

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 United States National Institute of Health tool. Random-effects meta-analyses were used to generate summary mean differences. In total, 44 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 to 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.

INTRODUCTION

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 – i.e. its concentration increases in response to inflammation – and retinol binding protein (RBP) is a negative acute phase protein – i.e. 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 World Health Organization (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, i.e. 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 Africa, while *P. vivax* accounts for 75% of infections in the Americas ⁽⁸⁾. Currently-used diagnostic methods include microscopy which visualizes parasites in stained blood smears; rapid diagnostic tests (RDT) that detect parasite antigen/s in blood samples; and polymerase chain reaction (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 alpha1-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 24th of September 2021: [CRD42021279974](https://doi.org/10.1017/S000714522000757). 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, 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, Philadelphia, United States of America). The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart of identified studies is illustrated in Figure 1.

Data extraction and statistical analysis

Data was 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 haemoglobin and soluble transferrin receptors are sometimes used to describe iron status, we did not include them in our analysis, as haemoglobin 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, 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 was 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 14 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, we reported the mean value of different groups (healthy control group, asymptomatic malaria group and clinical malaria group), as well as the 95% confidence interval (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 confidence interval, 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 versus vivax*), malaria endemicity profile of the country (low *versus* moderate *versus* high intensity of transmission, as defined by^(15; 16)), method of diagnosis of malaria (RDT *versus* microscopy or both), age of children (under *versus* over five years old) and design of the study (cross-sectional *versus* 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*⁽²⁴⁾ packages. The workbooks for meta-analysis (version 1.5) developed by Suurmond were used to perform the subgroup analyses⁽²⁵⁾. A p value of <0.05 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; 27; 28; 29; 30; 31; 32; 33; 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64; 65)(Figure 1). Twenty-nine studies were conducted in 15 African countries (10; 11; 13; 26; 28; 29; 30; 31; 38; 39; 40; 41; 46; 47; 49; 50; 51; 52; 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, 23 reported on ferritin and/or hepcidin concentrations in adults and/or children (7; 10; 13; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64), 13 reported on retinol or RBP concentrations in adults and/or children (11; 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 63) and eight reported on either ferritin, hepcidin, retinol or RBP concentrations in pregnant women (26; 27; 28; 29; 30; 31; 32; 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; 27; 28; 29; 30; 31; 32; 33; 34; 36; 37; 38; 39; 40; 42; 44; 50; 51; 52; 53; 54; 55; 57; 58; 59; 60; 62; 63; 64) whereas ten were prospective (7; 41; 43; 45; 46; 47; 48; 49; 56; 61). The predominant species of malaria was *P. falciparum* in 41 studies (7; 11; 13; 26; 28; 29; 30; 31; 32; 33; 34; 36; 37; 38; 39; 40; 41; 42; 43; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 59; 60; 62; 63; 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 (Supplementary table 1).

1. Asymptomatic malaria and ferritin concentrations in children and adults

Fifteen studies (7; 10; 49; 50; 51; 53; 57; 59; 60; 63; 64) (23 datasets) analysed the association between malaria and ferritin concentrations in asymptomatic children (4,309 children with malaria infection and 6,375 control children). Overall, ferritin concentrations were 28.2 µg/L (95% CI: 15.6, 40.9, $p < 0.001$) greater in children with asymptomatic malaria compared to control groups (Figure 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 figure 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 figure 2).

A greater ferritin concentration was also observed in non-pregnant adults with asymptomatic malaria (28.5 $\mu\text{g/L}$, 95% CI: 8.1, 48.8, $p=0.02$) (Figure 3). This mean difference was calculated for 234 adults with malaria and 400 control adults, from four 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 sub-group analyses. The heterogeneity was moderate (51%) and the sensitivity analysis showed the stability of the pooled result (Supplemental figure 3, Supplemental figure 4 and Table 4)

2. 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 $\mu\text{g/L}$ (95% CI: 162, 570) $p < 0.003$ greater in children with clinical malaria compared to control group (Figure 4). The sub-group analyses showed that the difference in mean ferritin was the greatest in settings with moderate transmission, compared to low transmission (Table 5). The sensitivity analysis showed the stability of the pooled results after the leave-one-out analysis (Supplementary figure 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 underreporting of small studies (Supplementary figure 6).

In terms of clinical malaria in adults, 269 adults were included in the analyses of five 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 $\mu\text{g/L}$ elevation in the clinical group compared to the control group). In the meta-analysis, the confidence interval was wide and included zero (493 $\mu\text{g/L}$, 95% CI: -219, 1206).

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).

3. **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 (4 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 to controls (Figure 5). No interaction was reported in the sub-group analyses. 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 figure 7 and Table 4). There was no significant publication bias, as assessed by the funnel plot and Egger's test (Supplementary figure 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.

4. **Malaria infection and ferritin concentrations in pregnant women**

Seven studies (26; 27; 28; 29; 30; 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 $\mu\text{g/L}$, CI: 5.8, 47.7, $p = 0.02$) (Figure 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 (Supplementary figure 9 and Supplementary figure 10). After excluding the study with high risk of bias, the difference in ferritin concentration was lower (+18.1 $\mu\text{g/L}$, 95% CI: 5.6, 30.7, $p = 0.01$), (Table 4).

5. 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 children (709 children with malaria and 1,383 control children). All studies were conducted in Africa and the species involved was always *P. falciparum*. Overall, retinol concentrations were lower, i.e. $-0.11 \mu\text{mol/L}$ (95% CI: $-0.22, -0.01$, $p=0.04$) in children with asymptomatic malaria compared to control group (Figure 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 figure 11, Supplementary figure 12 and Table 4).

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

6. Clinical malaria infection and retinol concentrations in children and adults

Six studies (35; 36; 39; 40; 41; 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 ($-0.43 \mu\text{mol/L}$, 95% CI: $-0.71, -0.16$, $p=0.01$) (Figure 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 sub-group 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 to healthy control adults, by $0.73 \mu\text{mol/L}$ (95% CI: $-1.11, -0.36$, $p=0.005$) (Figure 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 figure 13 and Supplementary figure 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 reduced concentration of retinol in pregnant women with malaria (-0.54, 95%IC: -0.67, -0.41, $p<0.001$).

7. **Malaria infection and RBP 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 (7 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 confidence interval 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 $\mu\text{g/L}$, across all settings and all groups of population. Considering that the mean value of ferritin concentration in control groups was c. 25 $\mu\text{g/L}$, this indicates that the ferritin concentration in asymptomatic malaria infection was approximately doubled compared to a control group. As a comparison, based on 16 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 $\mu\text{g/L}$ in the reference group to 50.8 $\mu\text{g/L}$ 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 $\mu\text{g/L}$, 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 $\mu\text{g/L}$).

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 > 1 g/L (57). In non-pregnant women, this proportion was even higher (65%). Similarly, de Mast found low circulating concentrations of CRP in Indonesian school-children 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 μ g/L vs. 11.1 ± 0.8 μ g/L; $P < 0.001$) (63). Muriuki found that children with malaria had greater ferritin concentrations at every decile of CRP, compared to 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 μ g/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 programs 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 pre-school 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 homeostasis, 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 to 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 individualized adjustment methods that can take into account the concentrations of acute phase proteins and the presence, and severity, of a malaria infection.

Contribution

F.S., E.J.M.J., S.F. and H.H. conceptualised the analytical protocol. F.S. and L.S.R. conducted the literature search. F.S. conducted the study data analyses with the support of A.MD. F.S. 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.

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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 ¹	Total cases	Clinical severity	Malaria species	Diagnosis of Malaria	Outcomes measured
Studies reporting on iron status indicators											
1	Barffour et al, 2018 ⁽¹⁰⁾	2012-2013	Zambia	CS nested in a cluster-randomised controlled trial	Children 4-8y	Moderate intensity, seasonal transmission; 2 cross-sectional surveys conducted, one during high and one in low transmission period	744	A and control	F	RDT (HRP2) and P ²	Ferritin
2	Beesley et al,	NA	Europe	Prospective	Adults	Imported	36	C	F	P	Ferritin

	2000 ⁽⁴⁵⁾			study		cases					
3	Burte et al, 2013 ⁽¹³⁾	2008 to 2010	Nigeria	CS as part of a larger prospective study	Children 6m-12y	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 1-12y	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 5-15y	Low intensity,	108	A and control	F and V	P	Ferritin, hepcidin

						year-round					
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 11-17y	Moderate intensity, year-round	17	A	F	P	Ferritin, hepcidin
11	Jeremiah et al, 2007 ⁽⁵⁰⁾	2005-2006	Nigeria	CS	Children 1-8y	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: n: 722 Adults: 396	A and C and control	F	P	Ferritin, hepcidin
13	Kivibidila et al, 1999 ⁽⁵²⁾	1991	DR Congo	CS	Children 6months-16y	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 1 case of P. Ovale	P and PCR	Ferritin
15 - 18	Muriuki et al, 2020 ⁽⁵³⁾ (73; 74; 75; 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	Children 2-6 y	Moderate intensity,	753	A and control	F	P	Ferritin, hepcidin

				prospective study		seasonal transmission; survey conducted during high transmission season						
		2012-2014	Kenya	CS as part of a prospective study	Children under 5y	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 a RCT	Children under 5y	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,	470	C and control	F	P	Ferritin	

						Year-round					
20	O'Donnell et al, 2009 ⁽⁵⁴⁾	1993-1996	Papua New Guinea	CS study	Children under 4y	Low intensity	495	C, severe malaria, control	F	P	Ferritin
21	Odunukwe et al, 2000 ⁽⁵⁵⁾	NA	Nigeria	CS study	Adults	High intensity, Year-round	300	A and control	F and Malaria e	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-8y and adults	High intensity Year-round	Children: 246 Adults: 92	A and C	F	RDT (HRP2) and P ³	Ferritin
24	Saad et al, 2012 ⁽³⁰⁾	2010	Sudan	CS study	Pregnant women	Low intensity	64	Severe, C and control	F	P	Ferritin
25	Seyrek et al, 2004 ⁽⁵⁸⁾	NA	Turkey	CS study	Adults	Very low intensity	31	C and control	V	P	Ferritin

26	Shulman et al, 1996 ⁽³¹⁾	1993	Kenya	CS study	Pregnant women	Low intensity, seasonal. Survey conducted during a low transmission period	275	C and control	F	P	Ferritin
27	Stoltzfus et al, 1997 ⁽⁵⁹⁾	1994	Zanzibar	CS	School children, 5-19y	Moderate intensity, Year-round	3605	A and control	F	P	Ferritin
28	Stoltzfus et al, 2000 ⁽⁶⁰⁾	1996	Zanzibar	CS	Children under 5y	Moderate intensity, Year-round	464	A and control	F	P	Ferritin
29	Uscategui et al, 2009 ⁽⁶¹⁾	2002	Colombia	Prospective case study	Children 4-10y	Very low intensity	89	C	F and V	P	Ferritin
30	Verhoef et al, 2001 ⁽⁶²⁾	1997	Kenya	CS study	Children 2-36 months	Moderate intensity, seasonal	318	A and control	F	P	Ferritin

						transmission; survey conducted during high transmission season					
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: 11y	Low intensity, seasonal transmission; survey conducted during high transmission	115	A, C and control	F and V	P	Ferritin

						n season					
Studies reporting on Vitamin A status indicators											
1	Barffour et al, 2018 ⁽³⁴⁾ ⁴	2012-2013	Zambia	CS nested in a cluster-RCT	Children 4-8y	Moderate intensity, seasonal transmission; 2 cross-sectional surveys conducted, one during high and one in low transmission period	744	A and control	F	RDT (HRP2) and P ²	Retinol
2	Carmona et al, 2008 ⁽³⁵⁾ ⁵	2002	Colombia	Prospective case study	Children 4-10y	Very low intensity	89	C	F and V	P	Retinol
3	Das et al, 1996 ⁽³⁶⁾	1992-1993	India	CS	Children 2-11y	Low intensity	173	A, C and	F	P	RBP, retinol

								severe malaria				
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 5y	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 study	Children 0-6 y	High intensity	116	C and control	F	P	Retinol	
8	Mfonkeu et	2007	Cameroon	CS	Children	High	139	C, MA,	F	P	Retinol	

	al, 2010 ⁽⁴⁰⁾		n		6 months-14y	intensity		CM, CM&M A and control				
9	Nussenblatt et al, 2002 ⁽⁴¹⁾	1998	Uganda	Prospective study	Children 1-10y	High intensity, Year-round	273	C	F	P	Retinol	
10	Raza et al, 2009 ⁽⁴²⁾	2006-2007	India	CS	Children 2-5y	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 study	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	Children 6-23	High intensity,	437	A and control	F	ELISA (HRP2)	RBP	

				RCT	months	Year-round					
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CS, cross-sectional; y, years; A, Asymptomatic malaria; F, Falciparum; RDT, Rapid Diagnostic Test; HRP2, histidine-rich protein 2; P, parasitaemia by microscopy; NA, Non available; C, Clinical- uncomplicated malaria; DC, disease control; CM, cerebral malaria; SMA, severe malarial anemia; V, Vivax; RCT, Randomised controlled trial; MA, malarial anemia; PCR, Polymerase Chain Reaction; ELISA, Enzyme-Linked Immunosorbent Assay; RBP, Retinol Binding Protein. 'Control' is defined as healthy children with no malaria detected by the study-specific diagnostic test. ¹Seasonality 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. 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. ²Malaria parasitaemia was detected by RDT and/or microscopy. ³Malaria diagnosis was by microscopy-confirmed RDT. ⁴Participants are the same as in ⁽¹⁰⁾. ⁵Participants are the same as in ⁽⁶¹⁾.

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

	Children	Non pregnant adults	Pregnant women	Total
Number of studies included	27	11	8	46 ¹
Number of participants in the included studies	14,330	985	3,438	18,753
Age in years (mean \pm SD) of participants in the included studies	6.2 \pm 4	29.6 \pm 9	24.1 \pm 5.1	-
Estimated malaria transmission intensity during the survey in the included studies²				
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%)

¹: two reports provided data for children and adults. ²: As defined in ^(15; 16)

Table 3: Results of subgroup analyses for ferritin concentration ($\mu\text{g/L}$) in children with asymptomatic malaria parasitaemia and control group (mean difference and 95% confidence interval)

	Number of datasets	Mean Difference	95 % Confidence Interval	I^2 for heterogeneity	
Endemic profile					p subgroup
					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					p subgroup
					0.06
<i>P. falciparum</i>	21	26.6	14.2, 39	99%	
<i>P. vivax</i>	2	43.3	25.5, 61.1	66%	
Age					p subgroup
					0.12
<60 months	12	30.6	16.8, 44.3	99%	
>60 months	11	23.3	3.4, 43.2	99%	
Diagnostic method					p subgroup
					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					p subgroup
					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 to 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)										
<i>Children</i>										
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
<i>Adults</i>										
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
<i>Pregnant women</i>	8	26.8	5.8, 47.7	100%	0.02	7	18.1	5.6, 30.7	99%	0.01
Hepcidin (nmol/L)										
<i>Children</i>										
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
<i>Adults</i>										
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 ($\mu\text{mol/L}$)										
<i>Children</i>										
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
<i>Adults</i>										
Clinical malaria	5	-0.73	-1.11, - 0.36	54%	0.005	1	-1.03	-1.40, - 0.66	NA	<0.001
<i>Pregnant women</i>	1	-0.54	-0.67, - 0.41	NA	<0.00 1	1	-0.54	-0.67, - 0.41	NA	<0.001

CI, Confidence Interval, NA, non applicable.

Table 5: Results of subgroup analyses for ferritin ($\mu\text{g/L}$) in children with clinical malaria parasitaemia and control group (mean difference and 95% confidence interval)

	Number of datasets	Mean difference	95 % Confidence Interval	I² for heterogeneity	
Endemic profile					p
					subgroup
					<
					0.001
Moderate intensity	5	688	457, 919	94%	
Low intensity	3	138	89, 188	96%	
Age					p
					subgroup
					0.13
<60 months	4	637	233, 1042	99%	
>60 months	5	304	119, 490	99%	
Study design					p
					subgroup
					0.57
Cross-sectional	7	389	209, 570	99%	
Prospective	2	665	-290, 1620	99%	

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)

	Number of datasets	Mean Difference	95 % Confidence Interval	I ² for heterogeneity	
Endemic profile					p subgroup 0.88
Moderate intensity	6	1.86	0.62, 3.1	98%	
Low intensity	5	1.77	1.25, 2.29	68%	
Malaria species					p subgroup 0.12
<i>P. falciparum</i>	10	1.77	1.04, 2.5	97%	
<i>P. vivax</i>	2	2.56	2.46, 2.66	0%	
Age					p subgroup 0.54
<60months	6	1.95	0.74, 3.16	98%	
>60 months	6	1.66	1.21, 2.11	60%	
Design of the study					p subgroup 0.46
Cross-sectional	9	1.95	1.14, 2.76	98%	
Prospective	3	1.58	0.73, 2.42	71%	

Table 7: Results of subgroup analyses for ferritin concentration ($\mu\text{g/L}$) in pregnant women with malaria parasitaemia and control group (mean difference and 95% confidence interval)

	Number of datasets	Mean Difference	95 % Confidence Interval	I² for heterogeneity	
Endemic profile					p subgroup <0.001
High intensity	2	54.8	-258, 367	99%	
Moderate intensity	2	22.3	-93, 138	96%	
Low intensity	3	16.5	-7.6, 40.6	74%	

Table 8: Results of subgroup analyses for retinol concentration ($\mu\text{mol/L}$) in children with clinical malaria parasitaemia and control group (mean difference and 95% confidence interval)

	Number of datasets	Mean Difference	95 % Confidence Interval	I² for heterogeneity	
Endemic profile					p subgroup 0.55
High intensity	3	-0.34	-0.70, 0.03	80%	
Low intensity	3	-0.47	-0.68, -0.27	0%	
Age					p subgroup 0.9
<60 months	4	-0.43	-0.75, -0.1	74%	
>60 months	2	-0.40	-0.45, -0.35	0%	

Supplementary table 1: Results of the risk of bias assessment using the US National Institute of Health quality assessment tool

Author	Selection bias	Information bias	Measurement bias	Confounding
Barrfour ⁽¹⁰⁾	No	Unclear	No	No
Barffour ⁽³⁴⁾	No	Unclear	No	No
Beesley ⁽⁴⁵⁾	No	Unclear	No	No
Burte ⁽¹³⁾	Unclear	Unclear	No	No
Carmona ⁽³⁵⁾	No	Unclear	No	No
Castberg ⁽⁴⁶⁾	No	Unclear	No	Yes
Cercamondi ⁽⁴⁷⁾	Unclear	Unclear	No	No
Das ⁽³⁶⁾	Yes	Unclear	No	Yes
Davis ⁽³⁷⁾	No	Unclear	No	Yes
de Mast ⁽⁷⁾	No	Unclear	No	No
de Mast ⁽⁴⁸⁾	No	Unclear	No	No
Diallo ⁽²⁶⁾	yes	Unclear	No	Yes
Diatta ⁽³⁸⁾	No	Unclear	No	Yes
Dreyfuss ⁽²⁷⁾	No	Unclear	No	No
Filteau ⁽¹¹⁾	No	Unclear	No	No
Glinz ⁽⁴⁹⁾	No	Unclear	No	No
Inocent ⁽³⁹⁾	No	Unclear	No	No
Jeremiah ⁽⁵⁰⁾	No	Unclear	No	No
Kabore ⁽⁵¹⁾	No	Unclear	No	No
Kivibidila ⁽⁵²⁾	yes	Unclear	No	Yes
Mfonkeu ⁽⁴⁰⁾	No	Unclear	No	No
Mockenhaupt ⁽²⁸⁾	No	Unclear	No	No
Muriuki ⁽⁵³⁾	Unclear	Unclear	No	Unclear
Mwangi ⁽²⁹⁾	No	Unclear	No	No
Nussenblatt ⁽⁴¹⁾	No	Unclear	No	Yes
O'donnell ⁽⁵⁴⁾	No	Unclear	No	No
Odunukwe ⁽⁵⁵⁾	Yes	Unclear	No	No

Philips ⁽⁵⁶⁾	Yes	Unclear	Unclear	Yes
Raza ⁽⁴²⁾	Yes	Unclear	No	Yes
Righetti ⁽⁵⁷⁾	No	Unclear	No	No
Saad ⁽³⁰⁾	No	Unclear	No	No
Seyrek ⁽⁵⁸⁾	Yes	Unclear	No	Yes
Shulman ⁽³¹⁾	No	Unclear	No	No
Stoltzfus ⁽⁵⁹⁾	No	Unclear	No	No
Stoltzfus ⁽⁶⁰⁾	No	Unclear	No	No
Stuetz ⁽³²⁾	No	Unclear	No	No
Tabone ⁽⁴³⁾	No	Unclear	No	No
Thurnhan ⁽⁴⁴⁾	Yes	Unclear	No	No
Uscategui ⁽⁶¹⁾	No	Unclear	No	No
Verhoef ⁽⁶²⁾	No	Unclear	No	No
Wessells ⁽⁶³⁾	No	Unclear	No	No
Wessells ⁽³³⁾	No	Unclear	No	No
Williams ⁽⁶⁴⁾	No	Unclear	No	No

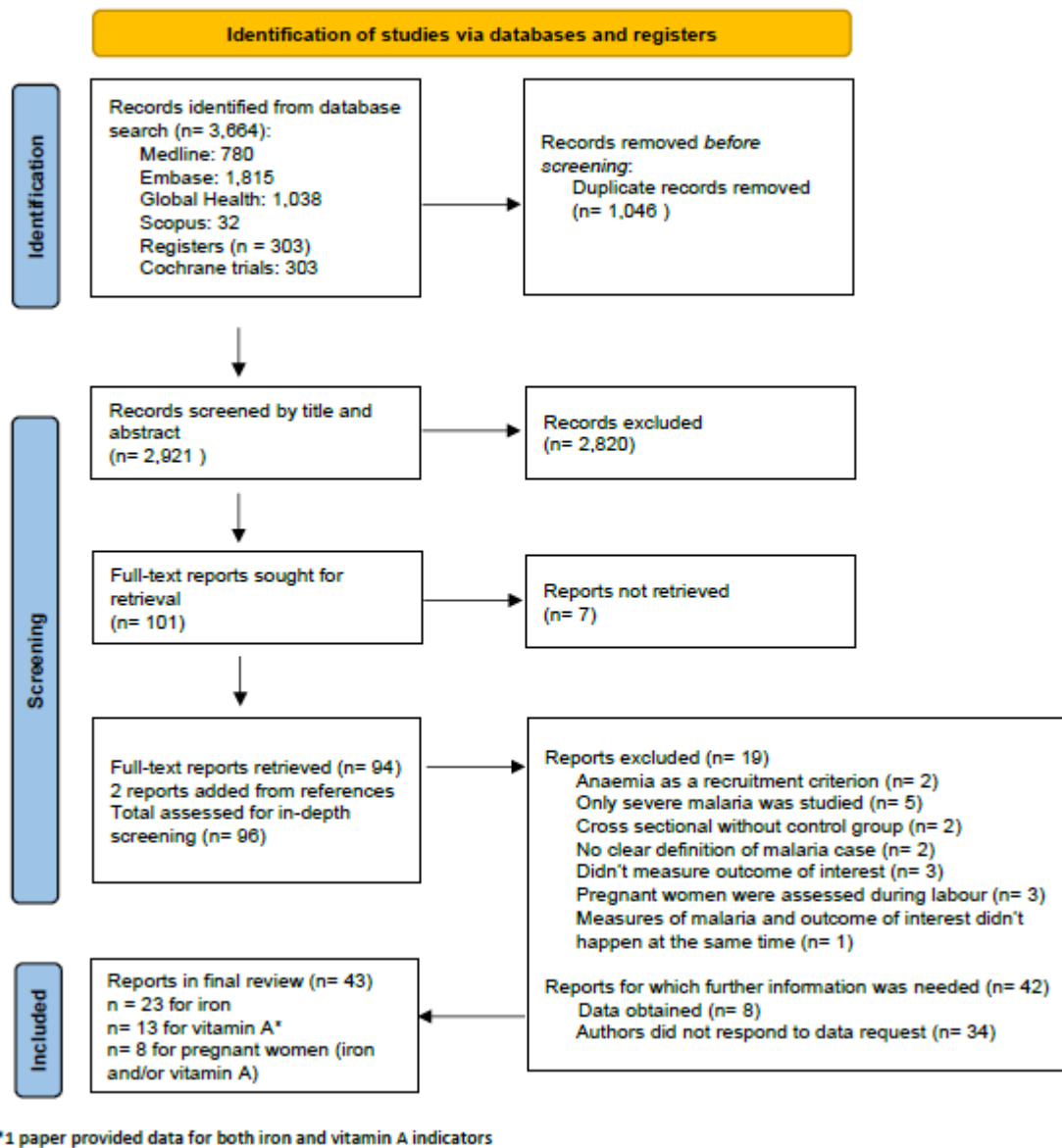


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flow diagram of publications screened in a systematic review of the impact of malaria infection on indicators of iron and vitamin A status.

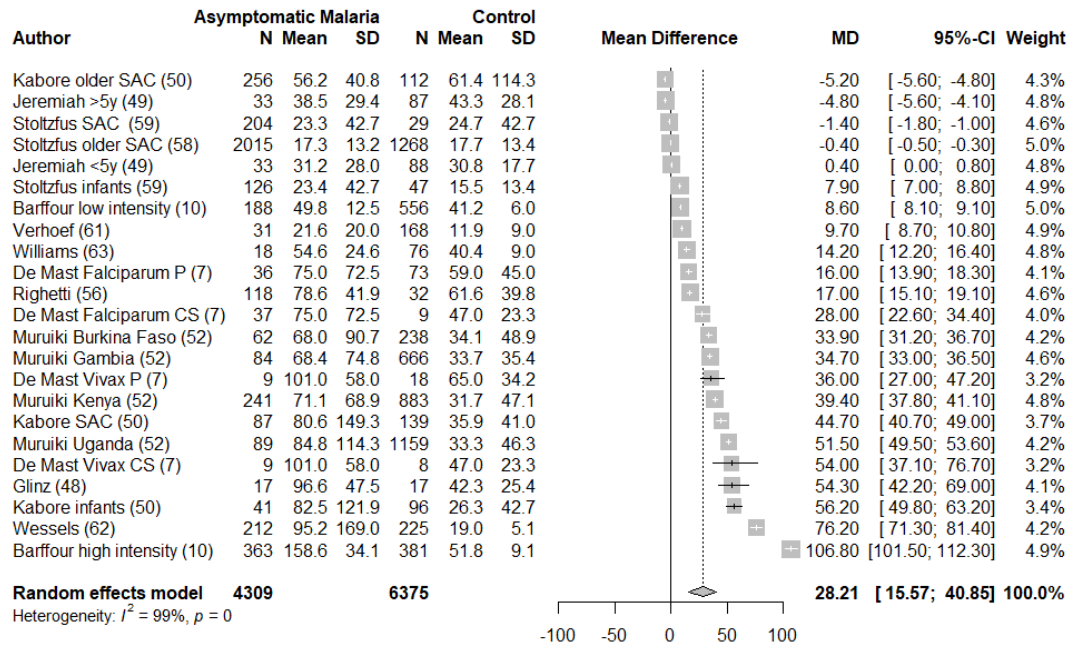


Figure 2: Forest plot of differences in ferritin concentrations ($\mu\text{g/L}$) between children with asymptomatic malaria and control group, using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. 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; SD, Standard Deviation; MD, mean difference; CI, Confidence Interval

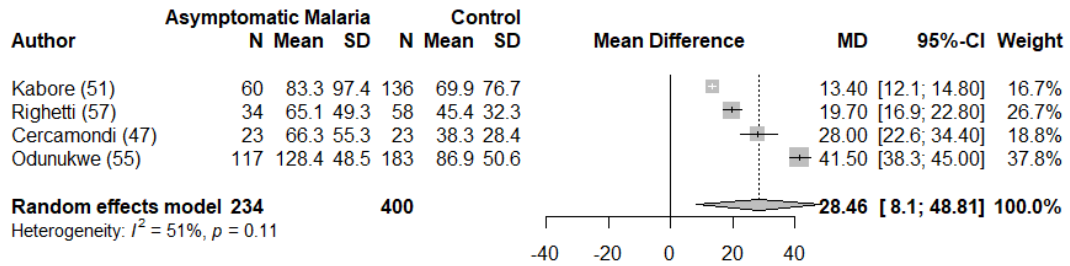


Figure 3: Forest plot for differences in ferritin concentrations ($\mu\text{g/L}$) between adults with asymptomatic malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. *SD*, standard deviation; *MD*, mean difference; CI, Confidence Interval

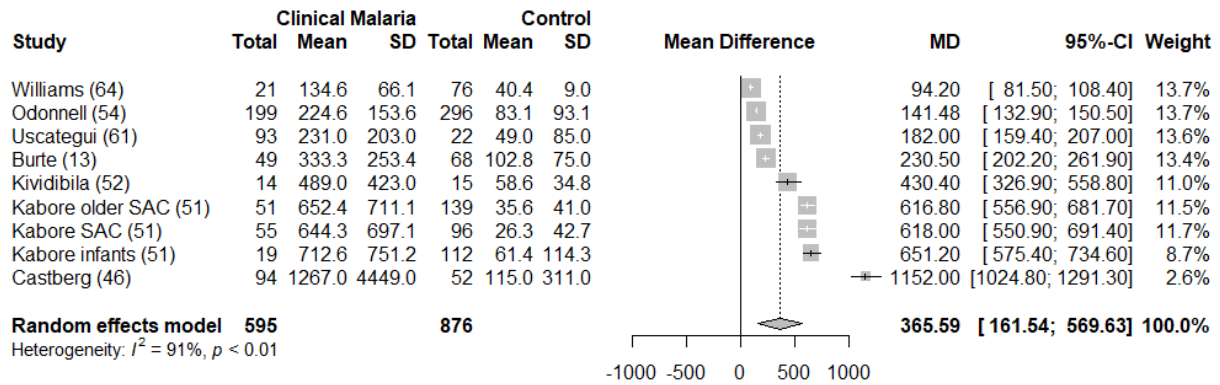


Figure 4: Forest plot for differences in ferritin concentrations ($\mu\text{g/L}$) in children between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. SAC, school-age children; *SD*, standard deviation; *MD*, mean difference; CI, Confidence Interval

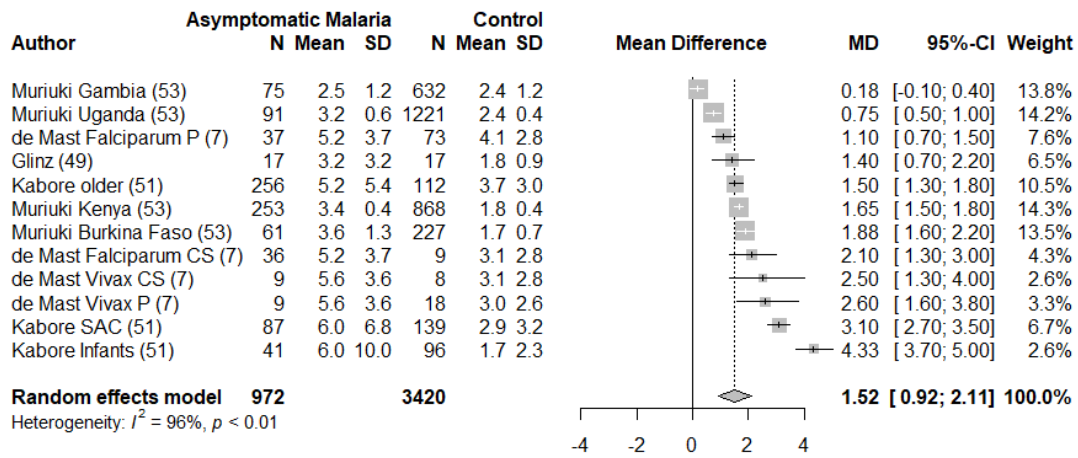


Figure 5: Forest plot for differences in hepcidin concentration (nmol/L) in children between malaria parasitaemia and control groups using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow 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; *SD*, standard deviation; *MD*, mean difference; *CI*, Confidence Interval

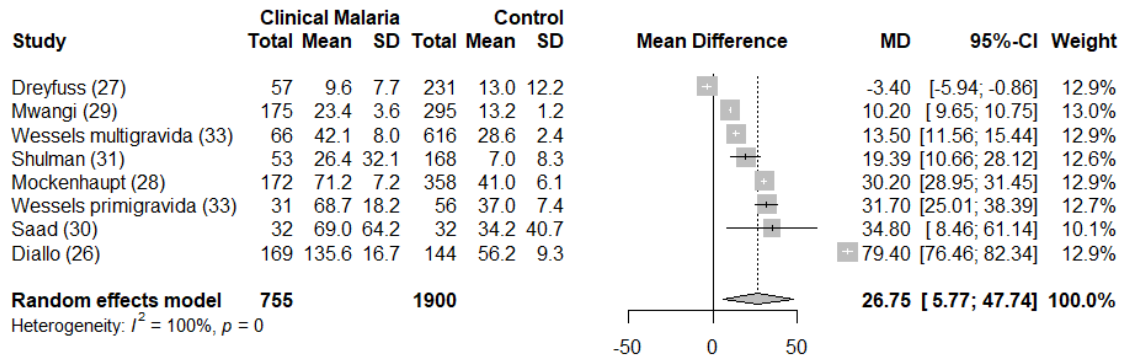


Figure 6: Forest plot for differences in ferritin concentrations ($\mu\text{g/L}$) in pregnant women between malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. *SD*, standard deviation; *MD*, mean difference; CI, Confidence Interval

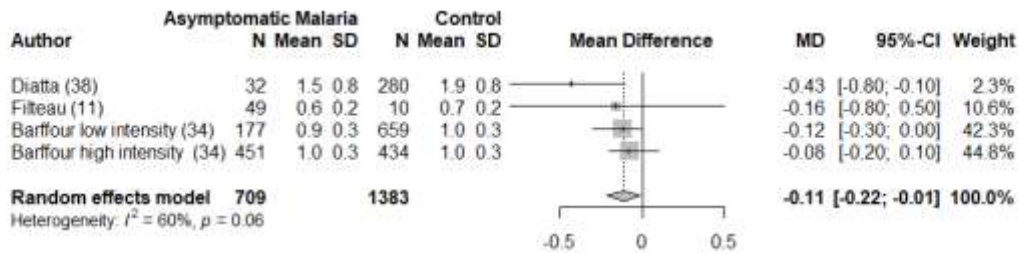


Figure 7: Forest plot for differences in retinol concentration ($\mu\text{mol/L}$) in children between asymptomatic malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. *SD*, standard deviation; *MD*, mean difference; *CI*, Confidence Interval

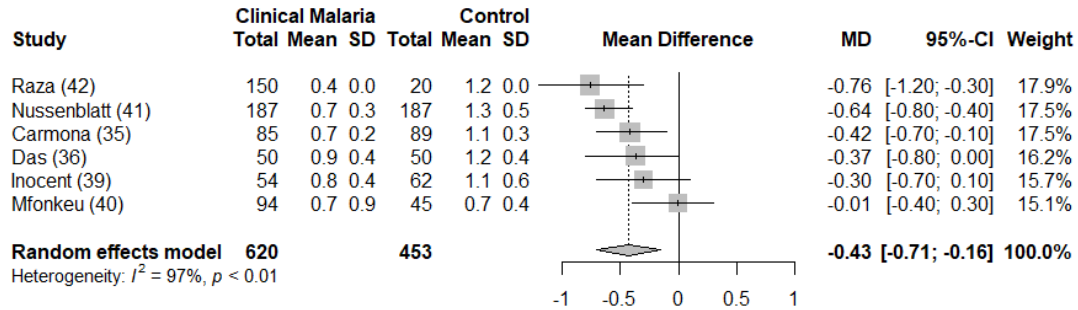


Figure 8: Forest plot for differences in retinol concentration ($\mu\text{mol/L}$) in children between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. *SD*, standard deviation; *MD*, mean difference; *CI*, Confidence Interval

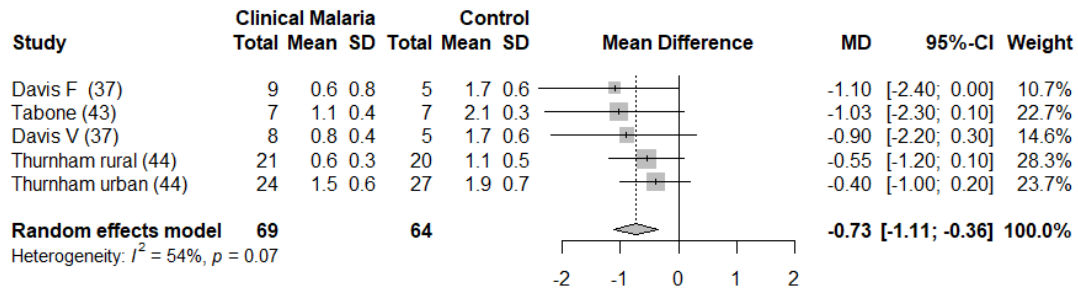
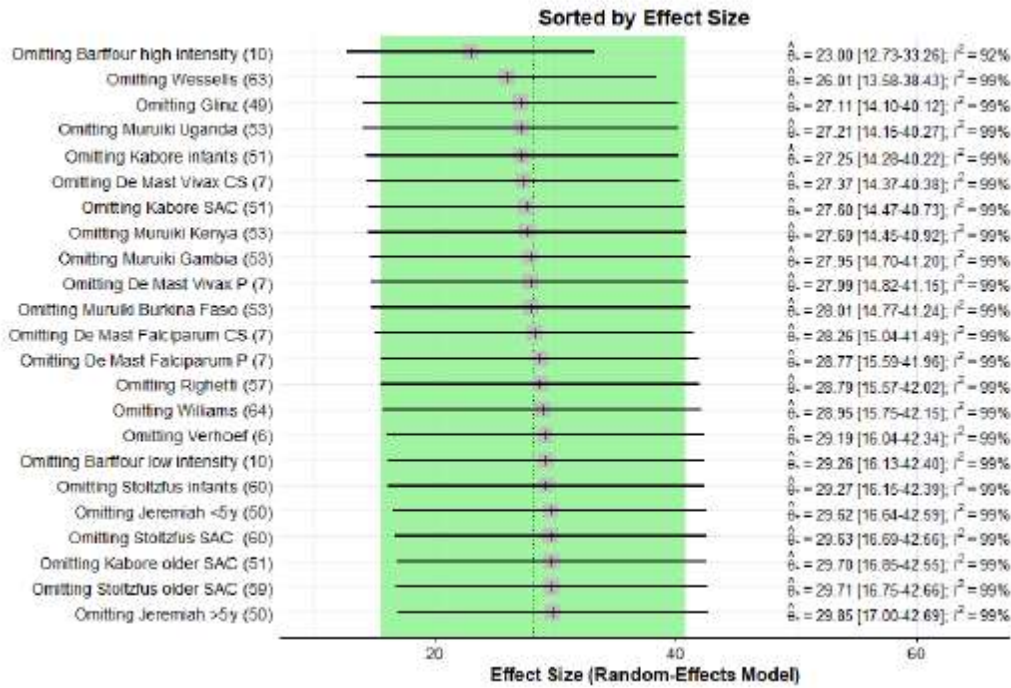
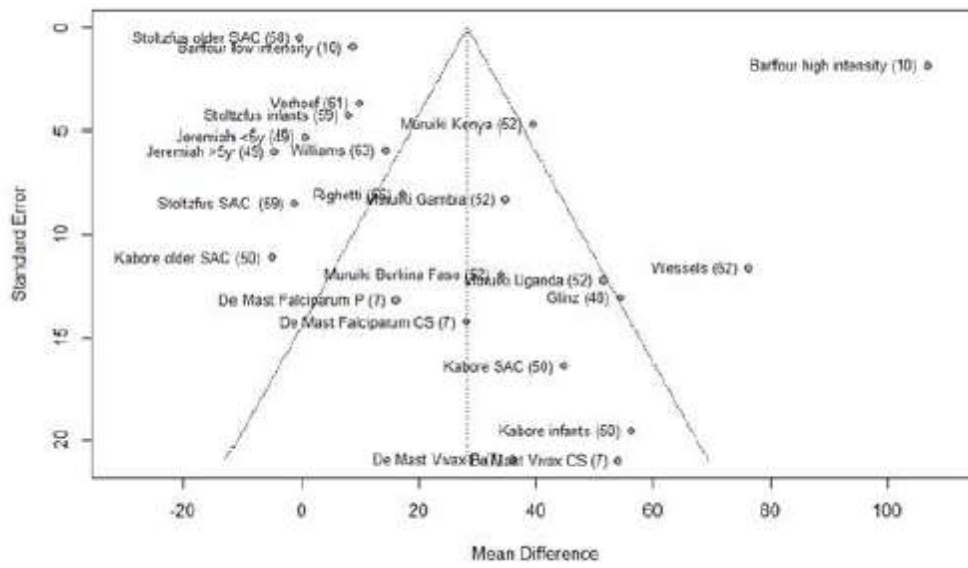


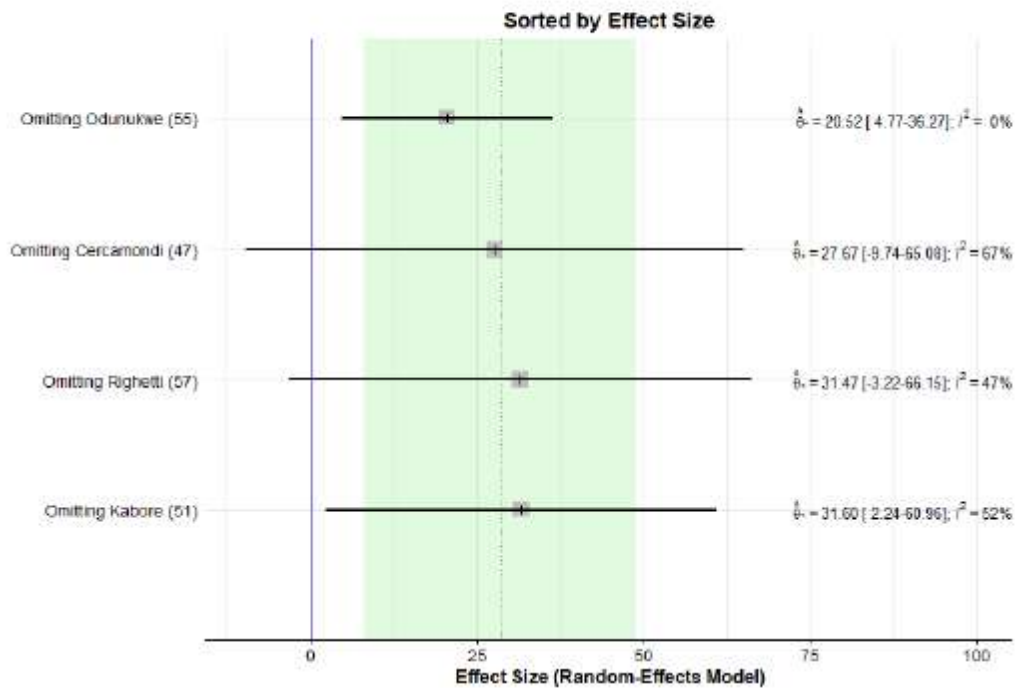
Figure 9: Forest plot for differences in retinol concentration ($\mu\text{mol/L}$) in adults between clinical malaria and control group using the random effect model. The grey squares represent the mean difference from each study, while the horizontal line represents the corresponding 95% CI. The hollow diamond represents the overall pooled effects while the left and right points of the diamond represent the corresponding 95% CI. F, Falciparum; V, Vivax; *SD*, standard deviation; *MD*, mean difference; *CI*, Confidence Interval



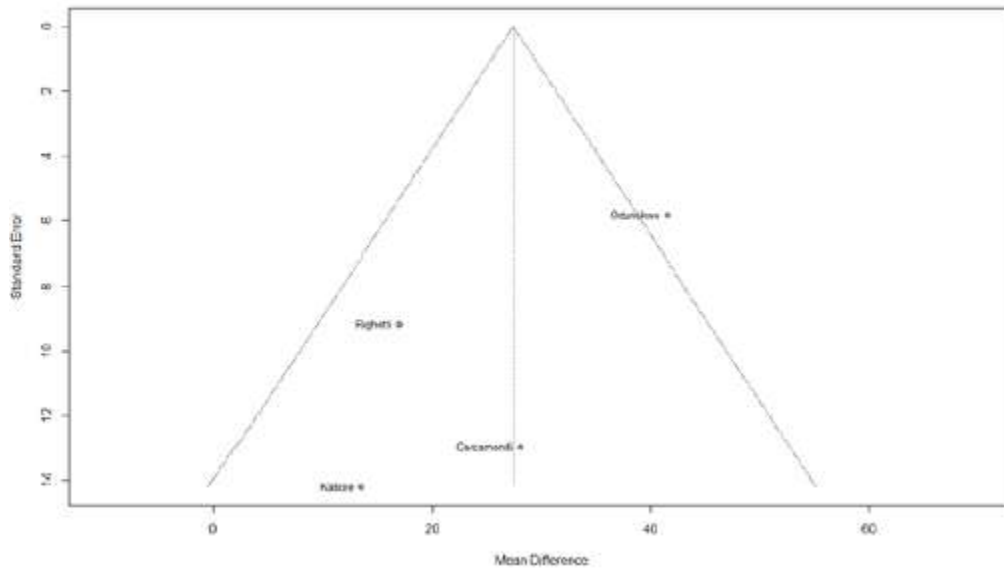
Supplementary figure 1: Sensitivity analysis of ferritin concentration ($\mu\text{g/L}$) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean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



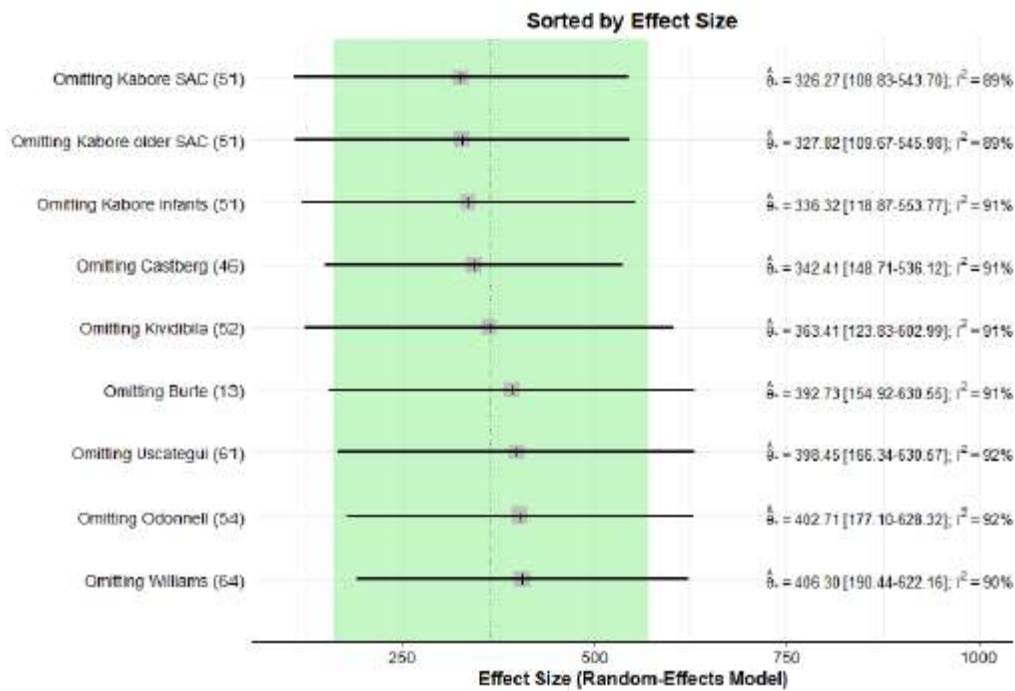
Supplementary figure 2: Funnel plot showing publications related to asymptomatic malaria and ferritin concentration ($\mu\text{g/L}$) in children. CS, cross-sectional; SAC, school-age children; P, prospective



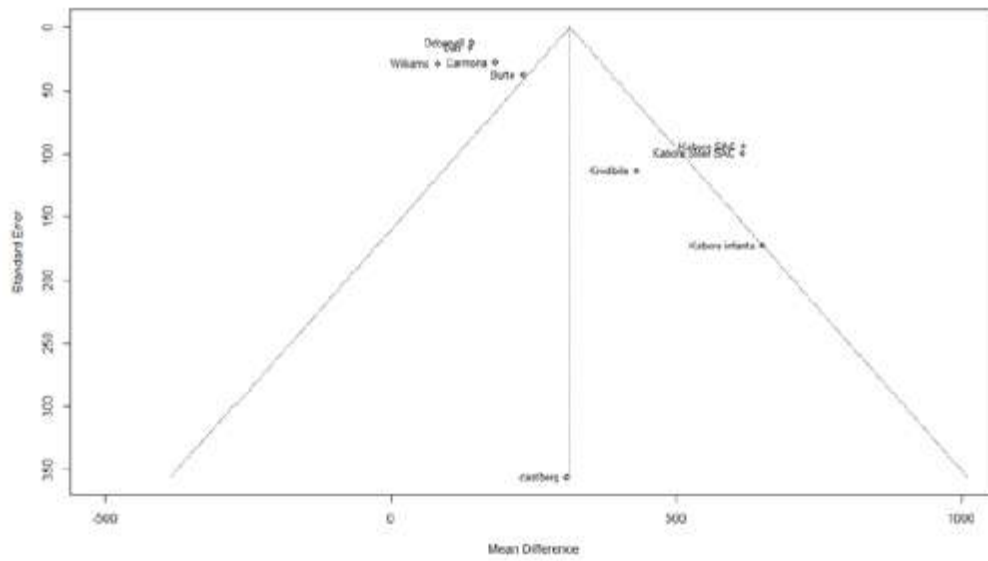
Supplementary figure 3: Sensitivity analysis of ferritin concentration ($\mu\text{g/L}$) between asymptomatic malaria and control in adults, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean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



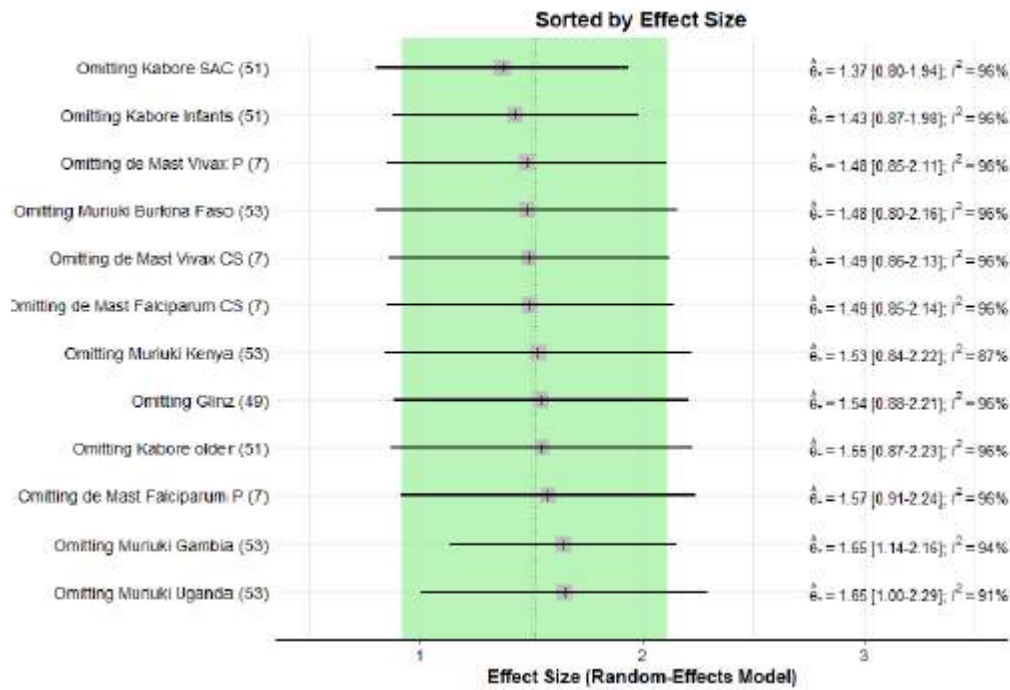
Supplementary figure 4: Funnel plot showing publications related to asymptomatic malaria and ferritin concentration ($\mu\text{g/L}$) in adults



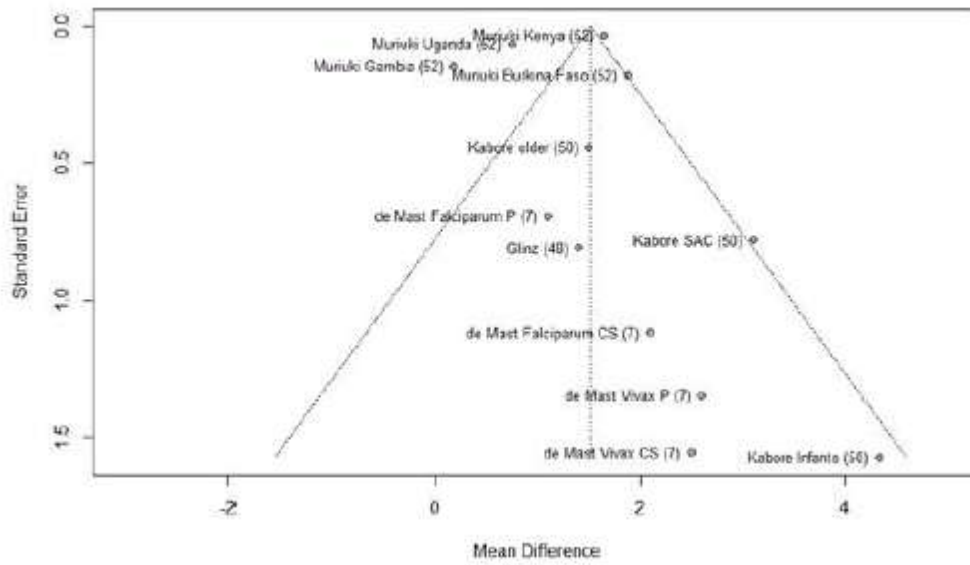
Supplementary figure 5: Sensitivity analysis of ferritin concentration ($\mu\text{g/L}$) between clinical malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean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



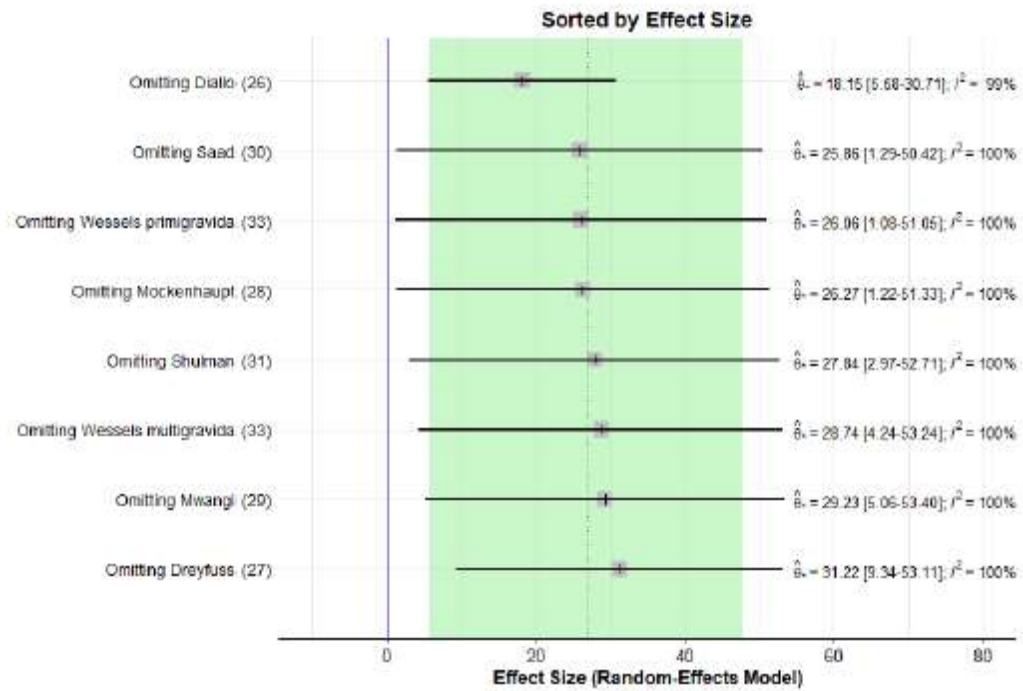
Supplementary figure 6: Funnel plot showing publications related to clinical malaria and ferritin concentration ($\mu\text{g/L}$) in children. SAC, school-age children



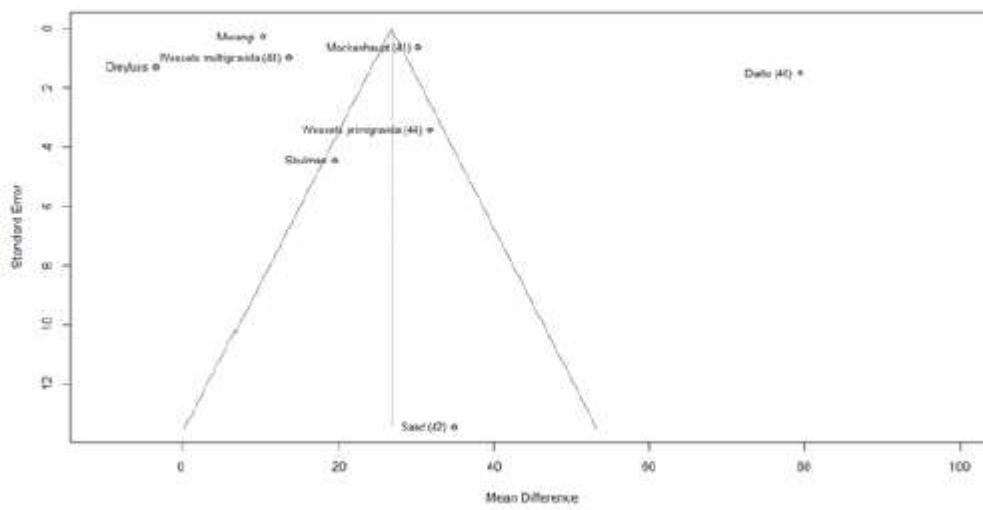
Supplementary figure 7: Sensitivity analysis of hepcidin concentration (nmol/L) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference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



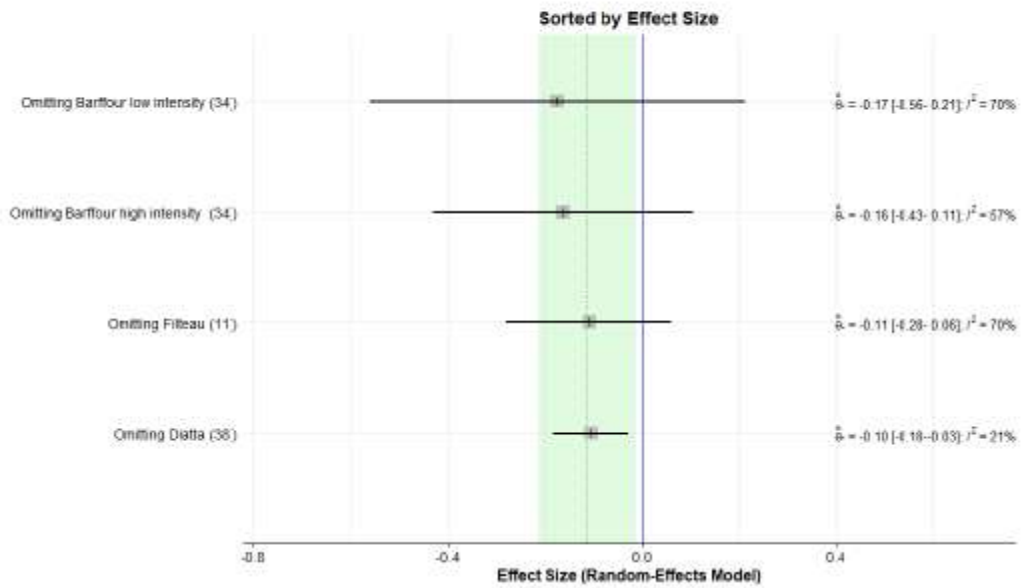
Supplementary figure 8: Funnel plot showing publications related to asymptomatic malaria and hepcidin concentration (nmol/L) in children. SAC, school-age children; P, prospective; CS, cross-sectional.



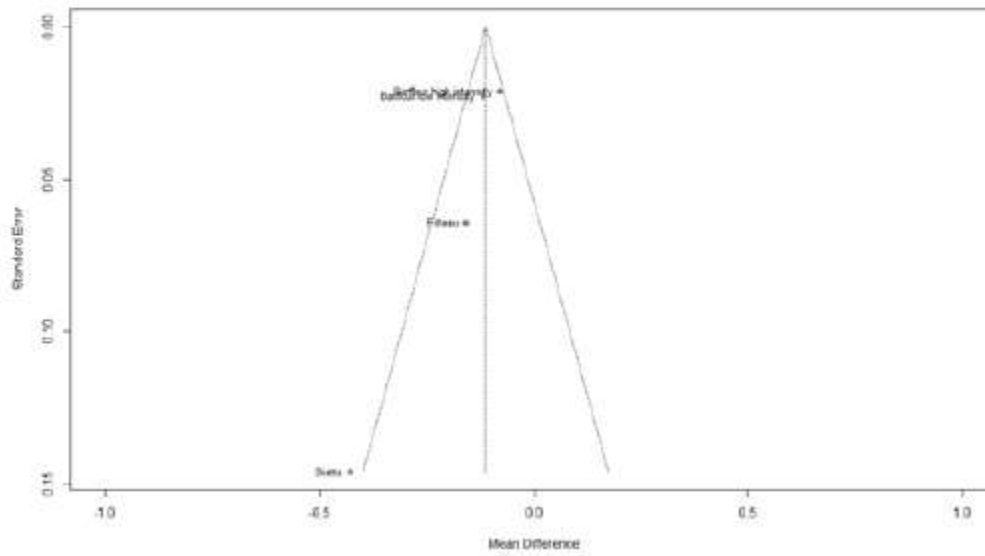
Supplementary figure 9: Sensitivity analysis of ferritin concentration ($\mu\text{g/L}$) between malaria and control in pregnant women, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled mean difference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



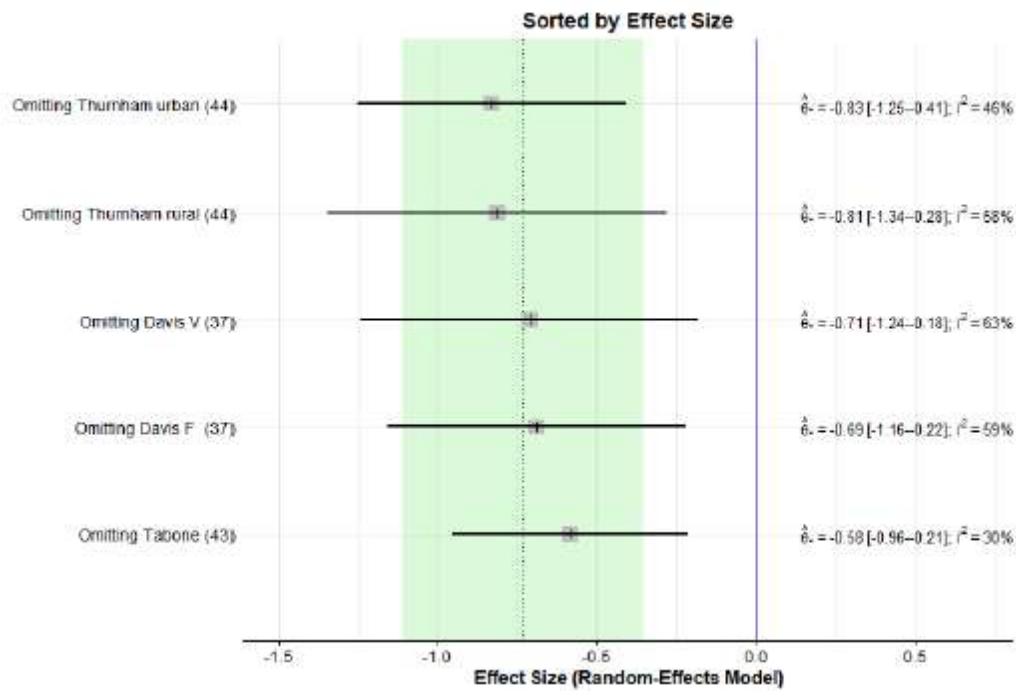
Supplementary figure 10: Funnel plot showing publications related to malaria and ferritin concentration ($\mu\text{g/L}$) in pregnant women



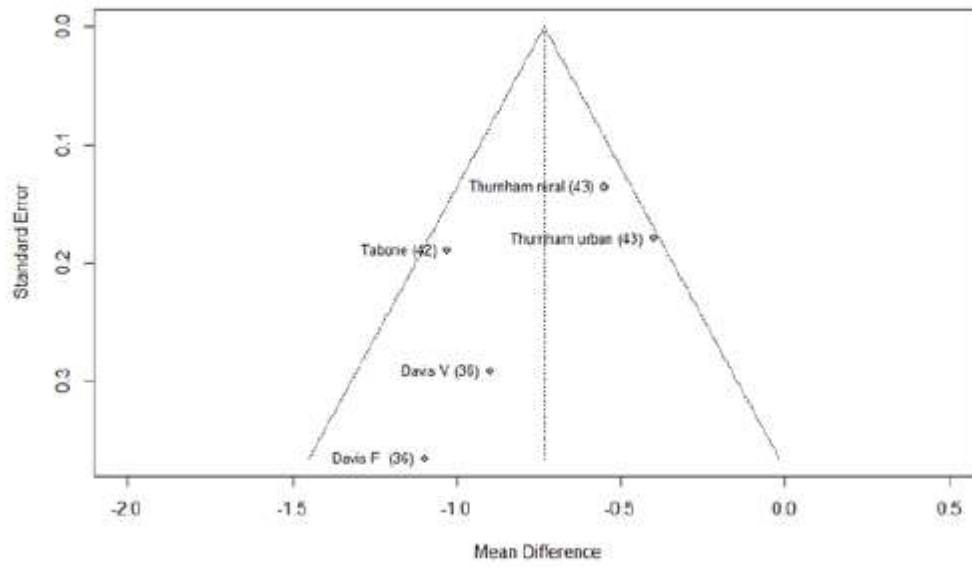
Supplementary figure 11: Sensitivity analysis of retinol concentration ($\mu\text{mol/L}$) between asymptomatic malaria and control in children, using leaving-one out analysis. Recalculated pooled effects, with one study omitted each time. The dashed line and shaded area represent the original pooled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



Supplementary figure 12: Funnel plot showing publications related to asymptomatic malaria and retinol concentration ($\mu\text{mol/L}$) in children



Supplementary figure 13: Sensitivity analysis of retinol concentration ($\mu\text{mol/L}$) 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 ($\mu\text{mol/L}$) in adults. V, Vivax; F, Falciparum