

Utilization of pre-existing energy stores of female *Aedes aegypti* mosquitoes during the first gonotrophic cycle

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Abstract

Pre-existing energy reserves may play an important role in regulating the utilization of blood meal proteins in female anautogenous mosquitoes. Determining the fate of reserves derived from the sugar meal and larval food during the first gonotrophic cycle would help to elucidate the relative contributions of larval and adult nutrition to survival and reproduction. We measured the allocation of pre-blood-meal reserves to egg production or energy production during the first gonotrophic cycle by using [^{14}C]-labeled female *Aedes aegypti* mosquitoes. Feeding adults [$3,4\text{-}^{14}\text{C}$]-glucose labeled the glycogen and sugar stores (~50%), lipid stores (~25%), and protein and amino acid stores (~25%). During the first gonotrophic cycle, about 60% of the glycogen and sugar stores were metabolized and all were used for energy production. About 33% of the labeled protein and 72% of the labeled amino acid stores were metabolized, with about 9% being transferred to the eggs and the rest oxidized. About 30% of the lipid was metabolized, with about 65% being transferred to the eggs and the rest oxidized. Feeding [$1\text{-}^{14}\text{C}$]-oleic acid to larvae effectively labeled adult lipid stores with about 75% of the label in lipid stores and 16% in proteins and 6% in glycogen. During the first gonotrophic cycle, about 35% of the labeled lipid stores were metabolized, with equal amounts being oxidized and transferred to the eggs. None of the other maternal stores labeled by fatty acid were metabolized during the first gonotrophic cycle. These results show that carbohydrate reserves are a critical source of energy during the first gonotrophic cycle, while lipid reserves are used equally for energy production and provisioning the eggs.

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1. Introduction

Numerous studies suggest that pre-existing energy reserves (including carbohydrates and lipids) before the first blood meal in female mosquitoes come from larval food supply and adult sugar feeding after eclosion. Larval food supply is of primary importance in determining imaginal body size and reserves (Briegel, 1990). Body size is covariant with energy reserves both at emergence and after reaching maximum levels from sugar feeding (Foster, 1995).

After starvation for 4–6 days post eclosion, the residual amount of reserves in female *Aedes aegypti* was 69–86% of the teneral proteins and 16–46% of the

teneral lipids, while all the carbohydrate reserves (less than one-fifth of the total calories) were consumed (Briegel, 1990), which suggested that the main component of energy reserves derived from larval stages should be lipid stored in adults before the first blood meal. Thus, an energetic advantage to utilization of a blood meal in female *Ae. aegypti* (\leq day 2 after emergence) (Naksathit et al., 1999a) may be provided mainly from teneral lipids, which were carried over from the larval stages. Briegel et al. (2002) further reported that in large female *Ae. aegypti*, a large segment of teneral lipids was invested in the ovaries during the first cycle independent of strong lipid synthesis from sugar and concluded that mosquito oogenesis depends primarily on the female's lipid status and not only on its protein acquisition. Although larval-stage-derived lipid reserves may play an important role in regulating the utilization of blood meal proteins for energy production and

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reproduction, little is known about the quantitative fate of larval-stage-derived lipid stores during the first gonotrophic cycle in mosquitoes.

Unlike the well-accepted role of larval-stage-derived lipid reserves in utilization of the first blood meal, the role of sugar feeding after eclosion remains somewhat controversial. On the one hand, a substantial number of studies show that sugar feeding is very important for building energy reserves (van Handel, 1965; Nayar and Sauerman, 1971, 1974, 1975a; Naksathit et al., 1999b) and improving egg production (Nayar and Sauerman, 1975b; van Handel, 1992, 1993; Gary and Foster, 2001; Briegel et al., 2001). On the other hand, several investigators have suggested that adult *Ae. aegypti* fail to feed on sugar in the field but use a strategy of multiple blood feeding to meet the need of survival and reproduction (Edman et al., 1992; Scott et al., 1993a,b; Day et al., 1994; Scott et al., 1997; Harrington et al., 2001). Our recent results showed that although sugar feeding after a blood meal did not influence the fecundity of female *Ae. aegypti* during the first gonotrophic cycle, it was important for building mosquito maternal energy reserves after the eggs were laid (Zhou et al., 2004). Although it has been reported that the initial sugar meal was very important for the longevity and subsequent oogenesis of female *Aedes communis* (Andersson, 1992), the role of sugar feeding or sugar-meal-derived energy reserves before the first blood meal in regulating the utilization of blood meal proteins during the first gonotrophic cycle has not yet been quantitatively studied.

A quantitative determination of the fate of larval food- and sugar-meal-derived energy reserves during the first gonotrophic cycle would greatly help to elucidate the relative importance of larval and adult nutrition (including sugar meal and blood meal) for different components of fitness, including maintenance, longevity and reproduction, in mosquitoes. Thus, an analysis of the allocation of pre-blood-meal reserves into egg production or energy production during the first gonotrophic cycle is timely.

The current study was designed to label the carbohydrate stores and triacylglycerol (TAG) stores of female *Ae. aegypti* mosquitoes with [^{14}C], and then determine the extent to which the glycogen and lipid reserves were used for energy production following a protein meal, and the extent to which these compounds were incorporated into eggs.

2. Materials and methods

2.1. Mosquitoes

Aedes aegypti (L.) (Rockefeller) were used. Larvae were maintained on a diet consisting of equal propor-

tions of rat chow (Sunburst Pet Foods, Phoenix, AZ), lactalbumin hydrolysate (USB, Cleveland, OH) and yeast hydrolysate (USB, Cleveland, OH) in batches of 2000 in a pan measuring $142 \times 76 \times 6$ cm, and containing about 4 cm of water. Female pupae were separated from males using a mosquito separator. Adult mosquitoes were routinely maintained at 28°C , 70–80% relative humidity and a photoperiod of 16:8 h (L:D) on 3% sucrose ad libitum for 3 days after eclosion.

2.2. Labeling of glycogen and sugar stores of adult female mosquitoes

Seventy teneral adult female *Ae. aegypti* mosquitoes were placed individually in 10-ml plastic scintillation vials, whose opening was covered with nylon mesh and secured with rubber bands, and starved (no sugar and no water) for 24 h. Fourteen microcurie of [$3,4\text{-}^{14}\text{C}$]-glucose (140 μl) (American Radiolabeled Chemicals, Inc., St. Louis, MO) were added to an 1.5-ml Eppendorf tube, dried under N_2 gas, and then dissolved in 348 μl of 3% sucrose and 2 μl of diluted blue food dye (Assorted Food Colors & Egg Dye, McCormick & Co., Inc., Hunt valley, MD) solution (blue food dye diluted 1:30 with 3% sucrose), and mixed well. A 5 μl drop of the labeled mixture was placed on the nylon mesh (0.2 $\mu\text{Ci}/\text{female}$). The mosquitoes were allowed to feed on the sucrose drop in a specially designed incubator ($\text{T}:28^\circ\text{C}$, $\text{RH}: 80\%$, $\text{L}:\text{D} = 16:8$ h) as described by Zhou et al. (2004). At 4, 8, and 12 h post-initial feeding, another 5 μl of unlabeled 3% sucrose was placed on the same site of the nylon mesh where the first sucrose drop was placed in order to dissolve any remaining [^{14}C]-glucose that had dried on the nylon mesh. This procedure facilitated the recovery of [^{14}C]-glucose in the mosquitoes. At 24 h post-initial feeding, the completely fed mosquitoes, as indicated by the presence of blue food dye, were transferred into a regular adult mosquito cage (18 cm diameter \times 19 cm depth and covered with nylon mesh) and provided with unlabeled 3% sucrose for another 24 h (those partially fed mosquitoes were eliminated from the experiment), and then fed with a pig blood meal by using an artificial feeder (Zhou et al., 2004). The total radioactivity incorporated into each female was $(160,000 \pm 10,000)$ dpm/ f ; the total radioactivity recovery in each female was 36%.

2.3. Labeling of lipid stores of adult female mosquitoes

Active dry brewer's yeast (Lallemand, Inc., Montreal, Canada) was ground to a powder using a 50-ml mortar and a pestle (Cole-Parmer Instrument Company, Vernon Hills, IL). Twenty-five microcurie of [$1\text{-}^{14}\text{C}$]-oleic acid (Amersham Pharmacia Biotech UK Limited, England) were added to 5 ml of ethyl ether and 2.5 g of

the dried yeast preparation and mixed well. The mixture was allowed to dry at room temperature under nitrogen gas and then ground to a powder. The powder was then added to 2 l of tap water containing 500 early fourth instar *Ae. aegypti* larvae. The larvae were allowed to feed on the mixture until pupation. The pupae were transferred into a clean 50-ml beaker containing 20 ml of tap water, and the beaker was placed in a clean adult mosquito cage. The eclosed females were separated into another clean cage of the same size and maintained as described above for 3 days, and then allowed to feed on a pig blood meal by using the artificial feeder. The total radioactivity incorporated into each female was $(15,000 \pm 1000)$ dpm/♀, with the fatty acids from TAG accounting for 77% of the radioactivity.

2.4. Analysis of utilization of the pre-labeled stores during the gonotrophic cycle

After the blood meal, four groups of three–five fed females which were frozen immediately after the blood meal were homogenized in 1 ml of PBS (phosphate buffered saline = 50 mM NaCl, 50 mM sodium phosphate buffer, pH 7.2) with a 2-ml automatic homogenizer (Cole-Parmer Instrument Company, Vernon Hills, IL), and then counted (Zhou et al., 2004). Another four groups of three mosquitoes each was analyzed by the micro-separation procedure to determine the radioactivity incorporated into glycogen, lipid, sugar, protein and amino acids (Zhou et al., 2004). In order to separate glycerol and fatty acid from TAG, we used 0.5 N KOH in 95% ethanol at 90 °C for 1 h. After the saponification reaction, the mixture was acidified with 10 N HCl and then extracted with hexanes, as described by Jackson and Arnold (1977). The aqueous phase (lower layer) contained glycerol and the organic phase (upper layer) fatty acids, and were counted, respectively. Three blood-fed females were used for $^{14}\text{CO}_2$ collection (Zhou et al., 2004). The rest were maintained with 3% sucrose until eggs were produced. After the eggs were laid, the female mosquito bodies were used for micro-separation to determine maternal storage, and the eggs were separated into egg proteins and egg lipids (Zhou et al., 2004).

2.5. Data analysis

Data for the various biochemical products were expressed as cpm/female, and then as the percentage of the original radioactivity present in the female immediately after the blood meal. All data were obtained from three independent experiments, each of which contained two parallel samples of 3–5 female mosquitoes. Curve-fitting was selected to describe the time-course of the percentage of radioactivity in CO_2 following a blood meal. Student's *t* test was used for comparison

of maternal proteins, amino acids, lipid, glycogen, and sugar between the start and the end of the first gonotrophic cycle. The significance level, α , was 0.05. All the statistical analyses and graphs drawn were carried out using Graph Pad Prism 3 (Graph Pad Prism Software, Inc., San Diego, CA).

3. Results and discussion

3.1. Utilization of carbohydrate stores during the first gonotrophic cycle in female *Ae. aegypti*

3.1.1. Labeling of carbohydrate stores with [3,4- ^{14}C]-glucose

In preliminary experiments, we fed females with D-[U- ^{14}C]-glucose (Amersham Pharmacia Biotech UK Limited, England) to label glycogen and sugar stores. The results showed that radioactivity was incorporated into proteins ($8 \pm 1\%$), amino acids ($2 \pm 1\%$), glycogen ($35 \pm 7\%$), sugars ($5 \pm 4\%$), and lipids ($50 \pm 7\%$). While these results were interesting in that they showed the wide variety of compounds that can be derived from glucose in mosquitoes, the fact that so much label was present in TAGs was expected to complicate our observations about the fate of pre-existing glycogen reserves during the first gonotrophic cycle. In order to reduce the labeling of fatty acids, we decided to use D-[3,4- ^{14}C]-glucose in place of D-[U- ^{14}C]-glucose, because the label in the third and fourth positions would be lost as CO_2 when pyruvate is converted to acetyl-CoA by pyruvate dehydrogenase.

The results of labeling female *Ae. aegypti* mosquitoes with D-[3,4- ^{14}C]-glucose (Table 1) showed that about 52% of the label was in carbohydrates, with 36% in glycogen and 16% in sugars. About 24% was found in lipid stores, with 6% in glycerol and 18% in fatty acids. About 24% was found in proteins and amino acids,

Table 1
Utilization of maternal reserves labeled with [3,4- ^{14}C]-glucose^a

Component	Start (%)	End (%)	$\Delta\%$	<i>P</i> value
Maternal proteins	16.2 ± 1.0	11.0 ± 0.8	5.2	0.0032
Maternal glycerol	6.3 ± 0.4	4.4 ± 0.3	1.9	0.0025
Maternal fatty acids	17.5 ± 1.6	12.8 ± 1.6	4.7	0.0238
Maternal glycogen	36.2 ± 0.5	15.4 ± 1.8	20.8	<0.0001
Maternal sugars	16.3 ± 1.8	4.2 ± 0.7	12.1	0.0010
Maternal amino acids	7.6 ± 0.4	2.1 ± 0.2	5.5	<0.0001
Egg lipids		4.1 ± 0.8		
Egg proteins		2.1 ± 0.4		
CO_2		44.0 ± 3.8		
Waste		0.0		

^a Of the maternal components pre-labeled by feeding [3,4- ^{14}C]-glucose, 50.2% was metabolized during the first gonotrophic cycle. Of that metabolized, 4.1% was converted to egg lipids and 2.1% was converted to egg proteins; 87.8% was oxidized to CO_2 and there was no detectable label in the waste.

with 16% in proteins and 8% in amino acids. Thus, the use of D-[3,4-¹⁴C]-glucose did significantly reduce the incorporation of label into lipid, but did not eliminate it. These results suggest that there must be another quantitatively important metabolic pathway in mosquitoes for the metabolism of glucose, in addition to glycolysis and glycogenesis. It has been reported in *Manduca sexta* larvae that hemolymph sugar formation is partially carried out by the pentose phosphate pathway (Thompson, 1999). If the pentose phosphate pathway is also important in mosquitoes, for either NADPH production for fatty acid synthesis or for oxidation of glucose, then carbon 3 of D-[3,4-¹⁴C]-glucose would be converted into carbon 2 or 3 of fructose-6-phosphate (F6P), while carbon 4 would be converted only into carbon 4 of F6P (Voet and Voet, 1995). When these F6P molecules re-enter the glycolytic pathway, carbon 2 of F6P, derived from the carbon 3 of the original glucose, would be converted into acetyl-CoA, which can account for fatty acid labeling. Although Thompson et al. (1995) concluded that under normal conditions the pentose pathway is not the principal source of triose phosphates for oxidative catabolism during larval development in *M. sexta*, our results showed that significant amounts of fatty acids were formed by feeding a [3,4-¹⁴C]-glucose meal, which suggests that the pentose pathway may play a role in the metabolism of meal sugar. The labeling of amino acids and proteins would also require labeling of the Krebs cycle. This could be accomplished via acetyl-CoA, as described above, or via pyruvate carboxylase, which is highly active in *Ae. aegypti* (Tu and Hagedorn, 1997). More work is needed to clarify these issues.

3.1.2. Metabolic fate of [3,4-¹⁴C]-glucose-labeled compounds during the first gonotrophic cycle

Table 1 shows the distribution of label at the end of the first gonotrophic cycle. About 44.0% of incorporated radioactivity was expired as CO₂; 19.6% was reserved as maternal carbohydrate stores (15.4% as glycogen and 4.2% as sugar); 17.2% was reserved as maternal lipid stores (4.4% as glycerol and 12.8% as fatty acids); 11.0% of maternal proteins and 2.1% of maternal amino acids remained; and 6.2% of the original radioactivity was transferred into egg components (2.1% as egg proteins and 4.1% as egg lipids).

Table 1 shows the change in the percentage of each component following the gonotrophic cycle, which can be used to construct a balance sheet. The lipids in the female decreased 6.6% (fatty acids + glycerol) with 4.1% being transferred to the eggs, while the remaining 2.5% was oxidized. Protein and amino acids in the female decreased 10.7% (5.2% in protein + 5.5% in amino acids) and 2.1% was transferred to the eggs. The remaining 8.6% was oxidized. That only 20% of the metabolized maternal protein and amino acids were

transferred to the eggs is consistent with labeled protein feeding studies (Zhou et al., 2004). Glycogen and sugar in the female decreased 32.9% and all were oxidized. The sum of the oxidized components was 44.0%, which matches the amount of CO₂ produced. The relative use of reserve stores for energy production, as deduced from the use of [3,4-¹⁴C]-glucose-labeled components, was 74.8% from carbohydrate reserves, 19.5% from protein reserves, and 5.7% from lipid reserves. On the other hand, the relative use of sugar-meal-derived carbohydrate reserves was 62.7% for energy production, and the rest (37.3%) for building maternal energy reserves (Table 2). These data show that carbohydrate reserves are an important energy source during the first gonotrophic cycle.

Yuval (1992) suggested that while a blood meal is a direct investment in reproductive success and a sugar meal is a somatic investment, there is evidence that sugar feeding may also contribute directly to reproductive success. Thus, our results quantitatively revealed that the sugar feeding before a blood meal is directly invested in egg production through synthesis of egg proteins and egg lipids. On the other hand, as mentioned in the Introduction of this article, several researchers have suggested that adult female *Ae. aegypti* failed to feed on sugar in the field, but used a strategy of multiple blood feeding to meet the need of survival and reproduction (Edman et al., 1992; Scott et al., 1993a,b; Day et al., 1994; Scott et al., 1997; Harrington et al., 2001). Considering that *Ae. aegypti* is a domestic mosquito species, an equivocal reason has been supposed, i.e. sugar is poorly available in the domestic environment. However, if the sugar availability could become a selective pressure on an anautogenous mosquito's hemophagic behaviour in the domestic environment, a question could be presented, i.e. how have domestic male mosquitoes been using only sugar as their food under such a selective pressure? In addition, it seems that insects may possess a much longer evolutionary history of sugar-feeding, which may date from the occurrence of the flowering

Table 2

Comparison of the allocation of pre-existing energy reserves (carbohydrate and lipid) and blood meal proteins to energy production, excreted waste, maternal energy reserves and egg components during the first gonotrophic cycle in female *Ae. aegypti* mosquitoes^a

Energy source	Energy production (%)	Waste (%)	Maternal reserves (%)	Egg components (%)
Blood meal	42.5	29.1	18.7	9.7
Carbohydrates	62.7	0.0	37.3	0.0
Lipids	17.9	0.8	63.4	16.6

^a The data are expressed as percentage of total pre-labeled carbohydrate reserves and lipid reserves, respectively. The data for [¹⁴C]-protein meal metabolism are from Zhou et al. (2004).

angiosperms (about 100–150 million years ago), compared to the hemophagic habit (Foster, 1995; Panda and Khush, 1995). Therefore, integrating all of this information into our results could allow us to suppose that female *Ae. aegypti* mosquitoes still retain a physiological and biochemical mechanism to balance the use of food sugar and blood for energy metabolism and reproduction when sugar is occasionally available in their habitat, after a very long period of evolution of the hemophagic habit. More studies on the ecology, physiology, and biochemistry of different mosquito species need to be conducted to clarify the role of sugar feeding in the biology of domestic and anautogenous mosquito species.

3.2. Utilization of lipid stores during the first gonotrophic cycle in female *Ae. aegypti*

3.2.1. Labeling of lipid stores with [$1-^{14}\text{C}$]-oleic acid

Following a procedure developed to label adult fat body lipids in *M. sexta* (Arrese and Wells, 1997), we labeled adult female mosquito lipids by feeding labeled fatty acids to larvae. In the fourth instar *Ae. aegypti* larvae, most of fatty acids are C18:0, C18:1 and C18:3 (Wallage et al., 2001), and the rate of lipid synthesis was highest in the early fourth instar larvae and decreased shortly before pupation (Timmermann and Briegel, 1999). Thus, we decided to use [$1-^{14}\text{C}$]-oleic acids as a component of larval diet in the early fourth instar larvae of *Ae. aegypti*.

The results of labeling female *Ae. aegypti* mosquitoes with [$1-^{14}\text{C}$]-oleic acid during the larval period (Table 3) showed that about 76% of the label was in lipids, with 75.2% in fatty acids and 0.8% in glycerol. It is important to point out that we measured only maternal TAGs or storage lipids and not maternal phospholipids in these experiments. About 17.3% of the label was found in proteins and amino acids with 15.8% in pro-

teins and 1.5% in amino acids. Only small amounts of label were found in glycogen (5.8%) and sugars (0.9%), most likely due to labeling of citric acid cycle intermediates by acetyl-CoA derived from oxidation of the labeled fatty acid. The results showed that larval dietary oleic acid can be efficiently carried over into adult females as TAG stores.

3.2.2. Metabolic fate of [$1-^{14}\text{C}$]-oleic acid-labeled compounds during the first gonotrophic cycle

Table 3 shows that there were no significant differences in proteins, amino acids, or carbohydrates (mainly glycogen) between the beginning and the end of the first gonotrophic cycle. Such a lack of utilization of proteins, amino acids, and carbohydrate stores is surprising in light of the results from carbohydrate labeling (see above) and from protein utilization studies (Zhou et al., 2004). However, if one considers the time of labeling—from the larval to the adult stage for lipid and only during the adult stage for carbohydrate and proteins—it is likely that under the conditions of our experiments, the labeled proteins are some structural proteins and/or storage proteins and the labeled glycogen might represent the residual core of the molecule. In both cases, these components would be expected to be relatively metabolically inert.

As shown in the column labeled $\Delta\%$ in Table 3, the only maternal component that changed significantly during the gonotrophic cycle was lipid. About 27% of the lipid stores were used during the gonotrophic cycle and of that used, almost 50% was used to make egg components (mainly egg lipids) and 50% was oxidized. These results show that during the first gonotrophic cycle, sugar-fed females use a substantial amount of lipid reserves derived from the larval diet to provision the eggs with lipids. It must be emphasized that the egg lipids in our experiments include both egg TAGs and egg phospholipids (see Zhou et al., 2004).

After the first gonotrophic cycle, about 50% of the maternal lipid store derived from the larval diet remained in the female. Briegel et al. (2002) reported that regular sugar meals allow the female *Ae. aegypti* to develop sufficient lipid stores to carry out six gonotrophic cycles. Our results suggest that maternal lipid reserves derived from the larval diet contribute to egg production during the first gonotrophic cycle and probably are also an important resource for subsequent cycles, along with lipids derived from sugar feeding. The fact that larval-derived lipids persist beyond the first gonotrophic cycle might provide an explanation for the steadily declining fecundity in older females (Briegel, 2003). This would be especially true if the larval-derived lipids contain essential fatty acids, which cannot be replaced by lipid synthesis from glucose. Clearly, determining the fate of the remaining maternal

Table 3
Utilization of maternal reserves labeled with [$1-^{14}\text{C}$]-oleic acids^a

Component	Start (%)	End (%)	$\Delta\%$	<i>P</i> value
Maternal proteins	15.8 ± 1.2	15.7 ± 2.3	0.1	0.9512
Maternal glycerol	0.8 ± 0.2	0.4 ± 0.1	0.4	0.0479
Maternal fatty acids	75.2 ± 2.9	47.8 ± 4.3	27.3	0.0022
Maternal glycogen	5.9 ± 0.7	6.1 ± 0.4	-0.2	0.7508
Maternal sugars	0.9 ± 0.2	0.6 ± 0.2	0.2	0.3962
Maternal amino acids	1.5 ± 0.2	1.4 ± 0.2	0.2	0.4885
Egg lipids		12.6 ± 3.0		
Egg proteins		0.8 ± 0.2		
CO ₂		13.6 ± 3.3		
Waste		0.8 ± 0.2		

^a Of the maternal components pre-labeled by feeding [$1-^{14}\text{C}$]-oleic acids, 27.9% was metabolized during the first gonotrophic cycle. Of that metabolized, 45.3% was converted egg lipid and 2.8% was converted to egg protein; 49.9% was oxidized to CO₂ and 3.0% was excreted as waste.

lipid store derived from the larval stages during subsequent gonotrophic cycles is necessary.

3.3. Comparison of the utilization of pre-existing energy stores with that of blood meal proteins during the first gonotrophic cycle

In Fig. 1, we compare the rate of CO₂ production from carbohydrate and lipid reserves (this study) with that from [¹⁴C]-labeled blood meal proteins (Zhou et al., 2004) over the first 60 h of the gonotrophic cycle. The time-courses of CO₂ expired during the first gonotrophic cycle all followed a cubic curve ($R^2 = 0.9968$, 0.9932, and 0.9988 for blood meal, sugar-derived reserves and larval-derived lipid reserves, respectively). Interestingly, the extent of substrate oxidation at the end of the first gonotrophic cycle was quite similar for labeled protein amino acids and for labeled glucose reserves (about 40%), but the extent of oxidation of labeled fatty acid reserves was lower (about 10%). These results support the important conclusion that lipid reserves are spared from oxidation and preferentially used for egg production.

Table 2 compares the allocation of maternal carbohydrate and lipid reserves and blood meal amino acids during the first gonotrophic cycle. Approximately 62.7% of the carbohydrate reserves and 42.5% of blood meal amino acids are used for energy production, respectively, while only about 17.9% of the lipid

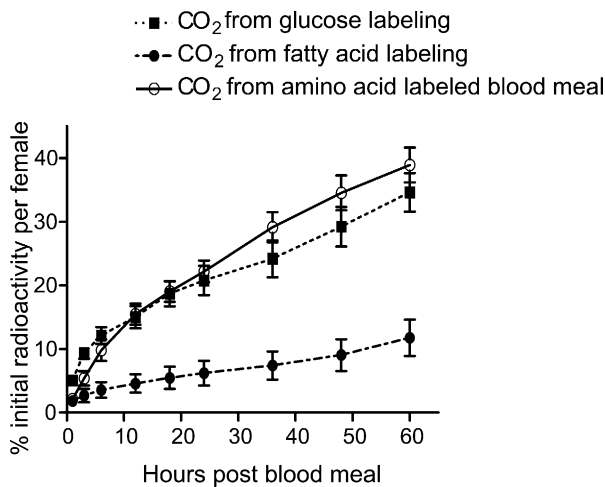


Fig. 1. Time courses of ¹⁴CO₂ production by female *Ae. aegypti* mosquitoes with pre-labeled [¹⁴C]-glucose or [¹⁴C]-oleic acid and after a blood meal with [¹⁴C]-protein. Three or five blood-fed mosquitoes were placed in a 20-ml scintillation vial containing a small piece of cotton soaked in 3% sucrose. At appropriate time intervals, ¹⁴CO₂ produced by the spent mosquitoes was trapped in 1 N KOH, and then counted, as described previously (Zhou et al., 2004). The data from each point are expressed as the percentage of original fed radioactivity converted to the expired CO₂ per female and represent the mean ± SD of three independent experiments. The data from [¹⁴C]-protein meal are derived from Zhou et al. (2004).

reserves are used for this purpose. About 30% of the blood meal amino acids are excreted as waste (see also Briegel, 1986), whereas very little of the carbohydrate and lipid reserves are metabolized by this route. The lipid reserves are retained in the female at the end of the gonotrophic cycle to a greater extent than the carbohydrate reserves, which are retained to a greater extent than reserves derived from blood meal amino acids. These results suggest that carbohydrate reserves and blood meal protein amino acids are the primary energy sources during the first gonotrophic cycle, while lipid stores are used primarily to provision the eggs with lipids or are retained as maternal lipid reserves. These results are consistent with the results reported by Briegel et al. (2002).

4. Conclusions

- Sugar feeding before a blood meal can be invested directly into egg production through synthesis of egg proteins and egg lipids during the first gonotrophic cycle.
- Most of the preexisting carbohydrate reserves are derived from a sugar meal and used for energy production during the first gonotrophic cycle.
- Larval dietary oleic acid can be carried over efficiently to the adult stage in female *Ae. aegypti* mosquitoes.
- During the first gonotrophic cycle, the sugar-fed females use a substantial amount of larval-derived lipid reserves for egg production.
- After the first gonotrophic cycle, about 50% of the larval-derived lipid reserves remain in the mosquito.
- The pentose pathway may play an important role in glucose metabolism in the mosquito.

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