P. falciparum gametocyte density and infectivity in peripheral blood and skin tissue of naturally infected parasite carriers in Burkina Faso

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Forty-word summary: *Plasmodium falciparum* gametocyte skin sequestration has long been hypothesized but never formally tested. We observed no evidence for higher gametocyte densities in skin tissue of naturally infected gametocyte carriers or blood meals of mosquitoes feeding on their skin.

ABSTRACT

Background. *Plasmodium falciparum* transmission depends on mature gametocytes that can be ingested by mosquitoes taking a bloodmeal on human skin. Although gametocyte skin sequestration has long been hypothesized as important contributor to efficient malaria transmission, this has never been formally tested.

Methods. In naturally infected gametocyte carriers from Burkina Faso, we assessed infectivity to mosquitoes by direct skin feeding and membrane feeding. We directly quantified male and female gametocytes and asexual parasites in: i) finger prick blood, ii) venous blood, iii) skin biopsies, and in pools of mosquitoes that fed iv) on venous blood or, v) directly on skin. Gametocytes were visualized in skin tissue by confocal microscopy.

Results. Whilst more mosquitoes became infected when feeding directly on the skin compared to venous blood (odds ratio 2.01; 95% CI 1.21 – 3.33, p = 0.007), concentrations of gametocytes in the subdermal skin vasculature were not higher compared to other blood compartments; only sparse gametocytes were observed in skin tissue.

Discussion. Our data strongly suggest that there is no significant skin sequestration of *P. falciparum* gametocytes. Gametocyte densities in peripheral blood are thus informative for predicting onward transmission potential to mosquitoes and can be used to target and monitor malaria elimination initiatives.

KEYWORDS: Plasmodium falciparum, gametocyte, sequestration, transmission, elimination, anopheles

BACKGROUND

Significant reductions in malaria burden in recent decades have stimulated malaria elimination initiatives [1] that may require approaches that specifically reduce malaria transmission [2]. Transmission of malaria depends on mature male and female gametocytes that circulate in the bloodstream and may be ingested by mosquitoes from the subdermal capillaries upon blood feeding. Interestingly, mosquito infections with *Plasmodium falciparum* have been observed from gametocyte donors whose low gametocyte density appears incompatible with transmission [3] and mosquito infection rates are typically higher when mosquitoes feed directly on skin of gametocyte carriers, as compared to feeding on venous blood through an artificial membrane [4, 5]. *P. falciparum* gametocyte aggregation [6] and sequestration could contribute to these observations by facilitating mosquito infections from low gametocyte densities. Skin sequestration has been observed for other human parasites, including *Trypanosoma brucei* [7], *Onchocerca volvulus, Mansonella, Leishmania infantum* and *L. donovani*, [8-11], where parasite burden in the skin is the best predictor of infectiousness.

Indirect evidence for skin sequestration of mature gametocytes in the microvasculature of the skin was first described in surveys conducted in the 1940s and 1950s in DR Congo: gametocyte prevalence in a survey using skin scarification was 3-fold higher compared to a survey 5 years earlier using finger prick blood [12]. In a follow-up study with 1243 paired samples, a 13.4% increase in *P. falciparum* parasite prevalence and 15.6% increase in gametocyte prevalence was observed when blood and dermal fluids from skin scarification were used for sample preparation instead of finger prick blood [13]. The hypothesized skin sequestration of intra-erythrocytic *P. falciparum* gametocytes may be related to mechanical retention in cutaneous capillaries [14, 15], analogous to the reversible rigidity that likely prevents immature gametocytes from entering circulation [16, 17]. Alternatively,

sequestration may be related to gametocyte cytoadhesive properties [18] mediated by parasite proteins on the infected red blood cell (iRBC) surface, analogous to adhesion of asexual *P. falciparum* parasites to receptors on human vascular endothelial cells by *P. falciparum* erythrocyte membrane-1 (PfEMP1)[19].

Whilst sequestration of mature gametocytes in the skin of naturally infected individuals remains speculative, it may play an important role in determining *Plasmodium* transmission efficiency [3, 15]. Here, we report on two independent studies in naturally infected gametocyte carriers from Burkina Faso where we quantified mature *P. falciparum* gametocytes in skin tissue, blood samples and mosquito blood meals in association with onward transmission to *Anopheles* mosquitoes.

METHODS

Ethics statement

Ethical approval was granted by the Ethical Review Committee of the Ministry of Health of Burkina Faso (Deliberation numbers 2016-03-033 and 2017-02-018) and the Ethics Committee of the London School of Hygiene and Tropical Medicine (#10489 and #11962). Individual written informed consent was obtained prior to enrolment. Malaria cases were treated according to national guidelines in Burkina Faso [20].

Study site and population

Study participants were recruited in the village of Balonghin (Saponé district, Burkina Faso) where malaria transmission is seasonal and intense with 51-84% *P. falciparum* parasite prevalence and 49-75% gametocyte prevalence by molecular methods [21].

Study design

Paired skin feeding and membrane feeding study

This study was conducted in October-December 2017. Eligible participants (15-50 years) were asymptomatic with *P. falciparum* mono-infection with gametocyte densities ≥1 gametocyte/500 leucocytes by microscopy (≥16 gametocytes/µL when assuming 8000 leucocytes/ µL) and haemoglobin concentration ≥8 g/dL (Table 1). Immediately after venipuncture in lithium heparin and EDTA tubes (BD Vacutainer™), 400-500µL of heparinized blood in duplicate (for infectivity) and 400-500µL EDTA blood (for gametocyte quantification in blood meals) was offered to 60 starved 4–5-day-old female *An. coluzzii* mosquitoes via an artificial membrane attached to a water-jacketed glass feeder maintained at 37°C [22]. After exactly 15 minutes of feeding in the dark, fully fed mosquitoes from heparin blood were transferred to storage cups and maintained for 6-8 days before dissection by two independent microscopists using 1% mercurochrome. From mosquitoes that fed on EDTA blood, 16 fully fed mosquitoes were sacrificed after feeding for exactly 15 minutes by sharp needle puncture of their midguts to release individual blood contents into 50µl of RNAprotect cell reagent for storage at -80°C. Immediately following membrane feeding, direct skin feeding took place. The participant's calves were exposed to 60 mosquitoes that were allowed to feed for exactly 15 minutes. From this group, 12 fully fed mosquitoes were immediately sacrificed, and their midguts punctured as described above. Remaining mosquitoes were maintained for dissection and oocyst screening, as above. In addition to membrane and direct skin feeding assays, K2EDTA blood was collected by venipuncture (BD Vacutainer™) and finger prick (BD Microtainer®).

Skin biopsy study

In the period September 2016-March 2017, adults (aged 18-50 years) were invited for study participation if they had *P. falciparum* mono-infection with microscopy detected gametocytes

(as above), Hb ≥11 g/dL, and no skin infections or conditions, history of vasovagal responses to blood sampling or biopsies or allergy to lidocaine/ prilocaine. Eligible individuals were asked to participate in sampling on two occasions, 4 days apart. At each occasion, skin biopsy samples including the dermis and hypodermis were taken the lower part of the forearm (antebrachium; n=2) and lower part of the calf (sura; n=2) using single use punchers (4mm Biopsy Punch; Miltex Inc. York, US). These locations are typically used for direct skin feeding experiments [23]. Procedures were performed by a qualified dermatologist 1 hour after application of a patch with an eutectic mixture of licodaine/prilocaine as anaesthetic (EMLA patch 5%; Aspen, France). Topical application was chosen to minimize vasoconstriction or dilution of tissue fluids that may occur upon injection of anaesthetics. Half of the biopsy samples (one each from arm and leg) were immediately immersed in 2 mL of 10% formalin and placed at 4°C overnight; following washing, samples were stored in 2 mL of 70% ethanol and stored at 4°C until further processing. Other biopsy samples were transferred to 1000 µL RNALater stabilization reagent (Qiagen), incubated overnight at 2-8°C and then transferred to -80°C. Finger prick and venous blood samples were collected in EDTA-coated tubes, as above.

Molecular analysis

Mosquito homogenates were pooled (4 mosquitoes in a total of 200µl RNAprotect) with 4 pools (16 mosquitoes) for membrane feeding experiments and 3 pools per skin feeding experiment (12 mosquitoes). Nucleic acids from mosquito pools and from 100µL venous and finger prick whole blood samples in RNAprotect Cell Reagent were isolated using MagNAPure LC automatic extractor (Total Nucleic Acid Isolation Kit—High Performance, Roche Applied Science). Ring-stage asexual parasites, female gametocytes and male gametocytes were quantified by individual quantitative reverse-transcription PCR (qRT-PCR)

assays targeting *sbp1* [24]; *Pfs25* [25] and *PfMGET* [26], respectively. RNA extraction from skin tissue was performed using the Qiagen RNeasy Plus Mini kit (Qiagen). In addition to qRT-PCR, the NanoString nCounter® platform was used to quantify genes differentially expressed in specific *P. falciparum* parasite stages, as described in detail elsewhere [27] and in more detail in the Supplemental Methods.

Histological analysis of skin samples

Skin biopsies were processed as described in the Supplemental Methods. Sections were stained mouse antibodies targeting CD31 (human endothelial cells) or rabbit antibodies targeting Pfs16 (gametocytes) [28] before adding goat anti-mouse IgG-AlexaFluor488 (ThermoFisher, A-11029) or goat anti-rabbit IgG-AlexaFluor647 (ThermoFisher, A-21245) secondary antibodies. Slides were viewed on a Nikon A1R inverted confocal microscope with Piezo Z-drive to acquire z-stacks. In addition to skin biopsies, clots of cultured *P. falciparum* parasites (strains Pf2004, 3D7 and NF54) were generated to act as positive and negative controls[28]. Images and movies were generated using Image J software.

Sample size justification

For the paired skin feeding-membrane feeding study, we assumed an average of 15% infected mosquitoes in patent gametocyte carriers with a standard deviation of 20% and a within subject correlation of the outcome of 0.5 [4, 29, 30]. If we then expected two-fold higher mosquito infection rates in direct skin feeding, 17 paired membrane feeding and skin-feeding experiments on patent gametocyte carriers would give 80% power to detect this difference at an alpha of 0.05. Sample size justification for skin-biopsy sampling was based on a paired comparison of the proportion of the total parasite population that is mature gametocyte. We expected that 73% of the skin snip biopsy samples had higher gametocyte concentrations,

based on a meta-analysis that demonstrated enhanced infectivity following skin feeding compared to venous blood membrane-feeding [4]. When assuming that 70% of infected adults have detectable malaria parasites in skin tissue and allow quantification of the proportion of parasites that is gametocyte, and a lower limit of the 95%-CI >50%, 45 paired skin snip samples and venous/finger prick blood samples would give 83% power with an alpha of 0.05 to detect a different in parasite stage composition. A go/no-go criterion was defined where an initial 10 gametocyte carriers were recruited for biopsy samples and additional participants would only be recruited if gametocytes were detected in ≥50% of all samples.

Statistical analysis

All statistical analyses were performed in STATA version 15.0 (Statacorp; College Station, TX, US). The proportion of infectious gametocyte carriers was compared between paired feeding experiments using McNemar's test; the proportion of infected mosquitoes was compared between direct skin feeding and membrane feeding using logistic regression controlling for study participant as a fixed effect. Spearman non-parametric correlation coefficients were calculated to assess associations between continuous variables; paired Wilcoxon rank-sum test was used to compare parasite densities between blood or tissue samples from the same participants. Gametocyte fraction was calculated as the sum of male and female gametocytes, expressed as a proportion of the total parasite biomass of asexual ring-stage parasites and gametocytes.

RESULTS

A total of 31 individuals aged 15-48 (median 29) participated in experiments with paired skin feeding and membrane feeding. The median number of dissected mosquitoes per experiment was 35 (interquartile range (IQR) 33-37) for direct skin feeding and 73 (IQR 69-82) for

membrane feeding. Of 31 paired experiments, 18 (58.1%) direct skin feeding and 22 (71.0%) membrane feeding experiments resulted in at least one infected mosquito (p=0.289). Total gametocyte density, quantified in venous blood by *Pfs25* and *Pfmget* qRT-PCR [26], was positively associated with the proportion of mosquitoes that became infected following direct skin feeding (ρ=0.415, p=0.0204) or membrane feeding (ρ=0.596, p = 0.0004) (Figure 1A). The proportion of infected mosquitoes was higher by direct skin feeding as compared to membrane feeding assays (odds ratio 2.01; 95% CI 1.21 – 3.33, p = 0.007). The medium number of oocysts was 4 (IQR 2-7.5; maximum 38) for mosquitoes that became infected after feeding directly on the skin and 2 (IQR 1-5; maximum 24) for mosquitoes that became infected after feeding on venous blood through a membrane feeder.

To examine whether this higher infectivity in direct skin feeding assays was related to higher ingested gametocyte densities, or to a higher gametocyte fraction in the blood meal, we directly quantified gametocytes and asexual parasites in mosquito blood meals. We quantified asexual parasites by skeleton-binding protein 1 *sbp1* qRT-PCR [24] and gametocytes (*Pfs25* and *Pfmget* qRT-PCR) in a median of 3 mosquito pools per participant, each containing 4 individual mosquitoes, from skin-feeding (range=2-3) and 4 pools per participant, each containing 4 individual mosquitoes, from membrane feeding (range=2-4). We observed strong correlations between parasite quantities in pools of mosquitoes that fed on skin or venous blood through artificial membranes for asexual ring-stage parasites (r=0.921, p<0.0001), male (r=0.790, p<0.0001) and female gametocytes (r=0.655, p=0.0001) (Figure 1B). Opposite to our hypothesis, densities of asexual ring-stage parasites (p=0.0021), female (p=0.032) and male gametocytes (p=0.0002) were lower in blood meals taken directly from the skin compared to venous blood (Supplemental Figure S1). We also expressed gametocytes as a fraction of the total parasite biomass. This fraction ranged from very low (<1% gametocytes in an individual with 21,086 ring-stage asexual parasites/µL and 179 gametocytes/µL) to

100% in 3 individuals without asexual parasites detected by qRT-PCR (Figure 1C). We observed no tendency towards a higher fraction of gametocytes in skin-fed mosquitoes or capillary blood compared to venous blood (Figure 1D) and a strong correlation between gametocyte fractions in the different compartments (Supplemental Figure S2).

In a complementary study, 9 adult gametocyte carriers participated in skin biopsy sampling. Male and female gametocytes and ring-stage asexual parasites were quantified by qRT-PCR to calculate the gametocyte fraction in finger prick blood (16 observations; 9 donors), venous blood (n=16; 9 donors), as well as skin tissue from the arm (n=13; 7 donors) and leg (n=12; 8 donors). Gametocytes were detected in all tissue and all blood samples by qRT-PCR; asexual parasites were detected in 17/25 tissue and in 30/32 blood samples. Gametocyte fraction was highly variable between donors (and between time-points) whilst estimates from the different compartments from the same donor and time-point showed strong correlation: gametocyte fraction in venous blood was strongly associated with that in finger prick blood ($\rho = 0.947$, $p<0.0001$), arm skin tissue ($\rho = 0.928$, $p < 0.0001$) and leg skin tissue ($\rho = 0.870$, $p=0.0002$) without any obvious bias towards higher gametocyte fraction in capillary blood or tissue samples compared to venous blood (Figure 2A). Parasite density estimates per microliter of blood or tissue were generally lower in the skin tissue compared to blood samples (Figure 2B) and not significantly different between venous or finger prick blood ($p\geq0.121$) or between leg skin tissue or arm skin tissue ($p\geq=0.116$). The same RNA aliquots were also processed for analysis by Nanostring expression array, a highly sensitive probe-based expression platform that we have optimized for use in *P. falciparum* [31, 32]. Using a previously defined stage-specific marker set for asexual rings and mature gametocytes [27, 32], there was no evidence for higher gametocyte transcripts in skin samples compared to blood samples (Figure 2C). The two approaches to quantify gene expression also showed a strong correlation for *sbp1* and *Pfs25* (Figure 2D).

To directly detect gametocytes in subcutaneous tissue, skin biopsy samples that were stored in formalin were processed for imaging. Given the low densities of gametocytes predicted based on the qRT-PCR quantification (estimated median of 55.0 gametocytes in arm tissue samples (IQR 28.2-153.0) and 36.9 gametocytes in leg tissue samples (IQR 11.6-98.3); we established a protocol to image 10µm sections by confocal microscopy, hence maximizing the detectability of sparse gametocytes (Figure 3A). Skin sections were initially analysed by haematoxylin and eosin staining and labelled with the endothelial marker CD31 (Figures 3B) to confirm integrity of the tissue. Evaluation of gametocyte markers identified Pfs16 antibodies [28, 33] as highly specific and sensitive using the confocal imaging protocol (Figure 3C). Screening of at least 12 sections per skin snip in arm and leg samples from each participant identified several putative gametocytes. A Pfs16 positive cell with a characteristic crescent shape, three-dimensional structure and nuclear stain is shown in close association with a vessel (Figure 3D and Supplementary movies 1 and 2). Based on these results, with low success gametocyte detection rates by this highly sensitive fluorescence microscopy protocol, no further gametocyte carriers were recruited as tissue donors.

DISCUSSION

Here, we tested a long-standing hypothesis of *P. falciparum* gametocyte sequestration in skin tissue in two populations of naturally infected individuals in Burkina Faso. By combining mosquito feeding assays and direct quantification of parasite populations in skin tissue, mosquito blood meals and blood compartments, we conclude that there is no evidence for significant skin sequestration of mature gametocytes.

Parasite sequestration in skin tissue is an intuitive explanation for how vector-borne parasites can maximize the likelihood of update by blood-feeding insects. This phenomenon, well demonstrated for a range of helminths [8-11] and protozoic trypanosomes [7], has

remained speculative for *Plasmodium* parasites [15]. Two recent studies in Cameroonian parasite carriers that used microscopy as diagnostic tool yielded conflicting results: one observed higher *P. falciparum* parasite prevalence in finger prick capillary blood compared to venous blood from hospital patients [34], the other found no differences for asexual parasites or gametocytes in gametocyte carriers [35]. The utility of finger prick blood to estimate parasite biomass in skin tissue is uncertain. Studies published in the 1940s and 50s reported superiority of skin scarification as compared to finger prick blood samples for parasite detection [12, 13, 36]. In the most extensive of these studies, in 1243 natural infections, 1 cm^2 skin of the scapular region was very slightly scarified with 4-5 light incisions, expressing a mixture of dermal fluids and capillary blood, with the first drop appearing richest in parasites [13]. This study demonstrated a 10-20% increase in prevalence of asexual parasites and gametocytes of *P. vivax, P. malariae and P. falciparum* but not *P. ovale.* Also parasite density, expressed as parasites per 15,000 examined white blood cells, appeared increased [13]. In the current study, we therefore not only collected venous blood and finger prick blood but we also directly quantified parasite stage composition in skin tissue of naturally infected donors and in blood meals of mosquitoes that naturally fed on the skin of the corresponding donor. We used a punch of 4 mm in diameter to get a cylindrical core of tissue extending through the epidermis and down into the subcutaneous adipose tissue. This core represents an ideal tissue section for our purposes [15] as was taken from two localities commonly used in direct skin feeding experiments [23]. We used the absolute quantity of gametocytes and the fraction of the total parasite biomass that is gametocyte as indicators of sequestration. In skin biopsy samples, we only sporadically encountered gametocytes by histology. We chose a fluorescence imaging protocol to image thick sections by confocal microscopy. This method allowed capturing of entire parasites and three-dimensional reconstruction of parasite and surrounding tissues. Using Pfs16 labelling we classified gametocytes by crescent shape, three-

dimensional structure (as opposed to non-specific speckles and autofluorescence, which is an inherent issue of this approach), nuclear stain and presence of a surrounding red blood cell. The frequency of immunofluorescence-detected gametocytes in our tissue samples was lower than that by molecular methods in a tissue sample taken during the same visit. The quality of the skin tissue, tested by analysing the tissue sections by haematoxylin and eosin staining, as well as by labelling for endothelial cells, clearly indicates they were processed and preserved well.

In contrast, molecular detection of gametocytes was successful for all tissue samples by qRT-PCR and for the majority of samples by Nanostring. Because the volume of blood is unknown in tissue samples and specifically gametocytes are hypothesized to be enriched in skin tissue [12, 13, 15], we compared the gametocyte fraction between different blood compartments and found no evidence for a biased gametocyte fraction. Gametocyte quantification in mosquito blood meals corroborated this finding and allowed a direct comparison of parasite densities. Again, we observed no evidence for higher concentrations of gametocytes in mosquitoes that fed directly on the skin of gametocyte donors compared to venous blood; estimated parasite densities were in fact higher in blood meals of mosquitoes that fed on venous blood. The reason for this is unclear an may be related to differences in mosquito blood meal volume. We observed a very strong association between gametocyte fractions from the different blood compartments. Although the sequestered parasite biomass may contain more mature parasite forms, our markers are specific of ring-stage parasites [24] or mature gametocytes with stable expression levels over time [26].

In the absence of clear evidence for skin sequestration, there must therefore be alternative explanations for the higher infection rates that we, in line with other studies [4, 5], observed in direct skin feeding experiments. Gametocyte activation may occur following phlebotomy and may reduce infection rates observed following membrane feeding. In

addition, anticoagulants used in phlebotomy can have a pronounced effect on mosquito infection rates [37]. Although heparin is the preferred anticoagulant [37], it may still have a disadvantageous impact on sporogonic development. In malaria-naïve individuals in whom *P. falciparum* gametocytes were induced during controlled human malaria infection studies, replacement of heparin plasma by serum resulted in increased mosquito infection rates [5]. Since human immune responses are unlikely to be of relevance in these gametocytaemic volunteers, this observation provides additional indirect evidence for a transmission modulatory effect of heparin.

We observed no evidence for gametocyte sequestration in skin tissue. Our findings argue against a long-standing hypothesis that never had a proposed mechanism. Since the deformability of erythrocytes infected with mature gametocytes is similar to that of uninfected erythrocytes [16, 38] and there is no evidence for antigens on the surface of mature gametocyte-infected erythrocytes [39, 40], it is perhaps unsurprising that gametocyte concentrations are similar in the different blood compartments. While direct skin-feeding assays tend to result in higher infectivity compared that observed in indirect feeding procedures using venous blood, our data demonstrate that any differences observed are based on technical rather than biological differences in the feeding procedure. Our findings also indicate that gametocyte levels in venous or finger prick blood can be used to predict onward transmission potential to mosquitoes. Our findings thus pave the way for methodologies to quantify the human infectious reservoir based on conventional blood sampling approaches to support the deployment and monitoring of malaria elimination efforts for maximum public health impact.

Supplemental Information

Supplemental Methods

Detailed methods for skin tissue processing and Nanostring nCounter technology.

Supplemental Figure S1. Bland-Altman plots (difference plots) for the density of parasites in mosquito blood meals. Presented is the average parasite density in mosquito blood meals taken from skin tissue by direct skin feeding or venous blood by membrane feeding (X-axis) versus the difference between the two estimates (Y-axis). Positive values on the Y-axis indicate higher parasite densities in skin-fed mosquitoes; negative values indicate lower densities in skin-fed mosquitoes. Colours indicate ring-stage asexual parasites by *sbp1* qRT-PCR (green), female gametocytes by *Pfs25* qRT-PCR (red) and male gametocytes by *PfMGET* qRT-PCR (blue). Dashed lines indicate the mean difference and 95% limits of agreement. For ring-stage parasites, there was strong evidence that correlation coefficient between the paired differences and means differed significantly from zero (Pitman's Test of difference in variance, r = -0.923, p < 0.001); for male (p = 0.251) and female gametocytes (p = 0.559) this was not observed.

Supplemental Figure S2. Correlation between gametocyte fraction in different blood compartments. Presented is the fraction of the total parasite population that is gametocyte in venous blood (X-axis) versus on the Y-axis gametocyte fraction in finger prick capillary blood (red; Spearman ρ=0.970; p<0.0001), mosquitoes that fed directly on the skin (green; Spearman ρ= 0.916; p<0.0001), mosquitoes that fed on venous blood (green; Spearman ρ=0.912; p<0.0001).

Supplementary movie 1 (3D movie): **3D projection of Z-stack of mature gametocyte in skin snip.** This movie shows the 3D reconstruction of the z-stack (step-size 0.2 micron) to illustrate the localisation of a mature gametocyte. The gametocyte is stained with Pfs16

(magenta), denoted by DAPI staining (cyan), and within an RBC (yellow. It is in close proximity to skin vasculature. The movie was generated using Image J software.

Supplementary movie 2 (Z stack): Z-stack of mature gametocyte in skin snip. Confocal z-stack of mature gametocyte taken across the whole thickness of the section (step-size 0.2 micron). Gametocyte stained with Pfs16 (magenta), with DAPI (cyan) nuclear staining. The movie was generated using Image J software.

Data availability

Data underlying this manuscript are available through https://datadryad.org/stash/share/_Di1z3S3jl2ahewKXHAXHfAtl7slSBGNAZmgueslqbI.

Conflict of interest statement

Authors report no conflicts of interest.

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TABLES

Table 1: Baseline characteristics of the study populations.

	Skin biopsy sampling	Paired skin and membrane feeding assays
N	9	31
Symptomatic status	asymptomatic	asymptomatic
Median age (interquartile range)	34.7 (30.7-36.4)	28.9 (18. 7-37.7)
Female sex % (n/N)	33.33(3/9)	51.6 (16/31)
Hemoglobin level median g/dL (interquartile range)	11.8(11.3-12.4)	14.2 (13.3-15.6)
Self-reported bednet use, % (n/N)	100 (9/9)	80.6 (25/31)

FIGURE LEGENDS

Figure 1. The density and infectivity of gametocytes in different blood compartments. A. Gametocyte density by qRT-PCR in venous blood in association with the proportion of mosquitoes that become infected when feeding directly on the skin of the blood donor (blue) or on venous blood offered through an artificial membrane feeder (red). Size indicates the number of examined mosquitoes; error bars indicate the 95% confidence interval around the proportion of infected mosquitoes. **B.** The density of ring stage asexual parasites (green), male gametocytes (blue) and female gametocytes (red) by qRT-PCR in mosquito blood meals when feeding directly on the skin (X-axis) versus venous blood offered through an artificial membrane feeder (Y-axis). Error bars indicate the standard error of density estimates in pools of mosquitoes fed directly on the skin (median 3 pools) or venous blood (median 4 pools). **C.** The fraction of the total parasite biomass that is gametocyte in finger prick capillary blood (red), mosquitoes that fed directly on the skin (green), mosquitoes that fed on venous blood (blue) or venous blood (purple). The box plot indicates median, interquartile range and range; dots indicate individual samples. **D.** Bland-Altman plots (difference plots) for the gametocyte fraction in different blood compartments. Red symbols indicate the difference in gametocyte fraction in capillary blood versus venous blood (Y-axis) in relation to average fraction in these two compartments (X-axis). Positive values indicate a higher gametocyte fraction in capillary blood compared to venous blood; dotted lines indicate 95% limits of agreement. Green symbols represent agreement in gametocyte fraction measured in blood meals from mosquitoes that fed directly on skin tissue versus venous blood; blue symbols represent agreement in gametocyte fraction in blood meals from mosquitoes that fed on venous blood versus measurements directly in venous blood.

Figure 2. qRT-PCR and Nanostring comparison of parasite densities in skin biopsy samples and blood samples. A. Bland-Altman plots (difference plots) for the gametocyte fraction in different tissue and blood compartments. Red symbols indicate the difference in gametocyte fraction in capillary blood versus venous blood (Y-axis) in relation to average fraction in these two compartments (X-axis). Positive values indicate a higher gametocyte fraction in capillary blood compared to venous blood; dotted lines indicate 95% limits of agreement. Green symbols represent agreement in gametocyte fraction measured in tissue samples from the leg versus venous blood; blue symbols represent agreement in gametocyte fraction in tissue samples from the arm versus measurements directly in venous blood. **B-D.** Relative numbers of asexual parasites and gametocytes in skin tissue from the arm, skin tissue from the leg, finger prick and venous blood based on qRT-PCR (**B**) and Nanostring (**C**). Nanostring data were normalized on the basis of background subtraction and expression of housekeeping genes. **D.** Correlation between estimates of ring-stage asexual parasites by *sbp1* and female gametocytes by *Pfs25* for qRT-PCR (X-axis) and Nanostring (Y-axis) showing good agreement but higher sensitivity of qRT-PCR.

Figure 3. Histological analysis of skin samples. A. 10µm cross section of a skin snip from leg with dimensions indicated. Sample was stained with CD31 and DAPI and a maximum projection across the depth of the section is shown. The insert represents a small section including several vessels stained with CD31. Scale bar = 500µm, insert = 10µm. **B.** 3µm section of a skin snip from arm stained with haematoxylin and eosin. Sections in A and B show the different layers of the epidermis on top, followed by the dermis with multiple vessels. **C.** Samples were stained with DAPI (cyan) and Pfs16 (magenta) for gametocytes. Representative images of asexual parasite (left), an immature (middle) and mature (right)

gametocyte images from control blood clots. Scale bar = 10μm. **D.** Representative image of a gametocyte in skin samples from arm. DAPI staining indicates several vessels in the vicinity of a gametocyte stained with Pfs16. XZ and YZ orientations are included to demonstrate the three-dimensional nature of the tissue section and the gametocyte. Scale bar = 10μm.

Figure 1

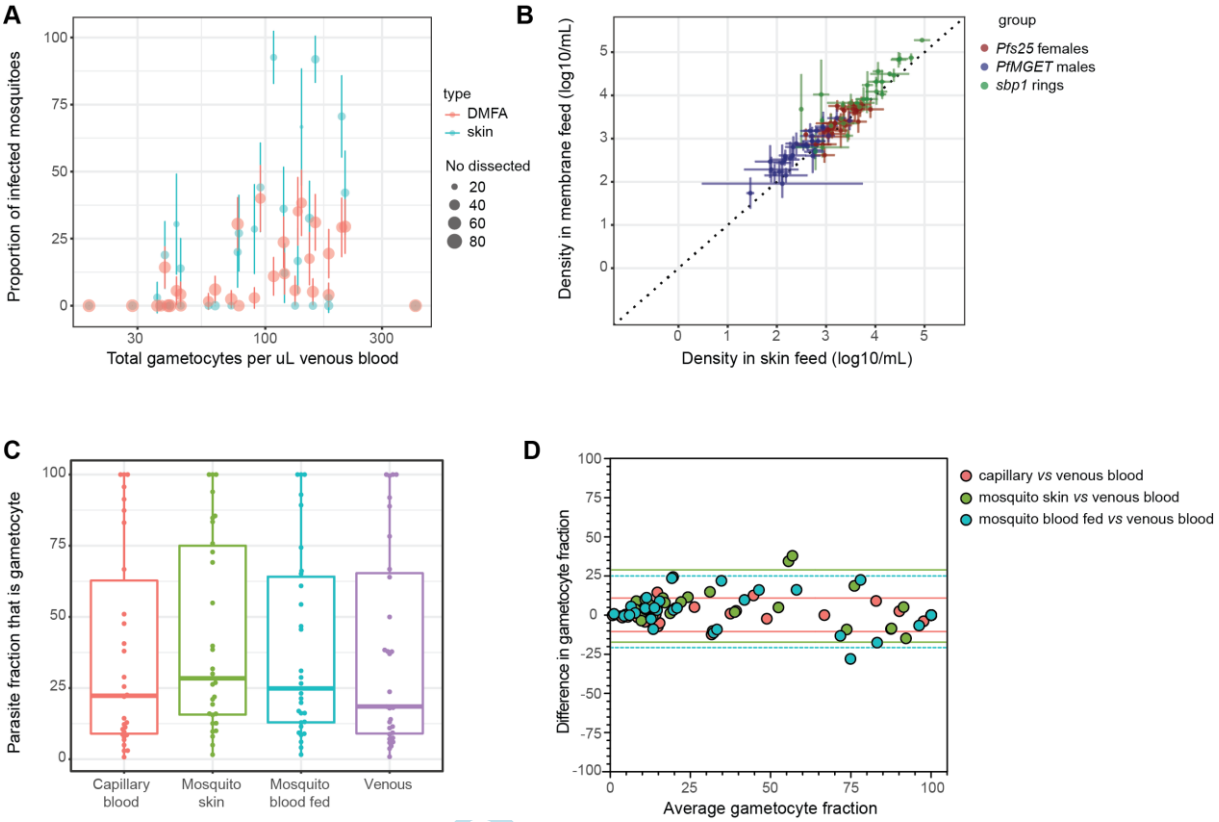

Figure 2

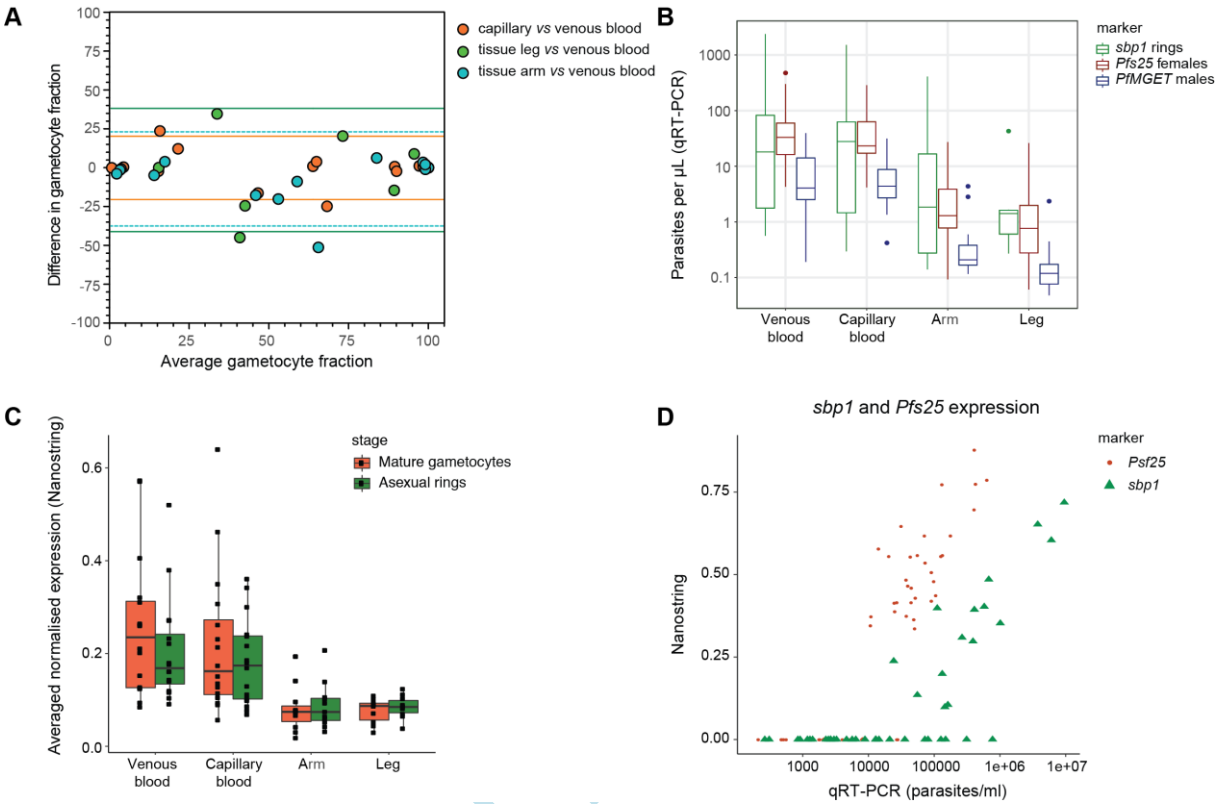

Figure 3