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Asymptomatic *Plasmodium* infection and glycemic control in adults: Results from a population-based survey in south-central Côte d'Ivoire

Ikenna C. Eze[a,b,*], *Clémence Essé*[c,d], *Fidèle K. Bassa*[e], *Siaka Koné*[a,b,c], *Félix Acka*[f], *Christian Schindler*[a,b], *Medea Imboden*[a,b], *Véronique Laubhouet-Koffi*[g], *Dinard Kouassi*[f], *Eliézer K. N'Goran*[c,e], *Jürg Utzinger*[a,b], *Bassirou Bonfoh*[a,b,c], *Nicole Probst-Hensch*[a,b]

[a] *Swiss Tropical and Public Health Institute, Basel, Switzerland*
[b] *University of Basel, Basel, Switzerland*
[c] *Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Côte d'Ivoire*
[d] *Institut d'Ethnologie, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire*
[e] *Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire*
[f] *Institut National de Santé Publique, Abidjan, Côte d'Ivoire*
[g] *Ligue Ivoirienne contre l'Hypertension Artérielle et les Maladies Cardiovasculaires, Abidjan, Côte d'Ivoire*

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ABSTRACT

AIMS: We investigated the cross-sectional associations of *Plasmodium* infection (PI) with fasting glucose (FG) and glycated hemoglobin (HbA1c) in malaria-endemic south-central Côte d'Ivoire.

Methods: We studied 979 participants (non-pregnant; no treated diabetes; 51% males; 18–87 years) of the Côte d'Ivoire Dual Burden of Disease study. Fasting venous blood was obtained for PI, FG, and HbA1c assessment. We defined PI as a positive malaria rapid diagnostic test (RDT) or microscopic identification of *Plasmodium* species. We applied multivariable linear regressions to assess beta coefficients (β) and 95% confidence intervals (CIs) of PI positivity for FG and HbA1c independent of diabetes risk factors.

Results: Prevalence of PI was 10.1% (5.5% microscopy; 9.7% RDT) without clinical fever. Prevalence of FG-based prediabetes (45.8%) and diabetes (3.6%) were considerably higher than HbA1c-based values (2.7% and 0.7%, respectively). PI was independently associated with FG among participants with higher body temperature (β 0.34, 95% CI 0.06–0.63, $p_{heterogeneity} = 0.028$), or family history of diabetes (β 0.88, 95% CI 0.28–1.47, $p_{heterogeneity} = 0.009$). Similar patterns observed with HbA1c were obliterated on accounting for FG. We also observed consistent associations with parasite density.

Conclusions: FG-based diabetes diagnosis in the presence of asymptomatic PI may misclassify or overestimate diabetes burden in malaria-endemic settings. Longitudinal studies are needed to confirm these findings and determine the risk for diabetes.

* Corresponding author at: Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland.
E-mail address: ikenna.eze@swisstph.ch (I.C. Eze).

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1. Introduction

The rising rates of non-communicable diseases (NCDs) in a background of prevalent infectious diseases, and in the context of weak health systems in low- and middle-income countries (LMICs) have sparked interest on the potential role of common infections as risk factors for NCDs [1–4]. LMICs now have the greatest burden of mortality for diabetes worldwide, in part because of delayed diagnosis [5]. Diabetes is of particular interest regarding a potential link to infections because of its association with altered immunity and inflammation.

Inflammatory responses to repeated infections with malaria parasites (*Plasmodium* species) may lead to sustained physiological changes that increase diabetes susceptibility [6]. *Plasmodium* infection (PI) induces systemic inflammation via the release of pyrogenic cytokines, including interleukin IL-1β [7] upon activation of inflammatory pathways such as NLRP3 inflammasome [8], which are also characteristic of type 2 diabetes [9]. Pro-inflammatory blood markers such as circulating C-reactive protein, IL-1β, IL-6, IL-8, and IL-10 were associated with both malaria severity [10] and insulin resistance [11]. IL-6 was higher in adults from a malaria-endemic setting than in their age-adjusted western counterparts [6]. *In utero* malaria is thought to direct fetal physiological pathways toward insulin resistance [12] and impaired fasting glucose (FG) in adolescence [13].

On the other hand, there are aspects that complicate the investigation of repeated malaria infection as a risk factor for diabetes. First, the stress-related glycemic responses to acute malaria infections may complicate its interpretation as an indicator for underlying diabetes in epidemiologic, screening, and diagnostic contexts in malaria-endemic settings. We have previously produced evidence on the need to postpone diagnosing underlying diabetes in tuberculosis patients until the end of treatment [14]. Fever, a cardinal symptom of malaria, like tuberculosis, induces stress-related hyperglycemia [14,15]. Second, diabetes patients are more prone to developing infections in general [16]. A higher prevalence of *P. falciparum* infection was found in participants with diabetes compared to those without diabetes [17], and participants with diabetes or obesity were reported to be at higher risk for severe malaria [18].

There is a general lack of population-based, even cross-sectional epidemiologic studies that investigate the association of PI, which is more prevalent in the asymptomatic form, with parameters of glycemic control or diabetes [3]. Population-based evidence on this link is therefore warranted for a deeper understanding of the diabetogenic effect of asymptomatic and potentially chronic carriage of PI. We investigated the association between PI and markers of glycemic control, including FG and glycated hemoglobin (HbA1c) in a population-based, malaria-endemic setting of south-central Côte d'Ivoire [1,19,20]. We considered effect modification by hypothesized susceptibility factors to better differentiate the acute vs. longer-term associations of PI with glycemic control.

2. Materials and methods

2.1. Study population

This study was carried out in 2017 in the frame of the Côte d'Ivoire Dual Burden of Disease (CoDuBu) study, which recruited 1019 adults selected at random, to investigate the concomitance of common infections and NCDs, and their molecular determinants [1,4] The CoDuBu study was done within the Taabo health and demographic surveillance system (HDSS) [19], which is equivalent to a civil registration system that allows random sampling from the population. The detailed study protocol, including sampling procedure, is described elsewhere [1]. In brief, we estimated that we would need at least 976 participants given an expected malaria-diabetes cooccurrence rate (assuming independence) of 2%, an error margin of 1%, and a non-response rate of 30%. Toward reaching this sample size, we first selected three out of 14 HDSS main villages and town (Amani-Ménou, Taabo-Cité, and Tokohiri), which would facilitate study execution and reflect urban-rural gradient. Based on the sampling frame, which included those meeting the inclusion criteria in each of three strata, we invited the participants in each stratum to the study in a random fashion until the desired sample size (reflecting the actual distribution in the three strata) was reached. At the end of the study, we recruited 252, 513, and 254 from Amani-Ménou, Taabo-Cité, and Tokohiri, respectively [1].

Participants had several tests (including FG, HbA1c, malaria rapid diagnostic test (RDT), and microscopy using EDTA-buffered venous blood). Blood, urine, and stool samples were aliquoted into a −80 °C biobank. Participants also responded to an interviewer-administered questionnaire covering their health, socioeconomic status, and lifestyle.

Ethics approvals for the CoDuBu study were obtained from the Ethics Committee of North-West and Central Switzerland (reference no. 2016-00143; May 2, 2016) and the National Ethics Committee for Research in Health of Côte d'Ivoire (reference no. 032/IMSHP/CNER-kp; March 24, 2017). Participants provided written informed consent before participating in the study. Inclusion criteria for the present analyses were (i) not being pregnant; (ii) not taking diabetes medication; and (iii) having complete data on all relevant covariates.

2.2. Assessment of Plasmodium infection

We used a combination of RDT and microscopy on EDTA-buffered fasting venous blood sample to identify cases of PI. RDT was done using the ICT malaria dual Pf/Pan antigen test kit (ICT Diagnostics; Cape Town, South Africa). This RDT is an *in vitro* immunochromatographic assay containing two monoclonal antibodies (*P. falciparum*-specific anti-histidine-rich protein (HRP) II and general *Plasmodium* anti-parasite lactate dehydrogenase antibodies) bound on a nitrocellulose test strip. These antibodies bind to *Plasmodium* antigens released on red blood cell lysis, forming a complex that signifies a positive test [21]. All tests were done according to standard protocol, and were considered valid if they also had the procedural control line. Microscopy was done by experienced laboratory

technicians using thick and thin blood films, which were air-dried, Giemsa-stained, and read under a light microscope at high magnification to identify *Plasmodium* parasites. Parasitized blood cells were counted against 200 leukocytes (or 500 leukocytes if parasite count <10), assuming a standard count of 8000 leukocytes per μl of blood [22]. Ten percent of the microscope slides, selected at random, were re-read by the senior technician for quality control, and revealed an inter-rater agreement of 100%.

We identified participants as having PI if they had a positive RDT or identification of *Plasmodium* spp. on microscopy. Since RDT can still detect HRP-II antigens a few weeks following parasite clearance [23], we also defined microscopic PI (limited to positive microscopy), which represents more recent and active PI.

2.3. Assessment of glycemic control parameters

We assessed FG (mmol/l) in EDTA-buffered venous blood sample using Hemocue 201+ RT and corresponding microcuvette (Hemocue; Ängelholm, Sweden) which applies an enzymatic reaction method and photometric quantification of glucose, and meets the International Federation of Clinical Chemistry standards. We assessed HbA1c (%) in EDTA-buffered venous blood using Alere Afinion AS100 analyser and HbA1c cartridge (Alere Inc.; Waltham, MA, USA) based on boronate affinity separation and the use of fluorescence quenching. This method meets the performance criteria for HbA1c, according to the "U.S. National Glycohemoglobin Standardization Program", with minimal interference from alternate hemoglobin (Hb) variants or derivatives and good performance in the tropics [24]. All devices were regularly controlled, microcuvettes/-cartridges were from same respective batch, and tests were performed according to standard protocols. Using a combination of FG, HbA1c, and questionnaire data, we defined prediabetes as FG 5.6–6.9 mmol/l or HbA1c 5.7–6.4% (39–46 mmol/mol) without clinician-diagnosed diabetes, and untreated diabetes as FG $\geq$ 7.0 mmol/l or HbA1c $\geq$ 6.5% (48 mmol/mol) or clinician-diagnosed diabetes, without use of diabetes medication [25].

2.4. Assessment of other covariates

We considered the following covariates measured by questionnaire: age (years), sex (male/female), formal education (none/primary/secondary/tertiary), wealth index computed using principal component analysis of household property items [19], smoking status (never/former/current), frequency of fruit intake (including seasonal and perennial fruits), and vegetable intake (including seasonal and perennial vegetables) in raw, cooked, dried, juice, or any other form (never, once per month, once per week, several days per week). We also considered study area, general susceptibility to illness (self-reported frequency of falling sick in comparison to people of same sex and age group in the community; lower or same frequency/higher frequency), family history of diabetes (FHD; yes/no), and sedentary lifestyle (hours per week of being in a sitting or lying position excluding sleeping hours).

We measured Hb in EDTA-buffered venous blood using Hemocue 301 System (Hemocue; Ängelholm, Sweden) and body temperature (three measurements, 1 min apart, highest reading recorded) using Omron auricular thermometer (Omron Healthcare; Kyoto, Japan). We also measured body weight (kg), height (cm), and waist circumference (WC; cm) using Omron weighing scale, body meter, and tape (SECA GmbH; Hamburg, Germany), respectively, and derived body mass index (BMI; kg/m^2).

2.5. Statistical analysis

We described categorical characteristics of study participants as counts and proportions, described continuous variables as means and standard deviations (SD), and compared these characteristics by FG levels. For the association between PI as independent binary variable and glycemic control parameters as endpoints, we performed linear regression models using the continuous outcome variables FG and HbA1c in order to differentiate the acute (stress-related glycemia) from the longer-term (related more to diabetes development) glycemic effect of PI. All regression models were adjusted incrementally, for potential confounders including age, sex, educational attainment, wealth index, and area, FHD, smoking status, fruit and vegetable intake, sedentary behavior, BMI, WC, and Hb. We assessed the stability of our models by (i) limiting PI cases to microscopic PI; (ii) excluding participants with potential clinical malaria defined as having PI and either body temperature $\geq$37.5 °C or anemia (Hb <120 mg/l in females and <130 mg/l in males); (iii) mutually adjusting the FG model for HbA1c and the HbA1c model for FG; and (iv) testing dose-response relationship of parasite density with FG and HbA1c, expressed as change in respective outcome per unit increase in parasite density (1 unit = 1000 parasites/μl of blood), among the microscopic PI cases.

We performed stratified analyses based on *a priori*-selected potential modifiers including susceptibility to illness, body temperature (cut-off at the median value, 36.5 °C), FHD alone, and FHD combined with having untreated diabetes (i.e., having either FHD or untreated diabetes vs. having neither). We tested subgroup differences by adding an interaction term between PI and the corresponding potential modifier to the model. The p-value of the interaction term, expressing the statistical significance of the effect modification will be denoted by $p_{heterogeneity}$. We also limited these effect modification analyses to microscopic PI, and tested the stability of results to additional adjustments for HbA1c in the FG model and for FG in the HbA1c model.

We performed all analyses with Stata version 14 (Stata Corporation; College Station, TX, USA) and considered the main associations and interaction terms to be statistically significant at alpha-levels of 0.05 and 0.1, respectively. Results are presented as beta coefficients (β) of PI positivity for FG and HbA1c, and their 95% confidence intervals (CIs).

3. Results

3.1. Summary of participants' characteristics

For the present analyses, we excluded 20 participants with treated clinician-diagnosed diabetes (to limit confounding of

effect estimates by medication use). There were 31 (3%) cases of clinician-diagnosed diabetes, 11 (35%) of which were untreated and hence, were eligible for inclusion. We also excluded 20 pregnant women, one of whom had untreated clinician-diagnosed diabetes. This study therefore included 979 (96%) CoDuBu participants, with a male-to-female ratio of 1.00:0.95. Mean (SD) age was 42 (13) years and 52% of participants were older than 40 years. Half of the participants lived in more rural areas, and 45% had no formal education. There was a low prevalence of smoking, and fruit and vegetable intake. Mean (SD) BMI and WC were 23 (4) kg/m^2 and 80 (11) cm respectively, and participants usually spent 33 h per week on average being sedentary. Compared to participants without elevated FG, those with elevated FG were more likely to smoke, to have lower socioeconomic status, lower fruit and vegetable intake, and higher BMI (Table 1).

Mean (SD) FG and HbA1c were 5.6 (1.0) mmol/l and 5.0 (0.6) % (31 (7) mmol/mol), respectively (Table 1). Spearman correlation (R) between FG and HbA1c was 0.03 (p = 0.32). Prevalence of prediabetes and diabetes (based on the composite definition) was 46.7% and 4.3%, respectively. Prevalence of prediabetes (45.8%) and diabetes (3.6%) based on FG was considerably higher than the prevalence of prediabetes (2.7%) and diabetes (0.7%) based on HbA1c (Table 1).

Ninety-nine participants (10%) had PI (RDT or microscopy) and 55% of these were microscopic PI. Participants with elevated FG had higher rates of PI than those with normal FG (11.8% vs. 8.6%; Table 1). PI cases were mostly due to *P. falciparum* alone, with only 11% being mixed infections. Median (IQR) parasite density among participants with microscopic PI was 192 (3 2 0) parasites/μl of blood. Mean (SD) and median (IQR) body temperature were 36.5 (0.5) °C. There was no case of clinical fever (range: 34.6–37.9 °C) but two (2%) PI-positive participants also had body temperature ≥37.5 °C. Mean (SD) Hb was 134 (16) g/l with 26 (26%) PI-positive participants having anemia. Among the 99 PI-positive participants, 27 (27%) had potential clinical malaria, having either anemia or body temperature ≥37.5 °C.

3.2. *Association between PI and glycemic control*

We observed statistically non-significant overall associations between PI and glycemic control in the fully adjusted models accounting for diabetes risk factors and Hb level. Associations of PI with FG were generally positive and stable to adjustments for potential confounders. Unadjusted estimate of difference in mean FG between PI-positives and negatives was 0.15 mmol/l (95% CI −0.06 to 0.36 mmol/l), whereas the difference in mean FG in the fully adjusted model was 0.13 mmol/l (95% CI −0.08 to 0.33 mmol/l). The adjusted estimate of difference in mean FG remained stable to restricting PI to microscopic PI, exclusion of participants with potential clinical malaria and additional adjustment for HbA1c levels (Table 2). We also observed a positive association between parasite density and FG among participants with microscopic PI. Adjusted estimate of mean FG increased by 0.09 mmol/l (95% CI −0.14 to 0.31 mmol/l) per unit increase in parasite density, being stronger and reaching borderline statistical significance on additional adjustment for HbA1c (β 0.13 mmol/l, 95% CI −0.02 to 0.28 mmol/l, p = 0.08).

In contrast, associations of PI with HbA1c tended to be negative. The unadjusted estimate of difference in mean HbA1c between PI-positives and negatives was −0.05% (95% CI −0.18 to 0.07%) whereas the difference in mean HbA1c in the fully adjusted model (also accounting for Hb level) was 0.02% (95% CI −0.10 to 0.14%). The adjusted estimate of difference in mean HbA1c remained on restricting PI to microscopic PI and was 0.09% (95% CI −0.07 to 0.25%). This difference became negative following the exclusion of potential clinical malaria cases (β −0.01%, 95% CI −0.15 to 0.12%) and on additional adjustment for FG levels (β −0.02%, 95% CI −0.13 to 0.08%) (Table 2). We also observed a negative trend between parasite density and HbA1c among participants with microscopic PI. Adjusted estimate of mean HbA1c decreased by 0.03% (95% CI −0.09 to 0.08%) per unit increase in parasite density. The negative association also became stronger and reached borderline statistical significance on additional adjustment for FG (β −0.07%, 95% CI −0.14 to 0.01%, p = 0.09).

3.3. *Modification of associations between PI and glycemic control*

We found associations between PI and glycemic control to be dependent on susceptibility factors. We observed statistically significant association between PI and FG among participants with higher illness susceptibility (β 0.34, 95% CI 0.0–0.68, $p_{heterogeneity}$ = 0.140) or with body temperature >36.5 °C (β 0.34, 95% CI 0.06–0.63, $p_{heterogeneity}$ = 0.028) or with a positive FHD alone (β 0.88, 95% CI 0.28–1.47, $p_{heterogeneity}$ = 0.009) or having either positive FHD or untreated diabetes (β 0.69, 95% CI 0.20–1.19, $p_{heterogeneity}$ = 0.023). Associations of microscopic PI with FG showed similar trends. We also observed statistically significant associations among participants with higher illness susceptibility (β 0.46, 95% CI 0.02–0.91, $p_{heterogeneity}$ = 0.038), with body temperature >36.5 °C (β 0.34, 95% CI −0.04 to 0.72, $p_{heterogeneity}$ = 0.057), a positive FHD (β 1.59, 95% CI 0.79–2.40, $p_{heterogeneity}$ < 0.0001) and having either positive FHD or untreated diabetes (β 1.60, 95% CI 0.88–2.32, $p_{heterogeneity}$ < 0.0001) (Fig. 1). These subgroup estimates of associations remained statistically significant in the sensitivity models additionally accounting for HbA1c levels (Table 3).

We found statistically significant positive associations of PI with HbA1c among those with positive FHD (β 0.37, 95% CI 0.03–0.72, $p_{heterogeneity}$ = 0.032). Associations of microscopic PI with HbA1c were also statistically significant and positive among participants with a positive FHD (β 0.59, 95% CI 0.11–1.06, $p_{heterogeneity}$ = 0.025) and among those having either positive FHD or untreated diabetes (β 0.59, 95% CI 0.13–1.05, $p_{heterogeneity}$ = 0.028) (Fig. 1). In contrast to FG, the interaction patterns observed with HbA1c were obliterated in the sensitivity models additionally accounting for FG levels (Table 3).

4. Discussion

In this population-based cross-sectional study, we found a considerably higher prevalence of diabetes and particularly pre-diabetes when defined based on FG compared to HbA1c, suggestive of prevalent transient hyperglycemia. The high prevalence of hyperglycemia defined based on more varying

Table 1 – Characteristics of included participants of the CoDuBu study in south-central Côte d'Ivoire (N = 979).

Categorical variables, n (%)	All	FG < 5.6 mmol/l	FG ≥ 5.6 mmol/l
Sex, Men	502 (51.3)	249 (50.2)	253 (52.4)
Sex, Women	477 (48.7)	247 (49.8)	230 (47.6)
Formal education, none	441 (45.0)	194 (39.1)	247 (51.1)**
Formal education, primary	233 (23.8)	122 (24.6)	111 (23.0)
Formal education, secondary	232 (23.7)	136 (27.4)	96 (19.9)
Formal education, tertiary	73 (7.5)	44 (8.9)	29 (6.0)
Wealth tertile, T1	336 (34.3)	145 (29.2)	191 (39.6)**
Wealth tertile, T2	324 (33.1)	149 (30.0)	175 (36.2)
Wealth tertile, T3	319 (32.6)	202 (40.8)	117 (24.2)
Smoking status, never	812 (82.9)	415 (83.7)	397 (82.2)**
Smoking status, former	79 (8.1)	30 (6.0)	49 (20.1)
Smoking status, current	88 (9.0)	51 (10.3)	37 (7.7)
Fruits, never	3 (0.3)	1 (0.2)	2 (0.4)**
Fruits, once per month	259 (26.5)	112 (22.6)	147 (30.4)
Fruits, once per week	626 (63.9)	330 (66.5)	296 (61.3)
Fruits, several days per week	91 (9.3)	53 (10.7)	38 (7.9)
Vegetables, never	130 (13.3)	35 (7.1)	95 (19.7)**
Vegetables, once per month	260 (26.6)	133 (26.8)	127 (26.3)
Vegetables, once per week	452 (46.2)	246 (49.6)	206 (42.6)
Vegetables, several days per week	137 (14.0)	82 (16.5)	55 (11.4)
Study area: Taabo-Cité	487 (49.7)	308 (62.1)	179 (37.1)**
Study area: Amani-Ménou	240 (24.5)	44 (8.9)	196 (40.6)
Study area: Tokohiri	252 (25.8)	144 (29.0)	108 (22.3)
Rural area	492 (50.3)	188 (37.9)	304 (62.9)**
Urban area	487 (49.7)	308 (62.1)	179 (37.1)
Higher susceptibility to illness	426 (43.5)	196 (39.5)	230 (47.6)**
Family history of diabetes	107 (10.9)	52 (10.5)	55 (11.4)
Anemia	202 (20.6)	112 (22.6)	90 (18.6)
Body temperature ≥37.5 °C	17 (1.7)	7 (1.4)	10 (2.1)
Plasmodium infection (rapid diagnostic test, RDT-PI)	95 (9.7)	41 (8.3)	54 (11.2)
Plasmodium infection (microscopy, microscopic PI)	54 (5.5)	25 (5.0)	29 (6.0)
Plasmodium infection (RDT-PI or microscopic PI)	99 (10.1)	42 (8.5)	57 (11.8)*
Prediabetes (fasting glucose, FG-based)	448 (45.8)	0 (0)	448 (100)**
Prediabetes (glycated hemoglobin, HbA1c-based)	26 (2.7)	13 (2.6)	13 (2.7)
Prediabetes (FG-based or HbA1c-based)	457 (46.7)	13 (2.6)	444 (91.9)**
Diabetes (FG-based)	35 (3.6)	0 (0)	35 (100)**
Diabetes (HbA1c-based)	7 (0.7)	0 (0)	7 (100)**
Diabetes (self-reported clinician diagnosis)	10 (1.0)	3 (0.6)	7 (1.5)
Diabetes (FG-based or HbA1c-based or clinician-diagnosis)	42 (4.3)	3 (0.6)	39 (8.1)**
Continuous variables, mean (SD)			
Age (years)	41.9 (13)	41.3 (13)	42.5 (13)
Body mass index (kg/m^2)	23.3 (4)	23.6 (4)	23.0 (4)**
Waist circumference (cm)	80.4 (11)	80.7 (12)	80.0 (10)
Body temperature (°C)	36.5 (0.5)	36.5 (0.5)	36.5 (0.5)
Sedentary behavior (hours/week)	32.5 (17)	32.5 (17)	32.6 (17)
Hemoglobin (Hb, mg/l)	134 (16)	134 (16)	134 (15)
Plasmodium parasite density (per μl)	547 (1735)	250 (2 3 8)	804 (2345)
Fasting glucose (FG, mmol/l)	5.6 (1)	5.0 (0.4)	6.2 (1.1)**
Glycated hemoglobin (HbA1c, % (mmol/mol))	5.0 (0.6) (31 (7))	5.0 (0.4) (31 (4))	5.0 (0.7) (31 (8))

CoDuBu: Côte d'Ivoire Dual Burden of Disease study. FG: fasting glucose. Prediabetes was defined as fasting glucose 5.6–6.9 mmol/l or glycated hemoglobin 5.7–6.4% (39–46 mmol/mol) without clinician-diagnosed diabetes. Diabetes was defined as fasting glucose ≥7.0 mmol/l or glycated hemoglobin ≥6.5% (48 mmol/mol) or self-reported clinician-diagnosed diabetes. Rural area comprises Amani-Ménou and Tokohiri whereas urban area comprises Taabo-Cité. Anemia was defined as Hb <130 mg/l in males and <120 mg/l in females. Higher susceptibility to illness was defined as a self-report of falling sick more frequently than people in the same age and sex group in the community. Summary estimates for *Plasmodium* parasite density were limited to the microscopic PI-positive participants.

* P-value of the difference in variable summary estimates between the FG groups <0.1.

¨ Respective p-value <0.05. Differences in categorical and continuous variables were tested using the χ^2 test and *t*-test, respectively.

FG could be the result of a high prevalence of repeated infections such as malaria in the tropical setting studied here. Indeed, we observed that asymptomatic PI was independently and positively associated with FG, but not HbA1c, in the presence of parameters suggestive of a setting characterized by more acute malarial infections (i.e., positive microscopic PI) or of an increased susceptibility to stress hyperglycemia (e.g., elevated temperature, higher susceptibility to symp-

Table 2 – Incrementally adjusted associations between *Plasmodium* infection and glycemic control in the CoDuBu study in south-central Côte d'Ivoire.

Estimate	Fasting glucose, FG (mmol/l) β (95% CI)	Glycated hemoglobin, HbA1c (%) β (95% CI)
Model 2	0.17 (−0.06 to 0.38)	−0.03 (−0.15 to 0.10)
Model 3	0.13 (−0.08 to 0.33)	−0.01 (−0.13 to 0.12)
Model 4	0.12 (−0.09 to 0.33)	−0.01 (−0.13 to 0.11)
Model 5	0.11 (−0.10 to 0.31)	−0.01 (−0.14 to 0.11)
Model 6	0.12 (−0.08 to 0.33)	−0.01 (−0.11 to 0.13)
Model 7a	0.13 (−0.08 to 0.33)	0.02 (−0.10 to 0.14)
Model 7b, RDT-PI	0.13 (−0.08 to 0.34)	0.004 (−0.12 to 0.13)
Model 7c, microscopic PI	0.09 (−0.19 to 0.36)	0.09 (−0.07 to 0.25)
Model 8	0.07 (−0.16 to 0.30)	−0.01 (−0.15 to 0.12)
Model 9	0.11 (−0.06 to 0.29)	−0.02 (−0.13 to 0.08)

CoDuBu: Côte d'Ivoire Dual Burden of Disease study. *Plasmodium* infection (PI) was defined as a positive malaria rapid diagnostic test or the microscopic identification of *Plasmodium* species. All beta-coefficients and 95% confidence intervals (CIs) derive from linear regression models excluding participants with treated diabetes, and represent increase or decrease in the mean of respective outcome in *Plasmodium* positive vs. negative participants. RDT-PI: *Plasmodium* infection defined as positive rapid diagnostic test. Microscopic PI: *Plasmodium* infection defined as positive microscopy. All models had sample size of 979 except model 8 where sample size was 952.

Model 1: Unadjusted.
Model 2: Model 1 + age and sex.
Model 3: Model 2 + educational level, wealth index, and area.
Model 4: Model 3 + family history of diabetes.
Model 5: Model 4 + smoking status, frequency of fruit and vegetable intake, and sedentariness.
Model 6: Model 5 + body mass index and waist circumference.
Model 7a: Model 6 + hemoglobin level (primary model).
Model 7b: Model 7a with PI defined only as positive RDT.
Model 7c: Model 7a with PI defined only as positive microscopy.
Model 8: Model 7a, excluding participants with potential clinical malaria (defined as having PI and either anemia or body temperature $\geq$ 37.5 °C).
Model 9: Model 7a, mutually adjusted i.e., FG model additionally adjusted for HbA1c, and vice versa.

tomatic infection, family history of diabetes, or presence of diabetes).

Suggestive evidence for the potential role of PI on more acute glucose derangements has been described. The inflammatory nature of PI promotes physiological stress, which may result in hyperglycemia. PI was shown to stimulate the promotion of inflammatory cytokines [7,10,26] and the activation of NLRP3 inflammasome [8], among other pathways. Indeed, these inflammatory markers could be a cause or direct consequence of hyperglycemia or diabetes [9]. PI is reported to increase permeability of gastric and intestinal linings to sucrose [27]. Compared to controls, adult patients with uncomplicated PI in a hospital-based study exhibited a 25% increase in glucose production, with parallel and significant increases in gluconeogenesis, counter-regulatory hormones, and inflammatory profiles [26]. Another study observed increases in FG, HbA1c, and insulin resistance during uncomplicated malaria in participants without diabetes. However, in this study, the positive associations with HbA1c had not been adjusted for FG, potentially explaining the difference in results for HbA1c. Interestingly, the reported insulin resistance was not compensated by insulin levels, suggesting a greater risk of diabetes following repeated PI [28].

The observed cross-sectional association between the presence of asymptomatic PI and hyperglycemia warrants future well-designed longitudinal studies to (i) follow-up participants (without PI) with and without diabetes for the incidence of new PI over a defined time period and (ii) follow-up participants (without diabetes) to see whether those with highest rates of PI and largest glycemic response are the ones at highest risk of developing clinical diabetes. Stated differently, longitudinal studies should test whether a hyperglycemia reaction in response to repeated or chronic infection is informing on the long-term diabetes risk, including age of diabetes onset. If in longitudinal studies, repeated PI infections are linked with a younger age of onset of diabetes, an adaptation of the target population for diabetes screening may be warranted in LMICs. Maternal malaria has in fact been associated with markers of insulin resistance in their babies [29]. Interestingly, these markers were also associated with low birth weight [29], which predisposes affected babies to metabolic diseases in later-life [30]. A considerable overlap between malaria and cardio-metabolic risk factors was also reported in African adolescents [31], and those who had *in utero* malaria exposure had raised FG levels [13]. Genetically determined susceptibility to a pro-inflammatory response should be tested for effect modification.

Our findings are of direct clinical and public health relevance. First, they point to current knowledge gaps on the potential influence of asymptomatic PI on glycemic control. Second, the observed association of PI and hyperglycemia emphasize the need for adapting health services toward more cost-effective integrated care in endemic countries facing a rapidly growing dual disease burden [32]. Third, if the transience of PI-related hyperglycemia is confirmed by longitudinal studies, this will expose the need for improvements in the estimation of diabetes prevalence in malaria-endemic settings and for the right time point for diagnosing an underlying

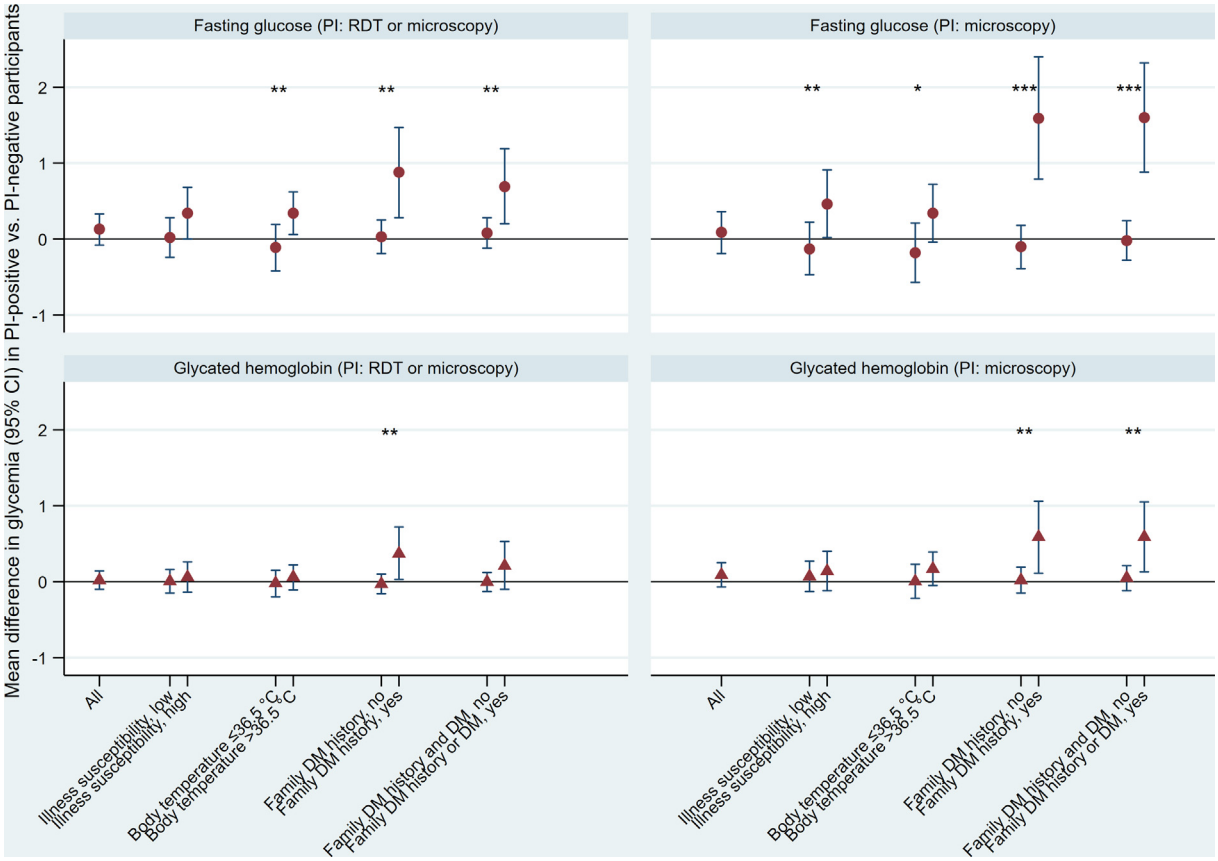

Fig. 1 – Modification of the association between *Plasmodium* infection (RDT or microscopy-diagnosed vs. microscopy-diagnosed) and glycemic control by different susceptibility factors, in the CoDuBu study in south-central Côte d'Ivoire. CoDuBu: Côte d'Ivoire Dual Burden of Disease study. PI: *Plasmodium* infection. RDT: rapid diagnostic test. All beta-coefficients and 95% confidence intervals (CIs) derive from multivariable linear regression models excluding participants with treated diabetes, and represent increase or decrease in the mean of respective outcome in PI-positive vs. PI-negative participants. All models were adjusted for age, sex, educational level, wealth index, area, family history of diabetes, smoking status, frequency of fruit and vegetable intake, sedentariness, body mass index, waist circumference, and hemoglobin level. PI was defined as a positive malaria RDT or the microscopic identification of *Plasmodium* species. DM: diabetes mellitus. N = 979; n (illness susceptibility, high) = 426; n (body temperature > 36.5 °C) = 458; n (family DM history, yes) = 107; n (family DM history or DM, yes) = 143. $^{*}P_{heterogeneity} < 0.1$; $^{**}P_{heterogeneity} < 0.05$; $^{***}P_{heterogeneity} < 0.0001$.

DM. This will involve the identification of most effective tests given the limitations of FG and HbA1c [33], and further exploration of the utility of oral glucose tolerance test (OGTT) and glycated albumin or their combinations in these settings [34]. In line with recent findings with tuberculosis [14], diagnostic testing for any underlying diabetes may need to be postponed to after malaria treatment or clearance of residual PI.

The strengths of our study include being the first population-based investigation shedding light on a direct link between asymptomatic PI and both short- and longer-term markers of glycemic control. We defined PI as a composite measure limiting exposure misclassification. We could control for several diabetes risk factors and explore effect modifications given the detailed phenotypic characterization of the cohort participants. The assessment of effect modification allowed confirming hypothesized susceptibility patterns in this population. The population-based setting of our study allowed the investigation of the impact of asymptomatic PI, which is quite prevalent in malaria-endemic areas, and these findings could be translated to similar settings undergoing epidemiological transition.

The major limitation of our study is its cross-sectional nature, precluding any inferences on direction of associations or causality. Although we applied RDT and microscopy for PI diagnosis, we may have missed some cases that we could have detected with more sensitive diagnostic tests, such as polymerase chain reaction (PCR). However, this potential misclassification has most likely biased our results toward null. We could not investigate associations with confirmed prediabetes or diabetes given the lack of OGTT in this study. Larger longitudinal studies including a PCR diagnosis of PI, and different diabetes tests would improve the understanding of the impact of PI on glycemic control and on underlying diabetes in LMICs.

In conclusion, asymptomatic PI in this malaria-endemic setting of south-central Côte d'Ivoire was associated with glycemic derangements, in conditions of background subclinical

Table 3 – Sensitivity analyses on the modification of the association between *Plasmodium* infection (RDT or microscopy-diagnosed vs. microscopy-diagnosed) and glycemic control by different susceptibility factors using mutually adjusted models (i.e., fasting glucose models additionally adjusted for glycated hemoglobin and vice versa), in the CoDuBu study in south-central Côte d'Ivoire.

			RDT or microscopy		Microscopy	
			Fasting glucose (mmol/l)	Glycated hemoglobin (%)	Fasting glucose (mmol/l)	Glycated hemoglobin (%)
Variable	Categories	N	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
All		979	0.11 (−0.06–0.29)	−0.02 (−0.13–0.08)	0.01 (−0.22–0.29)	0.06 (−0.08–0.20)
Susceptibility to illness	Low	551	0.01 (−0.21–0.24)	−0.0002 (−0.13–0.13)	−0.19 (−0.48–0.11)	0.10 (−0.07–0.28)
	High	426	0.29 (0.0–0.58)**	−0.04 (−0.22–0.13)	0.34 (−0.04–0.73)*	−0.02 (−0.23–0.22)
	$P_{heterogeneity}$		*0.140*	*0.684*	**0.031**	*0.457*
Body temperature	≤36.5 °C	521	−0.09 (−0.35–0.16)	0.01 (−0.14–0.16)	−0.19 (−0.52–0.15)	0.06 (−0.14–0.25)
	>36.5 °C	458	0.29 (0.05–0.54)**	−0.05 (−0.19–0.10)	0.19 (−0.13–0.52)	0.07 (−0.12–0.26)
	$P_{heterogeneity}$		**0.030**	*0.583*	*0.110*	*0.945*
Family history of diabetes	No	872	0.05 (−0.13–0.24)	−0.04 (−0.15–0.07)	−0.12 (−0.37–0.12)	0.05 (−0.09–0.20)
	Yes	107	0.55 (0.04–1.06)**	0.11 (−0.19–0.41)	1.08 (0.39–1.78)**	0.11 (−0.30–0.52)
	$P_{heterogeneity}$		**0.075**	*0.351*	**0.001**	*0.807*
Family history or diabetes diagnosis[a]	No	836	0.08 (−0.09–0.26)	−0.03 (−0.14–0.09)	−0.06 (−0.28–0.17)	0.05 (−0.09–0.20)
	Yes	143	0.53 (0.10–0.97)**	−0.002 (−0.28–0.28)	1.16 (0.52–1.80)***	0.10 (−0.31–0.59)
	$P_{heterogeneity}$		**0.056**	*0.869*	**<0.001**	*0.831*

CoDuBu: Côte d'Ivoire Dual Burden of Disease study. All beta-coefficients and 95% confidence intervals (CIs) derive from multivariable linear regression models excluding participants with treated diabetes, and represent increase or decrease in the mean of respective outcome in *Plasmodium* positive vs. negative participants. All models were adjusted for age, sex, educational level, wealth index, area, family history of diabetes, smoking status, frequency of fruit and vegetable intake, sedentariness, body mass index, waist circumference, and hemoglobin level. Fasting glucose models were additionally adjusted for glycated hemoglobin and glycated hemoglobin models were additionally adjusted for fasting glucose. *Plasmodium* infection was defined as a positive malaria rapid diagnostic test (RDT) or the microscopic identification of *Plasmodium* species.

[a] Combines family history of diabetes and diabetes diagnosis into one variable where participants with neither family history of diabetes nor diabetes diagnosis were designated no whereas those with either component were designated yes.

* $P < 0.1$.

** $P < 0.05$.

*** $P < 0.0001$.

inflammation. For a better estimation of diabetes burden and targeted diabetes control in LMICs, improvements in diagnostics and postponement of diabetes diagnosis in the presence of PI are warranted. These findings need confirmation by longitudinal studies, which should determine whether these glycemic derangements put persons at risk for clinical diabetes in the longer term and whether PI is a risk factor not only for hyperglycemia, but also for diabetes.

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Declaration of Competing Interest

The authors declare no conflict of interests.

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Author contributions

VLK, DK, EKN, JU, BB, and NPH obtained funding from the Novartis Foundation for the CoDuBu study. ICE obtained funding from the Research Funds of the University of Basel. ICE and NPH designed the present study, performed literature search and drafted the manuscript. ICE, CE, FKB, SK, FA, MI, VLK, DK, EKN, JU, BB, and NPH were involved in data collection. ICE, CS, and NPH analyzed the data. ICE, CE, FKB, SK, FA, CS, MI, VLK, DK, EKN, JU, BB, and NPH revised and finalized the manuscript. All authors read and approved the final manuscript.

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