Influence of placental and peripheral malaria exposure in fetal life on cardiometabolic traits in adult offspring

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ABSTRACT

Introduction Fetal malaria exposure may lead to intrauterine growth restriction and increase the risk of developing diabetes and cardiovascular diseases in adulthood. We investigated the extent to which fetal peripheral and placental malaria exposure impacts insulin sensitivity and secretion, body composition and cardiometabolic health 20 years after in utero malaria exposure.

Research design and methods We traced 101 men and women in Muheza district, Tanga region whose mothers participated in a malaria chemosuppression during a pregnancy study in 1989–1992. All potential participants were screened for malaria, hepatitis B and HIV to ascertain study eligibility. Seventy-six individuals (44 men, 32 women) were included in this cohort study. The participants underwent a thorough clinical examination including anthropometric measurements, ultrasound scanning for abdominal fat distribution, blood pressure, 75 g oral glucose tolerance test, an intravenous glucose tolerance test followed by a hyperinsulinemic euglycemic clamp and a submaximal exercise test.

Results Offspring exposed to placental malaria during pregnancy had significantly higher 30-minute plasma post-glucose load levels, but no significant difference in peripheral insulin resistance, insulin secretion or other cardiometabolic traits compared with non-exposed individuals.

Conclusions Using the state-of-the-art euglycemic clamp technique, we were unable to prove our a priori primary hypothesis of peripheral insulin resistance in young adult offspring of pregnancies affected by malaria. However, the subtle elevations of plasma glucose might represent an early risk marker for later development of type 2 diabetes if combined with aging and a more obesogenic living environment.

Significance of this study

What is already known about this subject?
► Malaria exposure in pregnancy increases the risk of low birth weight.
► Intrauterine growth restriction is a well-established risk factor of insulin resistance, type 2 diabetes and associated cardiometabolic diseases.
► The long-term consequences of exposure to placental and peripheral malaria in pregnancy on cardiometabolic health in adult offspring are unknown.

What are the new findings?
► No impact of fetal placental and peripheral malaria exposure on peripheral insulin sensitivity measured by the gold standard method in adulthood or on insulin secretion in relation to insulin sensitivity.
► No identified difference in cardiometabolic risk markers in adulthood by in utero placental and peripheral malaria exposure.
► Subtle elevated plasma glucose levels in placental malaria-exposed offspring may be an early risk marker for later development of type 2 diabetes.

How might these results change the focus of research or clinical practice?
► There is a need for larger and extended deep phenotyping studies, including direct measurements of hepatic insulin action, to explain subtle elevations of plasma glucose levels in adult offspring of women exposed to malaria in pregnancy.

INTRODUCTION

Exposure to malaria during pregnancy may cause fetal growth restriction resulting in low birth weight (LBW), especially in pregnancies with placental malaria infection.[1 2] The mechanism(s) behind this relationship is not fully understood, but a malaria-infected placenta has been shown to cause impaired blood flow to the fetus, which to some extent, compromised oxygen delivery, reduced nutrient supply and impaired placenta function by infiltration of the villi; all subjecting the fetus to suboptimal growth conditions.[3 4] However, systemic malaria effects may also contribute to LBW where *Plasmodium falciparum* and *P. vivax* have been shown to adversely affect birth weight even though the latter does not sequester in the placenta.[5] Since Hales and Barker launched their thrifty phenotype hypothesis three decades ago,[6] extensive knowledge has been created on the relationship between LBW and cardiometabolic diseases including,

insulin resistance, diabetes and hypertension.[7–10] A common physiological mechanism for cardiometabolic diseases is thought to be organ-specific insulin resistance most importantly affecting skeletal muscle and the liver.[11] A so-called thin-fat body phenotype, which refers to relative high body fat mass and low lean body mass may be a consequence of LBW and manifest itself in an adverse body composition with elevated abdominal adipose fat accumulation,[12] increasing the risk of developing insulin resistance. It is well known that pregnancy associated with malaria is associated with increased risk of preterm and LBW births[13] and a meta-analysis showed that malaria prevention in pregnancy can reduce neonatal mortality and LBW.[14] However, to the best of our knowledge, only one study has examined how fetal exposure to malaria impacts the long-term health consequences of the offspring. In this study, the authors examined exposure to malaria during pregnancy, assessed at delivery in peripheral and placental blood samples, in a Ghanaian birth cohort and found that malaria exposure was associated with increased fasting plasma glucose levels and a trend towards higher systolic blood pressure at 15 years of age.[15]

In the present study, we wanted to examine the long-term cardiometabolic health consequences of fetal exposure to placental and peripheral malaria with gold standard methods for the measurement of peripheral insulin sensitivity, insulin secretion, body composition and dysmetabolic traits in adult men and women living in Tanzania. We hypothesize that combined placental and peripheral malaria exposure in pregnancy is associated with a higher degree of insulin resistance and adverse cardiometabolic traits compared with peripheral malaria exposure only and non-exposed.

RESEARCH DESIGN AND METHODS

Offspring (mean age 19.6±1.0 years) of women who participated in a cohort study on malaria chemosuppression during pregnancy conducted in 1989–1992[16] in Muheza, northeastern Tanzania were retrieved from study document archives at the Amani Medical Research Centre and were contacted and informed about this follow-up study. In the original chemosuppression study, the participants were treated with chemoprophylaxis/chemosupressive regimens including proguanil and chloroquine. Additionally, clinical malaria attacks were treated with sulphadoxine–pyrimethamine and if failed with quinine. Written informed consent was obtained from the offspring after a thorough explanation of the currently planned study. Relevant data were retrieved from records including birth weight, mother's age and parity at the time of the chemosuppression study as well as her hemoglobin status during pregnancy. The follow-up study was performed in 2010–2011.

Malaria exposure during pregnancy was determined by examining Giemsa stained thick blood smear fortnightly for peripheral malaria, and by a similarly prepared placental thick blood smear at birth.[16]

All consenting participants were subjected to a rapid HIV (capillus test), hepatitis B (Hb-surface antigen test), and peripheral blood smear for malaria microscopy. A positive HIV or hepatitis B test was an exclusion criterion, while a positive malaria test postponed the eligibility to participate until clinical symptoms from the current infection had abated. Participants meeting the study inclusion criteria were instructed to abstain from alcohol, smoking and heavy physical labour for at least 72 hours prior to enrollment into the study. Anthropometric measurements including weight (kg), height (cm), and waist circumference (cm) were done with the participant barefooted and in light clothing. Body mass index (kg/m^2) was derived. Peripheral insulin sensitivity was measured by a hyperinsulinemic euglycemic clamp (40 mU/m^2/min). Prior to the clamp, an intravenous glucose bolus (0.3 g of 20% glucose per kg body weight) was applied to test first phase insulin secretion rate.

Visceral and abdominal subcutaneous adipose tissue distribution (cm) was measured by ultrasound scanning (Aquila Basic Unit, Esaote, Pie Medical Equipment, Maastricht, the Netherlands) with a 3.5/5.0 MHz transducer (Probe Article no. 410638 Curved Array HiD probe R40 Pie Medical Equipment), using a standard protocol.[17] Fasting blood glucose was measured, after which each participant was subjected to a standard 75 g standard oral glucose tolerance test (OGTT). Diabetes and prediabetes from the fasting or 2-hour blood glucose test (plasma-derived values) were determined based on the WHO 2006 criteria[18] using a HemoCue 201 RT system (HemoCue, Ängelholm, Sweden) according to a modified glucose dehydrogenase method. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as follows: ([(fasting plasma insulin (pmol/L)×fasting plasma glucose (mmol/L))/22.5]×0.144).[19] Fasting and 2-hour EDTA-plasma insulin (pmol/L) as well as fasting EDTA-plasma C-peptide (pmol/L) were analyzed according to the ECLIA-photon count method (Roche diagnostics). A standard plasma lipid profile (total cholesterol, Low Density Lipoprotein- Cholesterol (LDL-C), High Density Lipoprotein- Cholesterol (HDL-C), triglyceride, (mmol/L)) was analyzed by enzymatic identification–absorptionphotometry method (Roche diagnostics). Blood pressure (mm Hg) and resting pulse rate (beats/min) were measured on the left upper arm three times using a full-automatic device (Omron HEM-7120, Kyoto, Japan) and following 15 min of rest and with 2 min between each measurement and mean values were based on an average of the last two measurements. Maximal aerobic work capacity (watt-max) was assessed according to the method and formula by Andersen[20] using a Monark 828E stationary bicycle (Monark Exercise, Vansbro, Sweden) and calculated as VO$_2$/min/kg. For the entire period of data collection, investigators were blinded to the malaria exposure status during the fetal life of each participant.

We were able to trace 101 out of 337 women who participated in the original study. From these women, 76 offspring (aged 19–20 years at the study time point) with available birth weight measures were included in the study. The reasons for exclusion of 25 individuals were pregnancy (n=4), HIV positive (n=2), hepatitis B positive (n=9), not showing up on the examination day (n=5), mother left the original study prematurely (n=3), no placental blood smear analysis performed (n=1), or mother–child match not found (n=1).

Statistical analyses and calculations of index

Normally distributed data are presented as mean±SD and skewed data as median and IQR. Linear regression analyses (GLM procedures (general linear model)) were performed to examine differences in the outcome of interest and malaria exposure. Malaria exposure was divided into the following categories: (1) peripheral and placental malaria negative (M−) (2) peripheral malaria (PM+) at least once during pregnancy, and (3) both peripheral malaria and placental malaria (PPM+). For significant associations, the non-exposed group was tested if different from (1) PM+ and (2) PPM+ by t-test. The analyses were adjusted for sex due to a skewed gender distribution among the groups. All analyses were performed using SAS (V.9.4, SAS Institute) with p values of <0.05 determining statistical significance.

The following calculations were made from data derived from the clamp and the intravenous glucose tolerance

Table 1 Malaria exposure during pregnancy and offspring health in Tanzania (n=76)

	Malaria negative	Peripheral malaria	Peripheral+placental malaria	P value	P value adjusted
N	17	45	14		
Sex (male/female)	11/6	21/24	12/2	0.03	–
Mother's age (year)	25 (22–30)	23 (19–30)	20 (19–25)	0.21	–
Max malaria density in pregnancy (f/cc)—peripheral*	0	2640 (480–7720)	7320 (2640–36480)	0.16	–
Attack frequency*	0	2 (1–2)	2 (1–3)	0.79	–
Offspring					
Birth weight (kg)	3.0 (2.5–3.0)	2.8 (2.5–3.2)	2.6 (2.4–3.0)	0.38	–
Age (years)	20 (19–20)	20 (19–20)	20 (19–20)	0.94	–
Height (cm)	162.1 (157–165.5)	159.4 (155.2–165)	164.2 (158.–166.4)	0.60	0.81
Weight (kg)	54.3 (49.8–57.4)	52.1 (47.0–55.5)	51.0 (47.8–56.8)	0.71	0.74
Fat %	7.8 (6.7–10.5)	11.5 (7.6–17.4)	7.6 (4.6–13.0)	0.12	0.91
Waist circumference (cm)	70.5 (67.4–75.0)	71.7 (68.5–74.8)	73.2 (67.0–74.1)	0.82	0.78
Visceral fat (cm)	5.0 (4.4–6.39)	4.9 (3.7–5.7)	5.5 (4.6–5.8)	0.43	0.70
Subcutaneous fat (cm)†	0.94 (0.64–1.13)	0.92 (0.71–1.90)	0.98 (0.71–1.46)	0.64	0.59
Systolic blood pressure (mm Hg)	129 (122–136)	128 (116.5–134)	114 (111–132)	0.40	0.24
Diastolic blood pressure (mm Hg)†	75 (72–80)	78.5 (71–85)	73 (66–78)	0.16	0.39
Estimated aerobic fitness (mLO_2/min/kg)†	37.9 (29.5–50.6)	31.9 (23.1–43.7)	46.9 (41.8–51.2)	0.05	0.83
FPIR†	3630±1654	4686±3056	3172±2274	0.27	0.58
Phi1†	35.0 (29.9–46.2)	43.3 (27.1–74.7)	21.0 (19.–38.8)	0.16	0.39
M-value	9.9±2.3	10.3±3.0	9.9±3.0	0.93	0.72
DI:whole body†	29 063 (19 860–46 986)	35 416 (24 243–71 973)	32 877 (12 145–53 437)	0.25	0.51
DI:hepatic†	2534 (1753–3124)	2388 (1738–4212)	1950 (604–3296)	0.55	0.56

Normal distributed data are presented as mean±SD and skewed data as median and IQR. Linear regression analyses (GLM procedures) were performed to examine differences in the outcome of interest and malaria exposure.
P value adjusted for sex.
FPIR: (0–10 min ($AUC_{ins0-10}$–(ins0×10 min)). Phi1: AUC_{ins}/AUC_{glu}. M-value: mg glucose/min/kg FFM. DI:whole body: whole body insulin secretion DI–M-value×FPIR. DI:hepatic: hepatic insulin secretion DI: FPIR/HOMA-IR. N M-value: 10, 24 and 4 in each exposure group and N for DI:hepatic 9, 22, 2, respectively.
*P-test performed.
†P values from log-transformed data due to skewed residuals.
AUC, area under the curve; DI, disposition index; FFM, fat free mass; FPIR, first phase insulin response; GLM, general linear model; HOMA-IR, homeostatic model assessment of insulin resistance.

Table 2 Glucose, insulin and C-peptide concentrations during the OGTT

	Malaria negative	Peripheral malaria	Peripheral+placental malaria	P value	P value adjusted
N	13–16	36–43	11–14		
Fasting glucose (mmol/L)	4.5±0.6	4.5±0.6	5.8±0.7	0.21	0.29
Glucose 30 min (mmol/L)	6.7±1.2	6.6±1.0	7.9±1.5	0.002	0.01
Glucose 120 min (mmol/L)	6.6 (5.9–6.9)	6.1 (5.2-6-8)	6.6 (5.4–7.3)	0.35	0.33
Fasting insulin (pmol/L)∞	40 (22–57)	53 (35–65)	64 (30–70)	0.11	0.13
Insulin 30 min (pmol/L)∞	318 (174–539)	252 (101–414)	287 (218–389)	0.92	0.67
Insulin 120 min (pmol/L)∞	274 (197–337)	252 (165–451)	206 (106–333)	0.15	0.12
Fasting C-peptide (pmol/L)∞	424 (397–567)	443 (374–526)	362 (280–670)	0.76	0.96
C-peptide 30 min (pmol/L)∞	1562 (1018–1906)	1295 (964–1897)	1324 (1170–1569)	0.97	0.97
C-peptide 120 min (pmol/L)∞	1622 (1360–1081)	1666 (1293–2296)	1586 (1167–1975)	0.70	0.62
HOMA-IR∞	1.13 (0.62–1.81)	1.61 (1.10–1.92)	1.88 (0.88–2.15)	0.18	0.21

∞P values from log-transformed data due to skewed residuals.
HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; OGTT, oral glucose tolerance test.

test. M-value (peripheral insulin sensitivity): mg glucose/min/kg fat free mass (FFM). Disposition index (DI): whole body: FPIR: (first phase insulin response 0–10 min (Area under the curve $(AUC)_{ins0-10}$ − (ins0×10 min))). Phi1: AUC_{ins} /$AUC_{glu.}$ We calculated the DI based on the assumptions of a hyperbolic relationship between insulin sensitivity and insulin secretion and calculated both DI with peripheral insulin sensitivity and hepatic insulin sensitivity since they may provide different pathophysiological aspects.[21][22] Whole body insulin secretion DI: M-value×FPIR. DI:hepatic: Hepatic insulin secretion DI: FPIR/HOMA-IR.

RESULTS
All results on malaria exposure and offspring health are presented in tables 1 and 2. There were no differences in mean birth weight between the three groups. LBW defined as birth weight <2500 g was found in 11.7% in the M− group, 17.7% in PM+ and 35.7% in PPM+ group (p=0.27), respectively. Neither the amount nor the distribution of fat, blood pressure, or aerobic fitness level were different between the three groups. There was a tendency towards higher aerobic fitness level in the PPM+ group, but this was not sustained when the analysis was adjusted for unequal gender compositions between groups. No differences in insulin secretion, insulin resistance or in insulin secretion DIs were found between the groups. Malaria exposure in pregnancy did not significantly affect plasma HDL, LDL, total cholesterol, triglycerides, liver enzymes or high-sensitivity C reactive protein levels among the offspring at 20 years of age. Compared with the M− group, the PPM+ group had significantly higher plasma glucose levels at 30 min post-glucose load (M−: 6.7±1.2 mmol/L, PM: 6.6±1.0 and PPM+: 7.9±1.5 mmol/L; p=0.01), a tendency to higher fasting insulin and lower insulin levels at 120 min, but no significant differences in neither fasting glucose levels nor in HOMA-IR. In

subanalyses comparing non-malaria-positive individuals with malaria positive regardless of placental or peripheral exposure, we did not find any significant difference in fasting glucose, fasting insulin, glucose 30 min or M-value (p>0.05). However, when comparing individuals with placental malaria exposure versus no placental malaria exposure, we confirm our findings of elevated glucose levels 30 min following consumption of a standard glucose solution among exposed individuals (p=0.01).

Based on the OGTT, one individual had diabetes (in the PPM+ group), eight individuals had impaired glucose tolerance (two in M−, five in PM+, and one in PPM+ groups, respectively), and one individual in the PPM+ group had impaired fasting glycemia.

DISCUSSION
Using the state-of-the-art euglycemic clamp technique, we were unable to prove our a priori hypothesis of peripheral insulin resistance in young adult offspring of pregnancies affected by malaria. Although fasting plasma glucose levels did not differ significantly between groups, our finding of elevated 30-minute post-OGTT plasma glucose levels in the PPM+ group does, to some extent, support the Ghanaian data[15] and a role of malaria in pregnancy in developmental programming of cardiometabolic disease across generations. Neither in our study nor in the Ghanaian data could a significant difference in birth weight between malaria-exposed and non-exposed groups be demonstrated. This could either be due to the relatively small sample sizes, or that the exposure was not substantial enough to directly impact birth weight.

Due to the rural Tanzanian setting, we were unable to apply glucose tracers to measure hepatic insulin resistance influencing glucose metabolism primarily at low plasma insulin levels in the fasting state and during an OGTT.[21][23] Although not statistically significant, raw numbers indicate elevated HOMA-IR among offspring

affected by malaria which may suggest hepatic insulin resistance, supporting a need for future direct measures of hepatic insulin resistance. Indeed, studies have reported hepatic insulin resistance to precede peripheral insulin resistance in Caucasian LBW individuals.[10 24] Furthermore, recent data have shown that the pancreatic beta cell sense responds more closely to hepatic as opposed to peripheral insulin resistance.[22] Insulin secretion DIs, reflecting the capacity of insulin secretion to compensate for insulin resistance, should therefore optimally be calculated from accurate measures of hepatic insulin resistance. The lack of major metabolic differences between the exposure groups could also relate to the effective treatment of the pregnant women with malaria in the baseline study. Here, the women were tested every 14 days for peripheral malaria and treated if positive, which may have reduced the severity and potential harmful effects of malaria imposed on the fetus. Furthermore, according to the thrifty phenotype hypothesis,[25] the worst metabolic outcome will result from a mismatch between an adverse intrauterine environment and an enriched postnatal environment. Thus, even though the participants in the current study were young, non-obese and still living in a rural area, the documented subtle elevations of plasma glucose levels may be an early marker for later risk of developing type 2 diabetes as these individuals get older and potentially exposed to a more obesogenic environment.

The current study is unique with regard to using gold standard measure for peripheral insulin resistance, comprehensive long-term (~20 years) follow-up data with longitudinal data on malaria exposure during pregnancy as well as specific placental examinations. However, the study is limited in the small number of study participants, and furthermore the baseline study was not designed to follow up the offspring and examine impact of fetal malaria exposure on later metabolic health. Despite our efforts to match cases and controls as much as possible, we of course acknowledge that the results including the significantly elevated 30-minute plasma glucose concentration could have been influenced by undetected residual confounding from differences in genetic predisposition to diabetes and/or socioeconomic status, which we unfortunately were not able to determine precisely in the present study. Furthermore, we cannot exclude the possibility that some of our findings are influenced by selection bias, since we do not have any information about non-participating individuals. Thus, the current findings need to be verified in larger populations both living in rural and urban environments.

In conclusion, this study added new knowledge to the limited data on the long-term health consequences of exposure to malaria during pregnancy. No impact of malaria exposure on peripheral insulin sensitivity in adulthood or on cardiometabolic risk factors was observed, however the subtle change in plasma glucose might be an early risk marker for later development of type 2 diabetes.

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