

## ORIGINAL PAPER

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## Effect of fasting on the composition of the fat body lipid of *Dipetalogaster maximus*, *Triatoma infestans* and *Panstrongylus megistus* (Hemiptera: Reduviidae)

Accepted: 4 August 1998

**Abstract** Modifications in content and lipid composition induced by fasting were examined in fat bodies from adults of Triatominae, *Dipetalogaster maximus*, *Triatoma infestans* and *Panstrongylus megistus*. With fasting, total lipid stores dropped approximately 50% for *T. infestans* and more than 70% for *P. megistus*. Total lipids analyzed by thin layer chromatography and fractionated by column chromatography on Unisil showed triacylglycerols as the main component in the three species, although *P. megistus* showed high levels of diacylglycerols (31–46%). Cholesterol amounted to 8–15%. In diacylglycerol fractions, C<sub>16:0</sub>, C<sub>18:1</sub> and C<sub>18:0</sub> fatty acids were detected; their ratio varied with species but it was not dependent on nutritional status. In triacylglycerol fractions C<sub>18:1</sub> fatty acid was the major component at different times (48–68%); the ratio of monounsaturated to saturated in this fraction was 1.3, 2.6 and 1.2 for *D. maximus*, *T. infestans* and *P. megistus* respectively. The remarkable drop in lipid stores without noticeable changes in their relative composition would suggest that all types of lipid are used at similar rates. The higher content of diacylglycerols in *P. megistus* may be associated with the better flight performance of this species.

**Key words** Fat body · Fasting · Lipid composition · Reduviid bugs

**Abbreviations** DAG diacylglycerols · GLC gas liquid chromatography · GLC-MS gas chromatography-mass spectrometry · HDLp high density lipoprotein · TAG triacylglycerols · TLC thin layer chromatography · PUFAs polyunsaturated fatty acids

### Introduction

In insects, the fat body usually stores large amounts of lipids, and in some species the weight of this organ constitutes up to 50% of the fresh weight of the insect (Gilbert and Chino 1974). The lipidic composition of the fat body is a result of different processes including the storage of dietary lipids, de novo synthesis, degradation and subsequent release for mobilization to sites where they can be metabolized (Beenackers et al. 1985).

The nutritional requirements of insects are generally similar to those of vertebrates, with exception of cholesterol, which is required for cell membranes and for the synthesis of 20-hydroxyecdysone. In insects that feed on animals or animal products, cholesterol is obtained from the diet due to their inability to perform de novo biosynthesis of the steroid structure. However, phytophagous insects can produce sufficient cholesterol by converting C<sub>28</sub> and C<sub>29</sub> phytosterols via dealkylation of these phytosterols at C<sub>24</sub> (Svoboda and Feldlaufer 1991).

From a biochemical viewpoint, lipids, as stored triacylglycerols (TAG), play an essential role as a reserve of metabolic energy in addition to their role as membrane components. They are transported through the hemolymph by a selective mechanism as diacylglycerols (DAG) by high density lipoprotein (HDLp), and used as fuel by oxidation of their fatty acids during flight by most migratory insects (Bailey 1975; Beenackers et al. 1985; Arrese and Wells 1997).

In reduviid bugs, the obligatory hematophagous vectors of *Trypanosoma cruzi* (the aetiological agent of American trypanosomiasis), the blood meal and storage of metabolic reserves in the fat body are important ele-

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ments to cover needs of the insects at different stages, as well as for the survival of the adult bugs. However, the ability of these insects to endure long periods without feeding is well known (Szumlewicz 1976). Thus, the poor nutritional status of these vectors, access to blood sources and the changes in the weight/length ratio of the insects are closely associated with active dispersal by flight of the triatomine bugs when food is not available (Sjorgren and Ryckman 1966; Lehane and Schofield 1982; Schofield 1994).

Evidence concerning the role of lipids during starvation and flight in reduviid insects is basically limited to two studies. Ward et al. (1982) found that starvation produced a large increase in the lipid content of the flight muscle of *Rhodnius prolixus*, and that upon flight there was a large decrease in the amount of lipid stored in the flight muscle. On the other hand, by employing fat bodies from starved reduviid bugs, we previously reported that adipokinetic hormone promoted the generation of low density lipoprotein, the lipoprotein responsible for the delivery of lipids during flight (Canavoso and Rubiolo 1995). However, the relationship between the lipid reserves and their mobilization in these insects under starvation is scarcely known.

Therefore, the purpose of this work was to study the starvation-induced changes in the lipid content and lipid composition of the fat body of three vectors of American trypanosomiasis, *Dipetalogaster maximus*, *Triatoma infestans*, and *Panstrongylus megistus* (Hemiptera: Reduviidae).

## Materials and methods

### Insects

Colonies of triatomine bugs *D. maximus*, *T. infestans* and *P. megistus* (reared and kept in the insectary) were fed every 2 weeks on hen blood. For the experiments, adult male insects were fed on day 5 after final ecdysis and separated into groups of 10–12 insects with similar weights. These groups were fasted for 10, 20 and 30 days and maintained at  $28 \pm 1$  °C, 60–70% humidity and 8:16 h light:dark cycle as previously reported (Canavoso and Rubiolo 1993).

### Lipid extraction of the fat body

After 10, 20 and 30 days of fasting, the fat bodies were carefully dissected, rinsed with Ringer solution (120 mM NaCl, 15 mM KCl, 4 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 5 mM PIPES buffer, pH 7.0), dried on filter paper and then weighed individually with  $\pm 0.01$  mg precision. Total lipids were extracted according to Folch et al. (1957), and the material was taken up to constant weight in a nitrogen atmosphere.

**Table 1** Total lipids in Triatominae fat bodies at different times of fasting. Values are means  $\pm$  SD of the number of insects indicated in brackets

Time [days]	<i>Dipetalogaster maximus</i>	<i>Triatoma infestans</i> Lipids (mg/100 mg wet tissue)	<i>Panstrongylus megistus</i>
10	15.8 $\pm$ 1.2 [12]	14.7 $\pm$ 1.3 [12]	12.5 $\pm$ 1.1 [12]
20	12.6 $\pm$ 0.9 [12]	11.7 $\pm$ 1.0 [11]	6.7 $\pm$ 0.8 [10]
30	8.2 $\pm$ 0.5 [10]	7.3 $\pm$ 0.6 [10]	3.8 $\pm$ 0.7 [11]

### Analysis by thin layer chromatography and capillary gas liquid chromatography

Thin layer chromatography (TLC) of total lipids was performed using silica-gel 60 (Merck) with the solvent system hexane/ethyl acetate (9:1 v/v). The compounds were detected by spraying the plates with 10% H<sub>2</sub>SO<sub>4</sub> /0.04 M (NH<sub>4</sub>)<sub>6</sub> Mo<sub>7</sub>O<sub>24</sub> · 4H<sub>2</sub>O/3 mM Ce (SO<sub>4</sub>)<sub>2</sub> and heating to 200 °C. Quantification using internal standards was performed by densitometry (Bio Rad GS 670). Capillary gas liquid chromatography (GLC) was carried out with a Hewlett-Packard 5890 gas chromatograph with nitrogen as the carrier gas and GLC-mass spectrometry (GLC-MS) was performed on a TRIO-2VG MASSLAB or Shimadzu QP-5000 at 70 eV.

### Separation of total lipids

Separation of total lipids from fat bodies of the three species at the different times was performed by column chromatography on Unisil (200-325 mesh, Clarkson Chemical, South Williamsport, Pa, USA). The columns were equilibrated with hexane and eluted with hexane/ethyl acetate (10:1 v/v) and hexane/ethyl acetate (5:1 v/v) in order to obtain TAG and DAG/cholesterol fractions.

### Analysis of DAG and cholesterol

The fractions corresponding to DAG/cholesterol obtained from the Unisil column were analyzed by GLC as trimethylsilyl derivatives on an HP-1 capillary column (0.20 mm  $\times$  12 m). The temperature program was from 245 °C to 255 °C at 3 °C/min, and 255 °C to 320 °C at 9 °C/min. Trimethylsilylation was performed on 0.25–0.5 mg of lipids with 10  $\mu$ l of bis (trimethyl-silyl) trifluoroacetamide BSTFA (Sigma) and 20  $\mu$ l pyridine by heating at 70 °C for 15 min. Cholesterol was confirmed by GLC-MS.

### Analysis of fatty acids

Fatty acids from DAG and TAG fractions as well as total lipids from the fat body were analyzed as methyl ester derivatives by GLC, with previous saponification with 0.1 M NaOH in 90% ethanol for 1 h at 37 °C. After neutralization, the lipids were extracted with diethyl ether and methylated with 14% BF<sub>3</sub> in methanol, at 70 °C for 45 min before analysis. Fatty acids from total lipids, DAG and TAG were analyzed under the following conditions: (a) SPB-20 column (0.25 mm  $\times$  30 m), temperature programmed from 120 °C (1 min) to 290 °C at 6 °C/min, (b) HP-5 capillary column (0.32 mm  $\times$  50 m), oven temperature program from 180 °C (1 min) to 210 °C at 5 °C/min, and 210 °C (7 min) to 270 °C at 15 °C/min. Fatty acids were confirmed by GLC-MS.

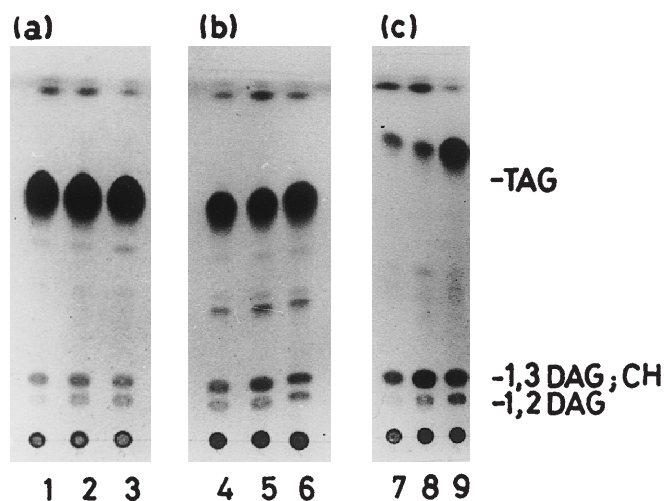
## Results

### Total lipid analysis

Total lipids stored in the fat bodies from three different species are shown in Table 1. After 30 days of fasting, lipid reserves had decreased to between half and one

third of the amounts found on day 10. The drop on day 30 represented about 50% in *T. infestans*, 48% in *D. maximus* and 70% in *P. megistus*.

Analysis by TLC of the total lipids from the fat body at different times post-feeding, showed that TAG were the major components in the three species, with percentages between 43% and 82% of the neutral lipids. No significant changes with fasting were observed in *T. infestans* and *D. maximus*, although *T. infestans* showed minor components with polarity between DAG and TAG (Fig. 1, Table 2). The lipid pattern in *P. megistus* showed slight differences with similar percentages of DAG and TAG on days 10 and 20, but at the end of the fasting period (day 30) the percentage of TAG was twice that of DAG. The 1,2 and 1,3 di-*O*-acylglycerols were detected in all cases. TLC with solvent hexane/ethyl acetate (9:1 v/v) does not discriminate between 1,3 di-*O*-acylglycerols and cholesterol, but they were separated by GLC. The presence of cholesterol was further confirmed by GLC-MS (see below). With the exception of *P. megistus*, DAG represented less than 15% of the neutral lipids (Table 2).



**Fig. 1a-c** Thin layer chromatography (TLC) on silica gel with solvent hexane/ethyl acetate (9:1 v/v) of the total lipids from fat bodies at 10, 20 and 30 days of fasting (a-c respectively) for *Dipetalogaster maximus* (lanes 1-3), *Triatoma infestans* (lanes 4-6) and *Panstrongylus megistus* (lanes 7-9). Standards were: triacylglycerols (TAG), 1,3 diacylglycerols (DAG), 1,3 di-*O*-palmitoylglycerol, 1,2 DAG, 1,2 di-*O*-palmitoylglycerol and cholesterol (CH)

## Analysis of DAG and cholesterol

DAG were eluted together with cholesterol from the Unisil column and were analyzed by GLC on HP-1 capillary column. The 1,2 and 1,3 di-*O*-acylglycerols were well separated by GLC. The major components of the DAG contained C<sub>16:0</sub> and C<sub>18:0</sub> or C<sub>18:1</sub> fatty acids (*Rt*, retention time 16.045 min and 16.531 min). The glycerides with C<sub>16:0</sub> and C<sub>18:0</sub> were not resolved from those containing C<sub>16:0</sub> and C<sub>18:1</sub>. Also, DAG with only C<sub>18</sub> fatty acid (*Rt* 18.528 min and 18.870 min, for the 1,2-diglycerides and 19.214 min and 19.570 min for the 1,3-diglycerides) were detected in all species (Fig. 2). The relative proportion between the DAG with C<sub>16/18</sub> and C<sub>18/18</sub> was 7:1, 2:1 and 4:1 for *D. maximus*, *T. infestans* and *P. megistus* respectively, and these were maintained through the fasting period (Table 3). The DAG with C<sub>16/16</sub> was not detected. A peak at 8.705 min, which corresponds to the *Rt* of cholesterol (Fig. 2), appeared in all insect species and was confirmed by GLC-MS. Percentages of DAG and cholesterol were calculated from TLC densitometry and GLC analysis; no changes were observed during fasting. Cholesterol amounted to 8-11% in *D. maximus* and *P. megistus*, whereas 12-15% was found in *T. infestans* (Table 2).

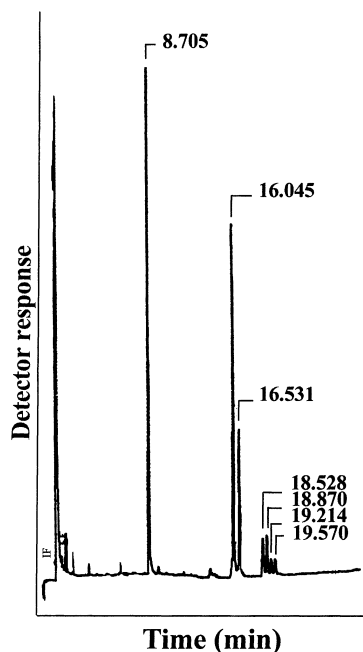
## Fatty acid composition

Fatty acids from DAG and TAG were analyzed as methyl esters by capillary GLC (Table 4). In DAG, C<sub>16:0</sub>, C<sub>18:1</sub> (two isomers) and C<sub>18:0</sub> fatty acids were detected. C<sub>16:0</sub> and C<sub>18:1</sub> were the major components of DAG in *T. infestans* and *P. megistus* while a ratio 2:1:1 was found in *D. maximus* for palmitic, oleic and stearic acids, respectively; these fatty acids were confirmed by GLC-MS. Two very close peaks both corresponding to C<sub>18:1</sub> were resolved (*Rt* 21.764 min and 21.843 min) (Fig. 3); they both gave the same mass spectra, which matched that of an authentic sample of oleic acid. The minor component co-chromatographed with a standard of elaidic acid, the trans isomer of oleic acid. The fatty acid composition is in agreement with the results obtained from the analyses of the DAG as trimethylsilyl derivatives.

In TAG, C<sub>18:1</sub> was the major component (49-68% at different times of fasting for the three species); C<sub>16:1</sub>, C<sub>1:60</sub> and C<sub>18:0</sub> were also detected and lauric, myristic and arachidic acids were found in low percentages (Table 4).

**Table 2** Triacylglycerol (TAG), diacylglycerol (DAG) and cholesterol (CH) from fat bodies at different times of fasting. Results expressed as percentages  $\pm$  SD ( $n = 4$ ), calculated by densitometry and GLC analysis

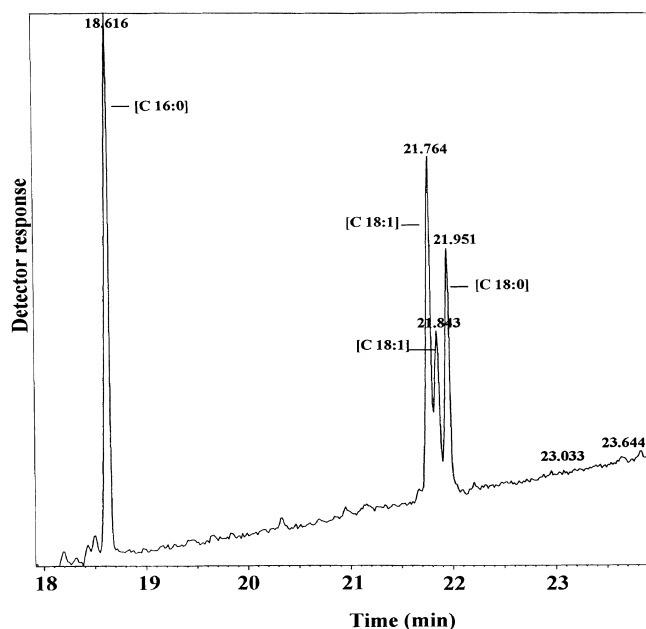
Time [days]	<i>D. maximus</i>			<i>T. infestans</i>			<i>P. megistus</i>		
	TAG	DAG	CH	TAG	DAG	CH	TAG	DAG	CH
10	80.5 $\pm$ 3.2	9.4 $\pm$ 0.9	10.1 $\pm$ 1.3	72.4 $\pm$ 3.1	12.6 $\pm$ 1.5	15.0 $\pm$ 1.7	54.2 $\pm$ 3.4	37.2 $\pm$ 2.8	8.6 $\pm$ 0.9
20	79.0 $\pm$ 2.5	10.0 $\pm$ 1.8	11.0 $\pm$ 0.8	70.6 $\pm$ 0.7	14.2 $\pm$ 0.9	15.2 $\pm$ 2.4	43.4 $\pm$ 2.2	46.2 $\pm$ 1.2	10.4 $\pm$ 1.5
30	81.8 $\pm$ 2.0	8.6 $\pm$ 2.5	9.6 $\pm$ 2.0	73.0 $\pm$ 2.3	15.0 $\pm$ 2.1	12.0 $\pm$ 3.2	60.8 $\pm$ 2.7	31.2 $\pm$ 3.1	8.0 $\pm$ 0.7



**Fig. 2** Gas liquid chromatography (GLC) analysis of DAG from fat body of *P. megistus*, separated on Unisil column and analyzed as trimethylsilyl derivatives on an HP-1 capillary column (0.2 mm  $\times$  12 m). The retention times correspond to the trimethylsilyl derivatives of DAG  $C_{16/18}$  (16.045 min and 16.531 min) and  $C_{18/18}$  (18.528, 18.870, 19.214 and 19.570 min). The peak at 8.705 min corresponds to cholesterol

**Table 3** DAG composition in fat body at day 10 of fasting. DAG composition expressed as percentages  $\pm$  SD ( $n = 4$ ). The results were similar at different times of fasting for the three species

DAG	<i>D. maximus</i>	<i>T. infestans</i>	<i>P. megistus</i>
$C_{16/18}$	87.2 $\pm$ 2.9	67.9 $\pm$ 3.1	80.3 $\pm$ 2.7
$C_{18/18}$	12.8 $\pm$ 2.1	32.1 $\pm$ 3.2	19.7 $\pm$ 1.5



**Fig. 3** GLC analysis of fatty acids from DAG analyzed as methyl ester derivatives on an SPB-20 capillary column (0.25 mm  $\times$  30 m). The retention times correspond to the methyl ester derivatives of  $C_{16:0}$  (18.616 min),  $C_{18:1}$  (21.764 min and 21.843 min) and  $C_{18:0}$  (21.951 min)

Fatty acid profiles of total lipids showed the same components as DAG and TAG (data not shown). In *P. megistus*, a very minor peak with a  $R_t$  coinciding with  $C_{20:4}$  was detected.

## Discussion

We have previously reported that the main lipidic component of the fat body of triatomine bugs are acylglycerols (Canavoso and Rubiolo 1995), and available

**Table 4** Fatty acid (FA) composition of the DAG and TAG from fat bodies at different times of fasting. Results expressed as percentages  $\pm$  SD ( $n = 4$ )

	<i>D. maximus</i>			<i>T. infestans</i>			<i>P. megistus</i>			
	Time [days]	10	20	30	10	20	30	10	20	30
<b>FA-DAG</b>										
$C_{16:0}$		49.0 $\pm$ 1.9	47.0 $\pm$ 1.3	45.8 $\pm$ 0.7	41.0 $\pm$ 0.8	39.6 $\pm$ 1.5	45.0 $\pm$ 2.3	44.0 $\pm$ 0.7	42.0 $\pm$ 1.1	46.5 $\pm$ 0.9
$C_{18:1}$		26.0 $\pm$ 0.9	27.0 $\pm$ 1.1	29.2 $\pm$ 1.3	43.2 $\pm$ 1.2	37.5 $\pm$ 0.9	36.9 $\pm$ 1.1	40.9 $\pm$ 0.5	43.0 $\pm$ 1.3	37.5 $\pm$ 1.6
$C_{18:0}$		25.0 $\pm$ 0.7	26.0 $\pm$ 1.1	25.0 $\pm$ 0.6	15.8 $\pm$ 0.7	13.7 $\pm$ 1.9	18.1 $\pm$ 1.5	15.1 $\pm$ 0.3	15.0 $\pm$ 0.9	16.0 $\pm$ 0.5
<b>FA-TAG</b>										
$C_{12:0}^a$		0.2 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1
$C_{14:0}$		0.8 $\pm$ 0.3	0.7 $\pm$ 0.3	0.7 $\pm$ 0.2	0.7 $\pm$ 0.4	0.5 $\pm$ 0.3	0.8 $\pm$ 0.2	0.7 $\pm$ 0.1	0.7 $\pm$ 0.3	0.8 $\pm$ 0.3
$C_{16:1}$		5.2 $\pm$ 1.4	5.7 $\pm$ 0.6	6.0 $\pm$ 1.7	5.7 $\pm$ 1.2	5.0 $\pm$ 0.6	4.0 $\pm$ 1.3	3.7 $\pm$ 1.2	5.0 $\pm$ 0.6	4.4 $\pm$ 0.3
$C_{16:0}$		36.6 $\pm$ 2.4	34.0 $\pm$ 1.9	35.0 $\pm$ 3.3	22.0 $\pm$ 1.4	20.3 $\pm$ 1.7	21.0 $\pm$ 2.2	36.0 $\pm$ 2.4	35.6 $\pm$ 1.3	33.7 $\pm$ 3.2
$C_{18:1}$		51.0 $\pm$ 3.0	51.6 $\pm$ 2.0	51.3 $\pm$ 3.6	64.8 $\pm$ 3.8	68.4 $\pm$ 2.1	66.0 $\pm$ 2.9	48.6 $\pm$ 0.2	48.7 $\pm$ 0.3	48.5 $\pm$ 0.5
$C_{18:0}$		6.0 $\pm$ 0.3	7.6 $\pm$ 1.0	6.3 $\pm$ 1.7	6.4 $\pm$ 0.7	5.4 $\pm$ 1.0	7.5 $\pm$ 1.7	10.6 $\pm$ 2.1	9.5 $\pm$ 1.7	12.1 $\pm$ 0.6
$C_{20:0}^a$		0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.5 $\pm$ 0.3	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1	0.5 $\pm$ 0.2	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.3 $\pm$ 0.2

<sup>a</sup> Fatty acids only identified by comparing their  $R_t$  from GLC analysis with those of authentic standards

data on the composition of the fat body lipids in several species of insects have been summarized (Bailey 1975). However, studies examining the influence of fasting on the lipid composition in Triatominae are scarce.

It is well known that lipids in the fat body are used as a source of energy in different events such as flight, reproduction, starvation or stress. However it is important to emphasize that flight activity in triatomine bugs is related to nutritional status (Gringorten and Friend 1979; Schofield 1994). Consequently, in the present work we studied possible modifications induced by fasting, in the fat body lipid reserves of three different species of Triatominae.

A significant drop in lipids stored in fat bodies of the analyzed species was induced by fasting, and this decrease was so striking that by day 30 *P. megistus* maintained only 30% of the amount found on day 10. Conversely, the relative proportions of neutral lipids were maintained throughout fasting, suggesting that these lipids are used at similar rates, with the exception of *P. megistus* which exhibited a different pattern. Thus, TAG represented about 71–82% of the total neutral lipids in *T. infestans* and *D. maximus* whereas it varied between 43% and 61% in *P. megistus*. In the latter species, the amount of DAG was significantly greater than that of the other two species (31–46%). From these interesting findings, we might safely infer that they may be associated with a better flight performance shown by *P. megistus* (Canavoso et al. 1997). In support of this idea, Chino et al. (1992) suggested that even in the presence of all components essential for flight, interconversion of lipophorins occurs when a large DAG pool is present, together with a TAG pool as a continuous source of DAG. Ganon and Bell (1984) also demonstrated that DAG rapidly and spontaneously translocate across membranes to give easy availability. As far as we know, *P. megistus* is the first species of insect with such a large amount of DAG in its fat body.

Cholesterol, the principal sterol which is obtained from the diet in hematophagous insects, is essential as a structural component of cell membranes and as a precursor of the ecdysteroids (Svoboda and Feldlaufer 1991). No significant differences in cholesterol levels of the fat bodies or variation with fasting time were observed between the species studied.

Analysis by TLC and GLC showed the presence of 1,2 and 1,3 di-*O*-acylglycerols in all three species. The latter isomer would be formed by the well known intramolecular acylmigration of 1,2 di-*O*-acylglycerol (Sjursnes and Anthonsen 1994). These isomers were also present in the main lipid carrier in hemolymph, the lipophorin (personal observation). The major components corresponded to DAG esterified with C<sub>16:0</sub> and C<sub>18:0</sub> or C<sub>18:1</sub>, and their composition did not change with fasting. Also, in DAG with only the C<sub>18</sub> fatty acid, at least one of them, C<sub>18:1</sub>, was detected in all species. The principal fatty acid in the DAG fraction of *D. maximus* was palmitic acid, whereas in the other two species C<sub>16:0</sub> and C<sub>18:1</sub> were found in similar amounts.

Fasting did not significantly affect the fatty acid composition of DAG and polyunsaturated fatty acids were not detected.

As expected for animal tissue lipids, the main fatty acids found in the TAG were oleic, palmitic, palmitoleic and stearic. Palmitic and oleic acid, which represented more than 80% of the total fatty acids in TAG, were also predominant in the fat body lipids of other insects (Nelson et al. 1967; Beenackers and Gilbert 1968; Balduz and Mutchmor 1988; Schneider and Dorn 1994). Moreover, it has recently been reported that these fatty acids are quantitatively the major components in the eggs of *T. infestans* (Juarez et al. 1996). In a review on the fatty acid composition in seven insect orders, Thompson (1973) described a similar pattern for the hemipterans *Triatoma phyllosoma* and *Rhodnius prolixus*. Polyunsaturated fatty acids were not detected in these species, although these kinds of lipids have been described in extracts from the whole-body of *T. infestans* (Tierno and Brenner 1978). The novo biosynthesis of linoleic and arachidonic acid (C<sub>20:4</sub>) could not be proved to occur in this insect (Brenner and Bernasconi 1987; Tierno and Brenner 1979). In our case the ratio for monounsaturated to saturated fatty acids in TAG was 1:3, 2:6 and 1:2 for *D. maximus*, *T. infestans* and *P. megistus* respectively. Also, in a study of four strains of *Aedes aegypti* mosquitoes, no polyunsaturated fatty acids were found (Miller and Novak 1985).

The importance of polyunsaturated fatty acids (C<sub>20</sub> PUFAs) as precursors of eicosanoids and their biological significance in cellular immune response of invertebrates is recognized (Stanley-Samuels 1993; Miller et al. 1994; Howard and Stanley-Samuels 1996). We need to know whether it is possible that absence of C<sub>20</sub> PUFAs from the fat bodies of triatomine bugs is related to their incapacity to elicit a cellular immune response. In this respect, the findings of a reduced immune response in experiments of xenograft rejection in *T. infestans* infected by *T. cruzi*, as well as the reactions of encapsulation and melanization inhibition in *T. infestans* infected by *Blastocrithidia triatomae*, will support this hypothesis (Bitkowska et al. 1982; Schaub 1992). Further investigations are required to clarify the role of eicosanoids in the susceptibility of the vectors to be colonized by parasites.

The results from this study show that lipid availability in the fat bodies of reduviid bugs is modified by fasting, principally affecting the total lipids of the organ; however, the ratio of their major components does not significantly change. More studies are necessary to elucidate the biochemical mechanisms that involve the lipid reserves and flight stimulation in these vectors to seek for blood sources and/or new habitats.

**Acknowledgements** We thank Dr. José Soulages for critical review of the manuscript. This research received financial support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Buenos Aires to R. M. de L. and from Secretaría de Ciencia y Tecnología-UNC (SECyT) and CONICOR to E. R. R.

## References

- Arrese EL, Wells MA (1997) Adipokinetic hormone-induced lipolysis in the fat body of an insect, *Manduca sexta*: synthesis of *sn*-1,2-diacylglycerols. *J Lipid Res* 38: 68–76
- Baldus TJ, Mutchmor JA (1988) The effects of temperature acclimation on the fatty acid composition of the nerve cord and fat body of the American cockroach, *Periplaneta americana*. *Comp Biochem Physiol* 89A: 141–147
- Bailey E (1975) Biochemistry of insect flight, part 2. Fuel supply. In: Candy DJ, Kilby BA (eds) *Insect biochemistry and function*. Chapman and Hall, London, pp 89–176
- Beenakkers AMT, Gilbert LI (1968) The fatty acid composition of fat body and haemolymph lipids in *Hyalophora cecropia* and its relation to lipid release. *J Insect Physiol* 14: 481–494
- Beenakkers AMT, Van der Horst DJ, Van Marrewijk WJA (1985) Insect lipids and lipoproteins and their role in physiological processes. *Prog Lipid Res* 24: 19–67
- Bitkowska E, Dzbenski TH, Szadziewska M, Wagner Z (1982) Inhibition of xenograft rejection reaction in the bug *Triatoma infestans* during infection with a protozoan, *Trypanosoma cruzi*. *J Invert Pathol* 40: 186–189
- Brenner RR, Bernasconi AM (1987) The occurrence of arachidonic acid in *Triatoma infestans*. *Comp Biochem Physiol* 87B: 815–819
- Canavoso LE, Rubiolo ER (1993) Hemolymphatic components in vectors of *Trypanosoma cruzi*. Study in several species of the subfamily Triatominae (Hemiptera:Reduviidae). *Rev Inst Med Trop São Paulo* 35: 123–128
- Canavoso LE, Rubiolo ER (1995) Interconversion of lipophorin particles by adipokinetic hormone in hemolymph of *Panstrongylus megistus*, *Dipetalogaster maximus* and *Triatoma infestans* (Hemiptera:Reduviidae). *Comp Biochem Physiol* 112 A: 143–150
- Canavoso LE, Stariolo R, Cano RC, Rubiolo ER (1997) Transformation of lipophorin induced by flight activity in vectors of Chagas' disease. *Mem Inst Oswaldo Cruz* 92 [Suppl 1]: 294
- Chino H, Lum PY, Nagao E, Hiraoka T (1992) The molecular and metabolic essentials for long-distance flight in insects. *J Comp Physiol B* 162: 101–106
- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497–509
- Ganong BR, Bell RM (1984) Transmembrane movement of phosphatidylglycerol and diacylglycerol sulfhydryl analogues. *Biochemistry* 23: 4977–4983
- Gilbert LI, Chino H (1974) Transport of lipid in insects. *J Lipid Res* 15: 439–456
- Gringorten JL, Friend WG (1979) Wing-beat pattern in *Rhodnius prolixus* Stal (Heteroptera:Reduviidae) during exhaustive flight. *Can J Zool* 57: 391–395
- Howard RW, Stanley-Samuelson DW (1996) Fatty acid composition of fat body and malpighian tubules of the tenebrionid beetle *Zophobas atratus*: significance in eicosanoid-mediated physiology. *Comp Biochem Physiol* 115B: 429–437
- Juarez MP, Ayala S, Brenner RR (1996) Methyl-branched fatty acid biosynthesis in *Triatoma infestans*. *Insect Biochem Mol Biol* 26: 599–605
- Lehane MJ, Schofield CJ (1982) Flight initiation in *Triatoma infestans* (Klug) (Hemiptera:Reduviidae). *Bull Entomol Res* 72: 497–510
- Miller S, Novak RJ (1985) Analysis of lipids by gas-liquid chromatography and complementary methods in four strains of *Aedes aegypti* mosquitoes. *Comp Biochem Physiol* 81B: 235–240
- Miller JS, Nguyen T, Stanley-Samuelson DW (1994) Eicosanoids mediate insect nodulation responses to bacterial infections. *Proc Natl Acad Sci USA* 91: 12418–12422
- Nelson DR, Terranova AC, Sukkestad DR (1967) Fatty acid composition of the glyceride and free fatty acid fractions of the fat body and hemolymph of the cockroach, *Periplaneta americana* (L.) *Comp Biochem Physiol* 20: 907–917
- Schaub GA (1992) The effects of trypanosomatids on insects. *Adv Parasitol* 31: 255–319
- Schofield CJ (1994) Triatominae. Biology & control. Zeneca Public Health, Fernhurst, UK
- Schneider M, Dorn A (1994) Lipid storage and mobilization by flight in relation to phase and age of *Schistocerca gregaria* females. *Insect Biochem Mol Biol* 24: 883–889
- Sjorgren RD, Ryckman RE (1966) Epizootiology of *Trypanosoma cruzi* in southwestern North America Part VIII: nocturnal flights of *Triatoma proctata* (Uhler) as indicated by collections at black light traps (Hemiptera:Reduviidae:Triatominae). *J Med Entomol* 3: 81–92
- Sjursnes BJ, Anthonsen T (1994) Acylmigration in 1,2-dibutylin. Dependence on solvent and water activity *Biocatalysis* 9: 285–297
- Stanley-Samuelson DW (1993) The biological significance of prostaglandins and related eicosanoids in insects. In: Stanley-Samuelson DW, Nelson DR (eds) *Insect lipids: chemistry, biochemistry and biology*. University of Nebraska Press, Lincoln, pp 45–97
- Svoboda JA, Feldlaufer MF (1991) Neutral sterol metabolism in insects. *Lipids* 26: 614–618
- Szumlewicz AP (1976) Laboratory colonies of triatominae: biology and population dynamics. In: Pan American Health Organization (eds) *American trypanosomiasis research*. Scientific Publication N° 318, Washington, pp 63–82
- Thompson SN (1973) A Review and comparative characterization of the fatty acid compositions of seven insect orders. *Comp Biochem Physiol* 45B: 467–482
- Tierno MA, Brenner RR (1978) Bioquímica del ciclo evolutivo del *Triatoma infestans* (Vinchuca). 1. Composición de los lípidos y ácidos grasos. *Acta Physiol Latinoam* 28: 41–52
- Tierno MA, Brenner RR (1979) Bioquímica del ciclo evolutivo del *Triatoma infestans* (Vinchuca). 2. Biosíntesis de ácidos grasos. *Acta Physiol Latinoam* 29: 87–94
- Ward JP, Candy DS, Smith SN (1982) Lipid storage and changes during flight by triatomine bugs (*Rhodnius prolixus* and *Triatoma infestans*). *J Insect Physiol* 28: 527–534

Communicated by G. Heldmaier