods

The methods have been described in detail in each research paper, but I will provide a brief description of the datasets that have been used.

2.3.1 Partnership with BRINDA

As part of this PhD, I have initiated a partnership with the BRINDA group (https://www.brinda-nutrition.org/). As above, BRINDA are working towards harmonization of techniques to adjust for inflammation. After I presented to them the results from the systematic review, we agreed to continue this research in collaboration. The collaboration presented many advantages, including access to datasets, support in the analyses and the potential to increase the uptake of the research findings.

2.3.2 Data used

Only datasets from nationally or regionally representative household surveys are considered in the BRINDA database. The inclusion criteria were surveys that 1) were conducted after 2004, 2) had target groups including preschool children, nonpregnant WRA, or both and 3) measured at least one marker of iron (ferritin or soluble transferrin receptor) or vitamin A status (retinol binding protein or retinol) and at least one marker of inflammation (AGP or CRP)[71]. Among these datasets, surveys included in our analysis are only those which include a diagnostic test for malaria infection (self reported indicators were not considered). I will focus on infections with *P. falciparum*, as it is the most common in sub-Saharan Africa. For pre-school children, eight datasets, all from sub-Saharan Africa, were usable (Table 1).

Table 1: List of datasets available from the BRINDA group for malaria and biomarker analysis in pre-school children.

Country	**Type of survey**	**Year**	**Sample size for biomarker analysis**	**Biomarkers in the dataset**	**Method of diagnosis of malaria**
Cote d'Ivoire	National nutrition survey	2007	746	Ferritin, sTfR, RBP	RDT and microscopy
Cameroon	National micronutrient survey	2009	792	Ferritin, sTfR, retinol, RBP	unknown
Kenya	Subnational nutrition survey	2007	896	Ferritin, sTfR, RBP	microscopy
Kenya	Subnational nutrition survey	2010	849	Ferritin, sTfR, RBP	microscopy
Liberia	National micronutrient survey	2011	1,434	Ferritin, sTfR, RBP	RDT (HRP2)
Malawi	National micronutrient survey	2016	1,102	Ferritin, sTfR, retinol, RBP	RDT (HRP2)
Nigeria	Subnational nutrition survey	2012	571	Ferritin, sTfR, retinol, RBP	Microscopy
Zambia	Subnational nutrition survey	2009	405	Ferritin, sTfR	Microscopy

sTfR: soluble transferrin receptors; RBP: retinol binding protein; RDT: rapid diagnostic test, HRP2: histidine rich protein 2. Both AGP and CRP were available in each dataset.

2.3.3 Ethics and data management

Ethics

This thesis only includes secondary data analyses. Participant consent and local approval were obtained by the survey manager prior to each individual survey. The datasets were de-identified at source and no attempt was made to identify participants.

LSHTM ethical approval was obtained before analysing the data (Appendix 1: Ethical approval). Research ethics training was completed on the 11th of June 2021 (Appendix 2: Certificate of completion: Research Ethics Training.).

Data management

The data and the scripts for analysis were stored on One Drive. I have worked with individual-level data with potentially sensitive information that were de-identified at source. Data management including storage and transfers conformed with UKRI and GDPR guidelines. I have respected dataset licensing conditions for reuse and sharing.

A data user agreement was signed with BRINDA that established the terms and conditions under which the data were used.

2.4 Candidate's involvement

The research question addressed in this thesis was my original idea, following work conducted with UNICEF on the analysis of the 2011 Liberia micronutrient survey[65], where we observed that inflammation-adjusted serum ferritin and RBP concentrations were different between malaria-infected children and uninfected children. I developed the research idea and discussed it with my primary supervisor Dr Edward Joy. Professor Suzanne Filteau and then Dr Heidi Hopkins joined the supervisory team. I conceptualized the approach, conducted the data analysis and wrote the three papers with regular guidance, support and advise from my three supervisors. I initiated the partnership with the BRINDA group with the support of Dr Edward Joy. For the multi-country analysis, I benefitted from the close support of Dr Amy MacDougall on the statistical analyses. Some additional concepts

were introduced by Dr Parmi Suchdev from BRINDA in the Malawi paper. Each author contribution is specified for each research paper.

This PhD was embedded in the Micronutrient Action Policy Support (MAPS) project funded by the Bill & Melinda Gates Foundation. The aim of the MAPS project is to co-create a web-hosted tool to estimate burdens of micronutrient deficiencies, and to explore pathways to improve nutrition. In this project, I lead the biomarker work. This involves preparing harmonized methods to analyse and report micronutrient deficiencies in populations, based on the analysis of individual biomarker data. We continuously update our methods through literature review, research activities, collaboration with other institutions such as WHO, UNICEF, BRINDA and potential users, so that the methods that we use in the tool to analyse and interpret biomarker data result from the latest available evidence and are useful to the users. Integrating a new malaria adjustment in the MAPS tool can be considered if the evidence that emerge from this thesis is sound and if targeted users express a need for it.

2.5 Publications & additional outputs

Published as part of this thesis.

Sandalinas F, Filteau S, Joy EJM, Segovia de la Revilla L, MacDougall A and Hopkins H. (2022) Measuring the impact of malaria infection on indicators of iron and vitamin A status: a systematic literature review and meta-analysis. *Br J Nutr* **129**, 1-70.

Not yet submitted

Sandalinas F, MacDougall A, Filteau S, Hopkins H, Blake T, Luo H, Suchdev PS, Laird R, Young MF, Joy EJM. Current or recent malaria infection is associated with elevated inflammation-adjusted ferritin concentrations in pre-school children: a secondary analysis of the BRINDA database (under CDC clearance)

Sandalinas F, Joy EJM, Hopkins H, Likoswe BH, Blake T, Luo H, Young MF, Bottomley C, Suchdev PS, Filteau S. Infection confounds inflammation-adjusted micronutrient biomarker concentrations in Malawi (under CDC clearance).

Participation in other publications during this PhD

Luo H, Geng J, Zeiler M, Nieckula E, **Sandalinas F**, Williams A, Young MF, Suchdev PS . (2023) A Practical Guide to Adjust Micronutrient Biomarkers for Inflammation Using the BRINDA Method. *J Nutr* **153**, 1265-1272.

Tang K, Eilerts H, Imohe A, Adams KP, **Sandalinas F**, Moloney G, Joy E, Hasman A. Evaluating equity dimensions of infant and child vitamin A supplementation programmes using Demographic and Health Surveys from 49 countries. BMJ Open. 2023 Mar 14;13(3):e062387.

Likoswe BH, Joy EJM, **Sandalinas F**, Filteau S, Maleta K, Phuka JC. Re-Defining the Population-Specific Cut-Off Mark for Vitamin A Deficiency in Pre-School Children of Malawi. Nutrients. 2021 Mar 5;13(3):849.

2.6 PhD timeframe

The PhD took place between January 2021 and April 2024. I completed the systematic review before going on maternity leave (from November 2021 to May 2022). I successfully upgraded in September 2022. I performed the multi-country analysis in the first semester of 2023 and the Malawi analysis in the second semester of 2023. I wrote the thesis between November 2023-February 2024.

2.7 Funding

I conducted this PhD as a staff member from the LSHTM. My salary and related costs were covered by the Micronutrient Action Policy Support (MAPS) project, funded by the Bill & Melinda Gates Foundation (INV-002855).

3 Chapter 3 Systematic review

Chapter 3 is a systematic review to summarize the evidence on the possible effects of malaria on the interpretation of micronutrient biomarkers for different population groups and different forms of malaria infection.

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RESEARCH PAPER COVER SHEET

Please note that a cover sheet must be completed for each research paper included within a thesis.

SECTION A – Student Details

Student ID Number	1811643	**Title**	Mrs
First Name(s)	Fanny		
Surname/Family Name	Sandalinas		
Thesis Title	Understanding the impact of malaria on the interpretation of micronutrient biomarkers		
Primary Supervisor	Edward Joy		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	British Journal of Nutrition		
When was the work published?	March 2022		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion			
Have you retained the copyright for the work?*	**Yes**	Was the work subject to academic peer review?	**Yes**

*If yes, please attach evidence of retention. If no, or if the work is being included in its published format, please attach evidence of permission from the copyright holder (publisher or other author) to include this work.

SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	
Please list the paper's authors in the intended authorship order:	
Stage of publication	Choose an item.

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualized the analytical protocol with the support of Edward Joy, Suzanne Filteau and Heidi Hopkins. I conducted the literature search with Lucia Segovia de la Revilla. I conducted the study data analyses with the support of Amy MacDougall. Edward Joy, Suzanne Filteau and Heidi Hopkins assisted in data analysis and interpretation. I was the primary writer.

SECTION E

Student Signature	Fanny Sandalinas
Date	07/02/2024

Supervisor Signature	Edward Joy
Date	07/02/2024

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Measuring the impact of malaria infection on indicators of iron and vitamin A status: a systematic literature review and meta-analysis

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Abstract

Inflammation and infections such as malaria affect estimates of micronutrient status. Medline, Embase, Web of Science, Scopus and the Cochrane library were searched to identify studies reporting mean concentrations of ferritin, hepcidin, retinol or retinol binding protein in individuals with asymptomatic or clinical malaria and healthy controls. Study quality was assessed using the US National Institute of Health tool. Random effects meta-analyses were used to generate summary mean differences. In total, forty-four studies were included. Mean ferritin concentrations were elevated by: 28·2 μg/l (95 % CI 15·6, 40·9) in children with asymptomatic malaria; 28·5 μg/l (95 % CI 8·1, 48·8) in adults with asymptomatic malaria; and 366 μg/l (95 % CI 162, 570) in children with clinical malaria compared with individuals without malaria infection. Mean hepcidin concentrations were elevated by 1·52 nmol/l (95 % CI 0·92, 2·11) in children with asymptomatic malaria. Mean retinol concentrations were reduced by: 0·11 μmol/l (95 % CI −0·22, −0·01) in children with asymptomatic malaria; 0·43 μmol/l (95 % CI −0·71, −0·16) in children with clinical malaria and 0·73 μmol/l (95 % CI −1·11, −0·36) in adults with clinical malaria. Most of these results were stable in sensitivity analyses. In children with clinical malaria and pregnant women, difference in ferritin concentrations were greater in areas with higher transmission intensity. We conclude that biomarkers of iron and vitamin A status should be statistically adjusted for malaria and the severity of infection. Several studies analysing asymptomatic infections reported elevated ferritin concentrations without noticeable elevation of inflammation markers, indicating a need to adjust for malaria status in addition to inflammation adjustments.

Key words: **ferritin: iron: retinol: vitamin A: malaria**

Micronutrient deficiencies are a major public health burden, especially in low-income countries, and accurate prevalence estimates are important to guide planning and monitoring of nutritional interventions[1]. However, prevalence of micronutrient deficiencies can be incorrectly estimated because certain micronutrient biomarkers are affected by inflammation and infections such as malaria[2]. Inflammation is characterised by the acute-phase response to infection, injury or environmental insults. Some acute-phase proteins are also micronutrient markers; for example, serum ferritin, the primary iron storage protein, is a positive acute-phase protein – that is, its concentration increases in response to inflammation – and retinol binding protein (RBP) is a negative acute-phase protein – that is, its concentration decreases in response to inflammation[2,3]. Whilst in the absence of inflammation, the concentration of plasma or serum ferritin is positively correlated with the size of the total body iron stores, during inflammation plasma/serum ferritin is raised and does not represent iron stores[2]. Infants and young children, as well as women of reproductive age, are at high risk of micronutrient deficiencies due to increased physiological needs[4]. They are also at considerably greater risk of contracting malaria, and developing severe disease, than other demographic groups[5]. According to the WHO, there were 229 million cases of malaria in 2019[5]. More than 90 % of these cases were located in the WHO African region. The presence of parasites can produce a chronic or mild acute-phase response[6]. In settings of higher and more holoendemic malaria transmission, more individuals in a population, especially non-pregnant adults, will have some degree of immunity to malaria. Asymptomatic malaria, that is, the presence of parasitaemia in the absence of fever or other malaria-related symptoms, is very common in malaria endemic areas, with some prevalence rates exceeding 50 %[7]. There are five well-established malaria parasite species that infect humans, namely *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*. *P. falciparum* accounts for 99·7 % of infections in sub-Saharan

Abbreviations: AGP, alpha-1-acid glycoprotein; CRP, C-reactive protein; PfPR, *Plasmodium falciparum* prevalence rate; RBP, retinol binding protein.

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Africa, while *P. vivax* accounts for 75 % of infections in the Americas[8]. Currently used diagnostic methods include microscopy which visualises parasites in stained blood smears, rapid diagnostic tests that detect parasite antigen/s in blood samples;and PCR which identifies the presence of specific malaria genes in a blood sample.

Recently, the WHO published an updated guide on the use of ferritin to assess iron status and the recommended adjustments for inflammation, measured on the basis of C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) concentrations in blood serum/plasma[9]. Ferritin values may differ by malaria infection status[10] after correcting for inflammation defined by raised CRP and/or AGP, and the updated WHO guidelines mention malaria as a possible factor for adjustment. Other biomarkers are also likely to be affected by malaria. Retinol, the predominant circulating form of vitamin A in the blood, is known to be affected by malaria infection[11]. In children aged 6–59 months in Ghana, increasing malaria parasite density was significantly associated with decreasing serum retinol concentrations[11]. These reductions have been attributed largely to the inflammatory response. As the measurement of serum retinol requires expensive laboratory equipment, some micronutrient surveys measure its carrier protein, RBP, instead of retinol itself. In young children in Liberia, Larson *et al.* found a significant added effect of malaria on RBP concentrations and vitamin A deficiency prevalence estimates even after adjusting for CRP and AGP using the regression approach[12]. There is also a growing interest in the impact of malaria on hepcidin, the iron regulatory hormone[13,14]. Hepcidin seems to be upregulated in malaria infection even in asymptomatic human infection[7]. This results in a blockage of iron absorption from the diet and a redistribution of iron into the body, away from the serum.

Taking into account the effect of malaria on micronutrient biomarkers has the potential to significantly modify the estimation of the prevalence of micronutrient deficiencies derived from large population-based surveys such as national micronutrient surveys. This research estimated the effect of malaria on several biomarker values (ferritin, hepcidin, retinol and RBP) by performing a meta-analysis of studies comparing biomarker values in individuals infected with malaria and individuals without malaria infection.

Methods

The protocol of this systematic review has been published on PROSPERO on the 24 September 2021: CRD42021279974. Ethical approval was not needed as the data used in the analysis are fully available in the public domain. We followed the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting checklist for this systematic review and meta-analysis.

Eligibility criteria

Randomised controlled trials or quasi-randomised controlled trials, prospective observational studies with data collection at multiple time points and cross-sectional studies with a control group that measured selected biomarkers in malaria-infected individuals were eligible. In children and non-pregnant adults, studies that distinguished asymptomatic and clinical malaria cases were included; studies in these populations that combined asymptomatic and symptomatic infections in a single malaria group were excluded. Studies that provided an intervention believed to impact the iron or vitamin A status of the participants were only included if data from a control group, or baseline data, could be extracted. Studies of human participants of any age and sex were eligible. As individuals suffering from severe malaria are often not sampled in large population-based surveys that measure micronutrient status, we decided not to include reports that only recruited participants with severe malaria. However, if individuals with severe malaria were included in papers that meet other selection criteria, they were analysed separately. For similar reasons, studies that recruited individuals based on their being anaemic or having another disease that is likely to affect iron or vitamin A metabolism (sickle cell disease and thalassemia) were not included.

Search strategy

Medline, Embase, Web of Science, Scopus and the Cochrane library were searched in April 2021. The search strategy included the use of Medical Subject Heading (MeSH) terms and text words, with the use of explosion technique. The complete search strategy, which was reviewed by a qualified librarian, is included in supplementary file 1. There was no restriction on the date of publication. Reports written in English, French and Spanish were eligible. Abstracts and unpublished studies were not considered. Two reviewers, FS and LSR, screened each record independently, in a two-stage process: first the reviewers examined titles and abstracts. The full texts were then retrieved and the reviewers examined the full-text reports for compliance with the eligibility criteria. After retrieval of articles from the search, the reference lists of all selected articles were checked for other potentially relevant articles; two additional papers were identified. Disagreements were discussed between the two reviewers until an agreement could be reached. References were managed in EndNote 20 (Clarivate Analytics). The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow chart of identified studies is illustrated in Fig. 1.

Data extraction and statistical analysis

Data were extracted into MS Excel by FS using a tool which included author name, study design, sample size, population group, age group, year when the study took place, country, malaria endemicity profile, method of diagnosis of malaria, clinical definition of malaria, malaria species, and the summary statistics of ferritin, hepcidin, retinol and RBP. Although Hb and soluble transferrin receptors are sometimes used to describe iron status, we did not include them in our analysis, as Hb is not a specific indicator of iron deficiency and soluble transferrin receptors concentrations are not reported as often as ferritin concentrations. The malaria endemicity profile was defined by the *Plasmodium falciparum* prevalence rate (PfPR) among children aged 2–10 years, as described in the Malaria Atlas Project[15]. The different categories were defined by the WHO[16]: PfPR < 1 %: very low intensity, PfPR ≥ 1 % and < 10 %: low intensity, PfPR ≥ 10 and < 35 %: moderate intensity,

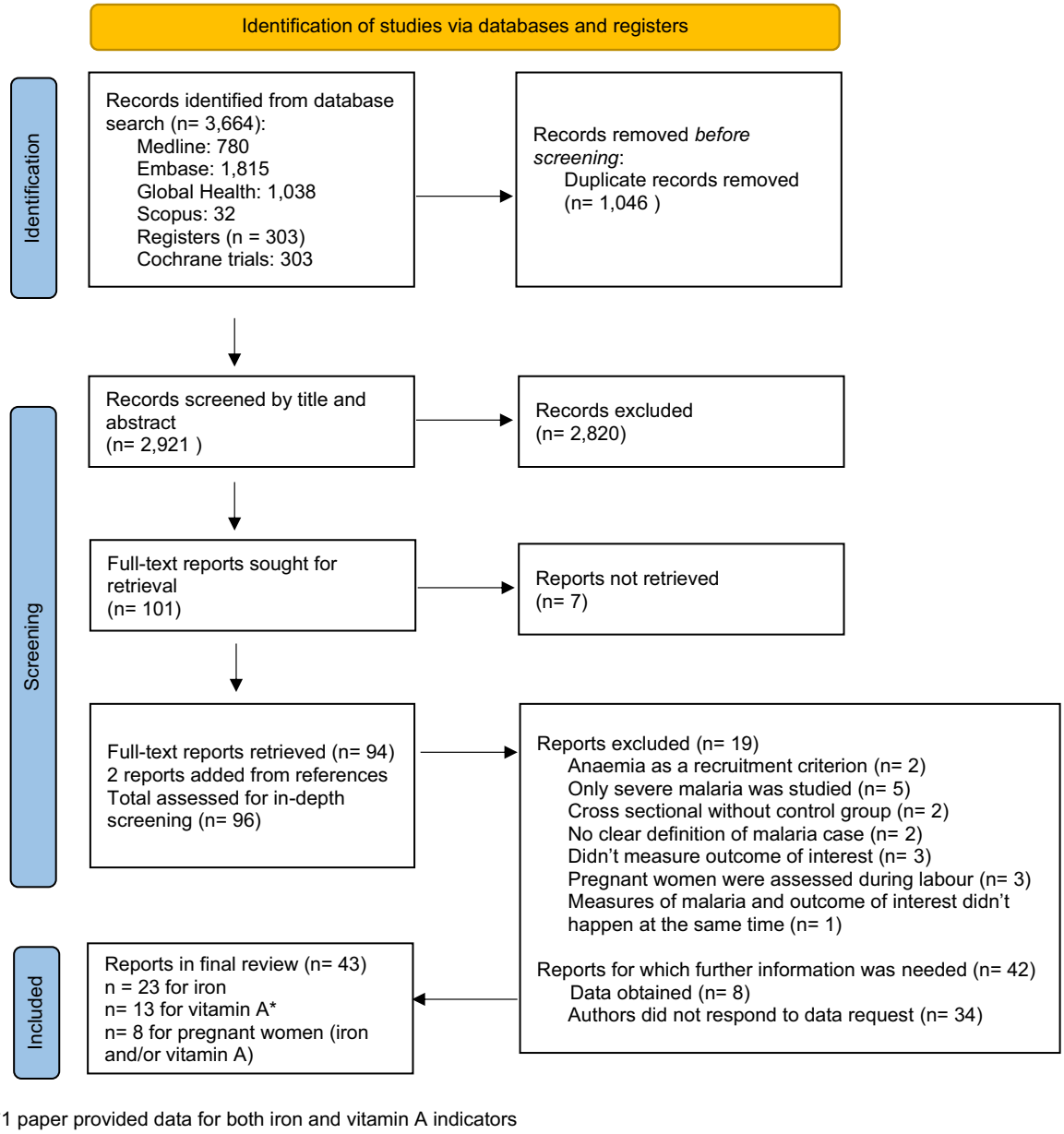

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram of publications screened in a systematic review of the impact of malaria infection on indicators of iron and vitamin A status.

PfPR ≥ 35 %: high intensity. The data from three clinical groups were included: healthy participants with negative malaria test results, asymptomatic participants with positive malaria test and without clinical sign of illness, and a clinical malaria group who had a positive malaria test and fever. For prospective studies, the biomarker measurement at admission was considered the measurement of the malaria group, and the measure at the final follow-up point was considered the measurement of the control group. We analysed separately three population groups: children, non-pregnant adults and pregnant women. When a study provided data for different malaria species, or for different age groups, the corresponding data were entered into different datasets to allow for subgroup analysis, which explains why there is a greater number of datasets than studies.

If the data from the same group were used for two comparisons in the same meta-analysis, we halved the number of participants from this group in each comparison, following the method described in the Cochrane Handbook[17]. Authors were contacted if the relevant information was not available in the report. The risk of bias of all included studies was assessed by FS, using the US National Institute of Health quality assessment tool for observational cohort and cross-sectional studies. This tool contains fourteen questions around key concepts for evaluating the internal validity of a study. They are not intended to create a final score but help to assess potential selection, information, measurement and confounding biases. The use of this tool for cross-sectional studies was recommended in a recent review by Ma *et al.*[18]. For the outcomes specified earlier,

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we reported the mean value of different groups (healthy control group, asymptomatic malaria group and clinical malaria group), as well as the 95 % CI or the standard deviation. We calculated the mean difference with 95 % CI between groups. We also attempted to calculate missing information from other reported measurements, if possible. When the geometric mean was provided, we transformed it into an arithmetic mean using the method explained by Higgins[19]. We generated meta-analyses based on the severity of the disease, either asymptomatic or clinical malaria and specific outcomes, such as ferritin or retinol concentrations. We first calculated a summary statistic for each study to describe the observed malaria effect. As our data were continuous, the summary statistic was a difference between means and a 95 % CI. When the data came from prospective studies, we followed the method described in the Cochrane Handbook to impute a standard deviation change from baseline to endline[17]. We then used a random effects meta-analysis for combining data, as we anticipated that there may be natural heterogeneity between studies attributable to the different populations and settings. The study weights were equal to the inverse of the variance of each study's effect estimate according to the methodology developed by DerSimonian and Laird[20]. We generated forest plots and we provided a CI, which communicates the precision (or uncertainty) of the summary estimate and a *P*-value.

When data were available, and when more than one study provided relevant data for meta-analysis, we conducted the following subgroup analyses: species of malaria (falciparum *v.* vivax), malaria endemicity profile of the country (low *v.* moderate *v.* high intensity of transmission, as defined by[15,16]), method of diagnosis of malaria (rapid diagnostic tests *v.* microscopy or both), age of children (under *v.* over 5 years old) and design of the study (cross-sectional *v.* prospective study). Heterogeneity was assessed using the I^2 statistic. Both a qualitative (funnel plot) and a quantitative (Egger's regression test) approach were used to examine potential publication biases. An influence analysis was conducted to determine the effect of removing each included study on the overall effect and 95 % CI using the technique described by Viechtbauer and Cheung[21]. The meta-analyses, funnel plot, Egger's regression test and influence analysis were conducted using RStudio software version 1.3.959[22] with the dmetar (v. 0.0.9000[23]) and meta-packages[24]. The workbooks for meta-analysis (version 1.5) developed by Suurmond were used to perform the subgroup analyses[25]. A *P*-value of $< 0{\cdot}05$ for meta-analyses was considered statistically significant.

Results

Of 101 full-text reports screened, 43 papers describing 44 studies conducted in 27 countries met selection criteria[7,10,11,13,26–65] (Fig. 1). Twenty-nine studies were conducted in fifteen African countries[10,11,13,26,28–31,38–41,46,47,49–53,55,57,62,63], nine were conducted in Asia[27,32,36,37,42,44,48,56,58], two in Oceania[54,64], three in Europe[43,45,48](two studied imported cases and one experimental malaria infection) and one in the Americas[35] (Table 1). Among the included studies, twenty-three reported on ferritin and/or hepcidin concentrations in adults and/or children[7,10,13,45–64], thirteen reported on retinol or RBP concentrations in adults and/or children[11,34–44,63], and eight reported on either ferritin, hepcidin, retinol or RBP concentrations in pregnant women[26–33]. Eight studies compared the ferritin or retinol level between groups with different severity of malaria[13,30,40,51,52,54,56,64]. Thirty-four studies were cross-sectional[11,13,26–34,36–40,42,44,50–55,57–60,62–64], whereas ten were prospective[7,41,43,45–49,56,61]. The predominant species of malaria was *P. falciparum* in forty-one studies[7,11,13,26,28–34,36–43,45–57,59,60,62–64], whereas *P. vivax* was either predominant or as present as *P. falciparum* in four studies[27,35,44,58]. A broad range of endemic profiles were represented. The pooled sample size for the analysis of children (*N* 14 330) was larger than for adults (*N* 985) (Table 2). The risk of bias assessment revealed that the majority of studies had a low or unclear risk of bias (online Supplementary Table 1).

Asymptomatic malaria and ferritin concentrations in children and adults

Fifteen studies[7,10,49–51,53,57,59,60,63,64] (twenty-three datasets) analysed the association between malaria and ferritin concentrations in asymptomatic children (4309 children with malaria infection and 6375 control children). Overall, ferritin concentrations were 28·2 μg/l (95 % CI 15·6, 40·9, $P < 0{\cdot}001$) greater in children with asymptomatic malaria compared with control groups (Fig. 2). The subgroup analyses did not reveal any differences (Table 3). There was strong evidence of between-study heterogeneity of effect ($I^2 = 99$ %). Heterogeneity was not explained by descriptive study factors (Table 3). The sensitivity analysis showed the stability of the pooled results after the leave-one-out analysis (Supplementary Fig. 1), and all studies had a low or unclear risk of bias (Table 4). The funnel plot and the Egger test did not show significant asymmetry, indicating no significant publication bias for this analysis (Supplementary Fig. 2).

A greater ferritin concentration was also observed in non-pregnant adults with asymptomatic malaria (28·5 μg/l, 95 % CI 8·1, 48·8, $P = 0{\cdot}02$) (Fig. 3). This mean difference was calculated for 234 adults with malaria and 400 control adults, from 4 studies conducted in sub-Saharan Africa in settings with moderate or high intensity of malaria transmission[47,51,55,57]. Given the limited number of studies, we did not perform subgroup analyses. The heterogeneity was moderate (51 %) and the sensitivity analysis showed the stability of the pooled result (Supplemental Fig. 3, Supplemental Fig. 4 and Table 4)

Clinical malaria and ferritin concentrations in children and adults

Seven studies[13,46,51,52,54,61,64] (nine datasets) analysed the association between clinical malaria and ferritin concentrations in children (595 children with clinical malaria and 876 healthy, control children). These studies were conducted in Africa, in Oceania and in the Americas. Overall, ferritin concentrations were 366 μg/l (95 % CI 162, 570) $P < 0{\cdot}003$) greater in children with clinical malaria compared with control group (Fig. 4). The sub-group analyses showed that the difference in mean

Table 1. Characteristics of the studies included in the systematic review of the impact of malaria infection on indicators of iron and vitamin a status

n	Author, publication year	Period the study was conducted	Country	Study design	Study participants	Malaria endemicity profile*	Total cases	Clinical severity	Malaria species	Diagnostic of Malaria	Outcomes measured
Studies reporting on iron status indicators											
1	Barffour *et al.* 2018[10]	2012–2013	Zambia	CS nested in a cluster-RCT	Children 4–8 years	Moderate intensity, seasonal transmission; two cross-sectional surveys conducted, one during high and one in low transmission period	744	A and control	F	RDT (HRP2) and P†	Ferritin
2	Beesley *et al.* 2000[45]	NA	Europe	Prospective study	Adults	Imported cases	36	C	F	P	Ferritin
3	Burte *et al.* 2013[13]	2008–2010	Nigeria	CS as part of a larger prospective study	Children 6 months–12 years	High intensity, year-round	117	DC, C, CM, SMA and control	F	P	Ferritin
4	Castberg *et al.* 2018[46]	2014–2015	Ghana	Prospective study as part of a larger study	Children 1–12 years	Moderate intensity, year-round	98	C	F	P	Ferritin, hepcidin
5	Cercamondi *et al.* 2010[47]	NA	Benin	Prospective study	Adults (women)	High intensity, seasonal transmission; survey conducted during high transmission season	23	A	F	P	Ferritin, hepcidin
6	de Mast *et al.* 2010[7]	NA	Indonesia	Prospective study	Children 5–15 years	Low intensity, year-round	108	A and control	F and V	P	Ferritin, hepcidin
7	de Mast *et al.* 2009[48]	1999–2003	Europe	Prospective experimental human malaria infection	Adults	Experimental infection	5	C and control	F	P	Ferritin, hepcidin
8	Diallo *et al.* 2020[26,65]	2011–2014	Burkina Faso	CS surveys included in a prospective study	Pregnant women and infants	High intensity, year-round	Women: 916	Positive malaria test and control	F	P	Ferritin
9	Dreyfuss *et al.* 2000[27]	1994–1997	Nepal	CS as part of an RCT	Pregnant women	Very low intensity, year-round	288	C and control	V	P	Ferritin
10	Glinz *et al.* 2015[49]	NA	Cote d'Ivoire	Prospective study	Children 11–17 years	Moderate intensity, year-round	17	A	F	P	Ferritin, hepcidin
11	Jeremiah *et al.* 2007[50]	2005–2006	Nigeria	CS	Children 1–8 years	High intensity, year-round	240	A and control	F	P	Ferritin
12	Kabore *et al.* 2020[51]	2016–2017	Burkina Faso	CS	Children (over 3 months) and adults	Moderate intensity, seasonal transmission; survey conducted during high transmission season	Children: 722 Adults: 396	A and C and control	F	P	Ferritin, hepcidin
13	Kivibidila *et al.* 1999[52]	1991	DR Congo	CS	Children 6 months–16 years	Moderate intensity	44	CM, C and control	F	P	Ferritin
14	Mockenhaupt *et al.* 2000[28]	1998	Ghana	CS	Pregnant women	High intensity	530	Positive malaria test and control	F and one case of P. Ovale	P and PCR	Ferritin
15–18	Muriuki *et al.* 2020[53,73–76]	2013	Burkina Faso	CS	Children 12 months	High intensity, year-round	348	A and control	F	P	Ferritin, hepcidin
		2001	The Gambia	CS as part of a prospective study	Children 2–6 years	Moderate intensity, seasonal transmission; survey conducted during high transmission season	753	A and control	F	P	Ferritin, hepcidin
		2012–2014	Kenya	CS as part of a prospective study	Children under 5 years	Low intensity, seasonal transmission; survey conducted during high transmission season	1484	A and control	F	P	Ferritin, hepcidin
		2003–2005	Uganda	CS as part of an RCT	Children under 5 years	Moderate intensity, year-round	1374	A and control	F	P	Ferritin, hepcidin
19	Mwangi[29]	2011–2014	Kenya	CS as part of an RCT	Pregnant women	Low intensity, year-round	470	C and control	F	P	Ferritin
20	O'Donnell *et al.* 2009[54]	1993–1996	Papua New Guinea	CS	Children under 4 years	Low intensity	495	C, severe malaria and control	F	P	Ferritin
21	Odunukwe *et al.* 2000[55]	NA	Nigeria	CS	Adults	High intensity, year-round	300	A and control	F and malariae	P	Ferritin
22	Phillips *et al.* 1986[56]	NA	Thailand	Prospective study	Adults	Very low intensity	23	C, CM and control	F	P	Ferritin
23	Righetti *et al.* 2013[57]	2010	Cote d'Ivoire	CS as part of prospective study	Children 6 months–8 years and adults	High intensity, year-round	Children: 246 Adults: 92	A and C	F	RDT (HRP2) and P‡	Ferritin
24	Saad *et al.* 2012[30]	2010	Sudan	CS	Pregnant women	Low intensity	64	Severe, C and control	F	P	Ferritin
25	Seyrek *et al.* 2004[58]	NA	Turkey	CS	Adults	Very low intensity	31	C and control	V	P	Ferritin
26	Shulman *et al.* 1996[31]	1993	Kenya	CS	Pregnant women	Low intensity, seasonal. Survey conducted during a low transmission period	275	C and control	F	P	Ferritin

Table 1. (*Continued*)

n	Author, publication year	Period the study was conducted	Country	Study design	Study participants	Malaria endemicity profile*	Total cases	Clinical severity	Malaria species	Diagnostic of Malaria	Outcomes measured
27	Stoltzfus *et al.* 1997[59]	1994	Zanzibar	CS	Schoolchildren 5–19 years	Moderate intensity, year-round	3605	A and control	F	P	Ferritin
28	Stoltzfus *et al.* 2000[60]	1996	Zanzibar	CS	Children under 5 years	Moderate intensity, year-round	464	A and control	F	P	Ferritin
29	Uscategui *et al.* 2009[61]	2002	Colombia	Prospective case study	Children 4–10 years	Very low intensity	89	C	F and V	P	Ferritin
30	Verhoef *et al.* 2001[62]	1997	Kenya	CS	Children 2–36 months	Moderate intensity, seasonal transmission; survey conducted during high transmission season	318	A and control	F	P	Ferritin
31	Wessells *et al.* 2014[63]	2009	Burkina Faso	CS data from an RCT	Children 6–23 months	High intensity, year-round	437	A and control	F	ELISA (HRP2)	Ferritin
32	Wessells *et al.* 2017[33]	2014–2015	Niger	CS as part of an RCT	Pregnant women	Moderate intensity	787	Positive malaria test and control	F	ELISA (HRP2)	Ferritin
33	Williams *et al.* 1999[64]	1994	Vanuatu	CS	Children, mean age: 11 years	Low intensity, seasonal transmission; survey conducted during high transmission season	115	A, C and control	F and V	P	Ferritin
Studies reporting on vitamin A status indicators											
1	Barffour *et al.* 2018[34]§	2012–2013	Zambia	CS nested in a cluster RCT	Children 4–8 years	Moderate intensity, seasonal transmission; two cross-sectional surveys conducted, one during high and one in low transmission period	744	A and control	F	RDT (HRP2) and P†	Retinol
2	Carmona *et al.* 2008[35]‖	2002	Colombia	Prospective case study	Children 4–10 years	Very low intensity	89	C	F and V	P	Retinol
3	Das *et al.* 1996[36]	1992–1993	India	CS	Children 2–11 years	Low intensity	173	A, C and severe malaria	F	P	RBP, retinol
4	Davis *et al.* 1993[37]	1991	China	CS	Adults	Very low intensity	27	C and control	F and V	P	Retinol
5	Diatta *et al.* 2013[38]	NA	Senegal	CS	Children under 5 years	Low intensity, seasonal transmission; survey conducted during high transmission season	312	A and control	F	P	Retinol
6	Filteau *et al.* 1993[11]	1990–1991	Ghana	CS as part of a randomised double-blind study	Children 6–59 months	High intensity, year-round	59	A and control	F	P	Retinol
7	Inocent *et al.* 2007[39]	2004–2005	Cameroon	CS	Children 0–6 years	High intensity	116	C and control	F	P	Retinol
8	Mfonkeu *et al.* 2010[40]	2007	Cameroon	CS	Children 6 months–14 years	High intensity	139	C, MA, CM, CM & MA and control	F	P	Retinol
9	Nussenblatt *et al.* 2002[41]	1998	Uganda	Prospective study	Children 1–10 years	High intensity, year-round	273	C	F	P	Retinol
10	Raza *et al.* 2009[42]	2006–2007	India	CS	Children 2–5 years	Low intensity	170	C and control	F	P	Retinol
11	Stuetz *et al.* 2005[32]	1998–2000	Thailand	Secondary samples analysis	Pregnant women	Low intensity, year-round	108	Positive malaria test and control	F	P	Retinol
12	Tabone *et al.* 1992[43]	NA	Europe	Prospective study	Adults	Imported cases	7	C	F	P	Retinol, RBP
13	Thurnman *et al.* 1991[44]	NA	Thailand	CS	Adults	Very low intensity	45	C and control	F, V and mixed	P	Retinol
14	Wessells *et al.* 2014[63]	2009	Burkina Faso	CS data from an RCT	Children 6–23 months	High intensity, year-round	437	A and control	F	ELISA (HRP2)	RBP

CS, cross-sectional; A, asymptomatic malaria; F, falciparum; RDT, rapid diagnostic test; HRP2, histidine-rich protein 2; NA, non-available; C, clinical-uncomplicated malaria; P, parasitaemia by microscopy; DC, disease control; CM, cerebral malaria; SMA, severe malarial anaemia; V, vivax; RCT, randomised controlled trial; MA, malarial anaemia; RBP, retinol binding protein. 'Control' is defined as healthy children with no malaria detected by the study-specific diagnostic test.

* Seasonality was defined by the author and the information was not systematically reported. The intensity of transmission was defined by the *Plasmodium falciparum* prevalence rate (PfPR) among children aged 2–10 years, as described in the Malaria Atlas Project[15]. As defined by WHO[16], PfPR < 1 %: very low intensity, PfPR ≥ 1 % and < 10 %: low intensity, PfPR ≥ 10 and < 35 %: moderate intensity, and PfPR ≥ 35 %: high intensity. The PfPR is the proportion of the population found to carry asexual blood-stage parasitaemia, a basis for the classical categorical measures of malaria transmission.

† Malaria parasitaemia was detected by RDT and/or microscopy.

‡ Malaria diagnosis was by microscopy-confirmed RDT.

§ Participants are the same as in ref. 10.

‖ Participants are the same as in ref. 61.

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Table 2. Characteristics of individuals and settings from studies included in the systematic review of the impact of malaria infection on indicators of iron and vitamin A status
(Number and percentages)

	Children		Non-pregnant adults		Pregnant women		Total	
	n	%	*n*	%	*n*	%	*n*	%
Number of studies included	27		11		8		46*	
Number of participants in the included studies	14 330		985		3438		18 753	
Age in years (mean ± SD) of participants in the included studies								
Mean	6·2		29·6		24·1			
SD	4		9		5·1			
Estimated malaria transmission intensity during the survey in the included studies†								
Very low (< 1 %)	2	7 %	4	37 %	1	13 %	7	16 %
Low (1–10 %)	7	26 %	0		4	50 %	11	14 %
Moderate (10–35 %)	8	30 %	1	9 %	1	13 %	10	23 %
High (> 35 %)	10	37 %	3	27 %	2	25 %	15	34 %
Imported cases treated in Europe or experimental infection	0		3	27 %	0		3	6 %
Seasonality of transmission in the included studies								
Holoendemic	12	44 %	2	18 %	4	40 %	18	40 %
Seasonal	6	22 %	2	18 %	1	20 %	9	19 %
Imported cases treated in Europe or experimental infection	0		3	28 %	0		3	7 %
Not specified by authors	9	34 %	4	36 %	2	40 %	15	34 %

* Two reports provided data for children and adults.
† As defined in ref. 15 and 16.

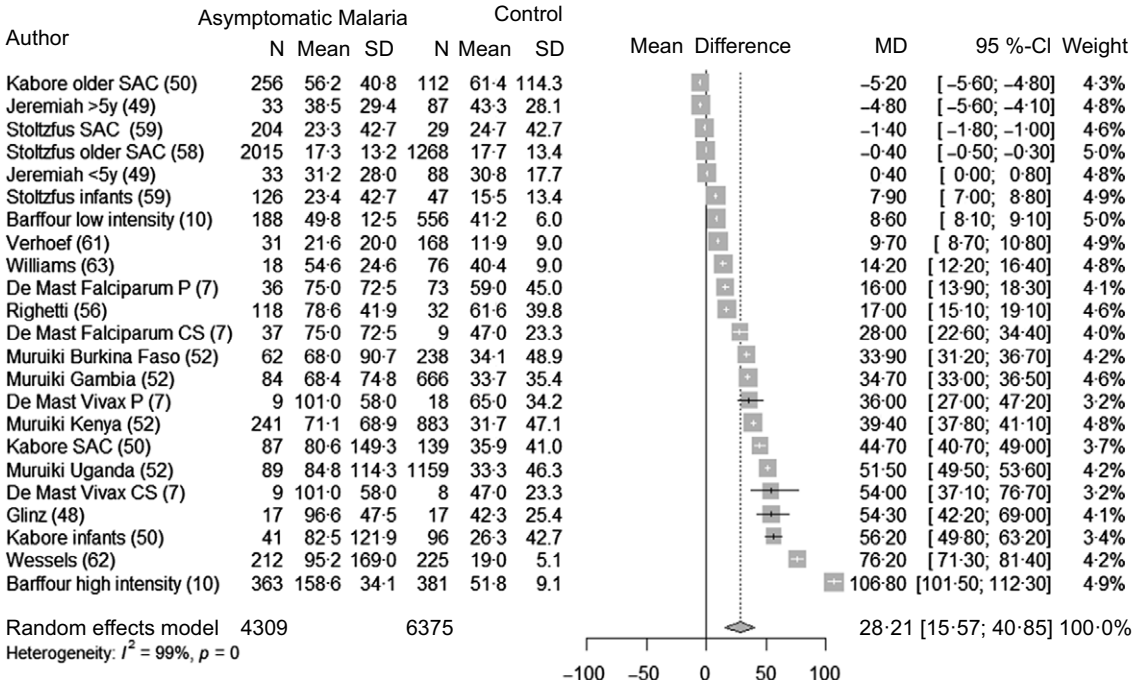

Fig. 2. Forest plot of differences in ferritin concentrations (μg/l) between children with asymptomatic malaria and control group, using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. SAC, school age children; P, prospective; CS, cross-sectional; MD, mean difference.

ferritin was the greatest in settings with moderate transmission, compared with low transmission (Table 5). The sensitivity analysis showed the stability of the pooled results after the leave-one-out analysis (Supplementary Fig. 5) and after removing the studies with high risk of bias (Table 4). The funnel plot revealed a publication bias for this analysis, with an under-reporting of small studies (Supplementary Fig. 6).

In terms of clinical malaria in adults, 269 adults were included in the analyses of 5 datasets, including an experimental infection and a study of imported cases in Europe. All the studies reported a higher ferritin concentration in the clinical malaria group (107 to 1554 μg/l elevation in the clinical group compared with the control group). In the meta-analysis, the CI was wide and included zero (493 μg/l, 95 % CI −219, 1206).

Table 3. Results of subgroup analyses for ferritin concentration (μg/l) in children with asymptomatic malaria parasitaemia and control group (Mean difference and 95 % confidence interval)

	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	$P_{subgroup}$
Endemic profile					0·69
High intensity	5	24·1	–4·34, 52·4	99 %	
Moderate intensity	12	28·6	9·8, 47·4	99 %	
Low intensity	6	30	18·4, 41·7	99 %	
Malaria species					0·06
P. falciparum	21	26·6	14·2, 39	99 %	
P. vivax	2	43·3	25·5, 61·1	66 %	
Age					0·12
< 60 months	12	30·6	16·8, 44·3	99 %	
> 60 months	11	23·3	3·4, 43·2	99 %	
Diagnostic method					0·51
Parasitaemia by microscopy	20	25·1	14·5, 35·7	99 %	
RDT or microscopy	2	57·7	–38·7, 154	99 %	
Design of the study					0·50
Cross-sectional	20	26·9	13·9, 39·9	99 %	
Prospective	3	34·7	12·9, 56·6	96 %	

RDT, rapid diagnostic test detecting histidine-rich protein 2.

Table 4. Summary of meta-analyses results by biomarker in children and adults with malaria parasitaemia compared with control group (Mean difference and 95 % confidence interval)

	All studies					Only studies with low or unclear risk of bias				
	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	*P*	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	*P*
Ferritin (μg/l)										
Children										
Asymptomatic malaria	23	28·2	15·6, 40·9	99 %	<0·001	23	28·2	15·6, 40·9	99 %	<0·001
Clinical malaria	9	366	162, 570	91 %	0·003	7	334	106, 563	92 %	0·01
Adults										
Asymptomatic malaria	4	28·5	8·1, 48·9	51 %	0·02	3	20·5	4·8, 36·3	0 %	0·03
Clinical malaria	5	493	–219, 1206	83 %	0·13	2	155	–758, 1069	57 %	0·28
Pregnant women	8	26·8	5·8, 47·7	100 %	0·02	7	18·1	5·6, 30·7	99 %	0·01
Hepcidin (nmol/l)										
Children										
Asymptomatic malaria	12	1·52	0·92, 2·11	96 %	<0·001	12	1·52	0·92, 2·11	96 %	<0·001
Clinical malaria	2	10·8	–18·1, 39·7	0 %	0·13	1	10·6	5·4, 15·8	NA	<0·001
Adults										
Asymptomatic malaria	2	0·3	–10·3, 10·9	52 %	0·78	2	0·3	–10·3, 10·9	52 %	0·78
Clinical malaria	2	6·3	–21·6, 34·3	52 %	0·21	2	6·3	–21·6, 34·3	52 %	0·21
Retinol (μmol/l)										
Children										
Asymptomatic malaria	4	–0·11	–0·22, –0·01	60 %	0·04	3	–0·10	–0·18, –0·03	21 %	0·03
Clinical malaria	6	–0·43	–0·71, –0·16	97 %	0·01	4	–0·36	–0·77, 0·06	93 %	0·07
Adults										
Clinical malaria	5	–0·73	–1·11, –0·36	54 %	0·005	1	–1·03	–1·40, –0·66	NA	<0·001
Pregnant women	1	–0·54	–0·67, –0·41	NA	<0·001	1	–0·54	–0·67, –0·41	NA	<0·001

NA, non-applicable.

The elevation of ferritin concentrations in clinical malaria patients increased with greater severity of malaria in all studies that included this analysis, in adults and children (data not shown).

Malaria infection and hepcidin concentrations in children and adults

Seven studies[7,49,51,53] (twelve datasets) were used to analyse the association between asymptomatic malaria parasitaemia and hepcidin concentrations in children. The studies were mainly conducted in Africa apart from one study (four datasets) that was conducted in Indonesia. Hepcidin concentrations were 1·52 nmol/l (95 % CI 0·92, 2·11, $P < 0·001$) greater in malaria-infected groups compared with controls (Fig. 5). No interaction was reported in the subgroup analyses (Table 6). The heterogeneity was high ($I^2 = 96$ %). The leave-one-out analysis and the sensibility analysis by risk of bias showed the stability of the pooled result (Supplementary Fig. 7 and Table 4). There

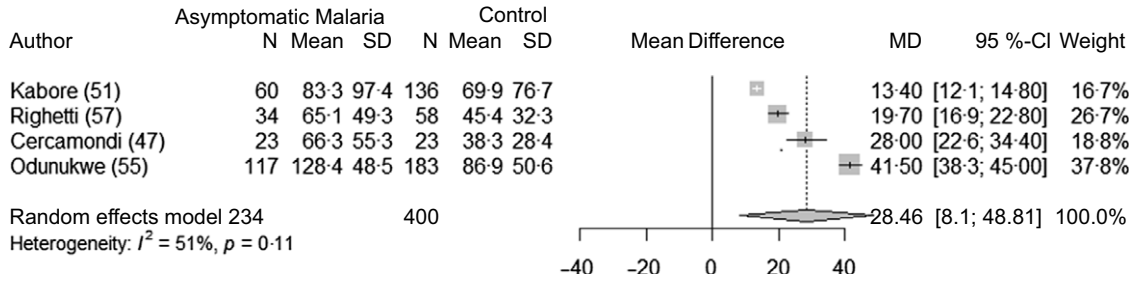

Fig. 3. Forest plot for differences in ferritin concentrations (μg/l) between adults with asymptomatic malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95 % CI. MD, mean difference.

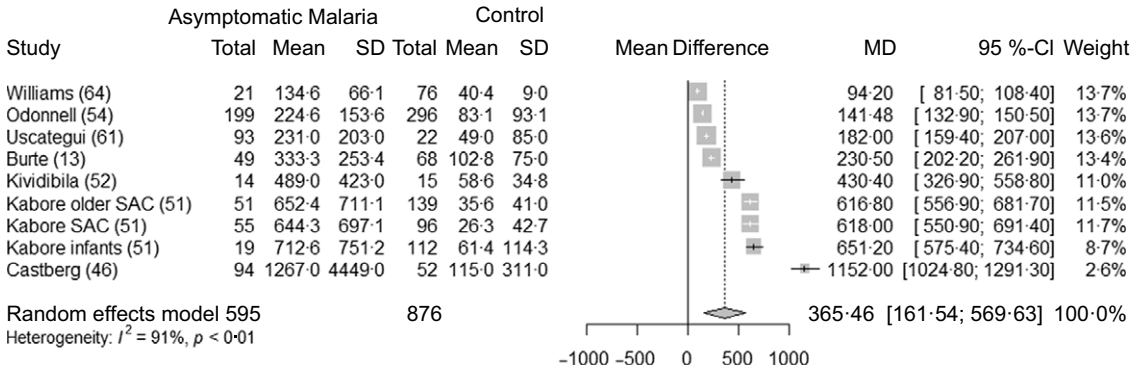

Fig. 4. Forest plot for differences in ferritin concentrations (μg/l) in children between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. SAC, school age children; MD, mean difference.

Table 5. Results of subgroup analyses for ferritin (μg/l) in children with clinical malaria parasitaemia and control group (Mean difference and 95 % confidence interval)

	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	$P_{subgroup}$
Endemic profile					<0·001
Moderate intensity	5	688	457, 919	94 %	
Low intensity	3	138	89, 188	96 %	
Age					0·13
<60 months	4	637	233, 1042	99 %	
>60 months	5	304	119, 490	99 %	
Study design					0·57
Cross-sectional	7	389	209, 570	99 %	
Prospective	2	665	−290, 1620	99 %	

was no significant publication bias, as assessed by the funnel plot and Egger's test (Supplementary Fig. 8).

Two studies[47,51] reported on asymptomatic malaria infection in adults and hepcidin concentrations, including 242 adults in total. There was a non-significant elevation in hepcidin in the malaria group of 0·3 nmol/l (95 % CI −10·3, 10·9). Two studies reported concentrations of hepcidin in clinical malaria infection in children[46,51] and reported a non-significant elevation in hepcidin of 10·8 nmol/l (95 % CI −18·1, 39·7) in the malaria group. In adults[48,51], there was also a non-significant elevation of hepcidin of 6·3 nmol/l (95 % CI −21·6, 34·3) in the malaria group.

Malaria infection and ferritin concentrations in pregnant women

Seven studies[26–31,33] analysed ferritin concentrations in pregnant women with or without malaria infection, as defined by a positive parasitaemia. The authors did not systematically report the presence of clinical symptoms. Pregnant women with malaria parasites had greater ferritin concentrations than control pregnant women without parasites (+26·8 μg/l, CI 5·8, 47·7, P = 0·02) (Fig. 6). The subgroup analysis revealed that the mean difference in ferritin was higher in settings with high malaria transmission (Table 7). Heterogeneity was high (I^2 = 100 %). The sensitivity analysis showed the stability of the pooled result

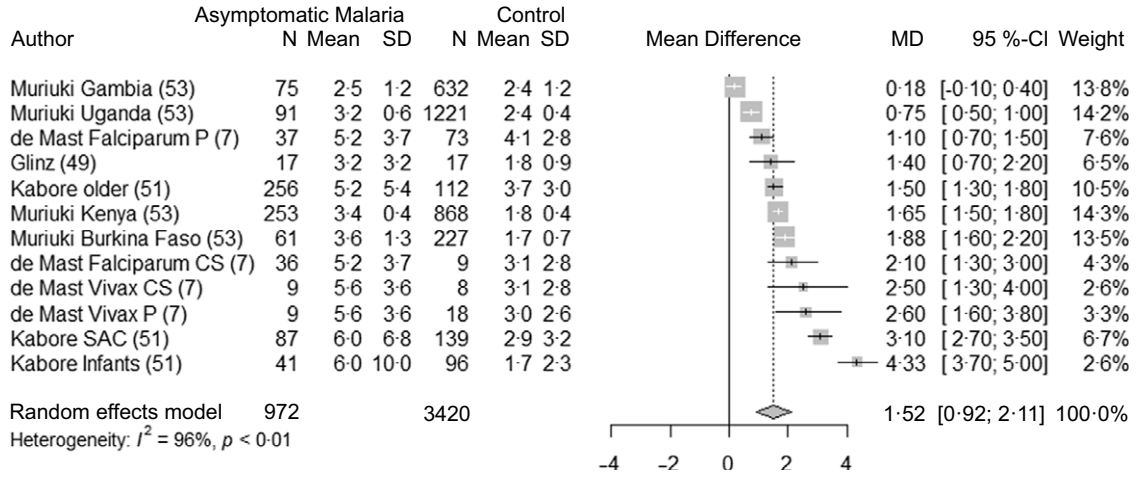

Fig. 5. Forest plot for differences in hepcidin concentration (nmol/l) in children between malaria parasitaemia and control groups using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. SAC, school age children; P, prospective; CS, cross-sectional; MD, mean difference.

Table 6. Results of subgroup analyses for hepcidin concentration (nmol/l) in children with asymptomatic malaria parasitaemia and control group (Mean difference and 95 % confidence interval)

Endemic profile	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	$P_{subgroup}$
Moderate intensity	6	1·86	0·62, 3·1	98 %	0·88
Low intensity	5	1·77	1·25, 2·29	68 %	
Malaria species					0·12
P. falciparum	10	1·77	1·04, 2·5	97 %	
P. vivax	2	2·56	2·46, 2·66	0 %	
Age					0·54
<60 months	6	1·95	0·74, 3·16	98 %	
>60 months	6	1·66	1·21, 2·11	60 %	
Design of the study					0·46
Cross-sectional	9	1·95	1·14, 2·76	98 %	
Prospective	3	1·58	0·73, 2·42	71 %	

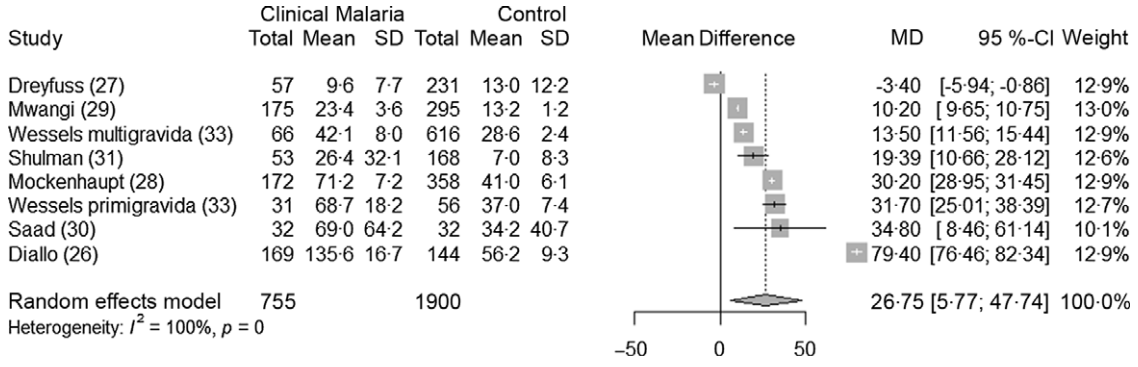

Fig. 6. Forest plot for differences in ferritin concentrations (μg/l) in pregnant women between malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. MD, mean difference.

(Supplementary Fig. 9 and Supplementary Fig. 10). After excluding the study with high risk of bias, the difference in ferritin concentration was lower (+18·1 μg/l, 95 % CI 5·6, 30·7, P = 0·01) (Table 4).

Asymptomatic malaria infection and retinol concentrations in children and adults

Three studies[11,34,38] (four datasets) analysed the association between malaria and retinol concentrations in asymptomatic

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Table 7. Results of subgroup analyses for ferritin concentration (μg/l) in pregnant women with malaria parasitaemia and control group (Mean difference and 95 % confidence interval)

Endemic profile	Number of datasets	Mean difference	95 % CI	I^2 for heterogeneity	$P_{subgroup}$
High intensity	2	54·8	−258, 367	99 %	<0·001
Moderate intensity	2	22·3	−93, 138	96 %	
Low intensity	3	16·5	−7·6, 40·6	74 %	

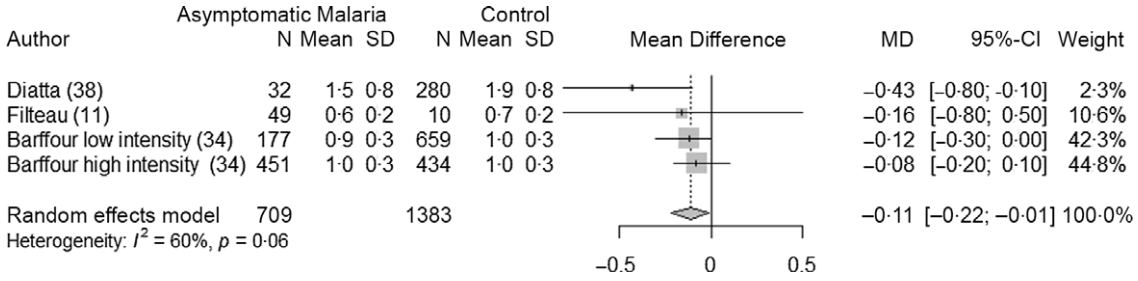

Fig. 7. Forest plot for differences in retinol concentration (μmol/l) in children between asymptomatic malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. MD, mean difference.

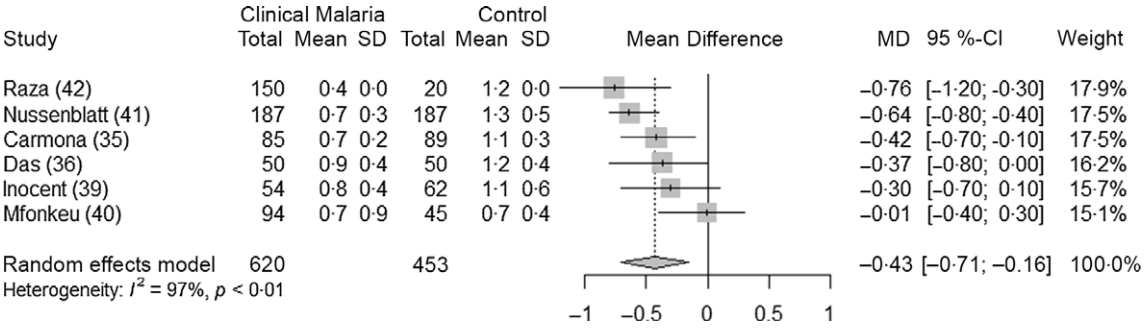

Fig. 8. Forest plot for differences in retinol concentration (μmol/l) in children between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. MD, mean difference.

children (709 children with malaria and 1383 control children). All studies were conducted in Africa and the species involved was always *P. falciparum*. Overall, retinol concentrations were lower, that is, −0·11 μmol/l (95 % CI −0·22, −0·01, $P = 0·04$) in children with asymptomatic malaria compared with control group (Fig. 7). We did not perform subgroup analyses because of the limited number of studies. The heterogeneity was moderate ($I^2 = 60$ %) and the sensitivity analysis showed the stability of the pooled result after the leave-one-out analysis, and after removing the studies with high risk of bias (Supplementary Fig. 11, Supplementary Fig. 12 and Table 4).

There were no studies on the associations between asymptomatic malaria and retinol concentrations in adults.

Clinical malaria infection and retinol concentrations in children and adults

Six studies[35,36,39–42] analysed the association between clinical malaria and retinol concentrations in children (620 children with malaria parasitaemia and 453 control children). The analysis showed that retinol concentrations were reduced during an infection (−0·43 μmol/l, 95 % CI −0·71, −0·16, $P = 0·01$) (Fig. 8). When the studies with high risk of bias were excluded, the mean difference in retinol concentration was no longer statistically significant (Table 4). There were no differences noted in the subgroup analyses (Table 8).

Three studies[37,43,44] (five datasets) analysed the association between clinical malaria and retinol concentrations in adults (69 adults with malaria and 64 control adults). Two of the studies were conducted in Asia, and they were both conducted in settings with very low intensity of transmission. One was conducted in Europe with imported cases, and the author did not specify the endemicity profile of the country of origin. Retinol concentrations were lower in adults with clinical malaria compared with healthy control adults, by 0·73 μmol/l (95 % CI −1·11, −0·36, $P = 0·005$) (Fig. 9). Due to the limited number of studies, we did not perform any subgroup analyses. The heterogeneity was moderate ($I^2 = 54$ %). The leave-one-out analysis showed the stability of pooled results (Supplementary Fig. 13 and Supplementary Fig. 14). Only one study in this analysis was considered at low risk of bias (Table 4).

Only one study[32] reported data on retinol concentrations in pregnant women with malaria. They found a significantly

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Table 8. Results of subgroup analyses for retinol concentration (μmol/l) in children with clinical malaria parasitaemia and control group (Mean difference and 95 % confidence interval)

	Number of datasets	Mean Difference	95 % CI	I^2 for heterogeneity	Psubgroup
Endemic profile					0·55
High intensity	3	−0·34	−0·70, 0·03	80 %	
Low intensity	3	−0·47	−0·68, −0·27	0 %	
Age					0·9
< 60 months	4	−0·43	−0·75, −0·1	74 %	
> 60 months	2	−0·40	−0·45, −0·35	0 %	

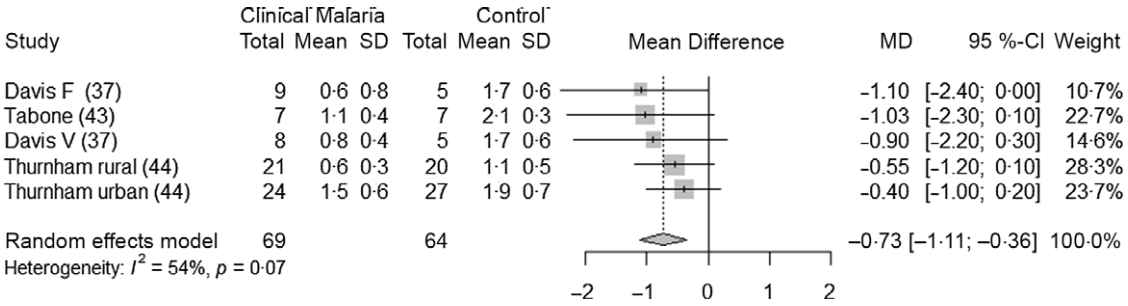

Fig. 9. Forest plot for differences in retinol concentration (μmol/l) in adults between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95 % CI. The hollow diamond represents the overall pooled effects, while the left and right points of the diamond represent the corresponding 95 % CI. F, falciparum; V, vivax; MD, mean difference.

reduced concentration of retinol in pregnant women with malaria (−0·54, 95 % CI −0·67, −0·41, $P < 0·001$).

Malaria infection and retinol binding protein concentrations in children and adults

One study[63] conducted in Burkina Faso with 262 children found that children with asymptomatic malaria had lower RBP values than the control group, and the mean difference was −0·13 (95 % CI −.17, −0·09, $P < 0·001$). One study[36] conducted in India with 100 children presented data on RBP concentration in children with clinical malaria and found that children with clinical malaria had lower RBP values than the control group and the mean difference was −1·52 (95 % CI −1·70, −1·35, $P < 0·001$).

Only one study reported RBP data in adults during clinical malaria[43], and the sample size was too small to report the data (seven patients).

Discussion

We conducted several meta-analyses to estimate associations between malaria infection and nutrition biomarkers by using data from cross-sectional and prospective studies. Although mostly based on data from observational studies, our analyses indicate that malaria infection is associated with increased ferritin and reduced retinol concentrations even in asymptomatic infections, when individuals might not have elevated markers of inflammation.

Association between malaria and iron indicators

The results provide strong and consistent evidence that malaria infection, asymptomatic or symptomatic, is associated with increased ferritin concentrations in children. This result was expected, as ferritin synthesis is highly upregulated by inflammatory cytokines and by infections including malaria[53,66,67]. In our analyses, the increase in ferritin concentration during an asymptomatic infection was similar in children and adults, and there were no differences noted in the subgroup analyses. The quality of the evidence for children seems strong as the sample size for analysis is large, the CI is relatively small and the sensitivity analysis did not reveal any significant influencer in the results or publication bias. Moreover, studies included in this analysis were considered of good quality based on the risk of bias analysis. The increase in ferritin concentration did not vary by age group. For residents in malaria-endemic areas, parasitaemia peaks in children younger than 5 years old and subsequently declines in an age-dependent manner[68]. In populations living under heavy exposure to malaria and frequent infection, individuals tend to develop partial immunity against the disease earlier in childhood, which may explain why children under 5 years old did not have a greater increase in ferritin than older children. However, this interpretation is limited by the fact that age groups were not particularly well defined and therefore, there might have been differences between age groups that we were not able to observe.

Overall, in the case of an asymptomatic malaria infection, the average increase in ferritin concentration is estimated at c. 28·5 μg/l, across all settings and all groups of population. Considering that the mean value of ferritin concentration in control groups was c. 25 μg/l, this indicates that the ferritin concentration in asymptomatic malaria infection was approximately doubled compared with a control group. As a comparison, based on sixteen datasets included as part of the BRINDA collaboration, Namaste and colleagues have assessed

the increase in ferritin concentrations during an inflammation process, based on elevated CRP and/or AGP[66]; both markers are elevated in large proportions of children in low- and middle-income countries[69]. This definition is imperfect, as inflammation is a complex process than cannot be captured simply by the elevation of these two acute-phase proteins. However, in the absence of other widely available biomarkers of inflammation in population-based surveys, we continue to rely on a definition on inflammation based on these two markers. Namaste reported that ferritin concentration increases from 19·5 μg/l in the reference group to 50·8 μg/l during the early convalescence phase of an inflammatory episode, which in this analysis was defined as when CRP and AGP concentrations are at their highest[66]. This represents an increase of about 30 μg/l, which is in the same range as the increase we see during a malaria asymptomatic infection. For women of reproductive age, they report an increase in the same range (c. 30 μg/l).

The recent WHO guidelines on the use of ferritin concentrations to define iron status recommend adjusting for inflammation and indicate that it is possible to adjust for malaria. There are, however, currently no details on why this adjustment should be made or whether some specificities, such as the severity of the infection, the population group or the endemic profile should be considered. An important question for micronutrient surveys is whether elevated acute-phase proteins fully capture the effects of malaria on micronutrient markers, as might be assumed from the similar differences in ferritin due to malaria or CRP plus AGP, or whether we should account for both inflammation and malaria. Several studies included in this review report that not all children with asymptomatic malaria have elevated CRP or AGP. In the study in Burkina Faso in children with asymptomatic malaria, Barffour reported that only half of the children with malaria also had elevated AGP during low malaria season, based on AGP concentrations >1 g/l[10]. Righetti found similar results in Cote d'Ivoire. Among children 6–8 years old with asymptomatic malaria, 55 % had neither CRP concentration > 5 mg/l nor AGP concentration > 1g/l[57]. In non-pregnant women, this proportion was even higher (65 %). Similarly, de Mast found low circulating concentrations of CRP in Indonesian schoolchildren with asymptomatic parasitaemia, and 84 % of them had CRP concentrations < 5 mg/l, the threshold to define inflammation[7]. These findings could be attributed to the use of thresholds for CRP and AGP and might mask a mild elevation in inflammatory markers. Several studies found that even after adjusting for raised CRP and/or AGP with the regression method, ferritin concentrations were higher in children suffering from asymptomatic malaria than in the control group[63,70]. In children 6–23 months old in Burkina Faso, Wessells found that after adjusting for acute-phase proteins, children with asymptomatic malaria had greater plasma ferritin concentrations than the control group (23·5 ± 1·5 μg/l *v.* 11·1 ± 0·8 μg/l; $P < 0·001$)[63]. Muriuki found that children with malaria had greater ferritin concentrations at every decile of CRP, compared with those without malaria. Even in the lowest decile of CRP, the difference in ferritin between children with malaria and without malaria was of about 20 μg/l. They found that malaria parasitaemia also increased ferritin levels independently of increased CRP and/or AGP in multivariable analyses[53].

In longitudinal studies looking at ferritin concentrations and inflammatory markers concentrations after a malaria infection, it is notable that, even if CRP and AGP concentrations are slightly elevated during an asymptomatic infection, their concentrations go back to normal rapidly once the malaria infection is cleared, while ferritin concentrations remain elevated for about 1 month after the infection[47,49]. Considering this, we can assume that individuals with an asymptomatic malaria infection are either not suffering from inflammation or have elevated CRP and AGP for a period of time that is shorter than the time needed for their ferritin concentration to return to normal.

The highest increase in ferritin is unsurprisingly seen in clinical malaria, even though we could not reach a conclusion on clinical infection and ferritin in adults, due to the high heterogeneity and the small number of adults included. In children, the highest increase in ferritin was observed in the countries with a moderate parasite rate. In pregnancy, there was also a significant increase in ferritin concentrations in pregnant women with malaria, of c. 27 μg/l. The difference in mean concentration was greater in settings with high transmission intensity.

Hepcidin concentrations were also increased during malaria infections. As for the ferritin data, these datasets included children with and without raised CRP or AGP, and we can assume that the increase in hepcidin concentration might be occurring through both an inflammatory[49] and a non-inflammatory pathway, as suggested previously[14,53]. Hepcidin reduces iron absorption from the gut and increases iron sequestration, resulting in a decrease of iron in the blood and a decrease in erythropoiesis[49]. Considering the high proportion of the population suffering from asymptomatic malaria infections in sub-Saharan Africa, this increase in hepcidin concentration could help to explain why iron deficiency prevalence remains high in population surveys and why iron supplementation and iron fortification programmes have been less effective than expected[71].

Associations between malaria and indicators of vitamin A deficiency

Children with an asymptomatic infection had lower values of serum retinol than the control group. In case of a clinical infection, the reduction was greater. In adults, lower values of serum retinol were also observed in case of a clinical infection. The reductions we observed in children and adults with a clinical infection (respectively 0·43 and 0·73 μmol/l) were greater than the reductions in retinol due to inflammation defined by elevated CRP and/or AGP, reported by the BRINDA collaboration. The BRINDA collaboration reported that inflammation reduces retinol by 0·27 μmol/l in preschool children and by 0·24 μmol/l in women of reproductive age[66]. However, the populations are different as BRINDA is more likely to include datasets coming from healthy participants while in these specific analyses, patients were ill and hospitalised due to malaria. Also, in these analyses, not all endemic profiles were represented. These results need to be interpreted with caution as the sample size for these analyses were small. The acute-phase response to either infection or inflammation affects retinol homoeostasis, and substantial vitamin A can be lost in the urine

during illness accompanied by high fever[3]. There may also be increased tissue retinol uptake due to increased needs for retinol in infection which could decrease plasma retinol concentrations[44]. An alternative explanation is that sick children tend to eat less, and malaria could lead to vitamin A deficiency in children who already had low reserves of vitamin A[72].

Implications for large-scale surveys interpretation

The magnitude of change in ferritin and retinol concentrations in the case of an asymptomatic infection is likely to affect the estimations of iron and vitamin A deficiency in population-based surveys. Asymptomatic individuals are often present in these surveys. They are likely to experience an increase in ferritin without a significant elevation of CRP and AGP values, and therefore their ferritin values would not be adjusted by the BRINDA method as it is recommended by WHO. With regard to retinol, even if BRINDA has recommended to adjust for inflammation in children, WHO does not currently recommend any adjustment. Children and adults with asymptomatic infection could be wrongly considered as iron replete or vitamin A-deficient, and the validity of the deficiency prevalence estimates could be affected. Adjusting for malaria is only referred to as a 'possible adjustment' in the WHO guidelines on ferritin, and there is no mention of asymptomatic infections. However, we observed that ferritin concentrations were elevated by 28·2 μg/l in asymptomatic children and by 366 μg/l in children with clinical malaria compared with healthy children. Retinol concentrations were reduced by 0·11 μmol/l in asymptomatic children and by 0·43 μmol/l in children with clinical malaria. These data suggest an important difference according to the severity of infection, and this could have important repercussions in the assessment of iron and vitamin A status in populations where different forms of malaria infections co-exist. Even if individuals with clinical malaria are not likely to be included in surveys, people recovering from clinical malaria might be. Applying a single correction factor to all forms of malaria, as is currently recommended in the WHO guidelines, would certainly affect estimates coming from micronutrient surveys and other surveys that assess the iron status of a sample of a population in a malaria endemic setting. More research should be done to confirm whether the study setting should be considered when applying an adjustment, as our data seem to indicate that a clinical infection could have different repercussions on ferritin concentrations depending on the endemic profile. We did not have enough datasets to analyse infections with *P. vivax*, and it requires further research. In our analyses, the use of different malaria diagnostic methods did not seem to impact the magnitude of the effects.

Limitations

The primary limitation to these analyses is the variability between studies, including the large age range among children in some of the datasets. Another limitation is the population used as a control group, as most studies had different strategies to recruit their control group. We did not investigate whether the children were suffering from hookworm or Schistosoma infections, which could have affected further their iron status. Another limitation is the generalisability of the results related to clinical infections. Most of the studies including clinical infections were conducted at the hospital, whereas in population-based surveys conducted to measure the micronutrient status of a population, participants would be sampled in the community. We also acknowledge that our search strategy, despite being large and inclusive of three languages of publication, did not include grey literature or regional databases, which might have introduced a selection bias in the systematic review. Finally, although there were many studies on the association of ferritin and malaria in children, fewer studies were included in other meta-analyses, and these included studies had higher risk of bias, suggesting caution is required in interpreting these results.

Conclusion

The findings of this systematic review and meta-analysis suggest that malaria infection should be measured and adjusted for in nutritional surveys of populations living in malaria endemic areas, particularly for assessments of iron status. Malaria test results should be reported in population-based surveys, as well as a measure of clinical symptoms in the participant. This will allow more accurate adjustment of serum ferritin concentrations to define individual iron status. Preliminary analyses indicate that retinol concentration also is affected by malaria, but not enough data are currently available to support firm conclusions for children and adults. Further research is needed to develop individualised adjustment methods that can take into account the concentrations of acute-phase proteins and the presence, and severity, of a malaria infection.

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FS, EJMJ, SF and HH conceptualised the analytical protocol. FS and LSR conducted the literature search. FS conducted the study data analyses with the support of AMDFS was the primary writer. All authors reviewed the content of the manuscript and provided feedback. None of the authors have any conflicts of interest to declare.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114522000757

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4 Chapter 4 Multi-country analysis

Chapter four is a multi-country analysis of micronutrient biomarker concentrations using inflammation-adjusted data and malaria data from available datasets. In this analysis, I also examined the effect of age and malaria endemicity.

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First Name(s)	Fanny		
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Thesis Title	Understanding the impact of malaria on the interpretation of micronutrient biomarkers		
Primary Supervisor	Edward Joy		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualized the research question and the analytical protocol with the support of my 3 supervisors Edward Joy, Suzanne Filteau and Heidi Hopkins. I conducted the data analysis with the statistical support of Amy MacDougall. All the authors reviewed the paper. I was the primary writer.

SECTION E

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Date	07/02/2024

Title: Current or recent malaria infection is associated with elevated inflammation-adjusted ferritin concentrations in pre-school children: a secondary analysis of the BRINDA database.

Journal considered: British Journal of Nutrition

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Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US Centers for Disease Control and Prevention.

Abstract

Inflammation and infections such as malaria affect micronutrient biomarker concentrations, and hence estimates of nutritional status. It is unknown whether correction for C-reactive protein (CRP) and α1-acid glycoprotein (AGP) fully captures the modification in ferritin concentrations during a malaria infection, or whether environmental and sociodemographic factors modify this association. Cross-sectional data from eight surveys in children aged 6-59 months (Cameroon, Cote d'Ivoire, Kenya, Liberia, Malawi, Nigeria and Zambia; n=7,886) from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) project were pooled. Ferritin was adjusted using the BRINDA inflammation adjustment method based on CRP and AGP, with adjusted values <12 µg/L indicating iron deficiency. The association between current or recent malaria infection, detected by microscopy or rapid test kit, and inflammation-adjusted ferritin was estimated using pooled multivariable linear regression. Age, sex, malaria endemicity profile (defined by the *Plasmodium Falciparum* infection prevalence), and malaria diagnostic methods were examined as effect modifiers. Unweighted pooled malaria prevalence was 26.0% (95% CI 25.0, 27.1) and unweighted pooled iron deficiency was 41.9% (95% CI 40.7, 43.1). Current or recent malaria infection was associated with a 44% (95% CI 39, 52; p<0.001) increase in inflammation-adjusted ferritin. In malaria-infected children, ferritin increased less with malaria infection as age and malaria endemicity increased. Adjustment for malaria status appears to significantly increase the prevalence of iron deficiency in one out of eight datasets. Additional information would be valuable to understand the underlying mechanisms of the role of endemicity and age in the association between malaria and ferritin.

INTRODUCTION

Micronutrient deficiencies underlie a large disease burden, especially in low-income countries[1]. Iron deficiency is estimated to affect 1.5-2 billion people worldwide[2], with considerable adverse health effects as iron is needed for energy production, oxygen transport and utilization, cellular proliferation, and pathogen destruction[3]. Iron deficiency affects all populations, but the most vulnerable are women and children due to their greater requirements[4]. Nutritional iron deficiency occurs when the diet supplies a chronic insufficiency of bioavailable iron to meet the body's requirements for growth and pregnancy and to replace iron lost from the gastrointestinal tract and skin, in the urine, and through menstruation[2].

There are various biomarkers that can be used to assess iron status. Serum ferritin is a measure of the amount of iron in body stores, although its concentration can be affected by concurrent infection or inflammation. This is believed to be due to the redistribution of iron during an infection[4] and can confound the proper assessment of iron deficiency in individuals experiencing inflammation. A correction method was developed by the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anaemia (BRINDA) group, whereby a linear regression is used to adjust the biomarker concentration by the concentrations of C-reactive protein (CRP) and α1-acid glycoprotein (AGP) in serum[5]. In 2020, the World Health Organization (WHO) updated the guideline on the use of ferritin concentrations to assess iron status in individuals and populations and mentioned malaria as another possible independent factor for adjustment[6].

Malaria is caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes and is a leading cause of morbidity and mortality in children. According to the WHO, there were a total of 247 millions cases of malaria in 2021, and 95% of these occurred in Africa[7]. *Plasmodium falciparum* accounted for 99.7% of infections in sub-Saharan Africa[8]. The level of malaria endemicity is defined as the degree of malaria transmission in an area[9], and the parasite prevalence in children aged 2-10 years old is commonly used to define levels of endemicity. Naturally acquired antibody responses against *P. falciparum* require repeated parasite exposure to attain protection. The rate of antibody acquisition against *P. falciparum* proteins is influenced by various factors, including age of the human host, transmission intensity, and the type of antigen. In general, antibody levels increase with both age and higher transmission intensity[10].

Currently used malaria diagnostic methods include microscopy, rapid diagnostic tests (RDT) that detect parasite antigens in blood, and polymerase chain reaction (PCR) which identifies the presence of specific malaria gene/s in a blood sample. The microscopic examination of blood films is typically considered a "gold standard" test that is used to directly detect parasitaemia in the blood. There are three antigens commonly targeted by RDT: histidine-rich protein 2 (HRP2), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase. HRP2 is specific to *P. falciparum*, while pLDH and aldolase are produced by all *Plasmodium* species [8]. HRP2-based assays can detect persistent antigenemia for up to several weeks after parasites have been eradicated, while other RDTs and microscopy detect only current infections[11; 12]. There are implications for the interpretation of ferritin values, as it has been shown that ferritin concentrations can stay elevated in the blood for several weeks after parasite clearance[13].

A recent study in Burkina Faso showed that asymptomatic malarial infections in young children in high-prevalence areas, measured by the presence of serum HRP-2 and the absence of fever, had an additive effect to elevated AGP and CRP on serum ferritin, resulting in inaccurate estimates of iron deficiency prevalence when only AGP and CRP adjustments were considered[14]. It is likely that ferritin

concentration can be increased during malaria infection independently of inflammation, although the specific pathways are not yet fully understood[15].

In 2017, Namaste et al. conducted a multi-country analysis of the effect of malaria on the interpretation of ferritin using the BRINDA datasets in pre-school children (PSC) and women of reproductive age (WRA)[16]. BRINDA datasets comprise nationally representative surveys focused on micronutrient biomarkers status, primarily conducted in low and low-middle income countries. Namaste et al found that malaria infection was independently associated with ferritin after adjusting for CRP and AGP. However, they concluded that when CRP and AGP are measured, there appears to be limited utility in measuring malaria status to adjust ferritin concentrations, because malaria adjustments alone or in addition to CRP and AGP adjustments did not considerably change the estimates of iron deficiency prevalence. A subsequent analysis by Luo et al confirmed that malaria should not be included in the BRINDA inflammation adjustment method as a binary variable[17]. In these analysis, malaria infection was considered as a dichotomous variable only, and the possible variations per malaria species, diagnostic methods, endemicity profile, or stage of infection were not studied, although these factors could modify the association between malaria and ferritin concentrations[18].

The objective of the current study analysis was to assess whether the adjustment for ferritin using CRP and AGP fully captures the modification in ferritin concentrations during a malaria infection, and whether environmental and sociodemographic factors, including acquired immunity, reflected by malaria endemicity and age, can modify this association. The findings may support the development of a new method to adjust ferritin in the context of malaria and ultimately result in more accurate estimates of iron deficiency prevalence.

METHODS

Biomarker datasets

Micronutrient biomarker data from national and regional surveys were accessed from the BRINDA project (www.BRINDA-nutrition.org). The protocol was reviewed and approved by the LSHTM ethics review committee. The methods for identifying datasets, inclusion and exclusion criteria and data management for the BRINDA project have been described in detail elsewhere[19]. The surveys were nationally or regionally representative, and the BRINDA inclusion criteria were surveys that were 1) conducted after 2004, 2) had target groups including PSC, WRA, or both and 3) used a similar laboratory methodology for the measurement of at least one biomarker of iron or vitamin A status and at least one biomarker of inflammation (AGP or CRP). We only included surveys in PSC for which the following were available: 1) the measurement of ferritin and at least one inflammation marker (AGP or CRP), and 2) a measure of malaria infection by a standardized method.

Among the 30 datasets available for PSC, ten had information on current or recent malaria infection. However, one of them (Burkina Faso) used retrospective measurement of malaria antibodies in serum to define malaria infection[20]. This method is unlikely to detect only recent or current infections, thus the dataset was not included in our database. The 2016 survey from Nepal did not detect any malaria case and was not included. In total, 7,886 PSC from eight surveys were available for inclusion. Nationally representative survey data were included from Cote d'Ivoire in 2007[21] , Cameroon in 2009[22; 23], Liberia in 2011[24] and Malawi in 2016[25]. Regional survey data were included from Kenya in 2007[26] and 2010[27], Nigeria in 2012[28] and Zambia in 2009[29]. *P. falciparum* was considered as the main *Plasmodium* species.

Ferritin, AGP, and CRP concentrations were assessed with the use of a sandwich ELISA at the VitMin Laboratory[30] in all surveys apart from Zambia where the same analytical method was used in the Tropical Diseases Research Centre in Ndola. Current malaria infection was assessed with the use of microscopy in Kenya (both surveys), Cote d'Ivoire, Nigeria and Zambia and current or recent malaria infection was assessed with the use of the Paracheck Pf rapid-diagnostic test (Orchid Biomedical System) in Liberia, the Malaria Ag CELISA kit (Cellabs Pty, Ltd.) in Cameroon, and the BIOLINE Malaria Ag *P.f*/Pan in Malawi. These three kits detect the presence of Histidine Rich Protein 2, a protein specific to *P. falciparum*[8].

Inflammation adjustment

For each dataset individually, ferritin values were adjusted for inflammation using the regression approach with the BRINDA package[31]. The regression approach has been described in detail elsewhere[16] and uses linear regression to adjust ferritin concentrations by the CRP and AGP concentrations on a continuous scale. All the ferritin observations that have a corresponding CRP value, and/or AGP value above the first decile of the considered biomarker were adjusted with linear regression. We decided to use the external deciles[19] instead of individual survey deciles for consistency and because two of the individual surveys (Ivory Coast and Zambia) had a very low first decile of CRP, suggesting a low level of inflammation.

Endemicity profile

When assessing malaria endemicity, parasite rate data constitute the bulk of the global information available[32] and are preferable to the use of prevalence data from surveys, which are likely to be varying according to multiple factors including the timing of the survey and the seasonality of transmission. The parasite rate is defined and validated by empirical data and constructed at a global scale[32]. A malaria endemicity profile was therefore assigned to each study, considering the year when the study was conducted and the location of the study (Table *1*). This profile was defined by the *Plasmodium falciparum* infection prevalence (PfP) among children aged 2–10 years, as described in the Malaria Atlas Project[33]. The infection prevalence data were extracted from the database hosted by the Malaria Atlas Project[33]. It was not possible to identify subnational levels in our dataset as the region or district names had been removed from the database. Therefore, subnational variations in endemicity were not taken into consideration. The data in the Malaria Atlas database were reported from 2010; therefore, the value for the year 2010 was used for surveys conducted before 2010. This was the case for Cameroon, Kenya, Zambia and Cote d'Ivoire. For these countries, other sources of data on malaria prevalence were consulted such as Demographic and Health Survey reports in order to confirm the prevalence data and no discrepancies were noted[34]. The different categories were defined by the WHO[35], thus: very low intensity (PfP <1%), low intensity (PfP ≥1% and <10%), moderate intensity (PfP ≥10% and <35%) and high intensity (PfP ≥35%).

Table 1: Endemicity profile of the survey settings

Moderate endemicity PfP ≥10% and <35%		**High endemicity** PfP ≥35%	
Study (location, year)	**Reported endemicity (location, year)**	**Study (location, year)**	**Reported endemicity (location, year)**
Cameroon (national, 2009)	Most regions: >10% and <35%, 2009	Cote d'Ivoire (National, 2007)	Most regions: >35%, 2010
Kenya (Nyando Division, Nyanza Province, 2007)	15% (Kisumu, 2010)	Liberia (National, 2011)	Most regions: >35%, 2011
Kenya (Nyando Division, Nyanza Province, 2010)	15% (Kisumu, 2010)		
Nigeria (Akwa Ibom State , 2012)	30% (Akwa Ibom, 2012)		
Malawi (National, 2016)	All regions: >10% and <35%, 2016		
Zambia (Central and Eastern provinces, 2009)	22% (Eastern,2010) 8% (Central, 2010)*		

PfP: *Plasmodium falciparum* infection prevalence. Data were extracted from the Malaria Atlas Project database[33]. *Zambia was considered moderate as one province was in the moderate category and the other province was in the upper end of the low endemicity category.

In three datasets (Ivory Coast, Kenya 2007 and Kenya 2010), the number of *P. falciparum* parasites were counted by microscopy per number of white blood cells. This was then converted into a parasitaemia level, which is the number of parasites per microliter of blood, on the bases of 8,000 white blood cells in one microliter of blood. A low level of parasitaemia was defined as a parasitaemia inferior to 1,000 parasites per microliter of blood, based on previous publications[36].

Statistical analysis

The outcome variable, inflammation-adjusted serum ferritin, was continuous. The exposure variable, 'malaria infection', was a binary variable (infected, uninfected). We examined the data to check for missing data, errors and inconsistencies and to gain an understanding of the distributions and patterns among the variables. We only included children who had a result for a malaria test and ferritin values. We explored the data by examining the associations between the exposure and each potential confounder and potential effect modifier (age, sex, rurality, malaria endemicity profile, and malaria diagnostic method), and between the outcome and each of these potential confounders and effect modifiers. Complex survey weights were accounted in the analysis for analyzing individual dataset. Weights were not applied for pooled estimates, as the pooled dataset is used to assess a biological association and is not supposed to be representative at any regional level; therefore, weights were also not used for the linear models which draw on the pooled dataset.

Linear model

A pooled multivariable linear regression analysis was conducted to estimate the association between malaria and inflammation-adjusted ferritin. To take into account the clustering effect by country in the pooled database, we included the survey identifier (survey ID) in the model as a fixed effect. The dependent variable serum ferritin was transformed (natural log) to improve the original skewed distribution. Consequently, the regression model was built on the logarithmic scale. If the estimated coefficient for malaria is $\hat{}$, then a malaria infection was associated with a $100 \times (e^{\hat{\beta}} - 1) = x$ percent

change in ferritin. The crude association between malaria and ferritin was assessed. The model was then adjusted for each potential cofounder, as follows: 1) individual characteristics [age (categorical, in age group: <2y and >2y), sex, rural or urban location, and 2) variables likely to modify the relation between ferritin and malaria (e.g., malaria endemicity profile and malaria diagnostic method). The confounders were selected based on plausible biological mechanisms and previously observed associations. Decisions about whether to include potential confounders in the final model were made according to whether their inclusion in the model changed the effect estimate for the main exposure. We limited the list of potential cofounders or effect modifiers to those available for all countries. For example, the presence of fever in the last 24 hour, an important variable to define the stage of malaria infection, was only available in three datasets. Maternal education was not available for two of the datasets. Similarly, information regarding iron supplement consumption was only available for 3 datasets. Two-factor interaction of each predictor variable with malaria infection were tested. Interactions with p>0.1 were removed from the model. Collinearity was examined with the variable inflation factor (VIF), that determines the strength of the correlation between independent variables. A VIF exceeding five indicated high collinearity. The coefficients from the linear model were used to calculate the malaria-adjusted biomarker concentrations and to estimate the prevalence of malaria-adjusted deficiency with the equation:

log(malaria-adjusted biomarker)=inflammation-adjusted biomarker + β(malaria) + β_i(interactions) + survey ID.

Model checking was based on residual and normal probability plots.

All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2022)[37]. Individual survey analyses, accounting for the complex survey design, were performed with the use of the 'survey' package in R 4.2.1 software[38]. Collinearity was assessed with the package 'car'[39]. The tables and graphs were made with the packages 'kableExtra', 'interactions' and 'sjPlot'[40; 41; 42].

The protocol was reviewed and approved by the LSHTM observation research ethics committee (study reference 28219).

Results

The pooled database included 7,886 PSC. Among them, 1,117 children did not have ferritin data and were excluded. Among the remaining children, 116 did not have results for a malaria test and were also excluded. These 116 children had significantly higher ferritin concentrations compared to children with a malaria test (geometric mean (μg/L) and 95% CI: 42 (39.5, 44.8) vs 32 (31.6, 33.2), p=0.003). Additionally, information on the sex of children was missing for 22 observations and the type of residence (urban or rural) was not reported for 10 children, but these children remained in the dataset. A total of 6,653 children were included in the final dataset.

The mean age of children was 26.4 months. The unweighted pooled prevalence of malaria was 26.0% (95% CI: 25.0,27.1). Iron deficiency, defined as inflammation-adjusted ferritin < 12 μg/L, ranged from 17.3% in Zambia to 72.4% in the Kenya 2007 dataset. The non-weighted pooled iron deficiency prevalence was 41.9% (Table 2).

The prevalence of malaria was higher in the older age group and in rural areas (Table 3).

Table 2: Participant characteristics (weighted percentage or mean).

Participants characteristics. Weighted percentage or mean.

	N	Male (%)	95% CI	Age in months (mean)	95% CI	Rural (%)	95% CI	Iron deficiency (%)	95% CI	Positive malaria test (%)	95% CI
Cote d Ivoire 2007 (6-59mo)	733	46.1	(42.3,49.9)	31.7	(30.4,32.9)	48.7	(36.7,60.9)	39.2	(35.0,43.5)	27.2	(22.0,33.0)
Cameroon 2009 (12-59mo)	771	49.8	(46.3,53.3)	30.7	(29.8,31.6)	41.3	(31.2,52.2)	34.6	(30.0,39.5)	25.8	(20.5,32.0)
Kenya 2007 (6-36mo)	888	47.4	(44.4,50.4)	19.9	(19.3,20.6)	100.0	(100.0,100.0)	72.4	(68.6,75.9)	19.7	(16.1,23.8)
Kenya 2010 (6-35mo)	843	49.7	(46.3,53.1)	21.4	(20.8,22.0)	100.0	(100.0,100.0)	53.1	(49.4,56.8)	32.5	(28.5,36.7)
Liberia 2011 (6-36mo)	1434	49.6	(46.8,52.4)	19.9	(19.5,20.4)	62.5	(54.8,69.6)	51.0	(47.0,55.0)	29.4	(25.9,33.1)
Malawi 2016 (6-59mo)	1084	50.9	(47.8,54.0)	32.5	(30.8,34.1)	90.2	(74.5,96.6)	21.2	(16.7,26.5)	27.9	(20.7,36.4)
Nigeria 2012 (6-59mo)	495	50.8	(45.1,56.5)	30.7	(29.1,32.2)	100.0	(100.0,100.0)	18.4	(14.0,23.7)	35.2	(29.6,41.2)
Zambia 2009 (6-59mo)	405	42.2	(36.4,48.3)	35.0	(30.8,39.2)	77.2	(58.9,88.9)	17.3	(12.1,24.1)	18.5	(13.2,25.4)
Total	6653	48.5	(47.3,49.7)	26.4	(26.1,26.8)	71.8	(70.8,72.9)	41.9	(40.7,43.1)	26.0	(25.0,27.1)

CI, confidence interval. Iron deficiency is defined by ferritin inferior to 12 microg/L. Ferritin concentrations were adjusted for inflammation with the BRINDA regression method. Malaria testing was done with microscopy or rapid diagnostic tests detecting histidine-rich protein 2.

Table 3: Prevalence of malaria by study characteristics (weighted percentage)

Prevalence of malaria by study characteristics

	number of observations	prevalence of malaria	95% CI	p
Sex				
male	3415	28.2	(26.6,29.9)	0.2
female	3216	26.5	(24.9,28.2)	
Age group				
6-24months	3020	23.2	(21.6,24.9)	<0.001
25-59months	3633	30.8	(29.2,32.4)	
Residence				
rural	4773	30.7	(29.3,32.1)	<0.001
urban	1870	16.9	(15.1,18.8)	
Malaria endemicity profile				
moderate endemicity	4486	26.7	(25.3,28.1)	0.1
high endemicity	2167	28.6	(26.6,30.7)	
Malaria diagnostic method				
RDT	3305	28.4	(26.7,30.2)	0.07
microscopy	3348	26.3	(24.8,27.8)	
Total				
Total	6653	26.0	(25.0,27.1)	

CI: Confidence Interval, RDT= Rapid diagnostic test.

Crude analysis

In the crude analysis, inflammation-adjusted ferritin concentration was significantly higher in malaria-infected children than in uninfected children (18.7 (95% CI: 17.9,19.4) µg/L vs 12.3 (95% CI: 11.9, 12.6) µg/L, p<0.001, analysis done on the log scale).

Inflammation-adjusted geometric mean ferritin concentrations were significantly different by country, age group, residence and malaria endemicity profile (Table *4*). The older age group had a higher inflammation-adjusted ferritin than the younger age group (17.5 vs 10.2 µg/L). Ferritin was higher in the rural areas and in the settings of moderate malaria endemicity profile compared to the urban areas and the settings of high endemicity profile, respectively. There was no significant difference by malaria diagnosis method.

Table 4: Inflammation-adjusted ferritin concentration (µg/L) per participant and study characteristics.

Adjusted ferritin concentration (microg/L) per participant characteristics

	number of observations	geometric mean	95% CI	p
Country				
Cote d Ivoire	733	15.0	(14.0, 16.0)	<0.001
Cameroon	771	14.5	(13.7, 15.3)	
Kenya 2007	888	6.6	(6.2, 7.0)	
Kenya 2010	843	10.3	(9.7, 11.0)	
Liberia	1434	10.8	(10.3, 11.3)	
Malawi	1084	23.3	(22.3, 24.4)	
Nigeria	495	21.9	(20.5, 23.3)	
Zambia	405	29.0	(26.5, 31.8)	
Sex				
male	3415	13.2	(12.8, 13.7)	0.2
female	3216	14.2	(13.7, 14.6)	
Age group				
6-24months	3020	10.2	(9.8, 10.5)	<0.001
25-59months	3633	17.5	(17.0, 18.0)	
Residence				
rural	4773	13.9	(13.6, 14.3)	<0.001
urban	1870	12.9	(12.4, 13.4)	
Malaria endemicity profile				
moderate endemicity	4486	14.5	(14.1, 15.0)	<0.001
high endemicity	2167	12.1	(11.6, 12.5)	
Malaria diagnosis method				
RDT	3305	14.9	(14.5, 15.4)	0.5
microscopy	3348	12.5	(12.1, 13.0)	

CI: confidence interval, RDT: Rapid diagnostic test. Inflammation adjustment was done with the BRINDA method.

Association between malaria and ferritin

The relative difference in inflammation-adjusted ferritin concentration between malaria-infected children and uninfected children was 44% (95% CI: 39, 52). The measure of the main association between ferritin and malaria is derived from model A, as it represents the main effect of malaria on ferritin, with the survey considered as a fixed effect (Table 5, model A). The difference in ferritin concentration between malaria-infected children and uninfected children was significant in both unadjusted analyses and those adjusted for each of the potential confounders (Supplementary Table 1). Age was the only potential cofounder that modified the effect estimate for the main exposure; therefore, age was kept in each model (+ 40%, 95% CI: 35, 46, p<0.001). Adjusting for multiple cofounders did not modify the value of the main estimate for malaria on ferritin concentration derived from model A (Supplementary Table 2).

Table 5: Difference in inflammation-adjusted ferritin concentration (log ferritin, µmol/L) between malaria-infected children and uninfected children in the multivariable linear regression models. Inflammation adjustment was done with the BRINDA method.

	Difference in log ferritin (95% CI)	**Difference in ferritin**	**p value**
Model A: *Multivariable linear regression analysis to estimate the association between malaria and inflammation-adjusted ferritin, adjusted for survey ID and age group*			
Malaria	0.37 (0.32, 0.41)	44% (39%-52%)	<0.001
Age group (> 2y vs under 2y)	0.34 (0.30, 0.38)	40% (35%-46%)	<0.001
R^2:0.26 P<0.001			
Model M *Multivariable linear regression analysis to estimate the association between malaria and inflammation-adjusted ferritin, including the main effect of age group and malaria endemicity profile and the interactions between age group and malaria and between malaria endemicity profile and malaria*			
Malaria	0.52 (0.44,0.61)	69% (55%,84%)	<0.001
Age group (> 2y vs under 2y)	0.54 (0.49, 0.6)	72% (63%,82%)	<0.001
Malaria endemicity profile (high vs moderate)	-0.07 (-0.13, -0.2)	-7% (-12%, -2%)	0.008
Interaction Malaria*age group (> 2y vs under 2y)	-0.16 (-0.26, -0.06)	-15% (-23%, -6%)	p for the interaction 0.002
Interaction Malaria*endemicity profile (high endemicity vs moderate endemicity)	-0.19 (-0.29, -0.08)	-17% (-25%, -8%)	p for the interaction <0.001
R^2:0.12 P<0.001			

Including endemicity profile in the model resulted in a high level of collinearity with study ID, therefore model M is run without the study ID.

Prevalence of iron deficiency after adjustment for inflammation and malaria

Adjusting for malaria as a continuous variable in addition to inflammation would increase the prevalence of iron deficiency in all countries, and this increase would be statistically significant in Kenya (from 61.4% to 53.1%, p<0.001) (Table 6).

Table 6: Prevalence of iron deficiency per country per adjustment method.

Prevalence of iron deficiency per country per adjustment method

	Iron deficiency (%) and 95% CI without any adjustement	Iron deficiency (%) and 95% CI using BRINDA adjustment only	Iron deficiency (%) and 95% CI using BRINDA, and malaria as a binary variable adjustment	Iron deficiency (%) and 95% CI using BRINDA, and malaria regression adjustment
Cote d Ivoire	11.7 (9.0,15.1)	39.2 (35.0,43.5)	44.3 (40.2,48.4)	41.4 (37.2,45.8)
Cameroon	14.9 (12.0,18.4)	34.6 (30.0,39.5)	39.6 (35.1,44.2)	39.8 (35.4,44.4)
Kenya 2007	38.9 (34.8,43.1)	72.4 (68.6,75.9)	75.8 (72.3,78.9)	76.4 (72.8,79.6)
Kenya 2010	19.2 (16.1,22.8)	53.1 (49.4,56.8)	61.1 (57.5,64.5)	61.4 (57.7,65.1)
Liberia	20.4 (17.9,23.2)	51.0 (47.0,55.0)	57.5 (53.7,61.1)	54.9 (51.0,58.8)
Malawi	10.8 (7.8,14.8)	21.2 (16.7,26.5)	24.0 (19.8,28.8)	23.9 (19.7,28.6)
Nigeria	5.3 (3.5,7.9)	18.4 (14.0,23.7)	23.6 (18.6,29.5)	24.0 (19.0,30.0)
Zambia	5.4 (2.8,10.3)	17.3 (12.1,24.1)	18.3 (13.1,24.8)	18.5 (13.4,25.0)

CI, Confidence Interval. Iron deficiency is defined by serum ferritin concentration below 12 microg/L. Inflammation adjustment is done with the BRINDA regression method. Surveys weights are applied to account for the survey design.

Effect modifiers in the association between malaria and ferritin

There were negative and significant interactions between malaria infection, defined as a dichotomous variable (infected or uninfected), and age and between malaria and endemicity profile on inflammation-adjusted ferritin concentration (Supplementary Table 2, model M). Among children with malaria infection compared to those uninfected, ferritin was less elevated in older children and in higher endemicity profile compared to younger children and children living in moderate endemicity profile (Figure 1 and Figure *2*). Adding other potential cofounders in the model did not modify the effect estimate for malaria on ferritin (Supplementary Table 2). None of the other tested interactions were significant (Supplementary Table 1).

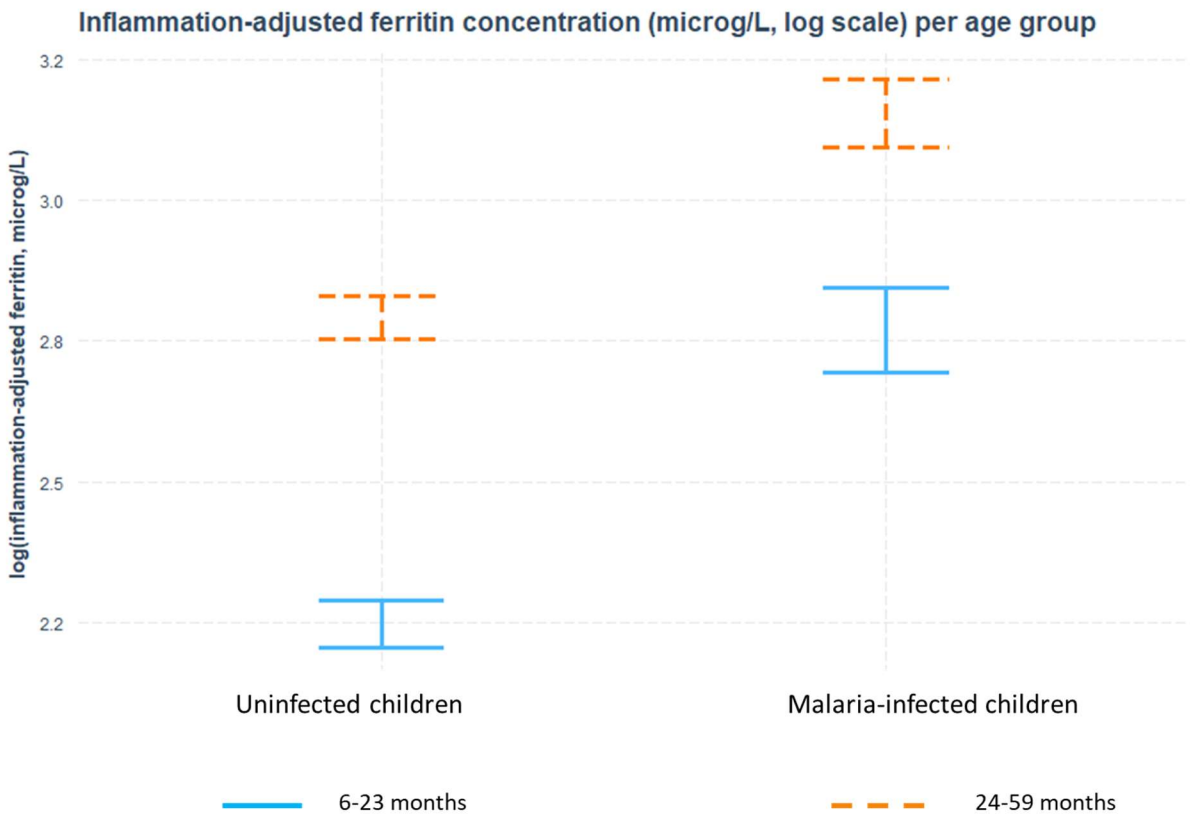


Figure 1: Mean inflammation-adjusted ferritin concentration (log scale, µg/L) by age group in malaria-infected children (n=1,733) and in uninfected children (n=4,920). Inflammation adjustment was done with the BRINDA method.

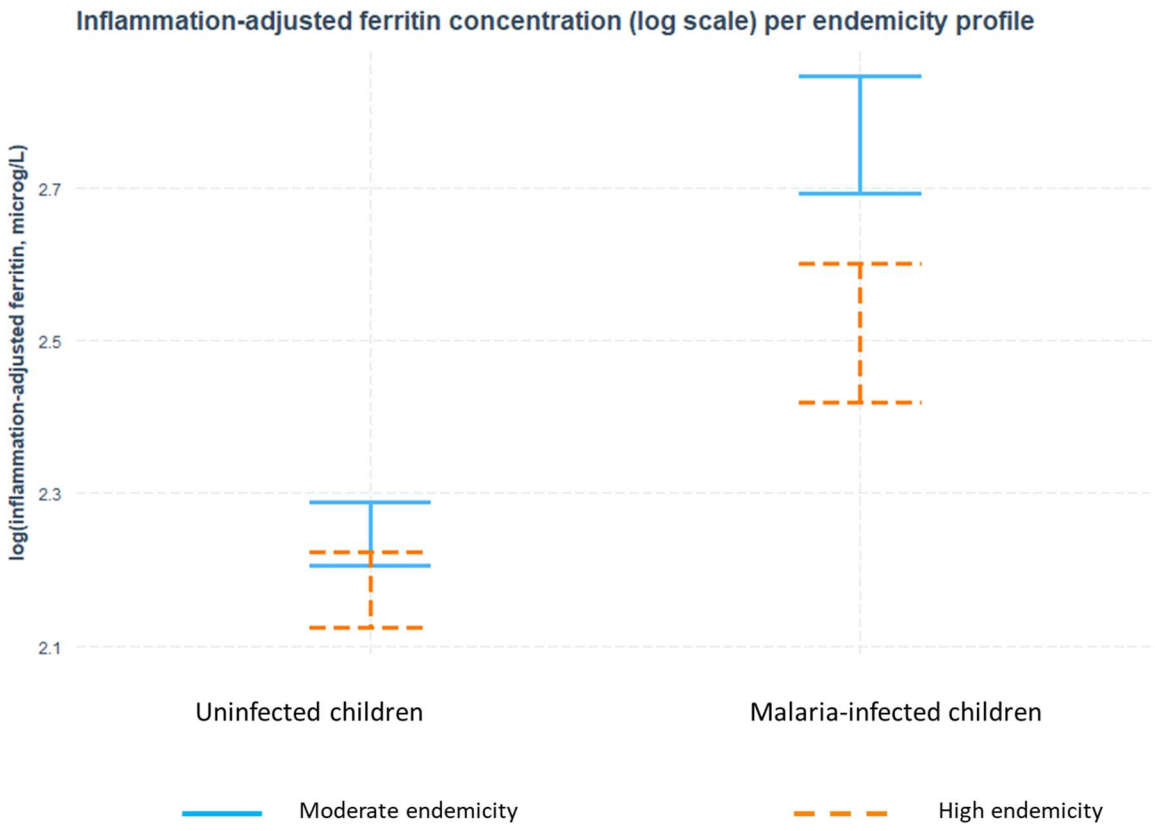


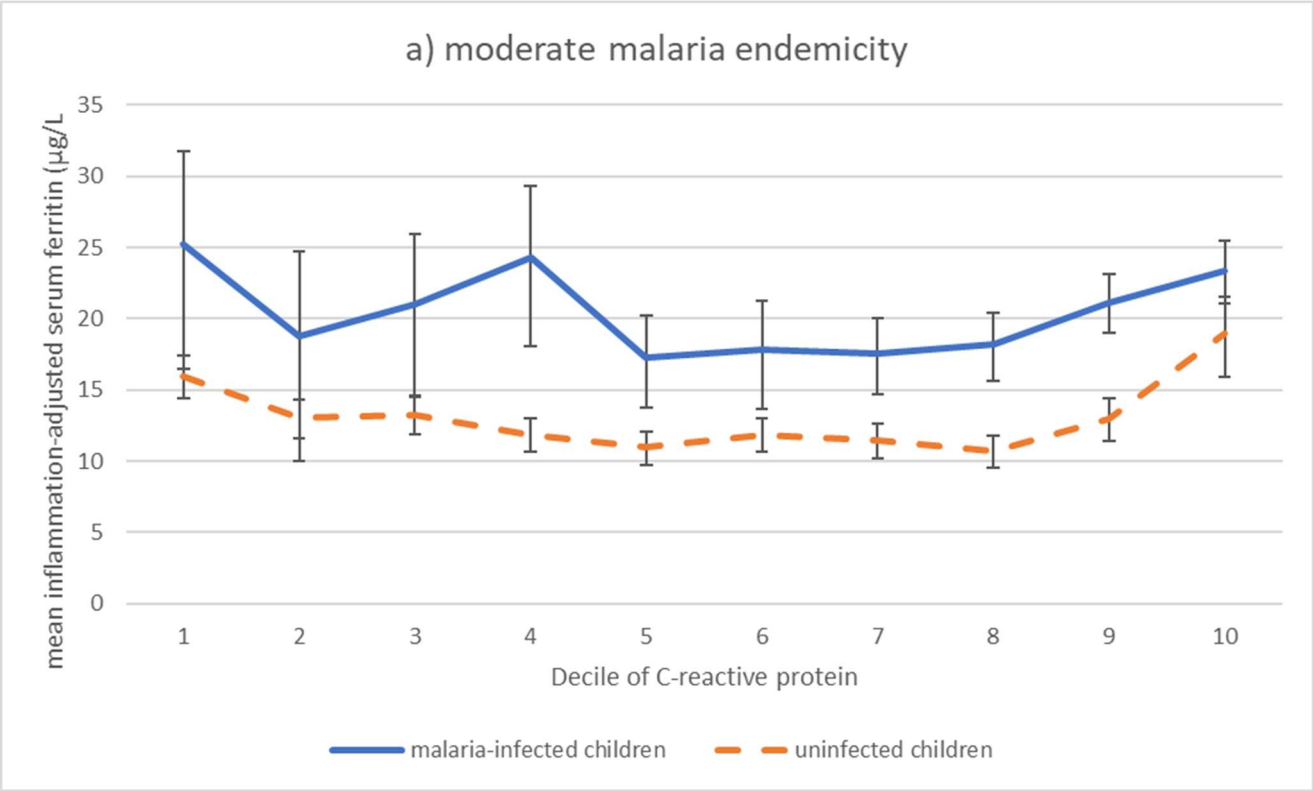
Figure 2: Mean inflammation-adjusted ferritin concentration (log scale, µg/L) per endemicity profile in malaria-infected children (n=1,733) and in uninfected children (n=4,920). Inflammation adjustment was done with the BRINDA method.

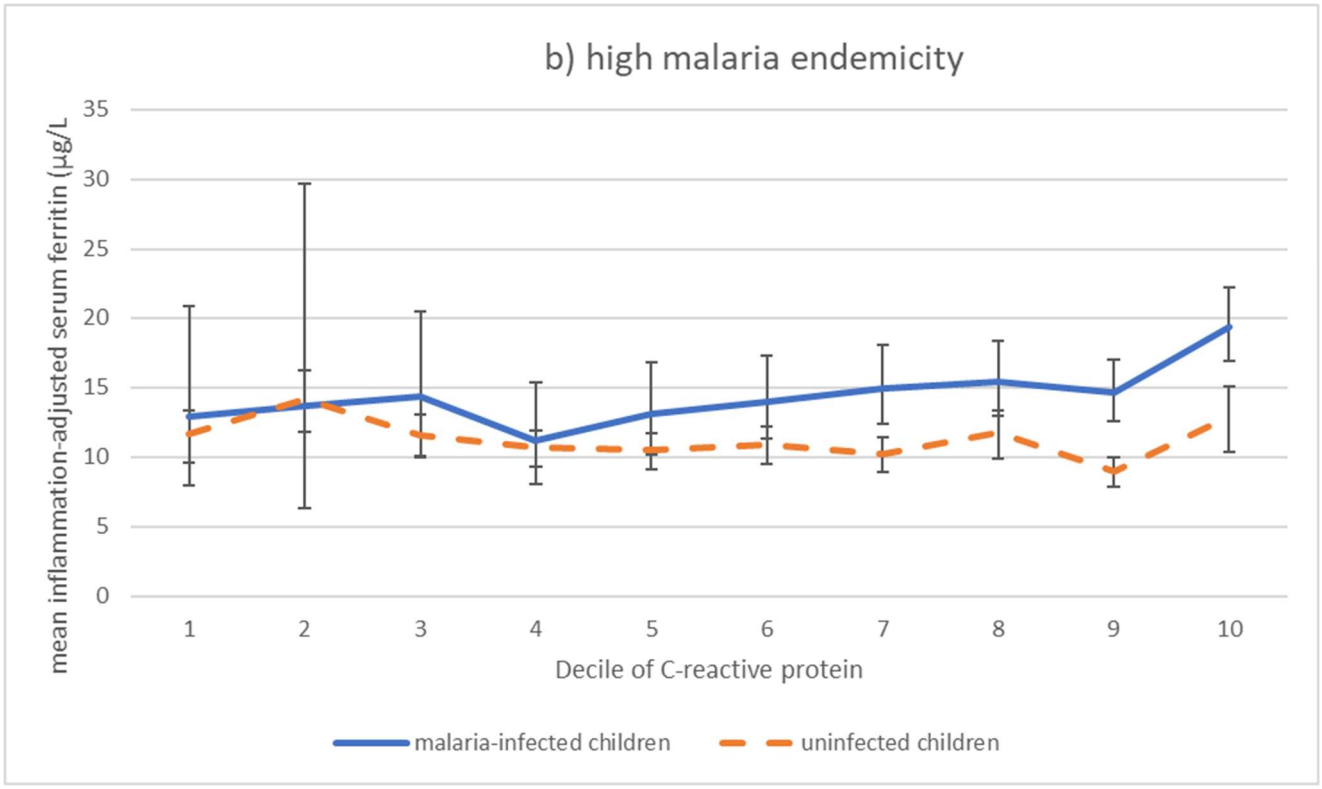
In both models A and M, the residual plots showed no fitted pattern and linear relationship between the predictors and the outcome variables was assumed. The residuals were spread equally along the ranges of predictors, suggesting no heteroscedasticity. The normality assumption was checked with the QQ plot of residuals.

Stratified analysis by endemicity profile

In moderate endemicity, inflammation-adjusted ferritin concentrations were consistently higher in malaria-infected children, at every decile of CRP (Figure 3). In high endemicity, inflammation-adjusted ferritin values between malaria groups were very similar at low level of CRP. From the 5^{th}-6^{th} decile of CRP, inflammation-adjusted ferritin concentrations started to differ between malaria-infected children and uninfected children. The same figure with non-adjusted ferritin concentrations showed a similar pattern, with visible increase in ferritin in malaria-infected children and uninfected children from the 5^{th} decile of CRP (Supplementary Figure 1).

Figure 3: Inflammation-adjusted ferritin concentration (µg/L, geometric mean and 95% confidence interval) per CRP decile in malaria-infected children and uninfected children, in a)moderate (n=4,486) and b)high endemicity profile (n=2,167). Inflammation adjustment was done with the BRINDA method.




b) high malaria endemicity
mean inflammation-adjusted serum ferritin (µg/L
35
30
25
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
Decile of C-reactive protein
malaria-infected children
uninfected children

Sub-group analysis on ferritin and malaria parasitemia

Three datasets had data on malaria parasitemia (number of parasites per µL of blood): Cote d'Ivoire, Kenya 2007 and Kenya 2010. The overall mean parasite density was 204 parasites/µL of blood and 63% of children had a low level of parasitemia (<1000 parasites/µL of blood).

In Kenyan children with low parasitemia, inflammation-adjusted ferritin concentration was elevated by 73% relative to uninfected children (p<0.001) whereas in Cote d'Ivoire, the increase was not as large (21%, p=0.07). In Cote d'Ivoire, only high parasitemia was associated with a significant increase in inflammation-adjusted concentration (100% relative increase, p=0.01) (Table 7).

Table 7: Malaria parasitemia and inflammation-adjusted ferritin concentration by country

	Kenya (combined 2007 and 2010 datasets)			**Cote d'Ivoire**		
Malaria parasitemia	**N**	**Mean inflammation--adjusted serum ferritin (µg/L)**	**p** (compared to uninfected children)	**N**	**Mean inflammation--adjusted serum ferritin (µg/L)**	**p** (compared to uninfected children)
Uninfected children	1,282	7.1 (6.8, 7.5)		527	14.2 (13.1, 15.4)	
Low (below 1,000 parasites/µL of blood)	151	12.3 (10.7, 14.2)	<0.001	126	16.6 (14.3, 19.2)	0.07
Intermediate (between 1,000 and 10,000 parasites/µL of blood)	156	11.3 (9.9, 12.7)	<0.001	65	16.5 (13.2, 20.7)	0.07
High (above 10,000 parasites/µL of blood)	142	13.2 (11.7, 14.9)	<0.001	15	27.8 (17.5, 44.2)	0.01

In Kenya, where the mean age of children was about 20 months, the difference in ferritin between children without infection and children with parasitemia was higher in young children compared to older children (p for interaction: 0.02). This was not the case in Cote d'Ivoire, where the mean age of children was 32 months.

Discussion

Our analysis showed that children with malaria infection had higher inflammation-adjusted ferritin concentrations than children without infection. This association held after allowing for the confounding effects of age, sex and rural-urban residency. These results are consistent with observations from studies in Zambia[15], Burkina Faso[14] and from a pooled analysis from children in Gambia, Uganda, Burkina Faso and Kenya[43]. This indicates that estimates of iron deficiency based on inflammation-adjusted ferritin should be interpreted with caution in malaria endemic areas. Based on our model, a malaria infection in pre-school children is associated with a 44% (95% CI: 39,52) increase in inflammation-adjusted ferritin concentration. Adjusting for malaria increased the prevalence of iron deficiency in all countries, and the increase was significant in one of the datasets in Kenya.

At least two mechanisms can be suggested to explain a greater inflammation-adjusted ferritin concentration in children with malaria infection. The first is that during a malaria infection, ferritin is increased with CRP and AGP but stays elevated for longer than CRP and AGP, possibly due to a longer half-life of ferritin[4]. The second is that ferritin can be elevated independently of CRP and AGP during a malaria infection. It could either be through an inflammatory pathway that is not captured by AGP and CRP, or via a non-inflammatory pathway.

The difference in ferritin concentration between children with malaria infection and children without was greater in the younger children (<2 years old) compared to the older children (2-5 years old), and greater in moderate endemicity settings compared to the high endemicity settings. This difference by age group was confirmed in the sub-group analysis per malaria parasitemia in the two datasets in Kenya, where we saw that ferritin increased more in younger children compared to older children when malaria parasitemia increased. Stratified analyses showed different patterns in moderate and high endemicity profiles. In moderate endemicity, ferritin concentrations were higher in children with malaria infection at every decile of CRP, suggesting that even infections causing a low level of inflammation – possibly asymptomatic infections – were associated with an increase in ferritin concentration. The difference in ferritin concentration between groups with malaria-infection and non-infection was fairly constant at every decile of CRP in areas of moderate endemicity. In areas with high endemicity, there was no difference in ferritin concentration between children with malaria infection and children without at low levels of CRP. A marked difference could be seen from the 5^{th}-6^{th} centile of CRP. The different pattern between high and moderate endemicity could be interpreted as an adaptation and acquired immunity to malaria in areas of high endemicity[44]. In high endemicity, it appears that asymptomatic infections were not associated with increased ferritin once ferritin was adjusted for CRP and AGP. A similar trend was observed in a study of pre-school children on Pemba Island, Zanzibar, where *Plasmodium falciparum* was holoendemic[36]. Serum ferritin was higher with higher malaria parasitaemia in younger children but there was no association between serum ferritin and malaria in older children. The authors speculated that age-dependent immune mechanisms might have protected older children. A study involving school-age children also conducted in Zanzibar[45] showed no relation between ferritin and malaria at low level of infections, characterized by low parasitemia (<1000 parasites/µL blood). Above this cutoff, ferritin increased slightly with parasitemia. This seems to mirror our findings from high endemicity settings, where malaria was only associated with increased ferritin at higher levels of CRP, reflecting a more severe infection.

The sub-group analysis per parasitemia showed different patterns in the two countries for which data were available. In Kenya, a country that belongs to the moderate endemicity category, even a low parasitemia was associated with an elevation in inflammation-adjusted ferritin, which corresponds to what was observed at low levels of CRP in moderate malaria endemicity. In Ivory Coast (a country with high malaria endemicity) only a high parasitemia was associated with a clinically relevant increase in

inflammation-adjusted ferritin. This corresponds to what was observed in high endemicity settings, where inflammation-adjusted ferritin was only increased at high levels of CRP. This is also consistent with the findings from the above-mentioned study in Zanzibar[45]. Although there are other factors that should be considered when comparing these two countries, such as younger mean age of children in Kenya compared to Ivory Coast, this analysis of parasitemia support our findings related to the importance of endemicity and acquired immunity. This analysis also indicates that measuring parasitemia may help to interpret the effect of malaria on iron status, or the severity of the malaria infection, as the relationship between parasitemia and inflammation-adjusted ferritin does not seem linear and comparable among different settings.

These results bring some nuance to the previous ferritin analysis of BRINDA datasets[16]. In 2017, five pre-school children datasets were analyzed and malaria infection was independently associated with ferritin after adjusting for CRP and AGP. However, adjusting for malaria alone, or adjusting for malaria in addition to CRP and AGP did not change the estimates of the prevalence of iron deficiency. While this conclusion was valid in the dataset considered, it is worth noting that the estimates of iron deficiency prevalence could be affected differently in other settings. Our analysis showed that age and endemicity modify the association between malaria and ferritin and therefore adjusting for malaria is likely to change the prevalence of iron deficiency in certain contexts, particularly in young children in moderate malaria endemicity settings. Our analysis shows that adjusting for malaria as a continuous variable would significantly increase the estimated prevalence of iron deficiency in Kenya (from 53.1% to 61.4%, Supplementary Table 3), whereas the effect was small in other surveys. Even moderate changes in the estimates of iron deficiency prevalence can have important consequences in terms of public spending, where decisions are made on the basis of the severity of the public health problems or on cost-benefit analyses, often relying on thresholds. Moreover, it is important to understand the factors that impact the concentration of serum ferritin, as it is the most used indicator of iron status and is often analyzed as a continuous variable in longitudinal analyses. Other factors could be considered such as parasite species and patterns of transmission. Although the use of different diagnostic methods for malaria can detect different stages of infections, the diagnostic method did not appear to modify the association between ferritin and malaria in our database. As RDT can be positive for several weeks after parasite clearance due to persistent antigenemia[12], we would expect to see higher values of ferritin concentrations in malaria cases detected by RDT compared to microscopy. However, it is usually considered that microscopy can detect earlier cases of malaria[12], and that would result in higher values of ferritin concentration as well, and therefore the two mechanisms might counteract each other. To truly assess the potential cofounding effect of malaria diagnostic test on the association between ferritin and malaria, we would need to analyze ferritin concentrations in surveys where the two diagnostic methods are used concomitantly.

Strengths and limitations

A major strength of this study is the availability of individual-level data on malaria infection and iron status from seven countries, resulting in a large-pooled sample size. Since the data are cross-sectional, we cannot be certain of the order of events, for instance whether children had higher ferritin at the time they were infected by malaria. However, prospective studies on malarial infection and indicators of iron status[13; 46; 47] suggested a causal effect of malaria on ferritin levels and showed that ferritin concentrations tend to go back to pre-infection level about three weeks after the infection. Furthermore, the categorization into moderate and high endemicity profile was done at the country level, as localization data were not available for national surveys. Subnational data on endemicity levels could add precision to the analysis. The datasets were limited to those with cofounders or effect

modifiers that were available for all countries; thus, fever in the last 24-hours, maternal education, and iron supplement consumption were excluded from the final model.

Conclusion

In our database of 7,886 pre-school-aged children from seven countries, malaria-infected children had higher inflammation-adjusted ferritin concentrations than uninfected children. The results suggest a specific serum ferritin response to malaria infection, that seems to be lower in older children and in settings with high malaria endemicity. Inflammation-adjusted ferritin might be a valid indicator of iron deficiency in population groups with high immunity to malaria but but in groups with lower levels of immunity, inflammation-adjusted ferritin using CRP and AGP might not address the independent effect of malaria on ferritin concentrationA similar analysis in other population group with higher immunity (school-age children and non-pregnant adults) could help check these assumptions. Analyzing the association between malaria infections and other indicators such as soluble transferrin receptors and hemoglobin could provide a more comprehensive picture of iron status in settings with different malaria endemicity.

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5 Chapter 5 Malawi analysis

In chapter five, I have focused on one micronutrient survey, the national 2015 micronutrient survey in Malawi. This study was chosen because the dataset was particularly rich and included several indicators related to malaria immunity: altitude, hemoglobinopathies and enzymopathy, and use of bed nets. It also included a variety of micronutrient biomarkers for three population age-groups.

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SECTION A – Student Details

Student ID Number	1811643	**Title**	Mrs
First Name(s)	Fanny		
Surname/Family Name	Sandalinas		
Thesis Title	Understanding the impact of malaria on the interpretation of micronutrient biomarkers		
Primary Supervisor	Edward Joy		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

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Where is the work intended to be published?	British Journal of Nutrition
Please list the paper's authors in the intended authorship order:	Fanny Sandalinas Edward Joy Heidi Hopkins Blessings Likoswe Tineka Blake

	Hanqi Luo Melissa Young Christian Bottomley Parminder Suchdev Suzanne Filteau
Stage of publication	**Not yet submitted**

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	I conceptualized the research question and the analytical protocol with the support of my 3 supervisors Edward Joy, Suzanne Filteau and Heidi Hopkins. I conducted the data analysis. All the authors reviewed the paper. I was the primary writer.

SECTION E

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Date	07/02/2024

Title: Malaria infection confounds inflammation-adjusted micronutrient biomarker concentrations in Malawi

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Abstract

Inflammation and infections such as malaria affect concentrations of many micronutrient biomarkers, and hence estimates of nutritional status. We aimed to assess the relationship between malaria infection and micronutrient biomarker concentrations in pre-school children (PSC), school-age children (SAC) and women of reproductive age (WRA) in Malawi, and to examine the potential role of malaria immunity on the relationship between malaria and micronutrient biomarkers. Data from the 2015/2016 Malawi micronutrient survey were used. The associations between current or recent malaria infection, detected by rapid diagnostic test, and concentration of serum ferritin, soluble transferrin receptor (sTfR), zinc, serum folate, red blood cell (RBC) folate and vitamin B12, were estimated using multivariable linear regression. Factors related to malaria immunity including age, altitude and presence of hemoglobinopathies were examined as effect modifiers. Serum ferritin, sTfR and zinc were adjusted for inflammation using the BRINDA method. Malaria infection was associated with 67%, 31% and 33% greater inflammation-adjusted ferritin in PSC, SAC and WRA respectively ($p<0.001$ for each). In PSC, the positive association was stronger in younger children than in older children, in high altitude compared to low altitude, and in children who were not carriers of the sickle cell trait. In PSC and SAC, sTfR was elevated (+ 25% (16, 29) and + 15% (9,22) respectively, $p<0.001$) while serum zinc was lower in malaria infection (-6% (-10, -1) and -5% (-0.4, -9), $p<0.001$ and $p=0.007$ respectively). Serum folate and RBC folate were elevated in malaria-infected WRA compared to uninfected WRA (+ 18% (3,35) and + 11% (1,23), $p=0.01$ and $p=0.003$ respectively). Malaria affects the interpretation of micronutrient biomarker concentrations and factors related to malaria immunity should be investigated.

Introduction

Micronutrient deficiencies underlie a large human disease burden, especially in low-income countries[1], where infections are also common. The biochemical changes in a person's body that are initiated in response to an infection, tissue injury, or physiologic stressor are termed the inflammatory response [2]. A common feature is a rapid fall in the blood concentration of several micronutrients, including iron, zinc and retinol[3]. Although these depressions are transient and reversible, they have the potential to affect the accurate estimation of micronutrient status if the level of inflammation is high during the sample collection period, or if a large proportion of individuals with inflammation are sampled in a population survey.

Changes in micronutrient biomarkers concentration associated with malaria

Methods exist to correct biomarker concentration for inflammation[4]. However, recent reports indicate that micronutrient biomarkers, especially ferritin, can be affected by malaria independently of inflammation[5; 6; 7; 8]. This is of particular concern in sub-Saharan Africa, where iron deficiency and malaria tend to co-exist. Some research indicates that malaria might also affect the concentration of serum soluble transferrin receptors (sTfR)[5; 9], serum retinol[6; 10; 11; 12] and serum zinc[13]. The mechanisms are largely unknown but could involve the incomplete capture of the acute phase response by C-reactive protein (CRP) and α1-acid glycoprotein AGP[11], increased erythropoiesis for sTfR[9], increased vitamin A requirements during malarial infection[12] or micronutrient redistribution[5]. Other micronutrient biomarkers such as serum folate, red blood cell (RBC) folate and serum vitamin B12 seem to not be affected by inflammation[14]; however, there have been reports of elevated folate status in children during malaria infection[15], probably due to the de-novo synthesis of folate by the malaria parasite. It is unknown if elevated folate status, or modified vitamin B12 status are common during malaria in women of reproductive age and if these could affect the estimates of folate and B12 deficiency prevalence, and therefore decisions on the implementation of programmes aimed at controlling these deficiencies.

Malaria naturally acquired immunity

In previous studies analysing malaria and micronutrient biomarkers, it has been hypothesised that the relationship between malaria and micronutrient biomarkers could be modified by malaria immunity[11; 16; 17]. Naturally acquired immunity is one of the factors that conditions the extent to which malaria can evolve towards severe illness in a given individual[18]. Naturally acquired antibody responses against *P. falciparum* require repeated parasite exposure to attain protection and therefore host immunity is determined mainly by age and exposure[19], which can be defined by the total number of infections experienced by an individual[20]. Malaria transmission intensity (number of infectious bites per person per year) varies with a number of factors including, for example, altitude and environmental temperatures, which affect the development of *P. falciparum*[21]. A lower malaria transmission intensity in high-altitude regions could therefore result in lower malaria immunity among people residing in those regions. Similarly, urbanization causes marked entomological, parasitological and behavioural changes that tend to result in reduced risks of malaria[22]. Additionally, the reduction in exposure associated with the use of mosquito nets could be associated with lower immunity to malaria[23].

Hemoglobinopathies and enzymopathies such as sickle cell disease, alpha-thalassemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency are common in sub-Saharan Africa[24; 25; 26]. While the genetic mutation that causes sickle cell disease can lead to early death in individuals who are homozygous for the mutation, in its heterozygous form (sickle cell carrier), it partially protects against severe malaria caused by *P. falciparum* infection[27]. Compared with persons with normal haemoglobin, individuals with sickle cell trait have a 50–90% reduction in parasite density[27]. A number of mechanisms have been proposed, including reduced parasite growth and enhanced removal of

parasitized cells through innate or acquired immune processes[28]. Alpha thalassemia is considered to be protective in cases of severe malaria but has no effect on asymptomatic parasitaemia[29]. G6PD-deficient alleles appear to confer a protective effect against malaria, although this protection seems to be limited to severe malaria[30].

Identifying whether malaria immunity can modify the relationship between malaria and micronutrient biomarker concentrations should improve our understanding of the impact of malaria on micronutrient biomarkers interpretation, and result in more accurate estimates of micronutrient status in individuals and populations.

Malaria natural immunity and micronutrient biomarker concentration in Malawi national micronutrient survey

Malaria and iron deficiency have historically coexisted in Malawi[31; 32], as reported in previous national micronutrient surveys conducted in 2001 and 2009[33; 34]. A national micronutrient survey was conducted in 2015 in order to report on the prevalence of micronutrient deficiencies in different population groups[35]. Although several analyses have focused on different factors impacting the level of micronutrient deficiencies in this survey[36; 37; 38], the potential impact of malaria on the interpretation of micronutrient biomarker concentrations has not been analysed, even though the prevalence of malaria was found to be 28% in pre-school children (PSC), 38% in school-age children (SAC) and 17% in women of reproductive age (WRA). In this analysis, we aimed to assess the relationship between malaria and micronutrient biomarker concentrations in these three population groups. We also aimed to examine the potential role of factors related to malaria immunity, such as age, altitude, rurality and presence of hemoglobinopathies, on the relationship between malaria and micronutrient biomarkers.

Methods

Data source

This analysis used data from the Malawi Micronutrient Survey (MNS) which was conducted in 2015–16[35]. Data were accessed from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) group (https://www.brinda-nutrition.org/). The study design was reported in the Malawi MNS report[35]. Briefly, the MNS represented a subsample of the wider Demographic and Health Survey (DHS), which was designed as a cross-sectional study, with a two-stage cluster sampling design in order to obtain nationally representative indicators.

Definition of variables

Deficiency in a specific micronutrient was defined as follows: iron deficiency by inflammation-adjusted serum ferritin[39] values below a cutoff of 12 µg/L in PSC and 15 µg/L in SAC and WRA[7], zinc deficiency by inflammation-adjusted serum zinc[39] values below a specific cutoff dependant on age, sex, fasting status and time of blood draw[40], folate deficiency by values below a cutoff of 14 nmol/L for serum folate to define risk of elevated homocysteine and 748 nmol/L for RBC folate to define risk of neural tube defects[41] and vitamin B12 deficiency by values below 150 pmol/L for serum vitamin B12[42]. Serum folate, RBC folate and vitamin B12 were available only for WRA.

In pre-school children, the presence of sickle cell disease or trait was determined as well as the presence of alpha-thalassemia trait and the deficiency in G6PD.

High altitude was defined as an altitude above 1,000 meters, as this threshold has been used before[43] and was close to the median value of altitude in this survey. Socio-economic status was defined based on a household assets score as low, medium or high, and maternal education was categorized into four

categories (some level of schooling, high school, at least 14 years of education, superior education). Whether the household owns a mosquito net for sleeping was asked in the DHS questionnaire. The question did not provide details on whether it was used, in good condition, or if the children were sleeping under it.

Per population group, only a subset of 50 samples had been analysed for serum retinol and this showed a poor correlation with retinol-binding protein measured in the entire group. Furthermore, issues related to data quality were raised[36]. For these reasons, we opted not to include retinol-binding protein data in our analysis.

Inflammation adjustment

For each dataset individually, ferritin, sTfR and zinc values were adjusted for inflammation using the regression approach with the BRINDA package[39]. The regression approach has been described in detail elsewhere[44] and uses linear regression to adjust biomarker concentrations by the CRP and AGP concentrations on a continuous scale. All the biomarker observations that had a corresponding CRP value, and/or AGP value above the highest decile of the considered biomarker were adjusted with the linear regression. We used survey-specific internal deciles for this analysis to account for the context-specific pattern of inflammation. Although this is not yet recommended, we adjusted the biomarker values of ferritin, sTfR and zinc in school-age children as well, as the correlations between biomarkers and CRP/AGP values were strong.

Laboratory analysis

Serum ferritin, sTfR, CRP and AGP were measured by sandwich ELISA at the VitMin lab (Willstaett, Germany)[45]. Zinc concentrations were analysed at the Children's Hospital Oakland Research Institute (CHORI) in Oakland, USA. Serum folate and RBC folate were measured with a microbiological assay (using *L. rhamnosus*) and vitamin B12 with an immunoassay in the CDC laboratory (Atlanta, USA). Malaria testing was done at the time the survey was conducted with an antigen-detecting rapid diagnostic test (RDT), the BIOLINE Malaria Ag *P.f*/Pan. Sickle cell, alpha-thalassemia and G6PD were diagnosed only in PSC with polymerase chain reaction (PCR) from a dried blood spot at the Community Health Sciences Unit laboratory in Malawi and the Cincinnati Children's Hospital Medical Center, USA.

Data on parasitemia levels were not available. The presence of fever in the last 24 hours was reported by the caregiver for pre-school children and school-age children. Children were considered to be malaria symptomatic if they had a positive malaria test and a reported fever in the last 24 hours. They were considered malaria asymptomatic if they had a malaria-positive test with no fever reported in the last 24 hours.

Statistical analysis

The outcome variables (ferritin, sTfR, zinc, folate, RBC folate and vitamin B12) were continuous. The exposure variable, 'malaria infection', was binary (infected, uninfected). We examined the data to check for missing data, errors and inconsistencies and to gain an understanding of the distributions and patterns among the variables. Sampling weights were used to describe the dataset and to give nationally representative estimates of iron deficiency and malaria prevalence. Weights were not applied for the linear models or for the measure of micronutrient status per infectious group, as the analyses were done to assess a biological association and were not supposed to be representative at any level. However, acknowledging that participants from the same cluster might have more similarities between them than participants from the entire sample, we conducted a sensitivity analysis using the clustering as a random effect in all linear models.

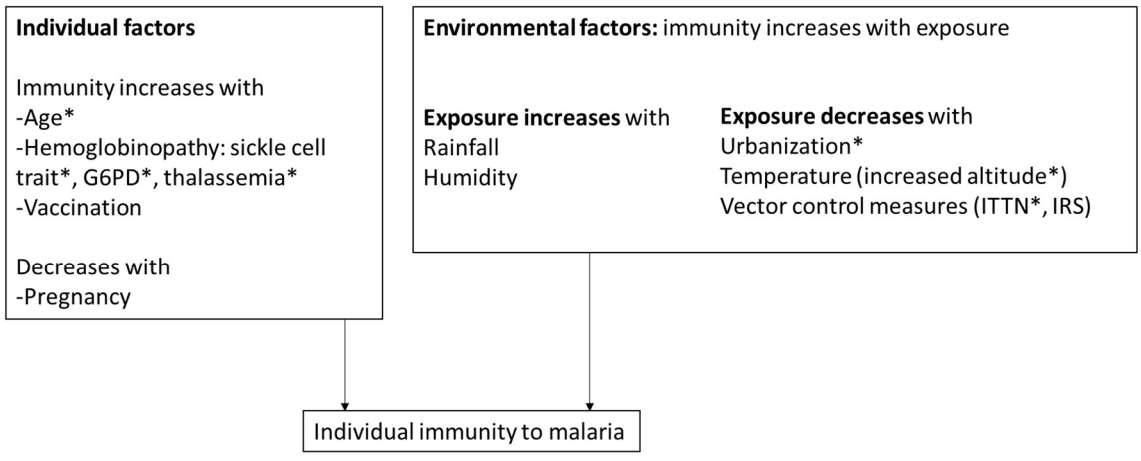
Linear model

Multivariable linear regression analyses were conducted to estimate the association between malaria and micronutrient biomarker concentrations. The dependent variables were transformed (natural log) to improve the original skewed distributions. Consequently, the regression models were built on the logarithmic scale. If the estimated coefficient for malaria was β, then a malaria infection was associated with a 100 × ($e^{\beta} - 1$) = per cent change in micronutrient biomarkers. The crude association between malaria and micronutrient biomarkers was assessed. The model was then adjusted for potential confounders: age (categorical, age group: <2y and ≥2y for PSC, <10y and ≥10y for SAC) or age in years for WRA, sex, rurality, socio-economic status, maternal or women's education, deworming in the last 6 months, altitude and presence of sickle cell trait and alpha-thalassemia. The list of cofounders was determined based on anticipated biological associations, particularly with regard to immunity to malaria (Figure 1). In WRA, further adjustments were made for the consumption of iron and folic acid supplements for iron, serum and RBC folate, and for CRP and AGP when analyzing serum and RBC folate concentrations, as the values were not adjusted for inflammation but correlations with these inflammatory markers were noticed. Two-factor interaction of each predictor variable with malaria infection was tested. The interactions with p> 0.1 were removed from the model. Interactions between malaria and rurality were not tested because of the very low number of cases of malaria in urban areas in all age groups. The coefficients from the linear model were used to calculate the malaria-adjusted biomarker concentrations and to estimate the prevalence of malaria-adjusted deficiency with the equation:

log(malaria-adjusted biomarker)=inflammation-adjusted biomarker +/- β(malaria) +/- β_i(interactions).

Model checking was based on residual and normal probability plots. All analyses were performed using R Statistical Software (2022.7.1.554; R Core Team 2022)[46]. Survey analyses, accounting for the complex survey design, with the use of the 'survey' package[47].

Figure 1: Identification of variables used to define malaria immunity



*data available in the dataset

G6PD: Glucose 6 phosphate dehydrogenase, ITN: Insecticide-treated nets, IRS: Indoor residual spraying.

Results

Micronutrient deficiencies affected all age groups and zinc deficiency was the most common deficiency. SAC had a higher malaria prevalence than PSC or WRA (Table 1). Among children with malaria, 18% of the PSC and 12% of the SAC were symptomatic.

Table 1: Participants' characteristics (mean (or %) and 95% CI)

	PSC	**SAC**	**WRA**
N	1,233	758	776
Age (mean)	31.9 (30.3, 33.4) months	9.6 (9.4, 9.7) years	28.1 (27.4, 28.9) years
Sex (% male)	50.0 (47.4, 53.0)	50.9 (47.3, 54.0)	-
Rurality (% rural)	90.2 (75.3, 97.0)	87.7 (80.1, 93.0)	90.9 (79.6, 96.0)
Socio-economic status Low (%) Medium (%) High (%)	 50 (44, 56) 41 (36, 45) 9 (6, 15)	 37 (31, 45) 45 (39, 52) 17 (12, 24)	 43 (36, 50) 45 (38, 51) 13 (8, 20)
Positive malaria test (%)	27.9 (21.0, 36.0)	37.6 (31.9, 44)	16.7 (12.7, 22.0)
Iron deficiency (%)	20.5 (15.5,26.7)	4.5 (3.3, 6.0)	17.1 (13.6, 21.0)
Zinc deficiency (%)	52.6 (46.4, 59.0)	39.4 (33.8, 45.0)	63.6 (56.5, 70.0)
Serum folate deficiency (%)	*Not measured in PSC and SAC*		34.2 (28.3, 41.0)
RBC folate insufficiency (%)			81.0 (74.2, 86.0)
Vitamin B12 deficiency (%)			13.0 (9.3,18.0)
Carriers of sickle cell trait (%)	9.1 (6,5, 12)	*Not measured in SAC and WRA*	
Carriers of alpha-thalassemia trait (%)	43.0 (37,6, 48.0)		

PSC: pre-school children, SAC: school-age children, WRA: women of reproductive age, RBC: red blood cell. Indicators of micronutrient deficiencies were adjusted for inflammation with the BRINDA method and the following cutoffs were used: 12 microg/L for ferritin in PSC and 15 microg/L in SAC and WRA; specific cutoff for zinc depending on age, sex, fasting status and time of blood draw for zinc; 14 mmol/L for serum folate; 748 mmol/L for red blood cell folate insufficiency; 150 pmol/L for vitamin B12. Data were weighted to account for survey design.

Table 2: Micronutrient biomarker concentrations by malaria infection in PSC, SAC and WRA (geometric mean)

	PSC				SAC				WRA			
	Uninfected (n=853)	Malaria-infected (n=307)	P[2]	Relative change (%, 95% CI)	Uninfected (n=468)	Malaria-infected (n=281)	P[2]	Relative change (%, 95% CI)	Uninfected (n=644)	Malaria-infected (n=113)	P[2]	Relative change (%, 95% CI)
Serum ferritin (µg/L)[1]	20.9	37.3	<0.001	+ 68 (51, 86)	36.3	44.1	<0.001	+ 28 (18,40)	28.1	36.0	<0.001	+ 34 (13,45)
Serum sTfR (mg/L)[1]	7.7	9.5	<0.001	+ 25 (16, 29)	6.6	7.7	<0.001	+ 15 (9,22)	6.6	6.9	0.3	No changes
Serum zinc (µg/L)[1]	625	580	<0.001	-6 (-10, -1)	654	622	0.007	-5 (-0.4, -9)	591	577	0.1	No changes
Serum folate (nmol/L)	*Not measured in PSC and SAC*								16.5	19.6	0.01	+ 18 (3,35)
RBC folate (nmol/L)									498	580	0.003	+ 11 (1,23)
Serum vitamin B12 (pmol/L)									289	301	0.5	No changes

PSC: pre-school children, SAC: school-age children, WRA: women of reproductive age, CI: confidence interval, sTfR= soluble Transferrin receptors, RBC: Red Blood cell. [1]: the concentrations of micronutrient biomarkers were adjusted for inflammation using the BRINDA method. Serum zinc was not adjusted in WRA because of the weak correlation between zinc and CRP/AGP. [2]: Tests for the difference between groups were done on the log scale for all biomarkers, after adjustment for potential confounders (age, sex, rurality, socio-economic status, maternal or women's education, deworming in the last 6 months, altitude and presence of sickle cell trait and alpha-thalassemia. In WRA, further adjustments were made for the consumption of iron supplements for all biomarkers, and for CRP and AGP for serum and RBC folate concentrations). Adding the clustering as a random effect did not change the significance of the results.

Inflammation-adjusted ferritin was significantly greater in malaria-infected individuals in all age groups (Table 2). In linear models, malaria was associated with 68% relative higher inflammation-adjusted ferritin concentration in PSC, 28% in SAC, and 34% in WRA (Table 2). In PSC and SAC, but not in WRA, sTfR was higher and zinc was slightly higher during malaria infection. In WRA, serum folate and RBC folate were higher during malaria. The concentration of serum folate was negatively correlated with AGP, whereas RBC folate concentration was not correlated with CRP nor AGP (Supplementary Table 1). No difference in vitamin B12 concentrations was noted.

Inflammation-adjusted ferritin was greater in PSC with symptomatic malaria compared to children with asymptomatic malaria. This was not the case in SAC (Table 3).

Table 3: Biomarker concentrations according to the stage of infection in PSC and SAC (geometric mean)

	PSC				SAC			
	Uninfected (n=853)	Asymptomatic malaria (n=253)	Symptomatic malaria (n=57)	P[2]	Uninfected (n=468)	Asymptomatic malaria (n=244)	Symptomatic malaria (n=34)	p[2]
Serum ferritin (µg/L)[1]	20.9[a]	35.3[b]	48.0[c]	<0.001	36.3[a]	43.7[b]	44.6[b]	<0.001
Serum sTfR (mg/L)[1]	7.7[a]	9.8[b]	8.3[a]	<0.001	6.6[a]	7.7[b]	7.9[b]	<0.001
Serum zinc (µg/L)[1]	625[a]	597[b]	508[c]	<0.001	654	622	621	0.07

PSC: pre-school children, SAC: school-age children, WRA: women of reproductive age, CI: confidence interval, sTfR= soluble Transferrin receptors. [1]: the concentrations of micronutrient biomarkers were adjusted for inflammation using the BRINDA method. [2]: Tests for difference between groups were done on the log scale for all biomarkers, after adjustment for potential confounders (age, sex, rurality, socio-economic status, maternal education, deworming in the last 6 months, altitude and presence of sickle cell trait and alpha-thalassemia)

Impact of factors related to immunity

-Ferritin

In PSC and SAC, there was a significant interaction with age, as ferritin was less elevated during malaria infection in older children compared to young children (p for interaction = 0.0495 in PSC and 0.02 in SAC) (Figure 2, a and b).

In PSC, there was also a significant interaction with altitude, as ferritin was greater at high altitude compared to low altitude in children with malaria (p for interaction=0.002) (Figure 2). Being a carrier of sickle cell trait was also associated with a smaller difference in ferritin concentration during malaria in PSC (p for interaction =0.009) (Figure 2). Being a carrier of the alpha-thalassemia trait was not associated with any differences in ferritin concentration in malaria-infected PSC.

Higher ferritin during malaria with increased age and/or lower altitude was observed in PSC and SAC but not in WRA.

-Other micronutrient biomarkers

In PSC, sTfR were less elevated during malaria in high altitude compared to low altitude (p for interaction = 0.03) (Supplementary Table 1). In PSC, being a carrier of the sickle cell trait was associated with a smaller reduction in serum zinc in malaria-infected children compared to non-carriers (p for interaction=0.01) (Supplementary Table 1). Factors related to immunity were not identified as effect modifiers in the relationship between malaria and zinc in SAC, nor between folate status and malaria in WRA.

Whether the household owned a mosquito net was not associated with any change in the relationship between malaria and inflammation-adjusted ferritin (data not shown).

Details of all linear models are presented in Supplementary Table 1.

a) PSC

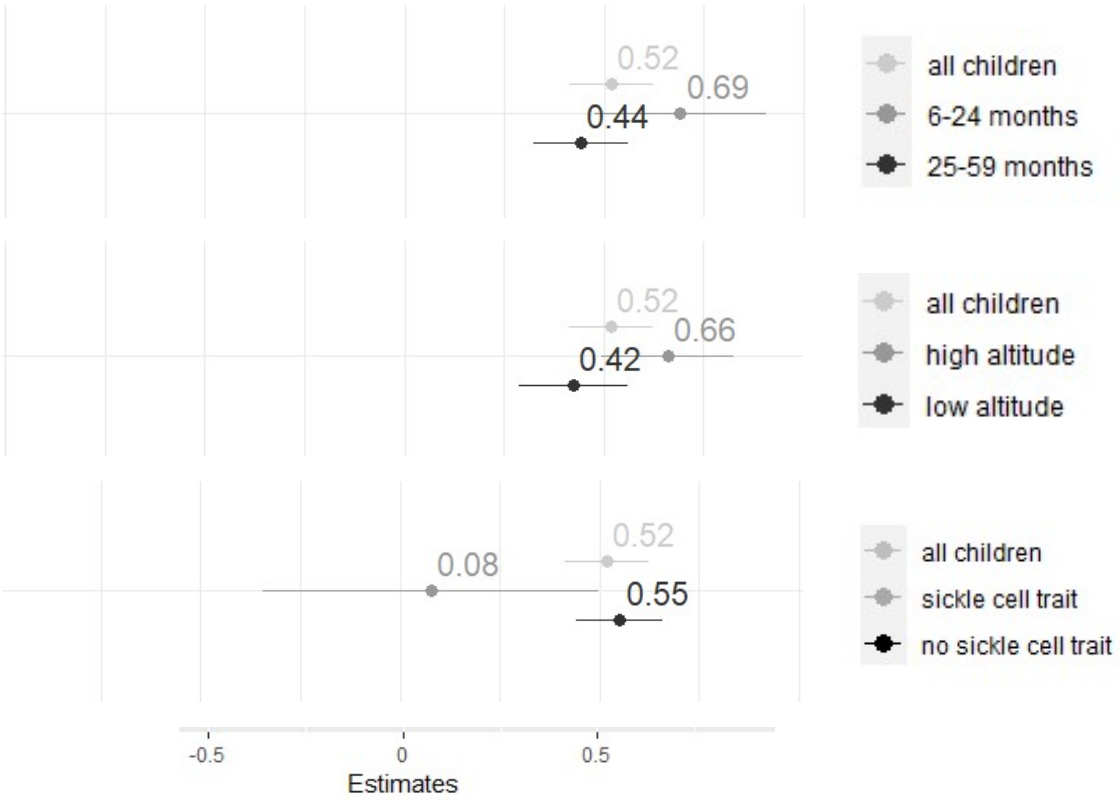
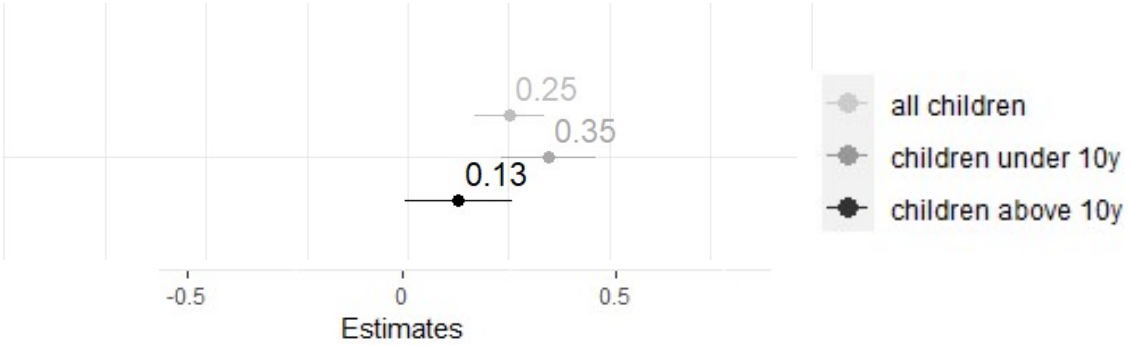


Figure 2: Difference in ferritin concentration (on the log scale) between malaria-infected and uninfected pre-school children (a) and school-age children (b) in different sub-groups of interest.

The circle represents the mean difference between malaria-infected and uninfected PSC while the horizontal line represents the 95% confidence interval. The concentration of ferritin was adjusted for inflammation using the BRINDA method. Linear models were adjusted for potential confounders (age group, sex, rurality, altitude, socio-economic status, maternal education, deworming, and presence of hemoglobinopathies). The different subgroups were created based on the significance of the interactions tested in the linear models.

b) SAC



The circle represents the mean difference between malaria-infected and uninfected PSC while the horizontal line represents the 95% confidence interval. The concentration of ferritin was adjusted for inflammation using the BRINDA method. Linear models were adjusted for potential confounders (sex, rurality, altitude, socio-economic status, maternal education, deworming). The subgroups were created based on the significance of the interactions tested in the linear models.

Adjustment for malaria

No significant changes were noted in the prevalence of micronutrient deficiencies after adjusting for malaria (Table 4).

Table 4: Malaria-adjustment impact on micronutrient deficiencies in three population groups

	Inflammation-adjustment only (prevalence in % and 95% CI)	**Inflammation and malaria adjustment (prevalence in % and 95% CI)**
PSC		
Iron deficiency	20.5 (15.5, 27)	23.8 (19.5, 29.0)
Zinc deficiency	52.6 (46.4, 59)	49.4 (43.5, 55.0)
SAC		
Iron deficiency	4.5 (3.3, 6.0)	5.1 (3.5, 7.0)
Zinc deficiency	39.4 (33.8, 45)	35.9 (30.7, 41.0)
WRA		
Iron deficiency	17.1 (13.6, 21.0)	18.4 (15.0, 22.0)
Zinc deficiency	63.6 (56.5, 70.0)	63.6 (56.5, 70.0)
Folate deficiency (serum folate)	34.2 (28.3, 41.0)	36.5 (30.3, 43.0)
RBC folate insufficiency	81.0 (74.2, 86.0)	82.2 (75.8, 87.0)
Vitamin B12 deficiency	13.0 (9.3, 18.0)	13.0 (9.3, 18.0)

PSC: pre-school children, SAC: school-age children, WRA: women of reproductive age. RBC: Red Blood cell. Indicators of micronutrient deficiencies were adjusted for inflammation with the BRINDA method and the following cutoffs were used: 12 microg/L for ferritin in PSC and 15 microg/L in SAC and WRA, specific cutoff for zinc dependent on age, sex, fasting status and time of blood draw for zinc, 14 mmol/L for serum folate, 746 mmol/L for red blood cell folate insufficiency, 150 pmol/L for vitamin B12. Data were weighted to account for survey design.

Relationship between indicators of iron status

Adjusting for malaria resulted in a better correlation between sTfR and ferritin in PSC (Table 5). The malaria adjustment did not modify the relationship between ferritin and sTfR in WRA.

Table 5: Coefficient of correlation (Spearman test) between indicators of iron status

	Inflammation adjusted ferritin	Malaria and inflammation-adjusted ferritin
PSC		
Inflammation adjusted sTfR	-0.27 (p<0.001)	
Malaria and inflammation-adjusted sTfR		-0.37 (p<0.001)
SAC		
Inflammation adjusted sTfR	-0.08 (p=0.02)	
Malaria and inflammation-adjusted sTfR		-0.13 (p<0.001)
WRA		
Inflammation adjusted sTfR	-0.45 (p<0.001)	
Malaria and inflammation-adjusted sTfR		-0.46 (p<0.001)

PSC: Pre-school children, SAC: school-age children, WRA: Women of reproductive age. sTfR: soluble transferrin receptors. Correlations were only tested between ferritin and sTfR if both variables were either malaria-adjusted or non-malaria-adjusted.

Discussion

In our analysis of the data from the 2015/16 micronutrient survey in Malawi, most micronutrient biomarkers were affected by current or recent malaria infection in all population groups studied, and factors related to immunity modified the relationship between malaria and iron biomarkers in children.

The difference in ferritin between malaria-infected individuals and uninfected individuals was greater in PSC compared to SAC and WRA. It was also greater in PSC with symptomatic malaria compared to PSC with asymptomatic malaria. In PSC and SAC, a greater difference in ferritin was seen in younger children compared to older children. The apparent absence of the effect of factors related to immunity on ferritin concentration during malaria in WRA could be due to the fact that immunity is already acquired by adulthood, although this would only explain the lack of age effect. In PSC, altitude was also found to be an effect modifier: during malaria infection, ferritin elevations were greater at higher altitudes compared to lower altitudes. Altitude is known to be a limiting factor for the reproduction of *P. falciparum*[21; 48] and this finding could result from lower immunity to malaria among children living in high altitudes. In children who carry the sickle cell trait, malaria was associated with a lower concentration of ferritin compared to malaria-infected children who were not sickle trait carriers. The partial immunity against malaria conferred by the presence of sickle cell trait could explain this result. It has been reported previously that sickle cell trait distribution was not homogenous in Malawi, with a very low prevalence in the south of the country[49]. This geographical distribution could result in iron deficiency prevalence being particularly under-estimated in the south of the country, where ferritin is likely to be more elevated during malaria compared to areas with a higher prevalence of sickle cell disease. Neither being a carrier of the alpha-thalassemia trait nor being deficient in G6PD was associated with a lower decrease in ferritin during malaria, which is consistent with earlier findings, as it has been shown that alpha-thalassemia and G6PD deficiency do not protect against asymptomatic malaria [29].

sTfR concentrations were higher in children with malaria but to a lesser extent at high altitudes. This seems contradictory as there is an increase of erythropoiesis during malaria and, in high altitudes, we should expect a further increase in sTfR. Conflicting findings on sTfR in malaria have been reported in the past[9; 50; 51]. The haemolysis associated with malaria infection could increase sTfR concentrations by stimulating erythropoiesis but there is also evidence of inhibition of erythropoiesis during acute malaria infection, which would be expected to decrease sTfR concentrations[52]. In Malawi, cases of malaria could be more acute at high altitudes because of lower immunity, which could explain the reduction in sTfR at high altitudes.

Implications for the assessment of iron status

The results from this analysis indicate that particular attention should be given to children with low immunity to malaria when assessing their iron status, whether this is at the individual level or when analyzing population-level data. Adjusting for malaria resulted in small non-significant differences in micronutrient deficiency prevalence. However, even small differences could be of public health importance, especially if policymakers are relying on thresholds to define the severity of micronutrient deficiencies, or are using these data for cost-effectiveness analyses to decide on allocation of spending. Moreover, identifying the factors that modify the relationship between serum ferritin and malaria can help to identify in which settings a malaria adjustment would be necessary, for example in areas of high altitude, and in young children. The fact that the iron indicators were more closely correlated after the malaria adjustment in PSC and SAC seems to indicate that this adjustment results in a more accurate estimation of iron status. This should be considered, especially when ferritin is analyzed as a continuous variable, for example when assessing the efficacy of an intervention to improve iron status. Elevated ferritin concentrations in children with low immunity were probably associated with elevated hepcidin, resulting in a blockage of iron absorption, and could help to understand why micronutrient

interventions are not always effective in improving population micronutrient status in apparently healthy individuals[53; 54].

Implications for other biomarkers

Malaria infection should be considered when reporting the folate status of the population. Even though the changes in biomarkers associated with malaria infection were modest in our analyses, the impact might be different in other contexts. Malaria was associated with higher serum and RBC folate, while inflammation was associated with lower serum folate. This suggests an inflammation-independent effect of malaria on folate metabolism. Furthermore, both serum and RBC folate were affected to the same extent, which could indicate both a short and long-term effect of malaria on folate status. This is, to our knowledge, the first time this has been reported in a national micronutrient survey. Population-level data on folate status are important to conduct and evaluate public health policies and programmes such as folic acid fortification. The elevation of folate during malaria has been previously observed in children and is believed to be due to either folate de novo synthesis by the pathogen, altered folate utilization in infected RBC, or reticulocytosis[15]. In settings where malaria prevalence is higher than in this survey, the impact on folate status could be important.

Reductions in plasma zinc concentrations have been reported in uncomplicated acute malaria[13] but not in asymptomatic malaria infections[11]. The small but similar reduction in both PSC and SAC serum zinc is consistent with earlier findings and could be due to the possible high proportion of asymptomatic infections in our sample, and may reflect an incomplete capture of the acute phase response by CRP and AGP. Many factors should be considered when interpreting indicators of zinc status, and the small reductions that we observed in our analysis do not seem to justify particular attention to malaria status when assessing zinc status with serum zinc. Further analysis in other contexts could help determine the impact of malaria on serum zinc.

Strengths and limitations

One of the major strengths of the study is the use of data from a large nationally representative survey with individual data on multiple micronutrients. There is also a consistency of findings with regard to factors related to immunity. All of the factors included in this analysis that are considered to correlate with malaria immunity (age, altitude, sickle cell trait) modified the relationship between malaria and ferritin in the same direction. The results were consistent across age groups and biomarkers in children.

One of the limitations for this study is the impossibility of determining whether relationships were causal since the data were cross-sectional. However, prospective studies on malarial infection and indicators of iron status[55; 56; 57] suggested a causal effect of malaria on ferritin levels. Additionally, the difference in inflammation-ferritin concentration between asymptomatic and symptomatic malaria cases suggests that the changes in ferritin are dependent on the severity of malaria infection, which also suggests a causal mechanism.

Malaria infections were detected by RDT and further analyses could be necessary to confirm that similar findings are observed in infections detected by other methods (e.g. microscopy or PCR, which may be used in other surveys). We should also note that some of the effects were small, and the limits of confidence were wide.

Conclusion

Our analysis showed that malaria infection in PSC, SAC and WRA was associated with changes in micronutrient biomarker concentrations, even after controlling for inflammation with CRP and AGP. The relationship between malaria and ferritin was the strongest and the most consistent across all age groups. Malaria-infected women had significantly higher serum folate and RBC folate than uninfected women. Malaria immunity-related factors were identified as modifying the relationship between

ferritin and malaria. More attention should be given to children with low immunity to malaria when assessing their iron status in malaria-endemic areas.

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6 Chapter 6 Dissemination

Chapter 6 relates to the dissemination of the research findings. Towards the end of this research work, I conducted a consultation with stakeholders who typically engage with micronutrient biomarker data to assess their professional opinion on the research findings. In addition to the dissemination through peer-reviewed articles, the results of this research have been disseminated through a technical brief that provides a step-by-step guide to perform the malaria adjustment, and oral and poster presentations.

6.1 Stakeholder consultation

6.1.1 Objectives

Although this research responds to a gap in evidence in terms of biomarker interpretation in the context of malaria, it was not originally designed to respond to an expressed need from stakeholders. Conducting an in-depth qualitative survey was not an objective of this thesis, but exploring ways to make the results of the research more accessible and useful for the stakeholders was deemed important. The objective of this consultation was to assess expert opinions on the micronutrient biomarkers malaria adjustment relevance and usability.

6.1.2 Methods

The consultation took place in December 2023 and January 2024 with representatives from the CDC, UNICEF, WHO, researchers, data analysts and stakeholders involved in data analysis at the country level. They were identified based on their:

- Experience with collecting, interpreting, or using micronutrient biomarker data, whether from a survey or research perspective;
- General knowledge of the effect of infections on biomarker concentrations, prior use of inflammation adjustment;
- Experience working with data from countries with malaria endemicity.

Prior to the consultation, the stakeholders received a 2-page technical brief (pages 120-121). The consultation was a free-flow conversation without systematic data collection, around the importance of malaria in the interpretation of micronutrient biomarker concentrations and the potential uptake of the thesis findings (Table 2).

The process was stopped when the discussions started to mainly repeat the findings from previous discussions and when no new concerns or areas of improvement were noted. An enquiry was made to the ethics committee to assess whether an ethical approval was needed for this work. I was advised that it was not necessary, because I was only engaging with stakeholders about their professional opinion.

Table 2: Theme and type of questions asked during the stakeholder consultation

Theme	**Type of questions asked**
Importance of malaria in the interpretation of micronutrient biomarker concentrations	Have you considered that malaria infection can affect the micronutrient biomarker data in the countries/dataset you are working with? Have you ever considered adjusting for malaria, or removing the participants with malaria from your dataset because of this potential effect?
Potential uptake of the thesis findings	Based on the results shared on the technical brief, would you consider applying a malaria adjustment to the dataset you're working with, or recommend that it's done? If you do not, why? o Not convinced by the importance o Lack of endorsement by WHO or another expert consensus o Lack of technical skills to perform the adjustment o Other

6.1.3 Results

Table 3: Main findings by stakeholders during the consultations

Theme	Users	Findings
Importance of malaria in the assessment of biomarker concentrations	CDC	Convinced of the importance. This research responds to a need of many stakeholders who reported that the BRINDA inflammation adjustment is not sufficient to account for the effect of certain infections, particularly malaria.
	UN agency (WHO)	Have not considered it yet but would like to see the publications.
	Researcher working on micronutrient status in malaria-endemic areas	Always been convinced that individuals with malaria have higher inflammation-adjusted ferritin, and that the different correction approaches are not suitable for individuals with malaria infection. It complicates the assessment of iron deficiency in malaria endemic areas.
	Clinician working in tropical infections and assessment of iron status	Very important. Ferritin can be elevated during infections, irrespectively of inflammation, and does not reflect patient's iron status.
	Country-level stakeholder involved in micronutrient survey data analysis	Thinks the topic is very important in their country and considers malaria as a confounding factor in the assessment of micronutrient status.
	Data analysts providing support to government to conduct and analyse micronutrient surveys	Very important, has always been an issue of importance for them and have been monitoring literature and recommendations to know what to do.

Scientific relevance of the findings	CDC	Very relevant, strong multi-country analysis with consistent and clear findings.
	UN agency (WHO)	The question is very interesting to them and will need to be examined by their independent expert committee (WHO/UNICEF Technical Expert Advisory group on nutrition Monitoring).
	Researcher working on micronutrient status in malaria-endemic areas	Very useful, particularly interested in the development of a statistical method to account for malaria. Questions whether the severity of malaria infection should be in the regression method (parasitaemia, presence of fever). What are the potential ways of validating the regression method? Effect on ferritin is large, very interesting especially when assessing ferritin as a continuous variable.
	Clinician working in tropical infections and assessment of iron status	Very interesting, especially regarding immunity. Results make sense biologically.
	Country-level stakeholder involved in micronutrient survey data analysis	Very relevant, consistent, valid. Would be good to replicate the study in pregnant women, and in vaccinated children. Results on folate in women of reproductive age are surprising. Should be explored further in new micronutrient survey.
	Data analysts providing support to government to conduct and analyse micronutrient surveys	Very relevant and in line with other publications. Agrees that previous publications from BRINDA did not say that malaria has no effect but that malaria adjustment does not change the deficiency prevalence in a specific context, which is very different.

Potential change to their interpretation of data	CDC	Need to see a change in micronutrient prevalence deficiency to justify a change in recommendations.
	UN agency (WHO)	Guidelines typically take a long time to be issued, but a technical brief could be developed in collaboration with the independent expert committee. They need to see the publications before taking further steps. Are more likely to consider issuing a recommendation if the work is endorsed by CDC/BRINDA. A malaria adjustment could be added in their online tool to analyse biomarker data.
	Researcher working on micronutrient status in malaria-endemic areas	Will apply the statistical method when working with this type of data. Very important as it will clear the confounding effect of malaria.
	Clinician working in tropical infections and assessment of iron status	Is already very cautious when assessing the iron status of an individual with malaria and usually waits until the infection is gone before re-assessing the iron status and deciding on supplements use for example. Will not change their practice but consistent with their idea of the role of infection on ferritin concentrations.
	Country-level stakeholder involved in micronutrient survey data analysis	The findings are important and relevant and might inform policies because they are specific to the country (Malawi). However, they would like to see the findings confirmed in the new upcoming micronutrient survey before considering changing practices. It is very useful to propose an adjustment method.
	Data analysts providing support to government	Questions how it would work in practice because the diagnosis of malaria is either complicated with

	to conduct and analyse micronutrient surveys	microscopy, or imprecise with Rapid Diagnostic Tests. They will circulate the 2-page brief in their team and consider a malaria adjustment when they work in malaria-endemic areas (upcoming micronutrient survey in Madagascar and Sierra Leone). Might need support to make sure the adjustment is done correctly.

6.1.4 Interpretation and implications of the results

The research was considered useful and relevant by all the stakeholders. They all agreed that malaria infection usually complicates the assessment of micronutrient status in malaria-endemic areas and that there are no methods to account for its potential effect. For most of them, the statistical adjustment is therefore considered as a solution to a problem that they had identified previously. How the results are likely to influence their interpretation of micronutrient status differs by individual/organisation. While researchers were ready to use the results immediately and apply an adjustment in their dataset, global organisations and country-level decision makers needed to see either scientific publications, endorsement by other institutions, or the results being validated in other surveys before changing their practices. These discussions have informed the identification of new areas of research and practical recommendations (paragraphs 7.5.2 and 7.5.3) and resulted in a modification of the technical brief to highlight the relevance of rapid diagnostic tests as diagnostic tools. The overall concern of the CDC that an adjustment is only relevant if it results in changes in micronutrient deficiency prevalence estimates was not echoed by other stakeholders when asked. Overall they agreed that the impact of an adjustment might differ depending on the context (age of the population, prevalence of malaria for example). They also thought that the iron status should not just be analysed in binary terms (deficiency vs non deficiency) but that mean ferritin concentrations are an indicator of interest to assess iron status and should be reported as accurately as possible.

Scientific points

There was a concern expressed about the validity of the malaria diagnostic method in relation to the development of the malaria adjustment. One of the stakeholders was aware that malaria could affect

the concentration of micronutrient biomarkers but had accepted that it was a general limitation in malaria-endemic areas. According to them, no method could effectively account for the effect of malaria because:

1) It is impractical in the field to perform malaria smears to detect malaria parasitaemia. The level of technical expertise needed to accurately read a smear is not readily available in all contexts.
2) The measure of malaria with RDT is not reliable as it detects malaria antigens, some of which persist longer in the bloodstream than malaria parasites, and therefore would classify participants as malaria positive when they may have had malaria several days of even weeks before the survey.

Therefore, even if a method was available to account for malaria, it could not be used in survey settings as no field-friendly devices would be available to measure current infection. This point was discussed in detail during the consultation with this stakeholder. It is believed from the recent literature, particularly from prospective studies and experimental studies, that changes in ferritin concentrations associated with malaria infection can last for up to 40 days. Ferritin concentrations appear to be elevated even after malaria parasite clearance. It would therefore not be an issue, and actually even more relevant, to use HRP2-based RDT as they would be more likely than other diagnostic methods to classify as malaria-positive children who still have raised ferritin because of a recent malaria infection. This might also be a concern for other stakeholders and therefore this has resulted in an addition about malaria diagnostic methods in the technical brief.

Another point that was discussed by one of the stakeholders working on research was the inclusion of the severity of the infection in the adjustment method, either by including the malaria parasitaemia or by including the presence of fever in the regression analysis. Referring to the conceptual model of immunity presented as Figure 1 of the Malawi paper (Chapter 5 Malawi analysis), we discussed that the presence of fever and the level of malaria parasitaemia were a manifestation of malaria immunity, rather than a determinant of malaria immunity. Not developing a fever, or being able to maintain a low level of malaria parasitaemia are likely to be the result of more effective acquired immunity because of repeated exposures for example. Therefore including the indicators on fever and malaria parasitaemia in addition to the indicators on age and exposure would likely result in an over-adjustment.

6.2 Technical brief

A technical brief was prepared in order to facilitate the dissemination of the findings to a technical audience. The intended users are data analysts and other professionals working with micronutrient biomarker data. The brief was reviewed by BRINDA and, once the clearance process is completed, will ultimately be co-authored with BRINDA/CDC and hosted on their website. The brief was also prepared in French to facilitate dissemination in francophone Africa (Appendix 6: Technical brief in French).

Accounting for malaria in the interpretation of serum ferritin concentration

BACKGROUND

Plasma or serum ferritin concentration is the most common indicator used to assess the prevalence of iron deficiency in populations. BRINDA (brinda-nutrition.org) and the WHO recommend that ferritin concentrations are corrected by inflammatory markers, as ferritin, a positive acute phase protein, is increased in the presence of inflammation[1,2]. Evidence suggest that malaria infection can have an additional, inflammation-independent effect on ferritin concentration[3].

This technical brief is for data scientists, analysts and public health professionals who collect, analyse and interpret data on iron status in population.

STUDY DESCRIPTION

In collaboration with the BRINDA project, we analysed cross-sectional data from national surveys that measured ferritin among preschool children (PSC) to examine the association between inflammation-adjusted ferritin concentration and malaria infection, diagnosed by Rapid Diagnostic Test or by microscopy. We also assessed the role of factors related to malaria immunity, as it has been hypothesised that the relationship between malaria and ferritin could be modified by malaria immunity[4].

MAIN FINDINGS

Table 1: Summary of findings from the analysis of inflammation-adjusted ferritin concentration and malaria infection from 8 cross-sectional surveys in PSC in Cameroon, Cote d'Ivoire, Kenya (x2), Liberia, Malawi, Nigeria, Zambia[5].

	Change in inflammation-adjusted ferritin associated with a malaria infection	Magnitude of the relative change (95% CI)
Biomarker		
Inflammation-adjusted serum ferritin	Higher in children with malaria infection compared to children without malaria infection	+44% (39, 52)
Effect modifiers		
Age	Lower in children over two years old with malaria infection, compared to children under two years old with malaria infection	-15% (-23,-6)
Malaria endemicity*	Lower in children with malaria infection living in settings with high endemicity, compared to children with malaria infection living in moderate endemicity	-17% (-25,-8)
Altitude**	Higher in children with malaria infection living in high altitude (>1000m) compared to children with malaria infection living in low altitude	+39% (13,70)
Sickle cell trait**	Lower in children with malaria infection and the sickle cell trait compared to children with malaria infection without the sickle cell trait	-67% (-144,-13)

CI: Confidence Interval. *Malaria endemicity is the degree of malaria transmission in an area and the parasite prevalence in children aged 2-10 years is a measure commonly used to define levels of endemicity **These analyses were done in the Malawian dataset only.

RECOMMENDATIONS TO INTERPRET SERUM FERRITIN IN THE CONTEXT OF MALARIA ENDEMICITY

Step 1: Assess the prevalence of malaria in PSC;
Step 2: Measure the association between malaria infection and inflammation-adjusted ferritin concentrations in the entire sample, adjusted for potential confounders. Recommended analyses include: stratified analysis by malaria status, bivariate association between ferritin and malaria, linear regression model;
Step 3: Measure the association between malaria infection and inflammation-adjusted micronutrient biomarker concentrations in population with low immunity to malaria (Table 1), adjusted for potential confounders;
Step 4: If the association(s) is(are) significant, consider adjusting for malaria in addition to inflammation adjustment with the use of a regression analysis (Box 2);
Step 5: Report the iron deficiency prevalence estimates with and without malaria adjustment.

Box 1: Example from the Malawi 2015 Micronutrient survey

Step 1. Prevalence of malaria in PSC: 27.9% (21.0, 36.0), diagnosed by Rapid Diagnostic Test (detecting Histidine-Rich Protein 2).
Step 2. In a linear model adjusted for potential confounders, malaria was associated with a 67% relative higher inflammation-adjusted ferritin concentration in PSC.
Step 3: In younger children with malaria (6-24 months), inflammation-adjusted ferritin concentration was 99% higher compared to children without malaria, and it was 55% in older children (25-59 months).
Step 4: Coefficient $\beta_{malaria}$ =0.36, $\beta_{\text{interaction malaria-age}}$: 0.22
Step 5: Iron deficiency prevalence **without** malaria adjustment: 20.5% (15.5, 27.0).
Iron deficiency prevalence **with** malaria adjustment: 23.8% (19.5, 29.0).

Box 2: How to use a linear regression model to adjust for malaria

1. Transform inflammation-adjusted serum ferritin on the natural log scale;
2. On the log scale, regress the inflammation-adjusted ferritin concentration on malaria infection, with adjustment for potential confounders (age, socio-economic status etc…) and obtain the coefficient β(malaria);
3. Consider adding interactions with factors related to immunity to obtain interaction coefficients;
4. Calculate log malaria-adjusted ferritin concentration using the coefficients from the regression such as:
   log(malaria-adjusted ferritin)=inflammation-adjusted ferritin +/- β(malaria) +/- β_i(interactions);
5. Calculate malaria-adjusted ferritin = $e^{(\log(\text{malaria-adjusted ferritin}))}$

Early findings suggest that Rapid Diagnostic Test detecting Histidine-Rich Protein 2 are suitable to identify individuals whose ferritin should be adjusted for malaria.

CONTACTS

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REFERENCES

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2. WHO, **2020**. ISBN: 978-92-4-000012-4
3. Wessells, K.R., et al. **2014**. 144: p. 2050-8.
4. Stoltzfus, R.J., et al. **2000**. 130: p. 1724–1733.
5. Sandalinas, F., et al. Poster presented at the Micronutrient Forum **2023.**

6.3 Oral and poster presentations

Posters of research in this thesis

Measuring the impact of malaria on the assessment of iron and vitamin A status in population. LSHTM poster day 2021

Current or recent malaria infection is associated with elevated inflammation-adjusted ferritin concentrations in pre-school children: a secondary analysis of the BRINDA database. Micronutrient Forum 2023

Oral presentation

Understanding the impact of malaria on the interpretation of micronutrient biomarkers. LSHTM Malaria showcase presentation, 2022.

Measuring the impact of malaria on micronutrient biomarker concentration: example of ferritin. Royal Society of Tropical Medicine and Hygiene annual meeting 2022.

7 Chapter 7 Discussion

7.1 Main findings

The systematic review indicated that current or recent malaria infection was associated with higher ferritin and lower retinol concentrations even in asymptomatic infections, when individuals might not have elevated markers of inflammation. The multi-country analysis found conclusive evidence that ferritin concentration was associated with malaria infection independently of inflammation. The relationship between malaria and ferritin concentrations was modified by immunity-related factors in children in both the multi-country analysis and the analysis of the Malawi micronutrient survey. In the analysis of the Malawi dataset, most micronutrient biomarker concentrations seemed to be affected by a current or recent malaria infection and the effect appeared greater in PSC compared to school age children and WRA.

7.2 Potential implications

7.2.1 On micronutrient status interpretation

While the evidence on the link between malaria and ferritin is confirmed by the systematic review, the multi-country analysis and the analysis of the Malawi dataset, the strength of the evidence of the relationship between malaria and the other biomarkers is not as strong as it comes mainly from the analysis of the Malawi dataset only and these findings should be validated in other datasets.

In both analyses, the impact size was modest in terms of changes in the estimates of micronutrient deficiencies (table 5, multi-country analysis and table 4, Malawi analysis). However, the impact on micronutrient deficiencies prevalence may depend on several factors:

- The prevalence of malaria in the population;
- The level of malaria immunity in the population;
- The distribution of biomarker concentration in the population.

Example of ferritin in PSC in Malawi

Although malaria was associated with a 68% relatively greater ferritin concentration in malaria-infected pre-school children in Malawi, the impact of the adjustment on the prevalence of iron

deficiency was modest in comparison (16% relative increase in iron deficiency prevalence). This was likely the case because most of the ferritin data points were distant from the cutoff to define iron deficiency. Looking in detail at the distribution of ferritin concentration in the Malawi PSC dataset (Figure 4), we can see that:

- In blue is the distribution of the values of inflammation-adjusted ferritin of malaria-infected children, before applying the malaria adjustment.
- In black is the distribution of the values of malaria-infected children, after adjustment for malaria.

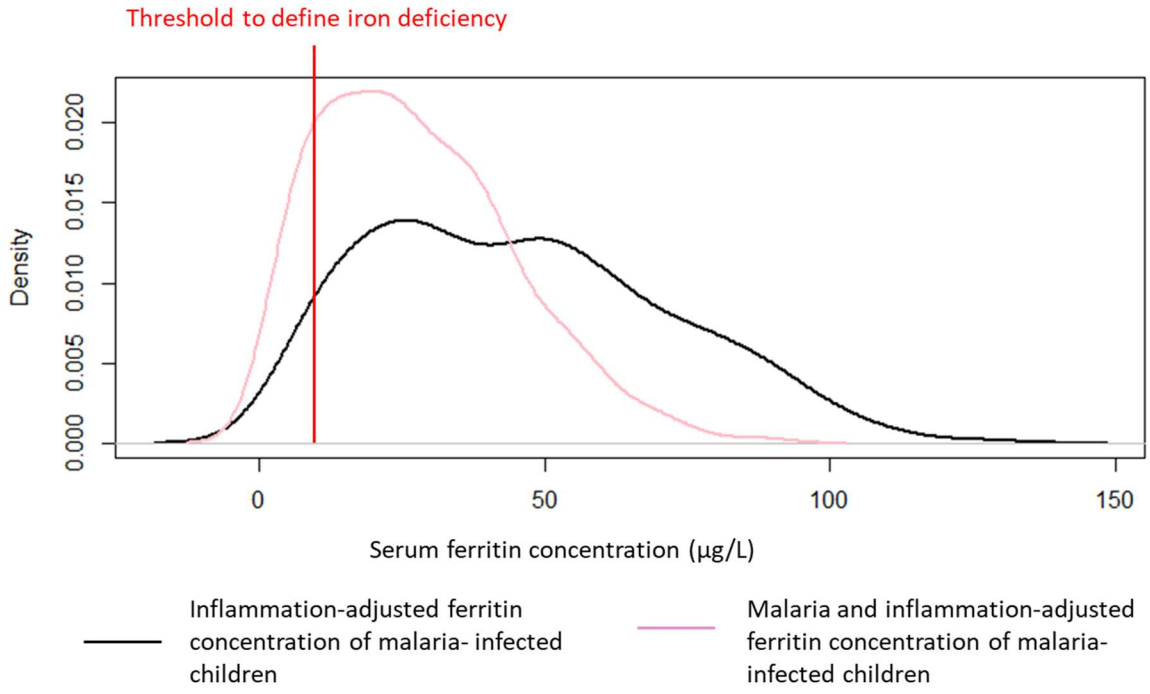

Figure 4: Kernel density plot of the distribution of inflammation-adjusted ferritin concentrations in malaria-infected pre-school children (n=290) before (in black) and after (in pink) malaria adjustment.

Even though the malaria-adjustment led to an average reduction in ferritin concentrations of 18.2 µg/L (range: 1.3, 54.4 µg/L), most of the corrected values were still above the threshold for deficiency, and therefore, the adjustment did not change significantly the prevalence estimate of iron deficiency. However, if most of the values were closer to the cutoff to define deficiency, or if we were interested in the values of ferritin on the continuous scale, the adjustment would make a difference.

Example of zinc in SAC

In SAC in Malawi, the distribution of inflammation-adjusted zinc concentration showed that many malaria-infected children had zinc concentration just below the cut-off for deficiency (about 600 µg/L, depending on fasting, time of blood draw and age) (Figure *5*). Hence, even a small change in zinc concentration due to the malaria adjustment (5% relatively lower serum zinc), led to classifying them as non-deficient, and the prevalence of zinc deficiency decreased by 10%.

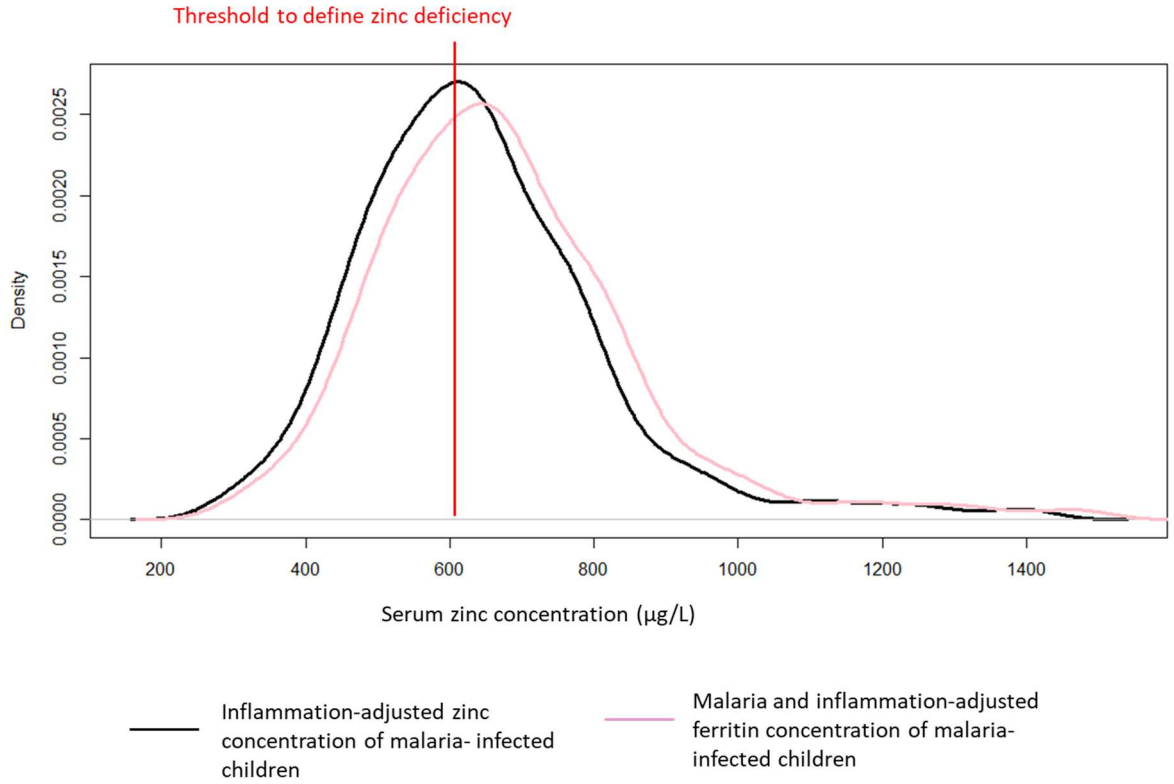

Figure 5: Kernel density plot of the distribution of inflammation-adjusted zinc concentrations in malaria-infected school-age children (n=277) before (in black) and after (in pink) malaria adjustment

These two examples show that it is challenging to predict how much a malaria adjustment can modify the prevalence of micronutrient deficiencies before applying it to the dataset. It also shows that even a small change can modify the distribution of micronutrient biomarker concentrations. These could result in differences in the interpretation of micronutrient status.

sTfR

Inflammation-adjusted sTfR were higher in malaria infection in PSC and SAC but not significantly in WRA. This probably reflects an increase in erythropoiesis during malaria infection in children. However, in PSC with fever, sTfR were not elevated significantly. This is consistent with other findings that showed that erythropoiesis was suppressed in symptomatic malaria in Indonesian children[33].

Folate

Serum folate and RBC folate were reported as being higher in malaria-infected WRA compared to uninfected WRA. A similar phenomenon has been observed in children and has been attributed to the de-novo synthesis of folate by the malaria parasite[70]. However, it has also been showed that high host folate status may favour growth of *P.falciparum*, as folate is needed by the malaria parasite[70]. If there is a direct causal relationship between folate status and malaria status, the direction is not clear and this should be further studied, particularly in the context of the fortification efforts to improve folate status in WRA.

7.2.2 On the evaluation of iron supplementation programmes

The results on the iron indicators should also be considered in view of the complex relationship between iron and malaria. The fact that inflammation-adjusted ferritin was elevated during a malaria infection should be considered biologically. The potential pathway is probably through elevated hepcidin, which would result in a blockage of iron absorption.

While it is known that iron is sequestered and iron absorption is impaired during infection as a defence mechanism, little is known about iron redistribution during asymptomatic malaria. In the multi-country analysis, in children without elevated inflammatory markers (likely in the first deciles of CRP distribution), ferritin concentrations were still elevated during a malaria infection. In the absence of a malaria test, these children would usually be considered healthy, and we would expect them to respond positively to iron supplementation. Understanding that these apparently healthy children, with no elevated AGP or CRP, could not respond to an iron intervention because of asymptomatic malaria could modify the interpretation of some iron supplementation trial results, iron fortification programmes, or simply the way iron supplementation is targeted. Considering the high prevalence of asymptomatic malaria at any time in sub-Saharan Africa, this raises concerns about the iron status of populations and the possible efficacy of strategies to control iron deficiency. This is particularly true

for young children, who are the most in need of iron, but who also tend to have lower malaria immunity. There have been suggestions to use hepcidin to guide iron treatment. Hepcidin is low when the iron stores are low and is elevated when iron stores are high or during infections. Low hepcidin level could therefore be a marker of the need and potential success of iron supplementation.

The safety of iron supplementation has been a long-standing concern among policy makers and clinicians in malaria-endemic areas. The widely cited, randomized large-scale trial on the island of Pemba found that iron supplementation in children was linked with an increase in overall morbidity and mortality[79]. Since then, the WHO recommends iron supplementation, or iron home-fortification in conjunction with effective malaria prevention and treatment strategies. Our analysis does not cover the safety of iron supplementation but suggests that iron metabolism is disturbed during malaria infection, and that effective malaria control could result in improved iron status.

7.2.3 On the interpretation of studies linking iron deficiency and reduced risk of malaria

Studies suggest that mild iron deficiency may provide a modest degree of protection against falciparum malaria[5] but these studies have largely used ferritin-based definitions of iron deficiency. Acknowledging the potential impact of malaria on ferritin levels, it is not surprising that malaria-infected children had higher ferritin concentration levels than uninfected children, and the assumption of causal relationship should be interpreted with caution.

7.3 Potential mechanisms

The possible mechanisms that can explain why malaria is associated with higher inflammation-adjusted ferritin concentrations are unknown but could involve:

- A malaria-specific redistribution of iron. It is already known that inflammation triggers the redistribution of iron in the body, in order to keep iron away from the serum. It the case of malaria, this redistribution could be accelerated, or intensified;
- An inflammation process that is not captured by AGP and CRP. Other proteins of inflammation such as interleukins 6 and 10 and/or fibroblast growth factor 23 could be triggered by malaria;
- An inflammatory process that is initiated with elevations of AGP and CRP, but that lasts even after CRP and AGP are back to pre-infection level. This would mean that ferritin concentrations

rise at the same time than AGP and CRP during malaria but stay elevated for longer. This has been observed in prospective studies. However, it has also been observed in the multi-country analysis that even at high levels of inflammation, ferritin concentrations are higher in malaria-infected individuals compared to uninfected individuals. This implies that malaria has an inflammation-independent effect on ferritin concentrations.

7.4 Role of immunity in the relationship between malaria and ferritin

The potential role of malaria immunity in the relationship between malaria and ferritin concentration was a novel finding of this research.

7.4.1 Age

In both the multi-country analysis and the Malawi analysis, age was a strong effect modifier in the relationship between malaria and inflammation-adjusted ferritin in children. In the multi-country analysis, the effect was analysed only within the group of pre-school children. In the Malawi analysis, we could observe that the difference in ferritin concentration between malaria-infected children and uninfected children was higher in pre-school children compared to school age children, and in women of reproductive age. We can hypothesize that, as children grow older and develop their immunity to malaria through repeated exposure and maturation of their immune system, their ferritin response to a malaria infection is reduced. It might be due to a regulation of the parasitaemia level, a reduced inflammatory response or a less intense redistribution of iron in the body. This hypothesis is supported by the work from Atkinson et al. in Kenya, who noticed that hepcidin concentrations were markedly higher in younger children compared to older parasitized children[80]. During adulthood, no effect of age was observed. It is possible that malaria immunity within a given endemic setting does not vary much in adulthood (apart from during pregnancy).

7.4.2 Exposure to malaria

Exposure to malaria, as measured by malaria endemicity in a given country, was also shown to modify the association between malaria and ferritin concentration. When malaria endemicity was high, meaning that children had repeated exposure to malaria, the difference between ferritin

concentration in malaria-infected children and uninfected children was lower than in areas with lower endemicity. This may reflect greater acquired immunity to malaria. As the analysis was done at the country level, it is possible that other factors could have confounded this association. In the Malawian dataset, altitude was associated with higher ferritin during malaria infection. This could be due to immunity being lower at higher altitudes because of less intensive exposure to malaria. Interestingly, none of the factors related to immunity modified the relation between malaria and ferritin in adults. This is in line with the assumption that people have acquired their immunity by adulthood and that little variation remains in terms of immunity between adults.

7.4.3 Hemoglobinopathies

The difference between ferritin concentration in malaria-infected children and uninfected children was lower in children with the sickle cell trait compared to children without sickle cell trait. The immunity conferred by the sickle cell trait is likely to be responsible for this difference. This was not the case for alpha-thalassemia. It is however consistent with previous findings that found a protective trait of alpha-thalassemia only in cases of severe malaria[54]. There was no association between the changes in ferritin during malaria and G6PD deficiency. This is also consistent with previous findings showing that G6PD deficiency does not modify the parasite density for *P. Falciparum* and only protects from severe malaria[56].

7.4.4 Fever

It is important to assess the presence of fever during malaria infection, as even after adjusting for inflammation, the presence of fever during malaria infection was associated with different elevations in ferritin concentrations. Ferritin was more elevated in uncomplicated malaria (malaria with presence of fever) than in asymptomatic parasitaemia. Although it is not known that the fever was due to malaria, it is a definition that is generally accepted. Malaria with fever is probably a manifestation of a higher parasite density and/or lower immunity, and the fact that this group had higher ferritin values than children without fever is consistent with the other findings related to immunity.

7.4.5 Potential mechanisms

The assumption that immunity could modify the relationship between malaria and micronutrient biomarkers has been verified in this research in different ways, and by different analyses. The mechanism is/are unknown but there are different assumptions that could be examined in future research:

- Malaria immunity, through an increased number of antibodies and response mechanisms, can maintain a lower level of parasitaemia (compared to people with low immunity). This seems to be confirmed in our dataset. In Cote d'Ivoire, a country with high malaria transmission where we can assume that most children have a high level of acquired immunity, 40% of infected children had a parasitaemia above 1,000 parasites/µL of blood. In Kenya, where the endemicity could be defined as moderate, the proportion was higher, as 66% of infected children had a parasitaemia above 1,000 parasites/µL of blood. In our dataset, it was however difficult to compare the ferritin concentration between Kenya and Cote d'Ivoire as the children in Kenya had overall a lower level of ferritin, certainly due to their younger age but also possibly due to other dietary factors. We have however observed that the difference in ferritin concentration between malaria-infected children and uninfected children was higher in Kenya compared to Cote d'Ivoire, suggesting a more intense response to malaria in Kenya. It would be interesting to compare two similar populations in terms of age and diet in different endemicity profile, to compare their parasitaemia and their level of ferritin, and verify if children with malaria in high endemicity maintain a lower level of parasitaemia, and lower levels of inflammation-adjusted ferritin.
- Regardless of the parasitaemia level, it is also possible that children with greater malaria immunity experience a rise in proinflammatory cytokines that is not as marked as in children with less immunity. The effect of immunity that we observe in our dataset is independent of inflammation defined by CRP and AGP. However, it is possible that other inflammatory cytokines trigger the ferritin response, and therefore it is still possible that the immunity mechanism that we observe is due to reduced inflammation.

7.5 Way forward

7.5.1 Elucidating the role of hepcidin in the relationship between malaria and ferritin

In areas with high malaria endemicity, the association between malaria and ferritin was weaker than in areas with moderate endemicity and the association was not significant at low level of inflammation. Hepcidin plays a central role in the relationship between malaria and iron status. It controls the absorption and distribution of iron and seems to be upregulated in malaria infection[33]. The systematic review showed that mean hepcidin concentrations were elevated by 1.52 nmol/L (95% confidence interval: 0.92, 2.11) in children with asymptomatic malaria in low and moderate endemicity profile, but there was no data in high endemicity[72]. Analysing the variation of hepcidin concentrations in different malaria endemicity profiles could strengthen our understanding of the complex relationship between malaria and indicators of iron status. The hypothesis is that hepcidin is not elevated in highly endemic areas, due to partial acquired malaria immunity. If this is confirmed, this would indicate that hepcidin could be used as a marker of immunity in population, and therefore be used to create an adjustment of ferritin for malaria, in a way that will be more context-specific and will take into account malaria immunity. One of the consulted stakeholders who has worked extensively on hepcidin agreed that this was a valid assumption that was worth exploring. Alongside the measure of hepcidin, it would be useful to identify direct or indirect indicators of malaria immunity for example the level of particular malaria antibodies, or the presence of fever during malaria parasitaemia.

7.5.2 Other areas of research

- Analysis of the relationship between malaria and micronutrient biomarkers in children vaccinated against malaria

It is believed that the immunity conferred by the malaria vaccine will result in a lower severity of the disease, and therefore a milder change in micronutrient biomarker concentrations during malaria. It will be interesting to include vaccinated children in future research work on malaria and ferritin. The stakeholder in Malawi confirmed that this type of work should be possible in Malawi as the roll-out of the vaccination program has started.

- Analysis of infants database

This work could be replicated in other population groups, such as infants and pregnant women, who are two groups with low immunity to malaria relative to others living in the same area.

- Method to adjust for malaria

The method to adjust for malaria suggested in the papers and in the technical brief is a regression analysis, similar to what is currently used by data analysts when they adjust micronutrient biomarkers for inflammation. Developing an excel macro or an R package could be the next step to facilitate the use of this adjustment. One stakeholder during the consultation also added that they would like to see a method for different statistical software, and also mentioned a sensitivity analysis that could be conducted in collaboration, using Box Cox transformation instead of log transformation for the ferritin concentration.

- Validation of the adjustment method

During the consultation, one stakeholder mentioned the lack of longitudinal data in the analysis. Following a cohort of children with malaria and analysing repeated measures of ferritin level during and after the infection could support the validation of the adjustment method.

7.5.3 Recommendations in practice

This research could inform the implementation of micronutrient surveys and the interpretation of biomarker data in the context of malaria.

- Malaria and fever

More research should inform whether malaria with fever (i.e. symptomatic malaria) is consistently associated with higher ferritin values compared to malaria without fever (i.e. asymptomatic malaria). If this is the case, measuring fever in malaria-infected children during any nutrition survey (in national micronutrient surveys or research settings) could be an easy and practical way to identify a group of children for whom a malaria adjustment should be necessary. The stakeholders involved in the implementation of micronutrient surveys confirmed that measuring temperature in participants should be doable.

- Diagnostic test

In our analysis, the particular malaria diagnostic test used was not associated with difference in the malaria impact on ferritin concentrations. However, it is challenging to conclude as the different diagnostic tests were used in different settings. Before making concrete recommendations on the

diagnostic tests, it would be useful to observe ferritin concentrations in malaria individuals who have been diagnosed with malaria by both microscopy and RDT, and those who have been diagnosed with malaria by only one of them.

- Impact of adding a new adjustment

Introducing a new adjustment for malaria has the potential to modify the estimation of the prevalence of micronutrient deficiencies, whether these estimates are derived from large population-based surveys, or from clinical research studies. Additionally, a whole range of studies that utilise these data could be affected, such as global burden of disease estimates, cost-effectiveness studies or modelling scenarios, and this could impact decisions on funding, choice and prioritisation of policies and programmes and research needs.

- Adding a malaria adjustment function in the online tools

One of the functions of the MAPS tool is to provide estimates of micronutrient deficiencies at national and subnational level, based on the analysis of micronutrient biomarker data. The current version includes an adjustment for inflammation following the BRINDA method[27]. In the future version of the tool, a function to adjust for malaria could be added. The users could use this functionality without statistical knowledge but would also be able to download the R script to document the statistical steps if needed, or to replicate the method. That would also be useful for users who bring their own data to the tool, as the malaria adjustment could be applied to their dataset, and they could compare the adjusted and non-adjusted data. The discussion with a representative for WHO indicated that adding a malaria adjustment in their survey analyzer tool could be done if the findings are endorsed by WHO.

- Changes in WHO recommendations on the interpretation of serum ferritin

Changing WHO recommendations is a lengthy process that relies on extensive review of the literature. However, as discussed with the WHO representative during the consultation, the question of malaria adjustment could be reviewed by the independent WHO/UNICEF committee on nutrition monitoring once the papers from this thesis are published, and they could issue a technical brief before changes in recommendations are made.

7.6 Strengths and limitations

7.6.1 Strengths

The main strength of this research is the use of nationally-representative individual level data from many countries. We could also observe a consistency of findings across biomarkers and age groups, and consistent, unidirectional relationships between immunity and ferritin concentrations in malaria-infected children.

7.6.2 Limitations

These data are cross-sectional and therefore it is not possible to establish a causal relationship between malaria and biomarker concentration data. The only datasets containing information on malaria testing are from Africa, which could limit the generalization of findings as typically only one malaria species is considered (*P. falciparum*).

It has been hypothesised that the physiological iron redistribution occurring during a malaria infection can facilitate the occurrence of other disease, by enhancing the host susceptibility to other parasites[81]. It is not impossible that children who were infected with malaria were also suffering from other infections, and that could confound the specific role of malaria in the biomarker fluctuations.

We did not have a gold standard measure of iron status in children, and we could not validate the findings and prove the necessity of a malaria adjustment. Following longitudinally children with malaria and analysing the fluctuations of ferritin level after they have recovered from malaria could support the validation of the adjustment method.

The risk of over-adjusting micronutrient status exists and should be considered when reporting micronutrient biomarker concentrations. This is particularly essential for retinol values, that are depressed during inflammation and malaria. Adjusting retinol values for malaria would therefore result in a decrease in the estimates of vitamin A deficiency, and this should be carefully considered, especially as there is evidence that some vitamin A is lost during an infection[82].

Overall conclusion

This research has highlighted the cofounding effect of malaria infection in the interpretation of micronutrient biomarker data, especially on serum ferritin. Ignoring the effect of malaria on these indicators can have consequences at the population level, by underestimating the prevalence of iron deficiency in a population, and at individual level, by misclassifying children and adults as iron replete when they are iron deficient. It can also compromise the evaluation of programmes designed to prevent and control iron deficiency, partly because the ability to respond to an iron intervention can be impaired by a current or recent malaria infection. Efforts should be made to consider the potential impact of malaria infection on micronutrient biomarker data and to understand the role of factors that can modify this association, particularly immunity-related factors. This would support efforts to accurately estimate iron status in populations, and the design of comprehensive interventions to improve iron status.

APPENDIX

1 Appendix 1: Ethical approval

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LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Mrs Fanny Sandalinas
LSHTM

27 November 2023

Dear Mrs Fanny Sandalinas,

Project Title: Multi-country analysis of micronutrient biomarker concentrations by stage of malaria infection

Project ID: 28219

Thank you for your annual report application for the continuation of your research dated 09/11/2023 16:56 , which has now been considered by the Chair on behalf of the Ethics Committee.

Confirmation of ethical opinion

This application is approved by the committee for a further year from the date of this letter.

Conditions of the favourable opinion

Approval is dependent on local ethical approval having been received, where relevant.

After ethical review

Any changes to the application must be submitted to the committee via an Amendment form.

The CI or delegate is also required to notify the ethics committee of any protocol violations and/or Suspected Unexpected Serious Adverse Reaction (SUSARs) which occur during the project by submitting a SUSAR and Protocol Violation form.

An annual report should be submitted to the committee using an Annual Report form on the anniversary of the approval of the study during the lifetime of the study.

At the end of the study, the CI or delegate must notify the committee using an End of Study form.

All aforementioned forms are available on the ethics online applications website and can only be submitted to the committee via the website at http://leo.lshtm.ac.uk.

Additional information is available at: www.lshtm.ac.uk/ethics.

Yours sincerely,

Professor David Leon and Professor Clare Gilbert
Co-Chairs

ethics@lshtm.ac.uk
http://www.lshtm.ac.uk/ethics/

Improving health worldwide

2 Appendix 2: Certificate of completion: Research Ethics Training.

This is to certify that

fanny sandalinas

successfully completed the

Research Ethics

e-learning course

with a score of

100.00 %

Comprising of modules covering:

- Introduction to the History of Research Ethics
- Fundamental Ethical Principles, including:
  - Respect for persons
  - Beneficence
  - Justice
- Responsibilities of Research Ethics Committees
- Understanding Vulnerability
- Privacy and Confidentiality

On

June 11, 2021

Provided by

London School of Hygiene & Tropical Medicine

This course meets the requirements for protection of human subjects training required by individuals involved in the design and/or conduct of National Institutes of Health (NIH) funded human subjects research.

3 Appendix 3: Supplementary materials from chapter 3

Supplementary file 1: Search strategy

1. malaria.mp. or exp Malaria Vaccines/ or exp Malaria, Falciparum/ or exp Malaria, Cerebral/ or exp Malaria, Vivax/

2. anophele.mp. or exp Anopheles/

3. exp Ferritins/ or ferritin*.mp.

4. ('iron indicator' or 'iron biomarker' or 'iron absorption' or 'iron deficiency').mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

5. exp Retinol-Binding Proteins/ or 'retinol binding protein*'.mp. or exp Vitamin A/

6. Plasmodium.mp. or Plasmodium/

7. 1 or 2 or 6

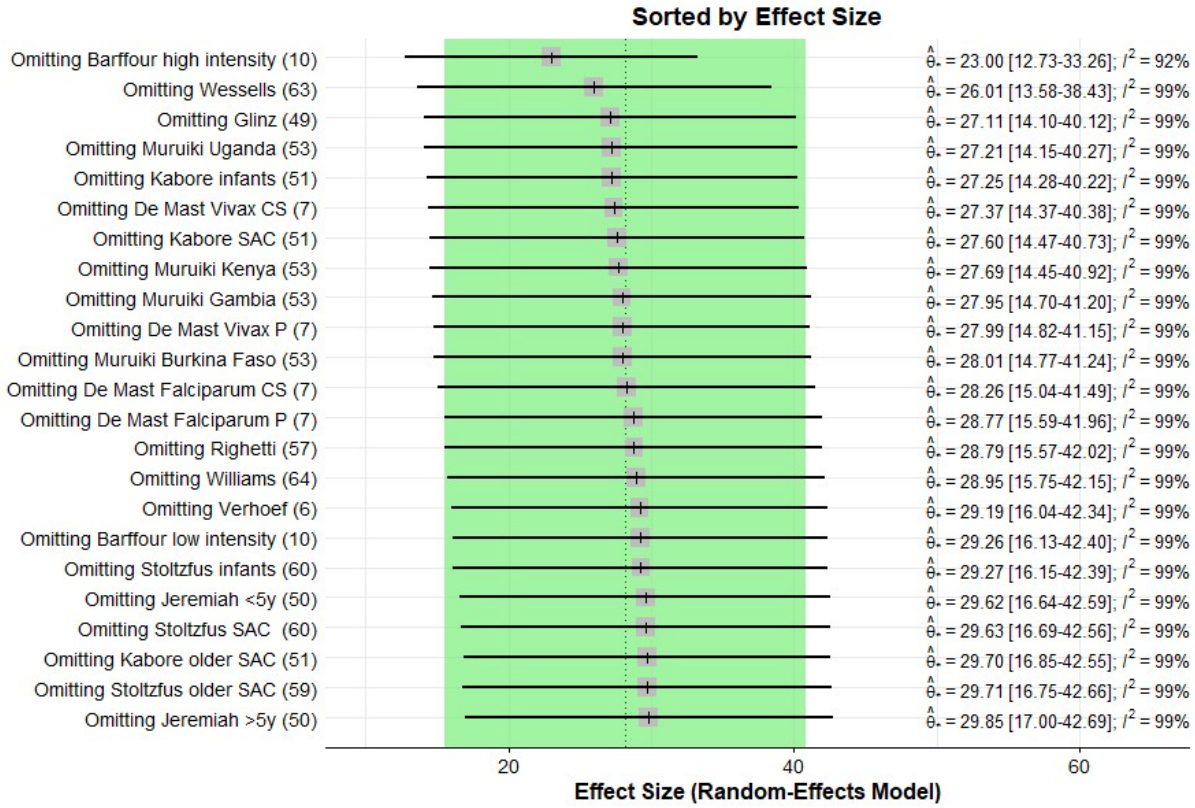
8. exp Hepcidins/

9. ("serum retinol" or "plasma retinol").mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]

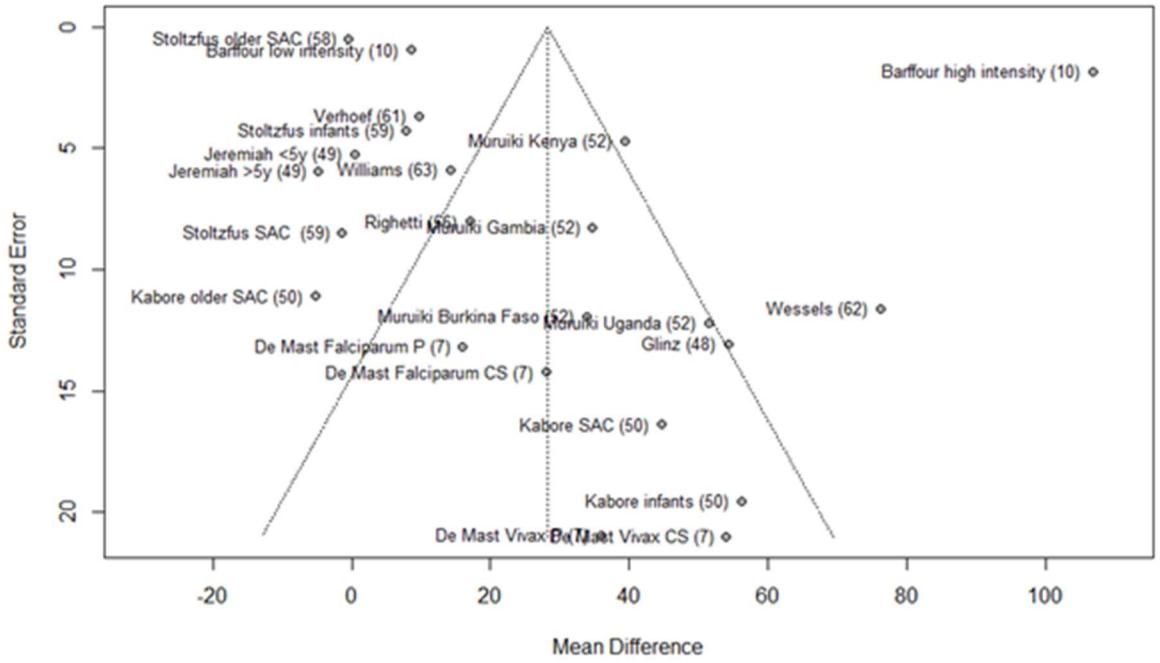
10. 3 or 4 or 5 or 8 or 9

11. 7 and 10

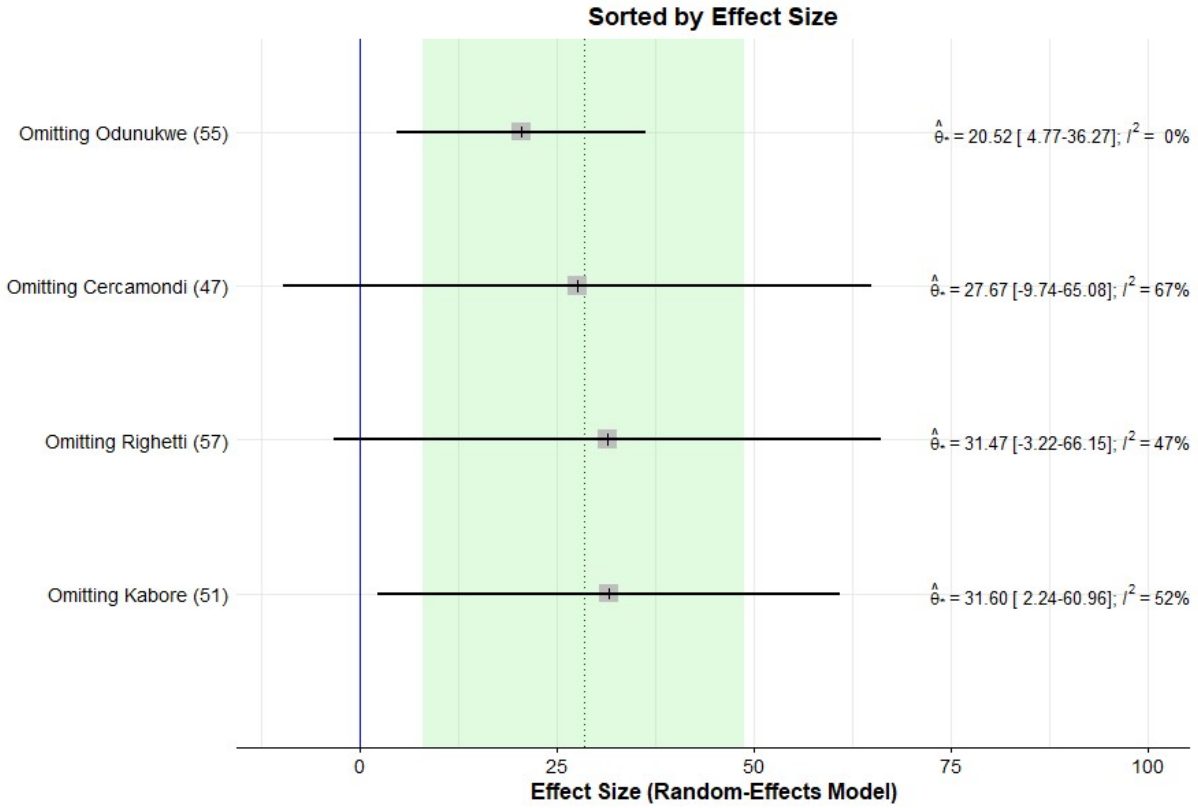
Filter: human studies, published in English, French and Spanish. There is no time or geographical limitation to the search.



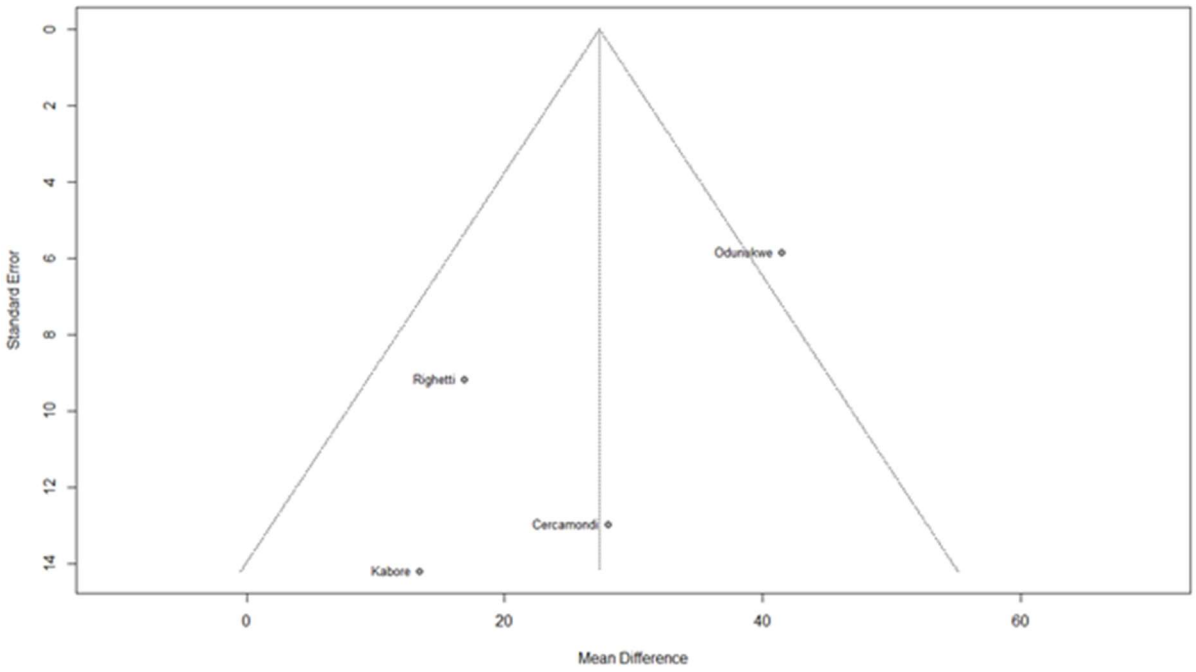
Supplementary Figure 1: Sensitivity analysis of ferritin concentration (µg/L) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval. CS, cross-sectional; SAC, school-age children; P, prospective



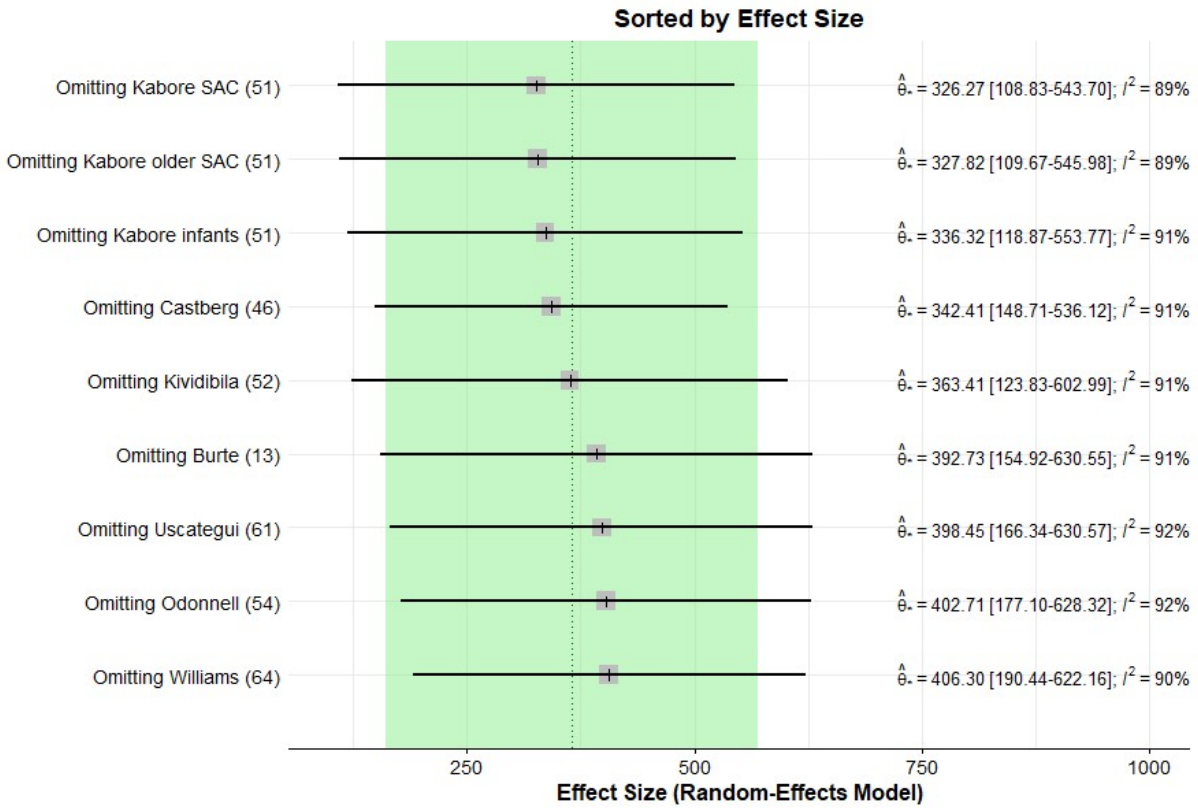
Supplementary Figure 2: Funnel plot showing publications related to asymptomatic malaria and ferritin concentration (µg/L) in children. CS, cross-sectional; SAC, school-age children; P, prospective



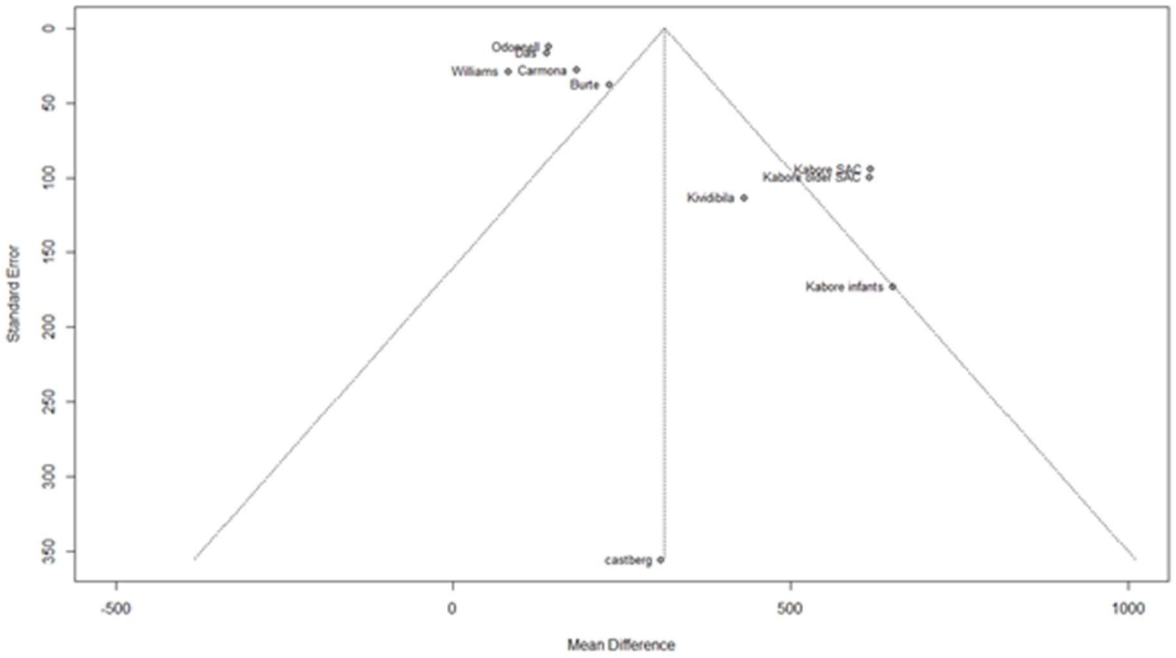
Supplementary Figure 3: Sensitivity analysis of ferritin concentration (µg/L) between asymptomatic malaria and control in adults, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval.



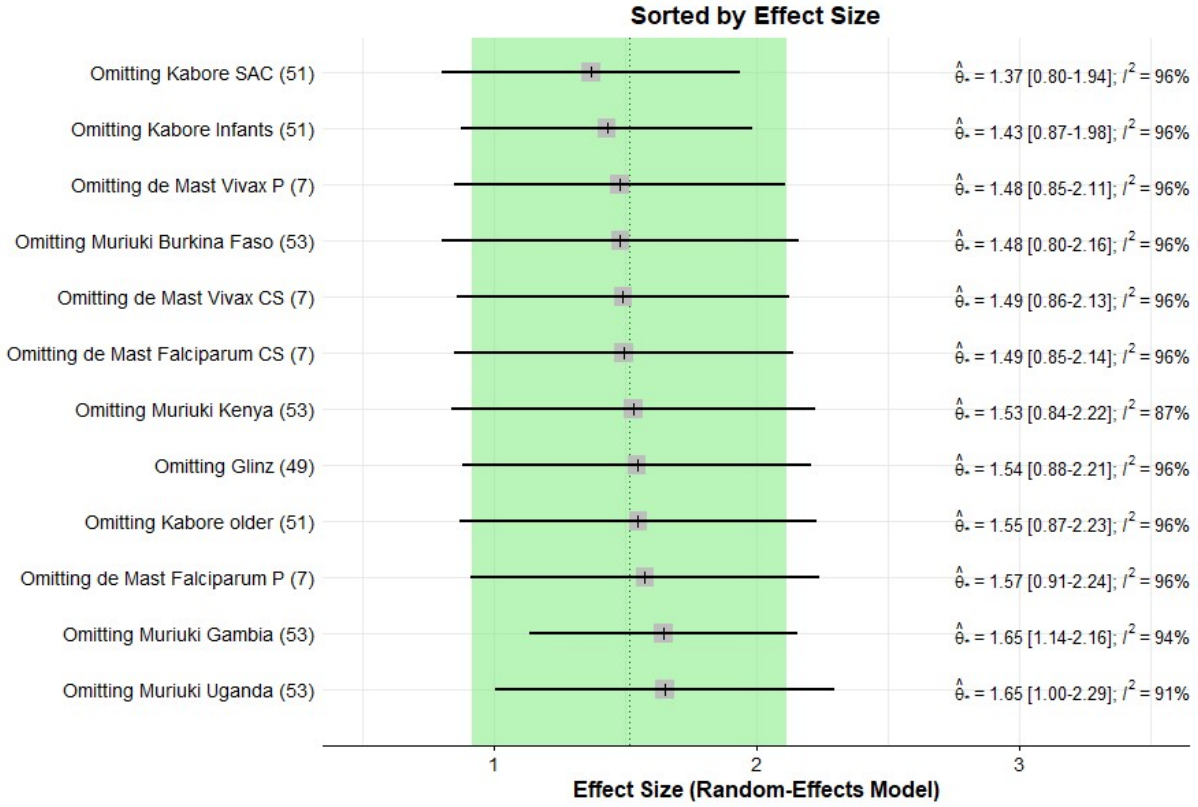
Supplementary Figure 4: Funnel plot showing publications related to asymptomatic malaria and ferritin concentration (µg/L) in adults.



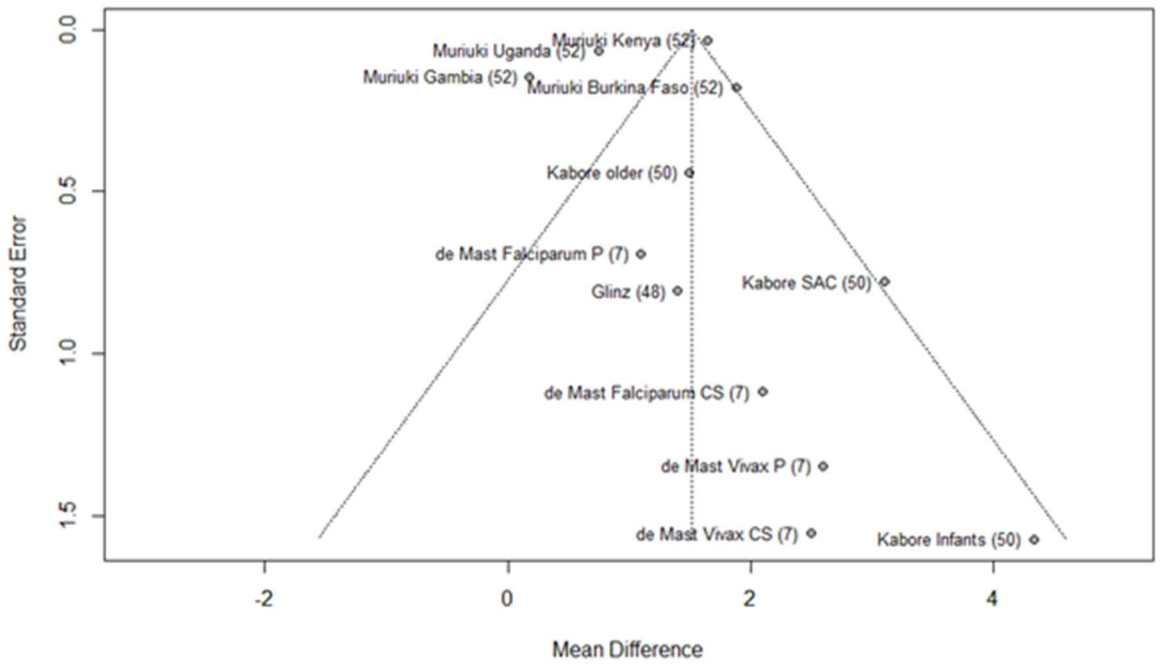
Supplementary Figure 5: Sensitivity analysis of ferritin concentration (µg/L) between clinical malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval. SAC, school-age children



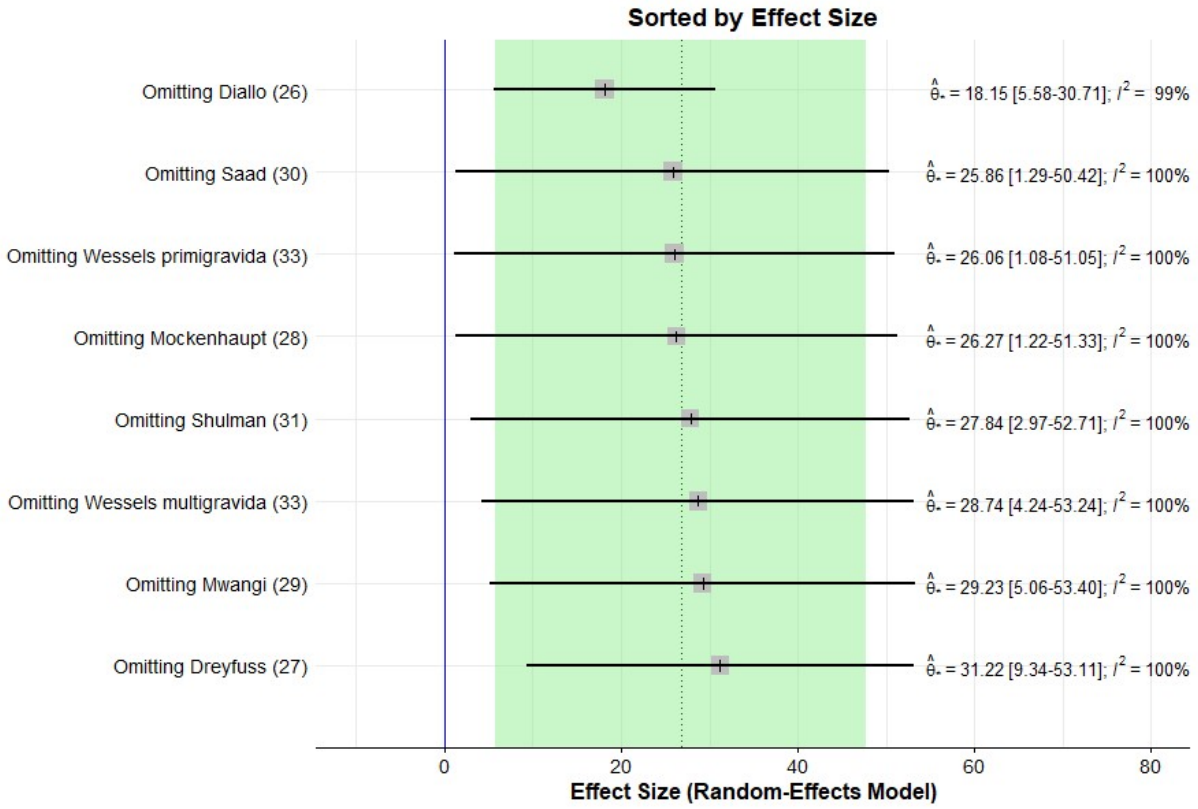
Supplementary Figure 6: Funnel plot showing publications related to clinical malaria and ferritin concentration (µg/L) in children. SAC, school-age children



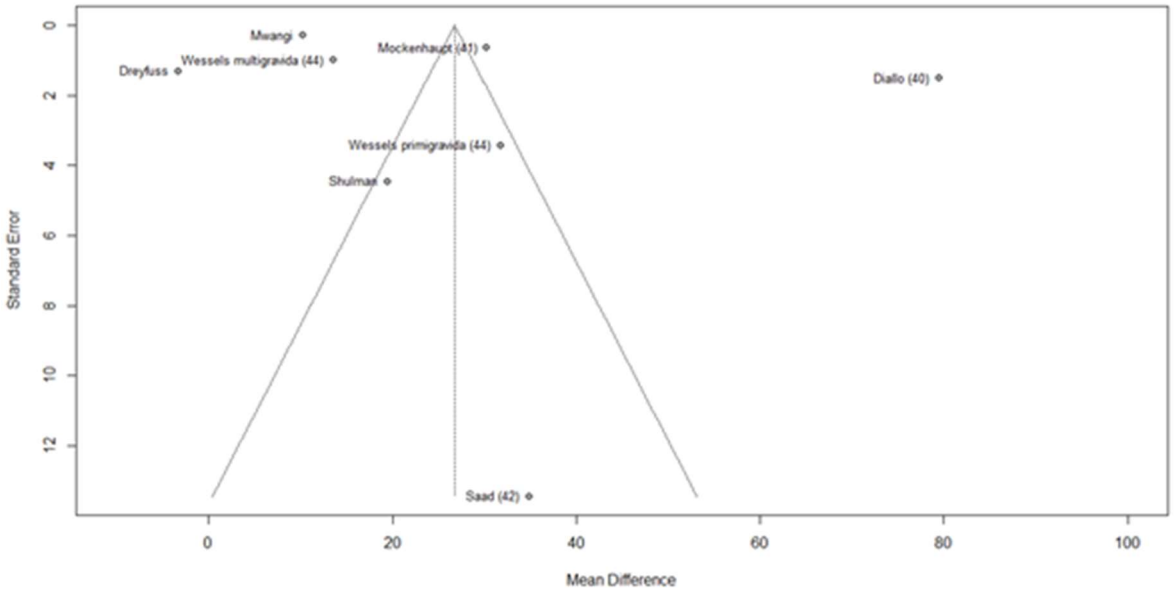
Supplementary Figure 7: Sensitivity analysis of hepcidin concentration (nmol/L) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval. SAC, school-age children; P, prospective; CS, cross-sectional.



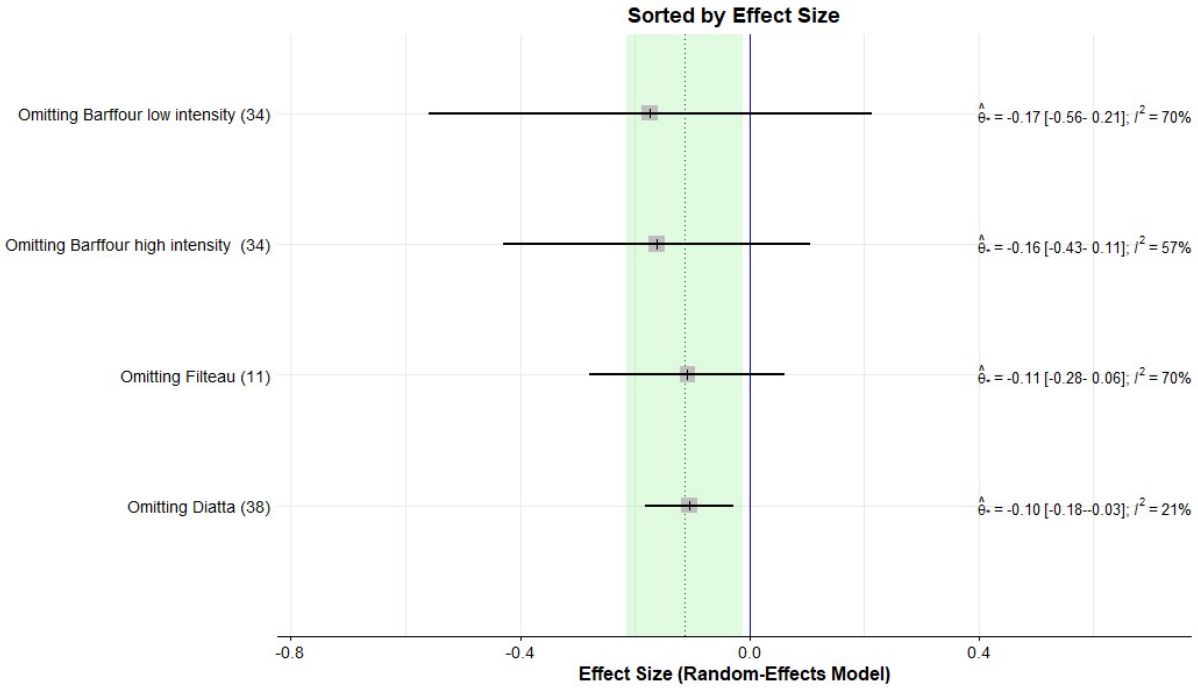
Supplementary Figure 8: Funnel plot showing publications related to asymptomatic malaria and hepcidin concentration (nmol/L) in children. SAC, school-age children; P, prospective; CS, cross-sectional.



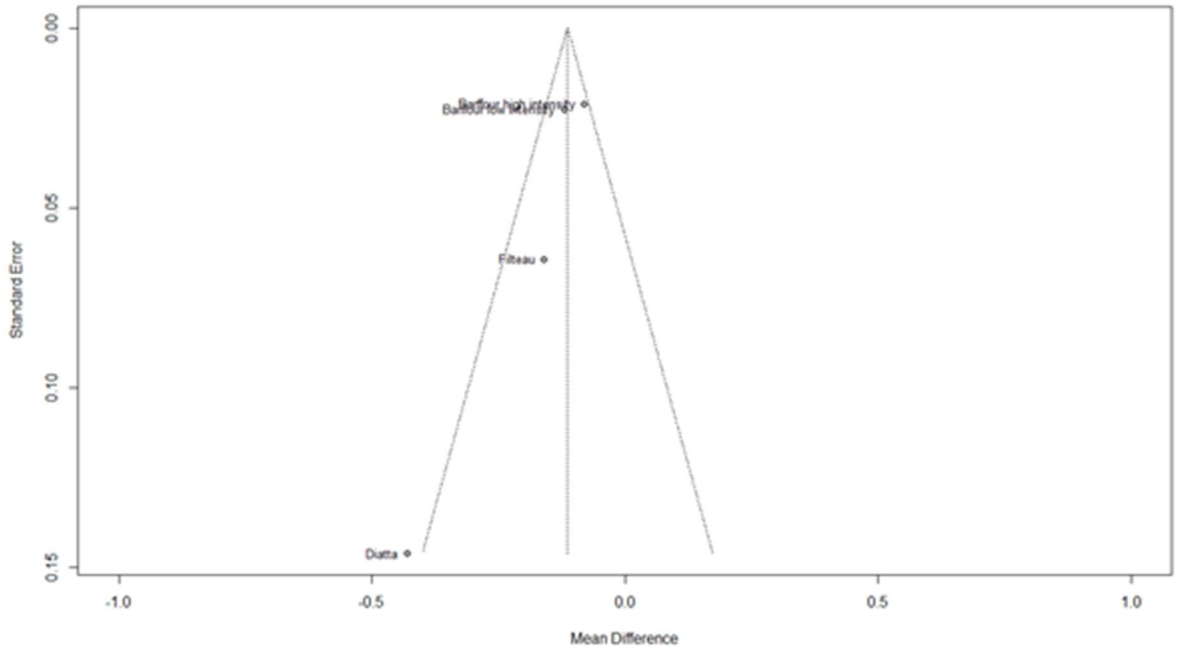
Supplementary Figure 9: Sensitivity analysis of ferritin concentration (µg/L) between malaria and control in pregnant women, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval.



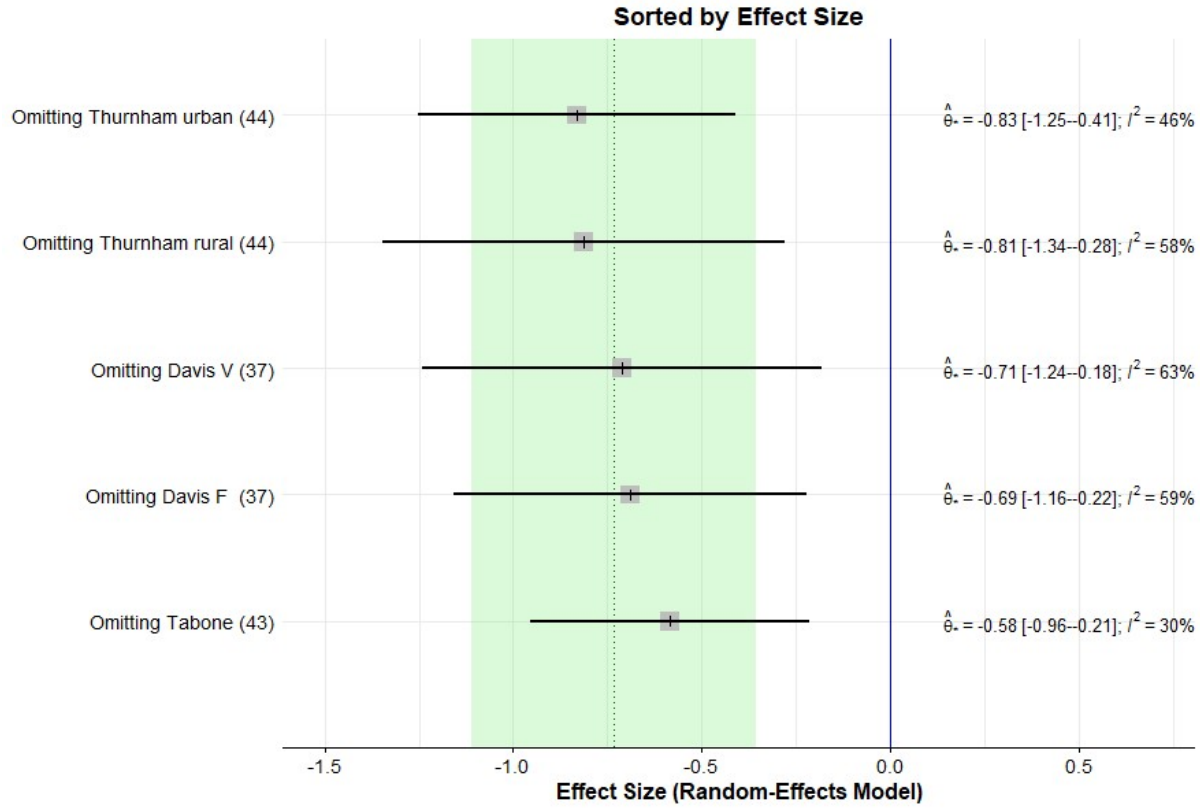
Supplementary Figure 10: Funnel plot showing publications related to malaria and ferritin concentration (µg/L) in pregnant women.



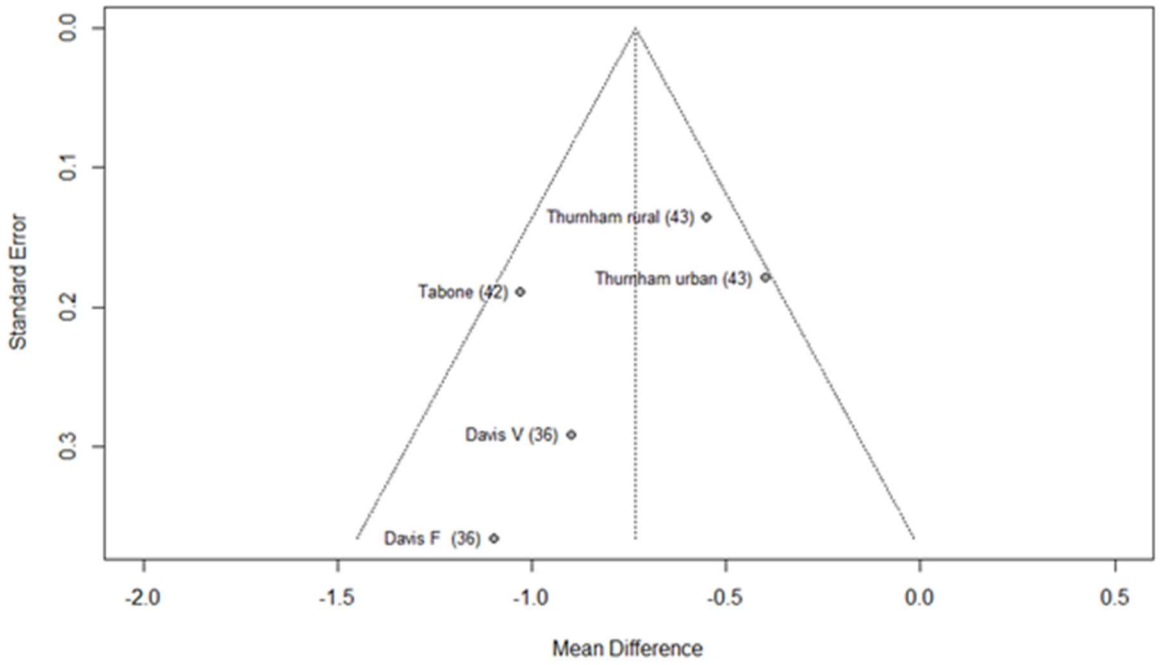
Supplementary Figure 11: Sensitivity analysis of retinol concentration (µmol/L) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval.



Supplementary Figure 12: Funnel plot showing publications related to asymptomatic malaria and retinol concentration (µmol/L) in children.



Supplementary Figure 13: Sensitivity analysis of retinol concentration (µmol/L) between malaria and control in adults, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference and its 95% confidence interval. V, Vivax; F, Falciparum



Supplementary Figure 14: Funnel plot showing publications related to malaria and retinol concentration (µmol/L) in adults. V, Vivax; F, Falciparum

4 Appendix 4: Supplementary materials from chapter 4

Supplementary Table 1: Difference in ferritin concentration (log ferritin, µmol/L) between malaria-infected children and uninfected children, adding each cofounder and each two-factor interaction in turn.

Model	**Potential cofounder**	**Difference in log ferritin concentration between malaria-infected children and uninfected children (95% IC)**	**Difference in ferritin concentration between malaria-infected children and uninfected children**	**p value of the model** (and p value of the interaction for model G-I)
Base	Study ID (n=8,292)	0.40 (0.35,0.44)	49 %	<0.001
A	Study ID, Age (n=8,292)	0.37 (0.32,0.41)	44 %	<0.001
B	Study ID, Sex (n=8,270)	0.40 (0.36, 0.45)	49 %	<0.001
C	Study ID, Residence (n=8,282)	0.39 (0.35, 0.44)	48 %	<0.001
D	Endemicity profile (n=8,282)	0.42 (0.37,0.47)	50%	<0.001
E	Diagnostic method (n=8,282)	0.34 (0.29, 0.39)	40%	<0.001
F	Study ID , Interaction malaria*age (n=8,292)	0.32 (0.26,0.37)	38%	<0.001 p for the interaction: 0.005
G	Study ID, Interaction malaria*sex (n=8,270)	0.40 (0.33, 0.46)	49%	<0.001 p for the interaction: 0.9
H	Interaction malaria*endemicity profile (n=6,653)	0.46 (0.40,0.53)	58%	<0.001 p for the interaction: 0.015
I	Interaction malaria*diagnostic method (n=8,282)	0.32 (0.25, 0.39)	40%	<0.001 p for the interaction: 0.5

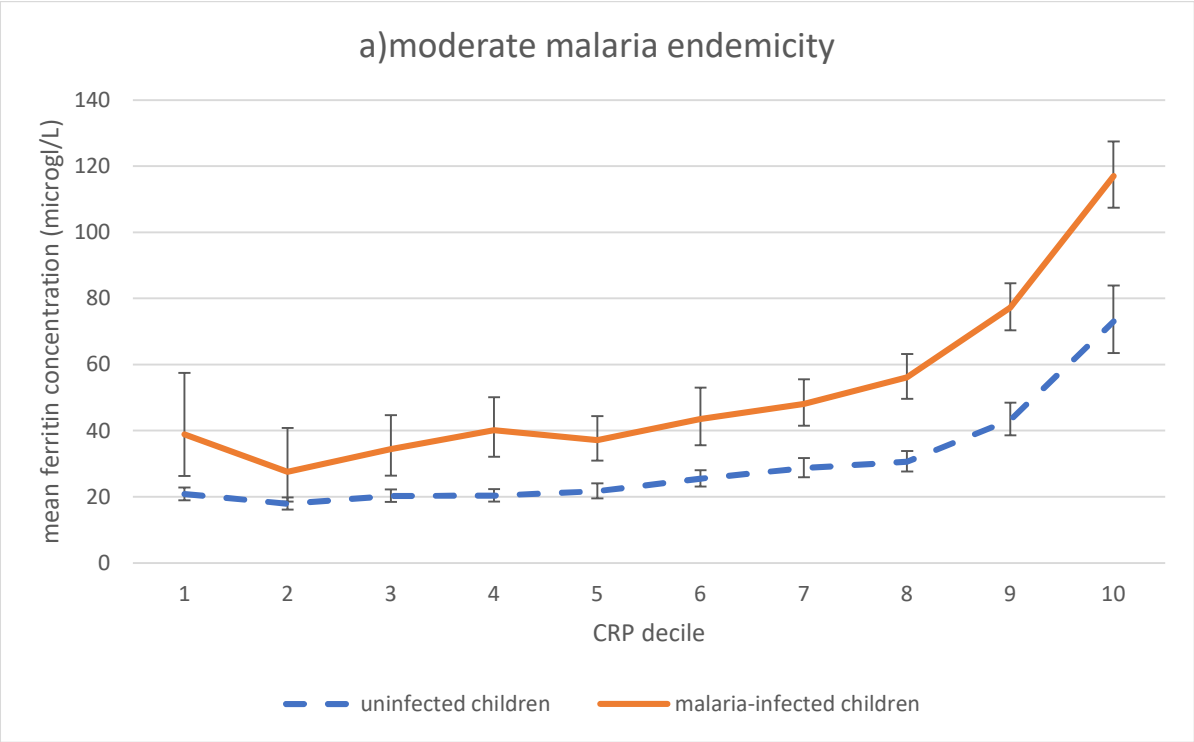
The variables endemicity profile and malaria diagnostic method had a high level of collinearity with the survey identifier. Therefore, models D, E, H and I were run without the fixed adjustment for survey identifier.

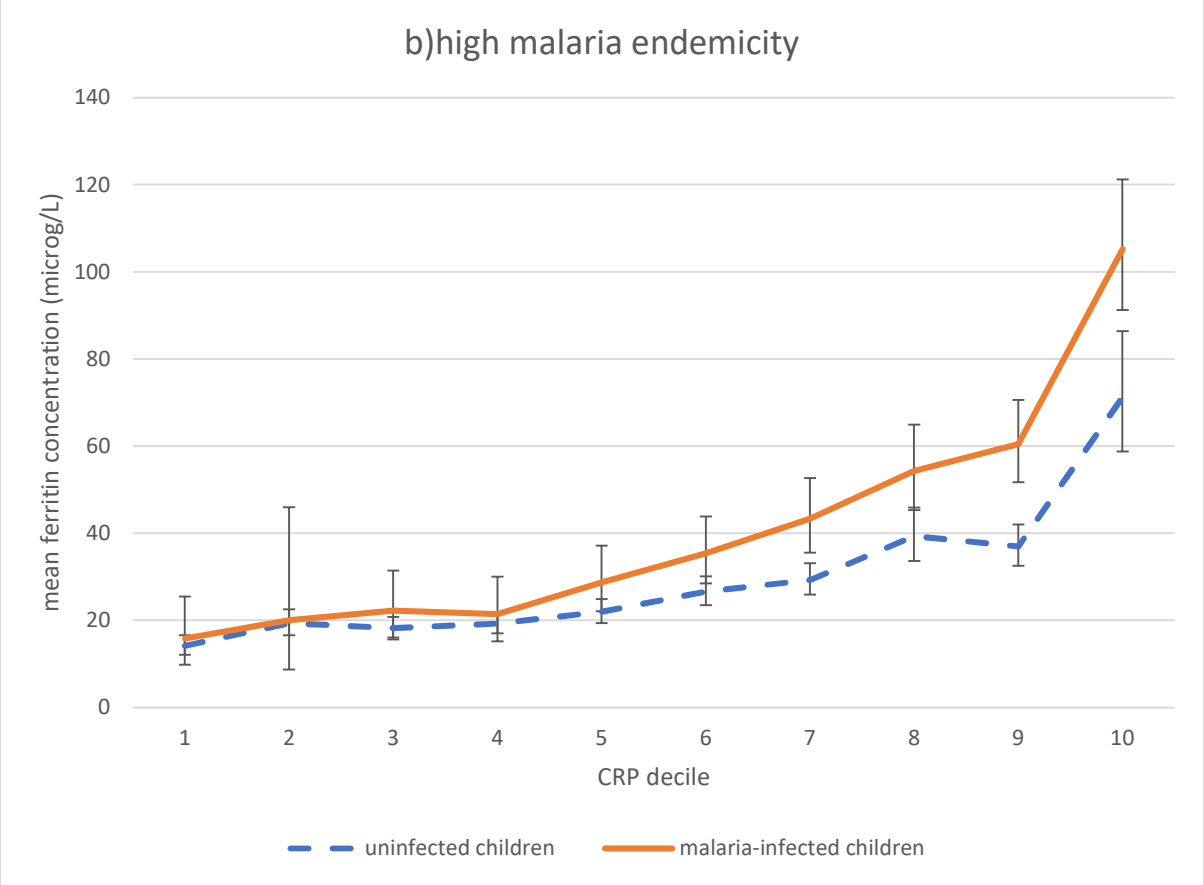
Supplementary Table 2: Difference in ferritin concentration (log ferritin, µmol/L) between malaria-infected children and uninfected children, adding multiple cofounders and two-factor interactions.

Model	**Adjusted for**	**N**	**Difference in log ferritin (95% IC)**	**Difference in ferritin**	**p for the model**	**p for the interaction**
J	Study ID, age, sex, residence	8,270	0.37 (0.32,0.41)	45%	<0.001	
K	Study ID, sex, residence, Interaction malaria* age	8,270	0.44 (0.37, 0.52)	56%	<0.001	0.004
L	Sex, residence Interaction malaria*age Interaction malaria*endemicity profile	6,621	0.53 (0.45,0.62)	70%	<0.001	Malaria*age: p=0.002 Malaria*endemicity profile p=0.002
M	Interaction malaria*age, Interaction malaria*end profile	6,653	0.52 (0.44,0.61)	69%	<0.001	Malaria*age: p=0.002 Malaria*endemicity profile P<0.001

Including diagnostic method and endemicity profile in the model resulted in a high level of collinearity with study ID, therefore models L and M were run without the study ID.

Supplementary Figure 1: Ferritin concentration (µg/L, geometric mean and 95% CI) per CRP decile in malaria-infected children and uninfected children, in a) moderate and b)high endemicity profile.





CRP: C-reactive protein, CI: confidence interval

5 Appendix 5: Supplementary materials from chapter 5

Supplementary Table 1: Results of the final linear model for each biomarker for each population group

PSC

	Difference in log ferritin (95% IC)	**p value**
Model A: log ferritin in PSC (main effect)		
Malaria (infected vs non-infected)	0.52 (0.41, 0.62)	<0.001
Age group (< 2y vs > 2y)	-0.66 (-0.75, -0.56)	<0.001
Sex (girls vs boys)	0.06	0.2
Altitude (high vs low)	-0.02	0.6
Rurality (rural vs urban)	0.14	0.09
Socio-economic status (ascending socio-economic status)	-0.02	0.7
Maternal education (ascending education)	0.004	0.9
Deworming in the last 6 months (yes vs no)	-0.03	0.6
Sickle cell carrier (carrier vs non-carrier)	-0.005	0.9
Alpha-thalassemia (carrier vs non carrier)	-0.06	0.2
R^2:0.26 P<0.001		
Model B: log ferritin in PSC (including interactions)		
Malaria (infected vs non-infected)	0.36 (0.21, 0.51)	<0.001
Age group (< 2y vs >2y)	-0.71 (-0.82, -0.60)	<0.001
Sex (girls vs boys)	0.05	0.3
High altitude (high altitude vs low altitude)	-0.1	0.06
Rurality (rural vs urban)	0.13	0.09
Socio-economic status (ascending socio-economic status)	-0.02	0.6
Maternal education (ascending education)	0.006	0.9
Deworming in the last 6 months (yes vs no)	-0.04	0.6

Sickle cell trait (carrier vs non carrier)	0.08	0.3
Alpha-thalassemia (carrier vs non carrier)	-0.05	0.3
Interaction Malaria*age group (< 2y vs >2y)	0.22 (0.001, 0.44)	p for the interaction 0.0495
Interaction Malaria*high altitude (high altitude versus low altitude)	0.33 (0.12, 0.53)	p for the interaction 0.002
Interaction Malaria*sickle cell trait (carrier vs non carrier)	-0.51 (-0.89, -0.13)	p for the interaction 0.009
R^2:0.27 P<0.001		

	Difference in log sTfR (95% IC)	**p value**
Model C: log sTfR in PSC (main effect)		
Malaria (infected vs non-infected)	0.23 (0.16, 0.29)	<0.001
Age group (< 2y vs >2y)	0.38 (0.32, 0.44)	<0.001
Sex (girls vs boys)	-0.04	0.1
Altitude (high vs low)	0.0002	0.9
Rurality (rural vs urban)	0.02	0.7
Socio-economic status (ascending socio-economic status)	0.06 (0.02, 0.11)	0.004
Maternal education (ascending education)	0.001	0.9
Deworming in the last 6 months (yes vs no)	-0.03	0.4
Sickle cell carrier (carrier vs non-carrier)	0.06	0.1
Alpha-thalassemia (carrier vs non carrier)	0.05	0.08
R^2:0.20 P<0.001		
Model D: log sTfR in PSC (including interactions)		
Malaria (infected vs non-infected)	0.28 (0.20, 0.35)	<0.001
Age group (< 2y vs >2y)	0.38 (0.32, 0.43)	<0.001

Sex (girls vs boys)	-0.04	0.1
High altitude (high altitude vs low altitude)	0.03	0.3
Rurality (rural vs urban)	0.02	0.7
Socio-economic status (ascending socio-economic status)	0.06 (0.02, 0.11)	0.003
Maternal education (ascending education)	-0.0007	0.9
Deworming in the last 6 months (yes vs no)	-0.03	0.4
Sickle cell trait (carrier vs non carrier)	0.07	0.1
Alpha-thalassemia (carrier vs non carrier)	0.04	0.1
Interaction Malaria*high altitude (high altitude versus low altitude)	-0.13 (-0.25, -0.01)	p for the interaction 0.03

	Difference in log zinc (95% IC)	**p value**
Model E: log zinc in PSC (main effect)		
Malaria (infected vs non-infected)	-0.07 (-0.10, -0.03)	0.001
Age group (< 2y vs >2y)	0.02	0.4
Sex (girls vs boys)	0..02	0.3
Altitude (high vs low)	0.02	0.2
Rurality (rural vs urban)	0.06	0.06
Socio-economic status (ascending socio-economic status)	0.03	0.05
Maternal education (ascending education)	0.02	0.3
Deworming in the last 6 months (yes vs no)	0.007	0.7
Sickle cell carrier (carrier vs non-carrier)	0.02	0.4
Alpha-thalassemia (carrier vs non carrier)	0.02	0.2
R^2:0.02 P<0.001		

Model F: log zinc in PSC (including interactions)		
Malaria (infected vs non-infected)	-0.08 (-0.12, -0.04)	0.0001
Age group (< 2y vs >2y)	0.02	0.3
Sex (girls vs boys)	0.02	0.2
High altitude (high altitude vs low altitude)	0.02	0.3
Rurality (rural vs urban)	0.06 (0.0004, 0.12)	0.049
Socio-economic status (ascending socio-economic status)	0.03 (0.001, 0.06)	0.04
Maternal education (ascending education)	0.02	0.2
Deworming in the last 6 months (yes vs no)	0.008	0.7
Sickle cell trait (carrier vs non carrier)	-0.01	0.7
Alpha-thalassemia (carrier vs non carrier)	0.02	0.2
Interaction Malaria*sickle cell (carrier vs non carrier)	0.21 (0.07, 0.36)	p for the interaction 0.004
R^2:0.03 P<0.001		

SAC

	Difference in log ferritin (95% IC)	**p value**
Model F: log ferritin in SAC (main effect)		
Malaria (infected vs non-infected)	0.25 (0.16, 0.34)	<0.001
Age in years (over 10y vs under 10y)	-0.04	0.4
Sex (girls vs boys)	0.003	0.9
Altitude (high vs low)	0.1 (0.03, 0.19)	0.008
Rurality (rural vs urban)	-0.11	0.1
Socio-economic status (ascending socio-economic status)	-0.05	0.2
Maternal education (ascending education)	0.09 (0.01, 0.16)	0.02
Deworming in the last 6 months (yes vs no)	0.005	0.9
R^2:0.07 P<0.001		
Model G: log ferritin in SAC (including interactions)		
Malaria (infected vs non-infected)	0.14 (0.01, 0.27)	0.03
Age in years (increasing age)	-0.11 (-0.22, -0.009)	0.03
Sex (girls vs boys)	0.004	0.9
High altitude (high altitude vs low altitude)	0.10 (0.02, 0.19)	0.01
Rurality (rural vs urban)	-0.11	0.1
Socio-economic status (ascending socio-economic status)	-0.04	0.2
Maternal education (ascending education)	0.08 (0.003, 0.15)	0.04
Deworming in the last 6 months (yes vs no)	0.01	0.9
Interaction Malaria*age (above 10y vs below 10y)	0.19 (0.03,0.36)	p for the interaction 0.02
R^2:0.08 P<0.001		

	Difference in log sTfR (95% IC)	**p value**
Model H: log sTfR in SAC (main effect)		
Malaria (infected vs non-infected)	0.14 (0.08, 0.20)	<0.001
Age (above 10 vs below 10)	-0.02	0.6
Sex (girls vs boys)	0.02	0.5
Altitude (high vs low)	-0.04	0.2
Rurality (rural vs urban)	0.04	0.4
Socio-economic status (ascending socio-economic status)	0.003	0.9
Maternal education (ascending education)	-0.01	0.6
Deworming in the last 6 months (yes vs no)	0.003	0.9
R^2:0.05 P<0.001		

	Difference in log zinc (95% IC)	**p value**
Model J: log zinc in SAC (main effect)		
Malaria (infected vs non-infected)	-0.05 (-0.1, -0.004)	0.03
Age in years (increasing age)	0.002	0.7
Sex (girls vs boys)	0.03	0.3
Altitude (high vs low)	0.005	0.8
Rurality (rural vs urban)	0.1 (0.04, 0.2)	0.004
Socio-economic status (ascending socio-economic status)	0.03	0.07
Maternal education (ascending education)	-0.007	0.7
Deworming in the last 6 months (yes vs no)	0.01	0.8
R^2:0.01 P=0.06		

WRA

	Difference in log ferritin (95% IC)	**p value**
Model K: log ferritin in WRA (main effect)		
Malaria (infected vs non-infected)	0.29 (0.13, 0.44)	<0.001
Age in years (increasing age)	0.004	0.2
Socio economic status (increasing socio-economic status)	0.001	0.9
Maternal education (increasing education)	-0.008	0.9
High altitude (high altitude versus low altitude)	0.14 (0.03, 0.25)	0.01
Rurality (rural against urban)	0.17 (-0.34, -0.01)	0.04
R^2:0.03 P<0.001		

	Difference in log folate (95% IC)	**p value**
Model L: log folate in WRA (main effect)		
Malaria (infected vs non-infected)	0.16 (0.03, 0.30)	0.02
Age in years (increasing age)	-0.003	0.3
Socio economic status (increasing socio-economic status)	-0.11 (-0.19, -0.04)	0.003
Education (increasing education)	-0.10 (-0.19, -0.01)	0.03
Iron supplementation (took iron vs did not take iron)	-0.01	0.9
Rurality (rural against urban)	0.17 (0.02, 0.31)	0.02
High altitude (high altitude versus low altitude)	0.24 (0.15,0.34)	<0.001
CRP (increasing CRP)	-0.004 (-0.01, 0.003)	0.3
AGP (increasing AGP)	-0.2 (-0.3, -0.01)	0.04
R^2:0.11 P<0.001		

CRP: C-reactive protein, AGP: α1-acid glycoprotein

	Difference in log red blood cell folate (95% IC)	**p value**
Model M: log red blood cell folate in WRA (main effect)		
Malaria (infected vs non-infected)	0.11 (0.07, 0.21)	0.04
Age in years (increasing age)	0.0005	0.8
Socio economic status (increasing socio-economic status)	-0.02	0.5
Education (increasing education)	-0.07 (-0.13, -0.005)	0.03
Iron supplementation (took iron vs did not take iron)	0.29 (0.03, 0.55)	0.03
Rurality (rural against urban)	0.12 (0.01, 0.22)	0.03
High altitude (high altitude versus low altitude)	-0.09 (-0.16, -0.02)	0.008
CRP (increasing CRP)	-0.002 (-0.01, 0.003)	0.4
AGP (increasing AGP)	-0.02 (-0.3, -0.01)	0.7
R^2:0.04 P<0.001		

6 Appendix 6: Technical brief in French

Prendre en compte l'infection au paludisme dans l'interprétation des valeurs sériques de ferritine

JUSTIFICATION

La concentration plasmatique ou sérique de ferritine est l'indicateur le plus couramment utilisé pour mesurer la prévalence de carence en fer. BRINDA (brinda-nutrition.org) et l'OMS recommandent que les concentrations de ferritine soient corrigées par des marqueurs inflammatoires, car la ferritine est plus élevée en cas d'inflammation[22; 83]. Des données suggèrent que l'infection paludéenne peut avoir un effet supplémentaire sur la concentration de ferritine[69].

Cette fiche technique est destinée aux professionnels qui collectent, analysent ou interprètent des données sur le statut en fer des populations.

DESCRIPTION DE L'ETUDE

En collaboration avec BRINDA, nous avons analysé les données d'enquêtes nationales mesurant la ferritine chez les enfants d'âge préscolaire (PSC) afin d'examiner l'association entre la concentration de ferritine ajustée pour l'inflammation et l'infection paludéenne. Nous avons également évalué le rôle des facteurs liés à l'immunité contre le paludisme, car il est possible que la relation entre le paludisme et la ferritine puisse être modifiée en cas d'immunité contre le paludisme[74].

RESULTATS PRINCIPAUX

Tableau 1: Résumé des résultats de l'analyse de la concentration de ferritine ajustée pour l'inflammation et de l'infection paludéenne dans huit enquêtes transversales chez les PSC au Cameroun, en Côte d'Ivoire, au Kenya (x2), au Libéria, au Malawi, au Nigéria et en Zambie[84].

	Changement dans la concentration de ferritine ajustée pour l'inflammation associée a une infection paludéenne	Magnitude du changement relatif (IC 95%)
Biomarqueur		
Ferritine ajustée pour l'inflammation	Plus élevée chez les enfants infectés par le paludisme que chez les enfants non infectés par le paludisme	+44% (39, 52)
Modificateurs		
Age	Plus faible chez les enfants de plus de deux ans infectés par le paludisme que chez les enfants de moins de deux ans infectés par le paludisme	-15% (-23,-6)
Profil paludéen*	Plus faible chez les enfants infectés par le paludisme vivant dans des zones à forte endémicité que chez les enfants infectés par le paludisme vivant dans une zone d'endémicité modérée.	-17% (-25,-8)
Altitude (>1000 mètres)**	Plus élevée chez les enfants infectés par le paludisme vivant en haute altitude que chez les enfants infectés par le paludisme vivant à basse altitude.	+39% (13,70)
Trait drépanocytaire**	Plus faible chez les enfants infectés par le paludisme et porteur du trait drépanocytaire que chez les enfants infectés par le paludisme mais non porteurs.	-67% (-144,-13)

IC: Intervalle de confiance. *Le profil paludéen est le degré de transmission du paludisme dans une zone, et la prévalence parasitaire chez les enfants de 2 à 10 ans est une mesure commune pour définir le profil paludéen. **Ces analyses ont été effectuées uniquement dans la base de données du Malawi.

RECOMMENDATIONS POUR L'INTERPRETATION DE LA FERRITINE SERIQUE EN CONTEXTE PALUDEEN

Etape 1: Mesurer la prévalence du paludisme chez les PSC

Etape 2: Mesurez l'association entre l'infection paludéenne et les concentrations de ferritine ajustées pour l'inflammation dans l'ensemble de l'échantillon, avec ajustement pour les facteurs de confusion potentiels. Les analyses peuvent être : une analyse stratifiée par statut paludéen, une association bivariée entre la ferritine et le paludisme, un modèle de régression linéaire ;

Etape 3: Mesurez l'association entre l'infection paludéenne et les concentrations de ferritine ajustées pour l'inflammation (avec ajustement pour les facteurs de confusion potentiels) chez les enfants à faible immunité (Tableau 1);

Etape 4: Si l'association est significative, considérez un ajustement en utilisant une régression linéaire;

Etape 5: Reportez les valeurs ajustées et non-ajustées.

Exemple de l'enquête nationale 2015 du Malawi

Etape 1. Prevalence du paludisme : 27.9% (21.0, 36.0)

Etape 2. Dans un modèle de régression linéaire ajusté pour les facteurs de confusion potentiels, le paludisme était associé à des valeurs de ferritine 67% supérieures.

Etape 3 : Cette valeur était supérieure chez les enfants en bas-âge (6-24 mois, 99%) comparée aux enfants plus âgés (25-59 mois, 55%)

Etape 4 : Coefficient $\beta_{malaria}$ =0.36, $\beta_{\text{interaction malaria-age}}$: 0.22

Etape 5: Prévalence de carence en fer **sans** ajustement pour le paludisme: 20.5% (15.5, 27.0) ; et **avec** ajustement pour le paludisme: 23.8% (19.5, 29.0).

Comment utiliser une régression linéaire pour ajuster pour le paludisme ?

1. Transformer les valeurs de ferritine sérique sur l'échelle logarithmique ;
2. Sur l'échelle logarithmique, effectuer une régression des valeurs de ferritine en fonction du statut paludéen, avec ajustement pour les facteurs de confusion potentiels, afin d'obtenir le coefficient β(paludisme) ;
3. Ajoutez les interactions avec les facteurs lies à l'immunité pour obtenir les coefficients interactions ;
4. Calculez les valeurs de log ferritine ajustées pour le paludisme en utilisant les coefficients de la manière suivante :
   log(ferritine ajustée pour le paludisme)=ferritine ajustée pour l'inflammation +/- β(paludisme) +/- β_i(interactions) ;
5. Calculez les valeurs de ferritine ajustées pour le paludisme = $e^{(\log(\text{ferritine ajustée pour le paludisme}))}$

Davantage de recherche doit permettre de comprendre s'il est nécessaire d'effectuer ce type d'ajustement dans d'autres populations, notamment chez les femmes enceintes.

CONTACTS

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