Lipid metabolism of *Aedes aegypti* females following blood feeding reveals similar changes in functionally related lipids

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Abstract

We conducted lipidomic analyses of the whole body of female *Aedes aegypti* mosquitoes at different time points over the course of feeding and reproduction. There were temporal

biphasic increases of most lipids. The first peak appeared at the time of feeding and then the abundance of many lipids dropped while body weight remained stable probably reflecting blood lipid digestion and the synthesis of vitellogenin in this period. Lipid amount increased at 30 hours post blood meal (a second peak) mostly representing biosynthesis by mosquitoes to support reproduction, and it was followed by a further decrease when approaching 120 hours after feeding. A concerted temporal pattern was particularly strong for signalling lipids, many of which increased more than ten-fold at the second peak. A different pattern emerged for lyso-glycerophospholipids when they were considered as a group, and further investigations found differences within this group were strongly functional related. Our study on the anautogenous insect *Ae. aegypti* helps in comparing the abundance of specific lipids between mosquitoes and human blood, and the results point to specific lipids likely to be important in the reproductive process with a role in the formation and growth of ovarian follicles.

Keywords

Lipidome; *Aedes aegypti*; reproduction; blood feeding

Introduction

The yellow fever mosquito, *Aedes aegypti*, is one of the major vectors that transmits arbovirus diseases in tropical and subtropical areas, such as dengue and Zika (Bhatt et al., 2013; Brown et al., 2014; Gould and Higgs, 2009). *Ae. aegypti* females are anautogenous: they must ingest human blood to obtain unique nutrients to produce their eggs, and diseases are transmitted through the blood ingestion process (Gulia-Nuss et al., 2015; Scott et al., 1997).

Reproduction in mosquito females is generally regulated through the provision of nutrition and the modulation of hormones (Attardo et al., 2005). Essential nutrients such as amino acids and cholesterol mainly come from human blood (Geoghegan et al., 2017; Hansen et al., 2004), and supports gene products involved in yolk development (Price et al., 2011). Apart from these changes of amino acids, cholesterol and yolk protein, the vittellogenic cycle is also expected to involve lipid metabolisms; lipids not only act as major energy resources in supporting egg development (Ziegler and Ibrahim, 2001), but also contribute 35% of ovarian dry weight (Briegel et al., 2002; Troy et al., 1975).

The development of ovaries can be separated into the pre-vitellogenic (before blood meal, BBM) and vitellogenic (post blood meal, PBM) stages depending on the timing of the blood meal. In the pre-vitellogenic stages, lipids are reserved in the fat body (Ziegler and Ibrahim, 2001), while during the vitellogenic stages, consumption of blood meal triggers insulin-like peptide 3 (ILP3) from the brain to stimulate oogenesis (Brown et al., 2008). The secretion of 20-hydroxyecdysone then increases the expression of vitellogenin genes in the fat body to support yolk protein accumulation and ovarian follicle development (Belles and Piulachs, 2015; Hansen et al., 2014), which is stimulated by gonadotropin (Klowden, 1997), and lipids are transferred from the fat body to follicles (Ziegler and Ibrahim, 2001). In the meantime, *Ae. aegypti* females also digest and absorb essential amino acids from human blood, where the concentration of amino acids increasing in haemolymph acts as a signal to activate egg development (Uchida et al., 1990) through the TOR pathway (Hansen et al., 2004).

Lipid reservation is essential to support *Ae. aegypti* reproduction (Zhou et al., 2004b), particularly when a sugar source is compromised. Starving female mosquitoes before or after their blood feeding will not reduce their fecundity but will significantly reduce the amount of lipids in mothers after laying eggs (Zhou et al., 2004a), suggesting a trade-off between reproduction and female survival. The source and the quality of blood that females are fed

can directly impact their fertility (Phasomkusolsil et al., 2013; Ross et al., 2019), reflecting variation in the essential nutrients obtained from human blood. Variation in nutritional quality cannot be fully explained based on our current understanding of amino acids and cholesterol (Caragata et al., 2013; Caragata et al., 2014), though several artificial blood substitute diets have been developed that rely on bovine proteins or blood (Dutra et al., 2017; Talyuli et al., 2015).

In this study, we used liquid chromatography coupled with the mass spectrometry (LCMS)-based lipidomic method and a comprehensive lipid standard mixture covering near all major lipid classes to identify and analyse lipid components of whole female individuals before the blood meal and post blood meal. Our study aims to understand lipid metabolism in *Ae. aegypti* during the reproductive process, how lipids support the development of eggs and to provide support for future studies about nutritional competition between *Ae. aegypti* and its parasites and symbionts.

Material and methods

Sample preparation

Aedes aegypti mosquitoes in this experiment were collected from *Wolbachia*-uninfected populations from Cairns, QLD, Australia and maintained in laboratory conditions. Samples were prepared by hatching *Ae. aegypti* eggs stored in an incubator (PG50 Plant Growth Chambers, Labec Laboratory Equipment, Marrickville, NSW, Australia) at 22-30 ± 1 °C, with a 12 h : 12 h photoperiod. Larvae were reared in reverse osmosis (RO) with yeast and fed with Hikari Sinking Wafers fish food tablets until adult. All female mosquitoes were blood fed by the same volunteer's forearm. The first blood meal was provided to mosquito females at 4 ± 1 days post-emergence. Females were isolated individually and observed for seven days to ensure all have similar body size, are able to take blood and to lay enough eggs

(>50). At the seventh day, a second blood meal was provided. Non- engorged individuals were removed from the experiment. Samples were then stored as different groups in their second vitellogenic cycle BBM and at different time PBM: 0 h PBM (just after blood meal), 4 h PBM, 16 h PBM, 30 h PBM, 72 h PBM, and 120 h PBM. Sugar and oviposition cups were provided during the entire period of the experiment.

Female mosquito measurement

Apart from analysis of the lipidome, we measured the weight of different female individuals (four to seven) at each time point from the additional collected samples using a Sartorius Analytical balance BP 210 D (Sartorius, Gottigen, Germany, Readability: 0.01 mg). After weighing, eight mosquitoes were randomly selected from all time points and their wing lengths were measured to indicate female body size (Briegel, 1990). To further understand the development of female ovaries, we hatched batches of mosquito eggs and took photos of ovaries using an NIS Elements BR imaging microscope (Nikon Instruments, Tokyo, Japan) every day PBM.

Lipid extraction

For each group, we extracted the whole body of six mosquito females, keeping individuals separate. We added 10 µL of Mouse SPLASH® LIPIDOMIX® Mass Spec Standard (cat. 330707, Avanti) after 1 in 4 dilution to each sample prior the extraction and then homogenised samples in 200 µL ice-cold 60% methanol (MeOH) containing 0.01% (w/v) butylated hydroxytoluene (BHT) by using TissueLyserII (Qiagen, Hilden, Germany) at 25 Hertz for one minute and incubating for 20 minutes in an ultrasonic bath (Soniclean Pty Ltd., Thebarton, Australia). Following the method previously reported by Lydic et al. (2015), 120 µL of MilliQ water, 420 µl of MeOH with 0.01% (w/v) BHT, and 260 µL of chloroform

($CHCl_3$) were added to each tube and vortexed thoroughly before incubating in a Bioshake iQ (Quantifoil Instrument GmbH, Germany) setting at 1,400 rpm for 30 minutes and centrifuged at 14,000 rpm for 15 minutes. Supernatant containing lipids was transferred to a new 2 mL tube. The remaining sediments were re-extracted with 100 µL of MilliQ water and 400 µL of $CHCl_3$: MeOH (1:2, v:v) containing 0.01% (w/v) BHT as described above. Supernatants from the first and second extraction were pooled in the same 2 mL tube and then dried by evaporation under vacuum using a GeneVac miVac sample concentrator (SP Scientific, Warminster, PA, USA). Before HPLC, dried lipid extracts were resuspended in 50 µL of $CHCl_3$: MeOH (1:9, v:v, containing 0.01% BHT).

Mass spectrometry analyses

Samples were analysed by ultrahigh performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) employing a Vanquish UHPLC linked to an Orbitrap Fusion Lumos mass spectrometer (Thermo Fisher Scientific), with separate runs in positive and negative ion polarity. Solvent A was 6/4 (v/v) acetonitrile/water with 10 mM ammonium acetate and 5 µM medronic acid and solvent B was 9/1 (v/v) isopropanol/acetonitrile with 10 mM ammonium acetate. Each sample was injected into an RRHD Eclipse Plus C18 column (2.1 × 100 mm, 1.8 µm, Agilent Technologies) at 50°C at a flow rate of 350 µl/min for 3 min using 3% solvent B. During separation, the percentage of solvent B was increased from 3% to 70% in 5 min, from 70% to 99% in 16 min, from 99% to 99% in 3 min, from 99% to 3% in 0.1 min and maintained at 3% for 3.9 min.

All experiments were performed using a Heated Electrospray Ionization source. The spray voltages were 3.5 kV in positive ionisation mode and 3.0 kV in negative ionisation mode. In both polarities, the flow rates of sheath, auxiliary and sweep gases were 20 and 6 and 1 ‘arbitrary’ unit(s), respectively. The ion transfer tube and vaporizer temperatures were

maintained at 350 °C and 400 °C, respectively, and the ion funnel RF level was set at 50%. In both positive and negative ionisation modes from 3 to 24 min, top speed data-dependent scan with a cycle time of 1 s was used. Within each cycle, a full-scan MS spectrum was acquired firstly in the Orbitrap at a mass resolving power of 120,000 (at m/z 200) across a m/z range of 300–2000 using quadrupole isolation, an automatic gain control (AGC) target of 4e5 and a maximum injection time of 50 milliseconds, followed by higher-energy collisional dissociation (HCD)-MS/MS at a mass resolving power of 15,000 (at m/z 200), a normalised collision energy (NCE) of 27% at positive mode and 30% at negative mode, an m/z isolation window of 1, a maximum injection time of 22 milliseconds and an AGC target of 5e4. For the improved structural characterisation of glycerophosphocholine (PC) in positive mode, a data-dependent product ion (m/z 184.0733)-triggered collision-induced dissociation (CID)-MS/MS scan was performed in the cycle using a q-value of 0.25 and a NCE of 30%, with other settings being the same as that for HCD-MS/MS. For the improved structural characterisation of triacylglycerol (TG) lipid in positive mode, the fatty acid + NH3 neutral loss product ions observed by HCD-MS/MS were used to trigger the acquisition of the top-3 data-dependent CID-MS3 scans in the cycle using a q-value of 0.25 and a NCE of 30%, with other settings being the same as that for HCD-MS/MS.

Lipid identification and quantification

LC-MS/MS data was searched through MS Dial 4.80 (Tsugawa et al., 2015; Tsugawa et al., 2020). The mass accuracy settings are 0.005 Da and 0.025 Da for MS1 and MS2. The minimum peak height is 50000 and mass slice width is 0.05 Da. The identification score cut off is 80%. Post identification was done with a text file containing name and m/z of each standard in Mouse SPLASH® LIPIDOMIX® Mass Spec Standard. In positive mode, $[M+H]^+$ and $[M+NH4]^+$ were selected as ion forms. In negative mode, $[M-H]^-$ were selected

as ion forms. All lipid classes available were selected for the search. The retention time tolerance for alignment is 0.1 min. Lipids with maximum intensity less than 5-fold of average intensity in blank were removed. All other settings were default. All lipid LC-MS features were manually inspected and re-integrated when needed. We also removed four other types of lipids: (1) lipids with only sum composition except PC and SM; (2) lipids identified due to peak tailing; (3) retention time outliers within each lipid class; and (4) LPA and PA artifacts generated by in-source fragmentation of LPS and PS. The shorthand notation used for lipid classification and structural representation follows the nomenclature proposed previously (Liebisch et al., 2020).

Relative quantification of lipid species was achieved by comparison of the LC peak areas of identified lipids against peak areas and quantities of the corresponding internal lipid standards in the same or similar lipid class (Table 1), and the concentrations of all lipids were reported at nmol/individual. Given that the commercially available stable isotope-labelled lipid standards are limited, some of the identified lipids were normalised against a standard from a different class or sub-class, and no attempts were made to quantitatively correct for different ESI responses of individual lipids due to concentration, acyl chain length, degree of unsaturation, or matrix effects caused by differences in chromatographic retention times compared with the relevant standards. The results reported here are for relative quantification and should not be considered to reflect the absolute concentrations of each lipid or lipid sub-class.

Results

Female body weight and wing length measurement

Aedes aegypti female mosquitoes were stored for lipidomic study in their second vitellogenic cycle before their blood meal (BBM) and at different time post blood meal (PBM): (0 h (just

after blood meal), 4 h, 16 h, 30 h, 72 h, and 120 h). We first weighed female mosquitoes from samples collected at each time point (Figure 1), with the difference between BBM and 0 h PBM representing the amount of blood a female engorged. Sampling time had a significant effect on female weight (ANOVA, $F_{7,41} = 13.06$, $P < 0.001$). The weights of engorged female mosquitoes were approximately double their weights BBM. The females then started to digest blood, and their weight dropped significantly from 0 h PBM to 4 h PBM, thereafter stabilising until 30 h PBM, and then dropping again further to the BBM level at 72 h PBM; this change in weight reflects the process of blood digestion, which takes around 32 hours after females are engorged (Mukabana et al., 2002; Oshaghi et al., 2006). The appearance of female ovaries was captured every day under a microscope (Supporting information 1). We also calculated the coefficient of variation (CV, CV = (Standard Deviation/Mean) × 100) for female wing length for the eight samples we measured (mean ± se = 2.07 ± 0.06 mm, CV = 8.71) to indicate the spread of mosquito body sizes in this study.

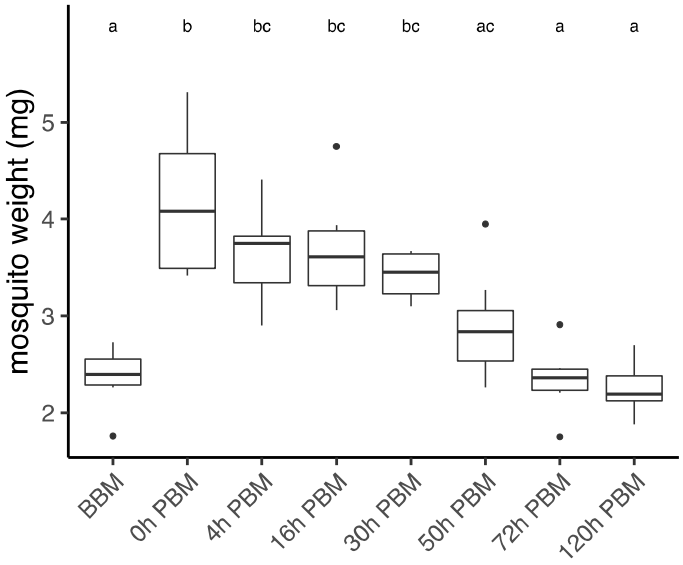

Figure 1. **Weights [illegible] over the course of feeding and reproduction**. Weights were measured at eight time points before blood meal (BBM) and post blood meal (PBM). The same letter at the top of the boxplots represents groups that are not significantly different following Tukey HSD post-hoc tests.

Lipids identification and their changes during female reproductive process

We measured lipidomic changes in individual females BBM and at different time points PBM, covering the entire vitellogenic cycle. In total, we identified 456 lipids (Table 1). We divided lipids into six functionally relevant groups: glycerolipids, diacyl-glycerophospholipids, lyso-glycerophospholipids, Cardiolipin (CL), Carnitine (CAR) and sphingolipids. The most abundant lipid group in female mosquitoes was the neutral glycerolipids, with 55 molecules making up approximately 80% of the total lipid amount. Glycerophospholipids accounted for 55% of the molecules identified in this group, with 193 diacyl-glycerophospholipids and 48 lyso-glycerophospholipids. We also identified 66 CL, 15 CAR and 69 sphingolipids.

Table 1. List of lipid standards, adduct type and number of lipids that were identified in each class.

Name (Abbreviation)	Standard	Adduct type	Number of lipids identified
Diacylglycerol (DG)	15:0-18:1(d7) DAG	$[M+NH_4]^+$	14
Triglycerides (TG)	15:0-18:1(d7)-15:0 TAG	$[M+NH_4]^+$	41
Phosphatidylethanolamines (PE)	15:0-18:1(d7) PE	$[M-H]^-$	43
Ether-linked PE (EtherPE)	15:0-18:1(d7) PE	$[M-H]^-$	18
Oxidized PE (OxPE)	15:0-18:1(d7) PE	$[M-H]^-$	8
Phosphatidylinositol (PI)	15:0-18:1(d7) PI	$[M-H]^-$	33
Ether-linked PI (EtherPI)	15:0-18:1(d7) PI	$[M-H]^-$	3
Phosphatidylserine (PS)	15:0-18:1(d7) PS	$[M-H]^-$	10
Phosphatidic acids (PA)	15:0_18:1(d7) PA	$[M-H]^-$	5

Phosphatidylglycerol (PG)	15:0_18:1(d7) PG	$[M-H]^-$	24
Phosphatidylcholines (PC)	15:0_18:1(d7) PC	$[M+H]^+$	52
Ether-linked PC (EtherPC)	15:0_18:1(d7) PC	$[M+H]^+$	7
Lyso-PE (LPE)	18:1(d7) Lyso PE	$[M-H]^-$	13
Ether-linked lyso-PE (EtherLPE)	18:1(d7) Lyso PE	$[M-H]^-$	2
Lyso-PI (LPI)	15:0-18:1(d7) PI	$[M-H]^-$	7
Lyso-PS (LPS)	15:0-18:1(d7) PS	$[M-H]^-$	6
Lyso-PA (LPA)	15:0_18:1(d7) PA	$[M-H]^-$	4
Lyso-PG (LPG)	15:0_18:1(d7) PG	$[M-H]^-$	7
Lyso-PC (LPC)	18:1(d7) Lyso PC	$[M+H]^+$	9
Cardiolipin (CL)	15:0_18:1(d7) PG	$[M-H]^-$	66
Carnitine (CAR)	15:0_18:1(d7) PC	$[M+H]^+$	15
Sphingomyelin (SM)	d18:1-18:1(d9) SM	$[M+H]^+$	26
Ceramides (Cer)	d18:1-18:1(d9) SM	$[M-H]^-$	23
Hexosylceramides (HexCer)	d18:1-18:1(d9) SM	$[M-H]^-$	4
Sulfatides (SHexCer)	d18:1-18:1(d9) SM	$[M-H]^-$	16

In order to obtain an overview of lipidomic changes during the reproductive process, we first undertook a principal component analysis (PCA) (Figure 2). This indicated that lipid components of female mosquitoes went through two main changes: the first occurred immediately after a blood meal (0 h PBM), and the second change occurred between 16 and 30 h PBM. Between these periods, the amounts of lipid in most groups also changed, except for lyso-glycerophospholipids, in which case the total amount remained relatively stable during the reproduction process (Figure 3).

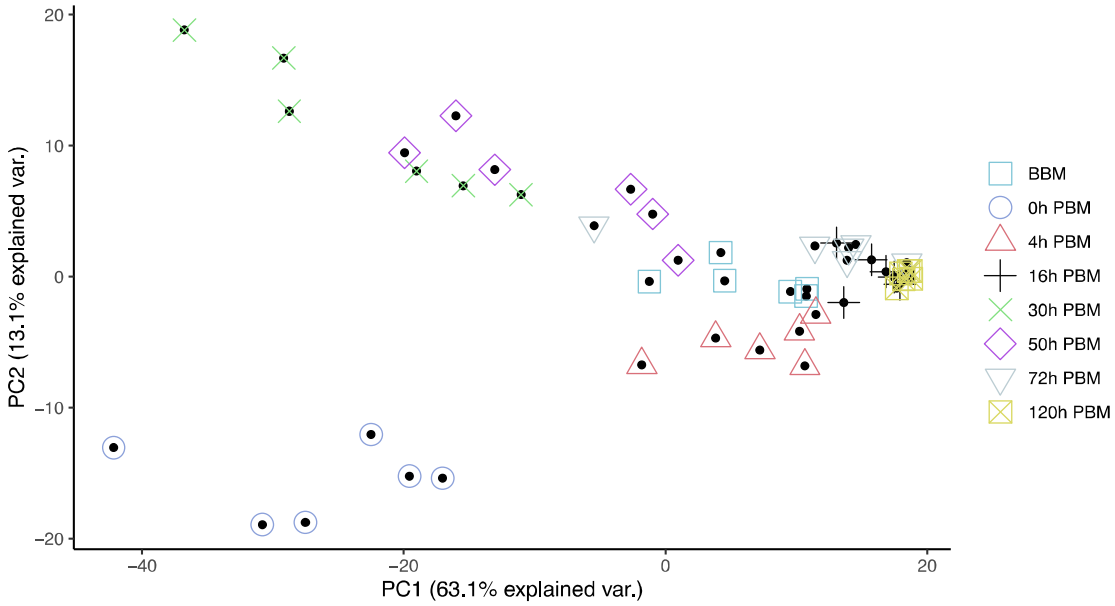

Figure 2. **Principal Component Analysis suggested lipidome differences of *Aedes aegypti* females at different time points over the course of feeding and reproduction.** Colour and shape symbols correspond to the eight time points before blood meal (BBM) and post blood meal (PBM). The first two principal axes explained 76 % of the variance.

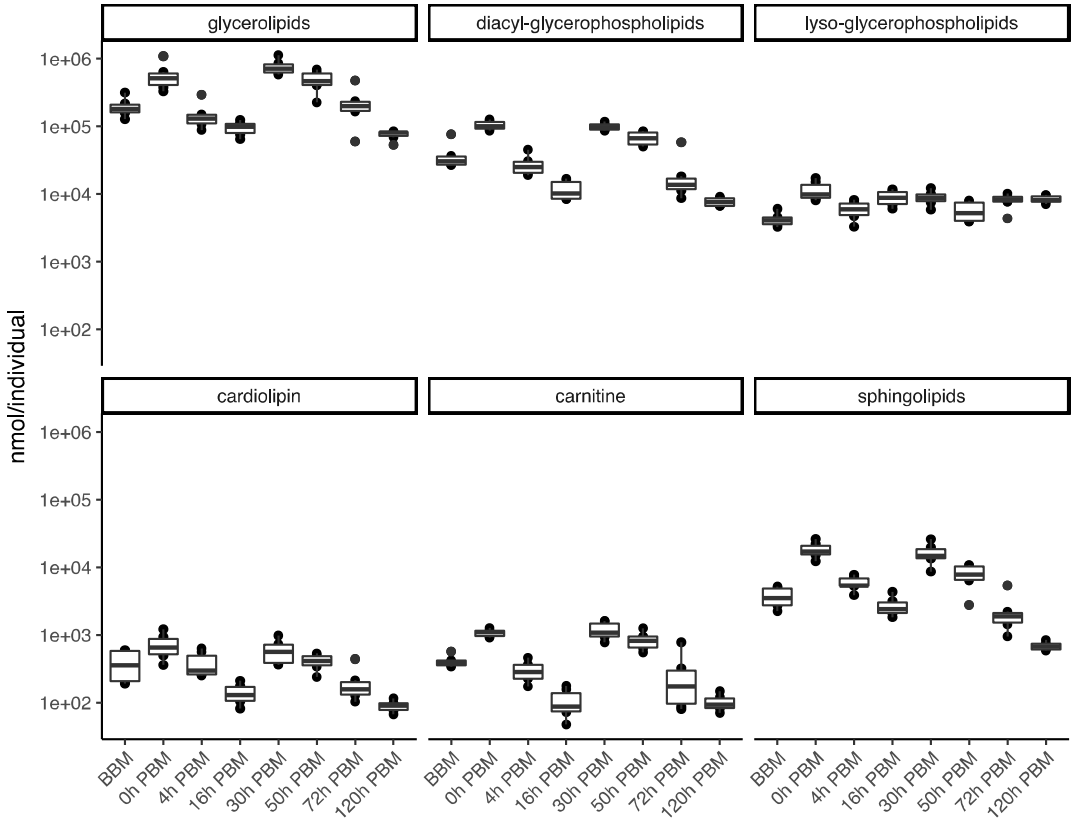

Figure 3. **Changes of lipid amounts in *Aedes aegypti* females over the course of feeding and reproduction.** The total amount (nmol/individual) of glycerolipids, diacyl-glycerophospholipids, lyso-glycerophospholipids, cardiolipins, carnitines and sphingolipids were measured at eight time points before blood meal (BBM) and post blood meal (PBM).

We then also did PCA analyses on specific groups of lipids (Figure 4); for most groups the first PCs explained more than half of the variation, with the exception of the lyso-glycerophospholipids where it only explained 31.8% of variation and there was no obvious change directly after blood feeding but there was a shift at a later time point, indicating that most changes in lyso-glycerophospholipid reflect mosquito metabolism rather than direct blood ingestion. However, all other lipid groups showed clear immediate changes in response to the blood meal as well as at a later time point.

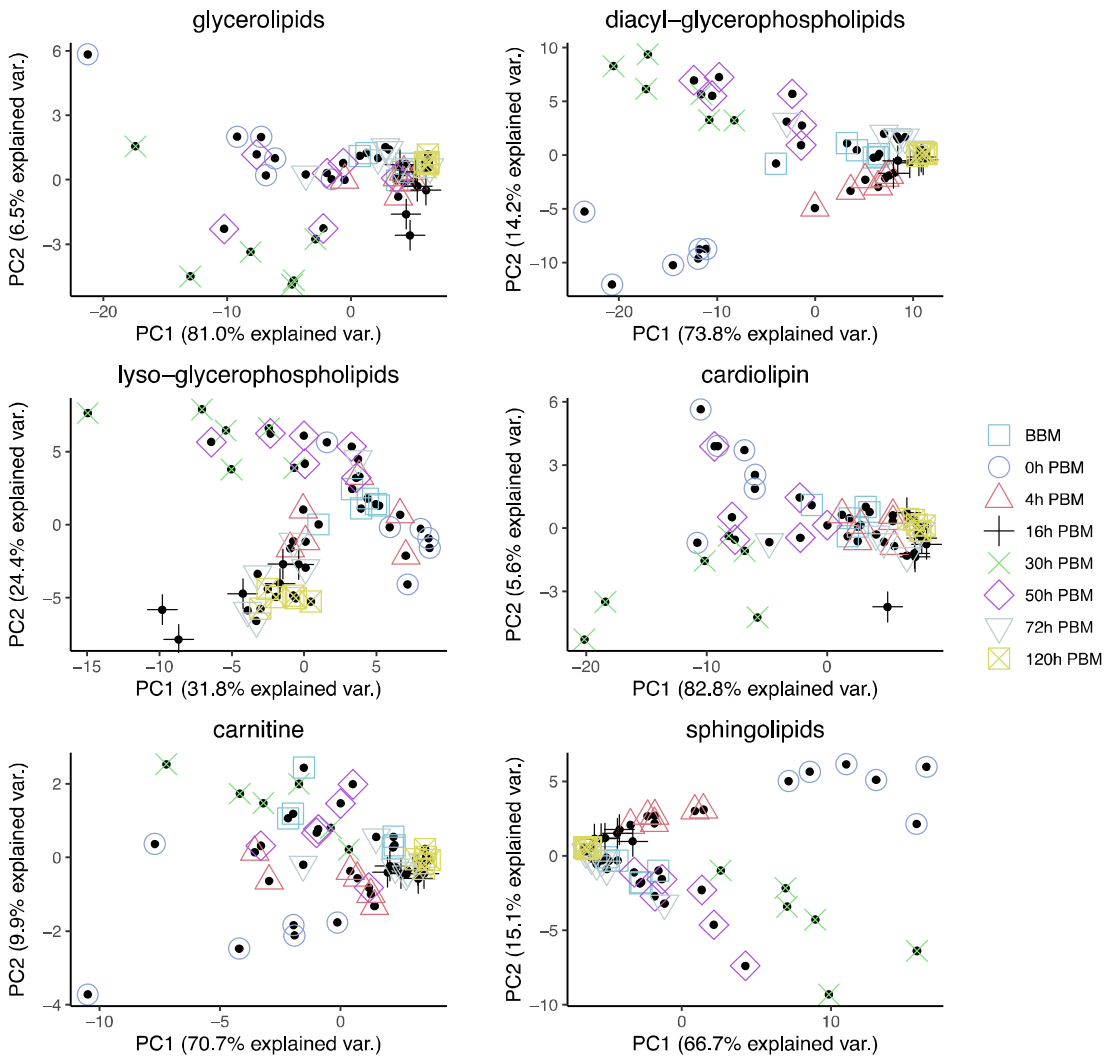

Figure 4. **Principal Component Analysis of different groups of lipids in *Aedes aegypti* females at different time points over the course of feeding and reproduction**. Colour and shape symbols correspond to the eight time points before blood meal (BBM) and post blood meal (PBM).

Identification of change patterns for specific lipids

The amount of blood a female mosquito takes in when fully engorged can be similar to the weight of the individual, or even nearly twice the individual's weight (Harrington et al., 2001;

Roy, 1936). We compared the time points BBM and 0 h PBM for individual lipid to determine which lipids were taken in with human blood. 86% of all the lipids identified significantly increased at the PBM monitoring time, while 14 (3%) of them significantly decreased and 50 (11%) did not change significantly (Supporting information 2). Specifically with reference to each lipid class, we obtained the following findings. (1) 75% (48 molecules) of lipids that did not increase are glycerophospholipids, and 29 are lyso-glycerophospholipids. The latter represent 60% of the lyso-glycerophospholipids identified, while the 19 diacyl-glycerophospholipids that did not increase only accounted for 10% of molecules in this class. (2) All ether-glycerophospholipids (EtherPE, EtherPI, EtherPC and EtherLPE) and PS increased significantly. (3) Tetradecasphingenine (C14) and hexadecasphingenine (C16) CAR did not change significantly, while most of the other carnitines (7 out of 11) increased only slightly (adjusted $0.05 > p > 0.01$); only one lipid (CAR 20:1) in this class increased highly significantly (adjusted $p < 0.001$). (4) Most glycerolipids, CL and sphingomyelins increased.

The second significant lipidomic changes happened during the period spanning from 16 to 50 h PBM, and we specifically considered the difference between 16 and 30 h PBM when most lipids increased (87.7%, 400 molecules). These changed lipids may be used by female mosquitoes for the growth of ovarian follicles. 12 lipid molecules (2.6%) decreased significantly, and 44 lipid molecules (9.6%) did not change significantly (Supporting information 3). Of those lipids that increased, 392 showed increases of at least twice from 16 to 30 h PBM. These lipids are expected to be particularly important in mosquito egg production. For each group, we noted the following. (1) 60.7% (34 molecules) of those that did not increase are lyso-glycerophospholipids (constituting 70.8% of lyso-glycerophospholipids molecules), including all LPC and LPE. Interestingly, all LPA identified increased more than ten-fold from 16 to 30 h PBM. (2) For diacyl-

glycerophospholipids, all EtherPI, OxPE, PA, PE, PG, PI and PS increased; 30 out of 43 PE and 18 out of 33 PI identified increased more than ten-fold. (3) All CAR and most glycerolipids, CL and sphingolipids increased. Specially, 42 out of 66 CL increased more than ten-fold and the four HexCer identified increased about ten-fold.

We found that the lyso-glycerophospholipids, representing a group composed of various sub-groups of lipids with different functions, had a different pattern of change in the above analyses. Specifically, the two most abundant lyso-glycerophospholipids LPE and LPC, which play an important role in membrane composition, were relatively stable in terms of total amount during reproduction. LPA showed a dramatic increase from 16 to 30 h PBM, a pattern similar to that of most lipids in other groups. And LPI, LPG and LPS only had one significant increase at time period from 0 to 16 h PBM, the period when most of lipids in other groups decreased.

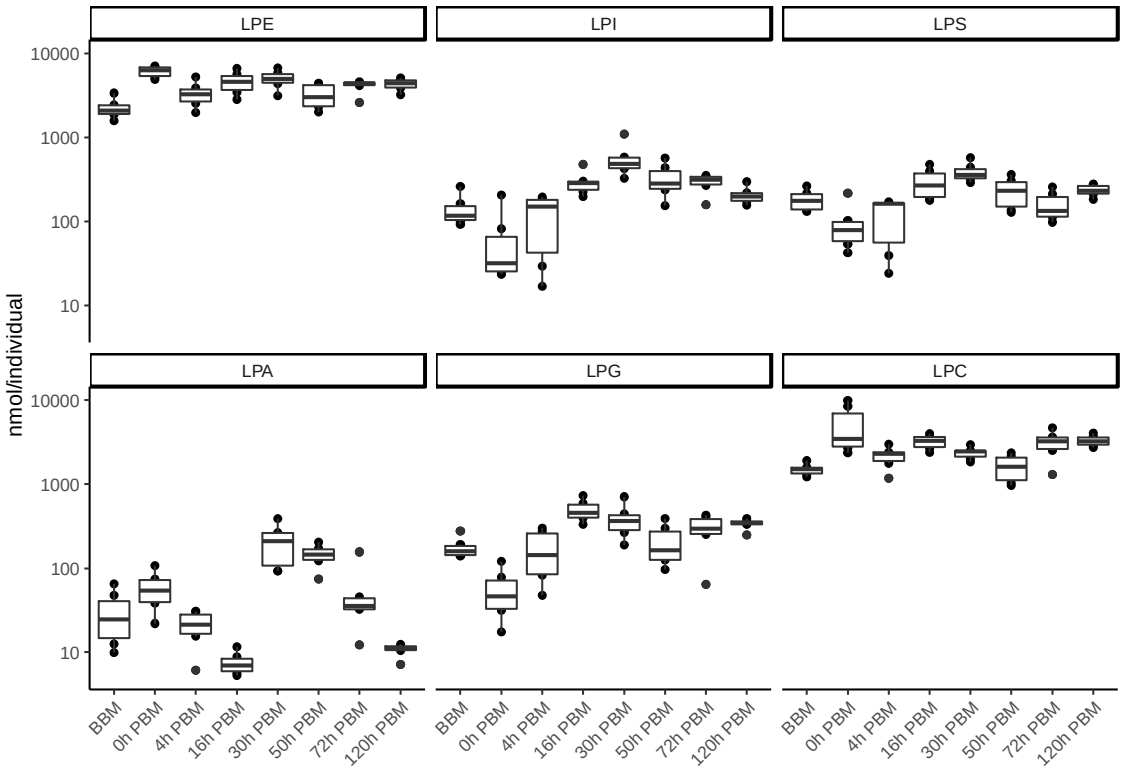

Figure 5. **Changes of lipid amounts in the class of lyso-glycerophospholipids in *Aedes aegypti* females over the course of feeding and reproduction.** The total amount

(nmol/individual) of LPE; LPI; LPS; LPA; LPG and LPC were measured at eight time points before blood meal (BBM) and post blood meal (PBM).

By comparing the differences between the two periods when most increases occurred, we found that most lipids we identified were present in both human blood and mosquitoes, and also being affected by mosquito ovarian follicle development. Nevertheless, some lipids seemed to be present at a stable level in mosquitoes and were not significantly increased after blood feeding, such as tetradecasphingenine (C14) and hexadecasphingenine (C16) CAR, and most of the LPG (Figure 5). Some lipids seemed to exist at a much higher abundance in human blood, which we define here as those that increased more than ten-fold after blood feeding but did not increase significantly (adjust $p > 0.05$) at the later period. Such as some Oxidized PE and Sphingomyelin.

Discussion

In the current study, we focused on lipidomic changes of whole female mosquitoes during the reproductive process. Whole-body investigations provide an overall picture of lipid changes that might not be evident when focusing only on organs or cells. We found biphasic increases in most lipids at 0 h PBM and 16 to 30 h PBM. The amount of lipids dropped between these periods of increases, as also observed in the fat body (Pinch et al., 2021). The weight of the mosquitoes did not decrease at the first time point when lipids decreased, suggesting that lipids were transformed into different metabolites, such as LPI, LPG and LPS that were observed to increase in this period. However, these only constituted a very small component of total lipids in *Ae. aegypti* females. Many metabolites can be produced within this period such as glycogen, and fatty acids (Hou et al., 2015; Toprak et al., 2020), but it is more likely that the decrease of lipids from 0 to 16 h PBM reflects the synthesis of vitellogenin in the fat

body, which is triggered by blood feeding. Previous studies found that the secretion of vitellogenin in the fat body reaches its highest peak at around 18 h PBM, and decreases to the previtellogenic level at around 30 h PBM (Hagedorn et al., 1973; Sun et al., 2000). Pinch et al. (2021) observed a relatively lower level of TG in the *Ae. aegypti* female fat body at around 30 h PBM, while our study of whole body lipidome showed highest level of TG at 30 h PBM. The discrepancy may reflect the transportation of lipids from the fat body or midgut into oocytes during the insect reproductive process through lipophorin, a major lipid-carrying protein (Kawooya and Law, 1988). *Ae. aegypti* and some other Diptera predominantly transport TG via lipophorin, not DG as is the case of most other insects (Arrese et al., 2001; Ford and Van Heusden, 1994; Pennington et al., 1996). Moreover, a general observation for insects is that the midgut can convert DG to TG in the presence of high concentrations of potentially toxic fatty acids; this conversion could maintain low intracellular levels of DG and fatty acids to prevent lipotoxicity (Canavoso et al., 2001; Canavoso and Wells, 2000; Toprak et al., 2020). Additionally, there are other possibilities for lipid movement not based on DG transport including evolutionarily conserved mechanisms for movement of fatty acids (Black and DiRusso, 2003).

Lyso-glycerophospholipids were highlighted as a lipid group of interest by the present study due to their unusual temporal pattern in abundance. Lyso-glycerophospholipids have diverse functions and they are also found in cell membranes (Rietschel et al., 1994); as precursor of phospholipid biosynthesis, they may act as signal molecules involved in a broad range of biological processes (Ishii et al., 2004; Tan et al., 2020). However, they might also reflect a highly regulated reservoir of lipids with accessible utility and concentration-dependent lipid toxicity liability (Arouri and Mouritsen, 2013), which can result from lipid overload generated by dietary excess (Levental et al., 2020) as might be the case when mosquitoes blood feed. LPE and LPC incorporate into membranes and change membrane permeability

and stability. The change in permeability and stability can result in membrane toxicity unless concentrations of lyso-glycerophospholipids are kept below a cytotoxic limit (Arouri and Mouritsen, 2013), which may explain the stability of these two lipid groups. Also, we found substantial increases in cell membrane lipids between 16 and 30 h PBM as in the cases of PE and PC, reflecting the growth of embryos during the reproductive process. Moreover, lyso-glycerophospholipids could act as a collective reserve which provides acyl chains for beta-oxidation when needed (Black and DiRusso, 2003), or they could be a source of partially assembled molecules for further biosynthesis, which may explain the unusual increase of LPI, LPG and LPS between 0 and 16 h PBM followed by increase of near all major lipid groups from 16 to 30 h PBM. It is worth noting that LPA, the only group from lyso-glycerophospholipids showing a bimodal pattern with increases from 16 to 30 h PBM, are essential signalling molecules (Luquain et al., 2003).

We also found that many lipids from groups PI and CL increased dramatically from 16 to 30 h PBM. These lipids are closely connected with metabolic activity and signalling. For example, PIs are important in regulating phototransduction, cell growth and the developmental process of fruit flies (Garcia-Murillas et al., 2006; Gupta et al., 2013; Milligan et al., 1997). And CLs are integral to mitochondrial membranes, with important roles in ATP-synthase and phospholipid remodelling (Acehan et al., 2011; Schlame and Ren, 2009). Moreover, SM play significant roles in mammal cells being involved in diverse signalling pathways (Hannun and Bell, 1989; Hannun and Obeid, 2008), whereas for dipteran insects such as *Drosophila*, there is a lack of SM and instead they synthesize the SM analogue phosphoethanolamine ceramide (Acharya and Acharya, 2005). Though we did not look into phosphoethanolamine ceramide in the present study, we also note that most SMs were either relatively constant or changed only slightly between 16 h and 30 h PBM.

Finally, it is worth noting that approximately a quarter of the lipids, including almost all SHexCer we identified, contained odd chains. Pinch et al. (2021) investigated the fat body of mosquitoes feeding on bovine blood and supposed that odd-chain lipids may come from ruminal microflora or microbes in larval rearing water or branched chain amino acids from haemoglobin. Our study showed that the last hypothesis is most likely correct, as we observed these odd-chain lipids from human blood and these also increased in mosquitoes between 16 h and 30 h PBM. These odd chain fatty acid-containing lipids did not show any distinct change patterns comparable to even chain fatty acid-containing lipids from the same lipid group.

Data analysis

All data were analysed through R v. 3.6.0. Female weight was analysed by ANOVA across time followed by posthoc Tukey HSB tests. The concentrations of different lipid classes (nmol/individual) were used as variables for principal component analysis (PCA). For specific comparisons between treatments for particular lipids, concentration was natural log-transformed before undertaking paired t-tests, and p values were corrected for lipid number considered in a comparison by the Benjamin-Hochberg method.

Ethics statement

Female mosquitoes were blood fed on a volunteer in this experiment, with ethics approval from the University of Melbourne Human Ethics committee (approval 0723847).

Supporting information

Supporting information 1. Ovarian appearance of female mosquitoes dissected at (A) BBM, (B) 1 d PBM, (C) 2 d PBM, (D) 3 d PBM, (E) 4 d PBM and (F) 5 d PBM. There is some

variation in the stages of development of the ovaries among individuals as captured by variation in weight (Fig. 1).

Supporting information 2. List of individual lipids and their changes before and after female *Aedes aegypti* mosquitoes blood fed (between BBM and 0 h PBM).

Supporting information 3. List of individual lipids and their changes between 16 and 30 h after female *Aedes aegypti* mosquitoes blood fed (between 16 and 30 h PBM).

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