RESEARCH NOTE

Adipokinetic Hormone Causes Formation of Low Density Lipophorin in the Hemolymph of *Triatoma infestans*

Lilian Etelvina Canavoso/[+], Edilberto Rene Rubiolo/[*]

Dpto. Bioquímica Clínica, Fac. Ciencias Químicas, Universidad Nacional de Córdoba, Suc. 16, CC 61, 5016, Córdoba, Argentina * Servicio Nacional de Chagas, Min. Salud Pública y Acción Social, Córdoba, Argentina

The importance of the presence of lipids in insects has been taken into account for many years. The lipids travel throughout the hemolymph from the midgut to the fat body and other organs, and this also occurs in mammals in a similar way; they associate with proteins transforming into the hemolymphatic lipoproteins or lipophorins (H Chino & KJ Kitazawa 1981 J *Lipid Res 22:* 1042-1051, AM Beenakkers et al. 1985 *Prog Lipid Res 24 :* 19-67).

In insects, the major lipophorin is a high density lipophorin (HDLp), conformed by two apolipoproteins: Apolipophorin I (ApoLp-I) and Apolipophorin II (ApoLp-II) with an apparent molecular weight of 240 and 80 kDa respectively. Both apolipophorins are present in all the class Insecta and are highly conserved throughout the phylum Arthropoda (N Hunnerland & WS Bowers 1989 *Comp Biochem Physiol 92* (B): 137-141). The adipokinetic hormone (AKH), a blocked decapeptide released from the corpora cardiaca during flight, greatly stimulates the loading of diacylglycerols by HDLp from the fat body and it becomes a larger and more heterogeneous particle with minor density range, called low density lipophorin (LDLp) (H Chino et al. 1989 *J Lipid Res 30:* 571-578, D Konopinska et al. 1992 *Int J Peptide Prot Res 39:* 1-11). The formation of LDLp involves the association of a third apolipophorin, ApoLp-III, with a weight of 20 kDa. This interconversion event is possible in insects that have ApoLp-III and use lipids as metabolic fuel for flying (RO Ryan 1990 *J Lipid Res 31:* 1725-1739).

In the last years, the flight activity in vectors of *Trypanosoma cruzi* has been studied in laboratory models as well as in field (JP Ward & PS Baker 1982 *Bull Ent Res 72:* 522-528, CJ Schofield et al. 1992 *Med Vet Entomol 62:* 51-56). We recently confirmed the presence of ApoLp-III in adults of three species of triatomine bugs (LE Canavoso & ER Rubiolo 1991 *Mem Inst Oswaldo Cruz 86* (Suppl. I): 244) as has been previously reported in *Rhodnius prolixus* (K Gondim et al. 1989 *Insect Biochem 19:* 153-161).

This work aimed to show that the vector *T.infestans* has the essential molecular constituents for generating LDLp from HDLp.

Adults of *T.infestans* were used for these experiments whose rearing conditions and fast to hemolymph collection have been described previously (LE Canavoso & ER Rubiolo 1993 *Rev Inst Med trop São Paulo 35:* 123-128). Hemocyte-free hemolymph was dialysed and then incubated in a Dubnoff incubator with the presence of dissected fat bodies. The system was finished with Ringer insect solution (120 mM NaCl, 15 mM KCl, 4 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM PIPES buffer, pH 7.0) and 100 nM of locust AKH (Peninsula Laboratories, Ca.).

The incubations were performed at 30°C in a humid oxygen-saturated chamber for 180 min and shaken at 60-80 cycles/min. The controls were carried out either without AKH or with AKH without fat bodies. After incubation and in order to isolate lipophorins, the fat bodies were extracted and the incubation medium (in some cases pre-stained with Sudan Black B) was subjected to KBr density gradient ultracentrifugation for 24 hr at 12°C (JL Kelley & AW Kruski 1986 *Meth Enzimol 128* (Part A): 170-181). The tube contents were then fractionated from the top into 1 ml fractions and the density was determined by gravimetry and/or refractometry.

The HDLp was isolated in a density range between 1.120 and 1.140 $g.ml^{-1}$ and the LDLp between 1.060 and 1.070 $g.ml^{-1}$. Before the survey of the lipidic and proteic composition, both fractions were extensively dialysed. In order to analyse apolipophorins, the delipidation procedure was performed according to Scanu and Edelstein (1971 *Anal Biochem 44:* 576-588) and the materials obtained were solubilized with buffer pH 7.4 (TRIS 10 mM, NaCl 100 mM, NaN_3 1 mM, EDTA 1 mM, Guanidine Hydrochloride 2 M) and submitted to SDS-PAGE on a gradient gel (UK Laemmli 1970 *Nature 227:* 680-685), previous quantification of proteins (MM Bradford 1976 *Anal Biochem 72:* 248-253).

This study was supported in part by a grant from SECyT. UNC.

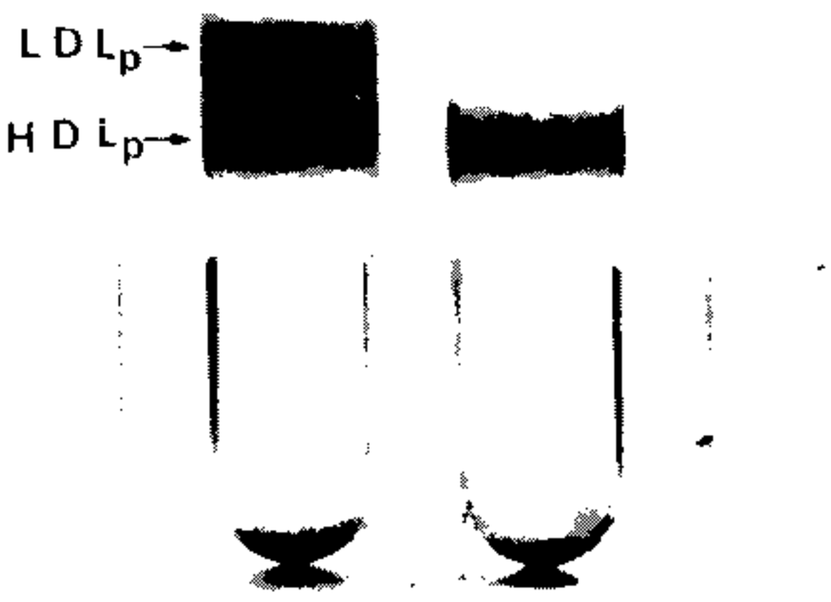
+ Research fellow from CONICET

* Corresponding author

Received 19 August 1993

Accepted 16 December 1993

Figure shows the hemolymph fractionation of *T.infestans*, incubated with fat bodies and AKH in a 100 nM final concentration. The HDLp partial interconversion with 1.130 g.ml $^{-1}$ density to a LDLp with a density 1.062 g.ml $^{-1}$ can be observed.



KBr density gradient ultracentrifugation of the hemolymph of *Triatoma infestans* pre-stained with Sudan Black B after incubation with fat body and adipokinetic hormone (1) or without adipokinetic hormone (2).

The load effect of acylglycerides from the fat body to HDLp for its transformation was proved by the variations in lipidic composition in both lipophorins. Thus, the change in acylglycerides was remarkable with the de-crease of the phospholipids content. Acylgly-cerides in HDLp were found in 48.5% vs 74.6% in LDLp; cholesterol in HDLp was found in 6.2% vs 3.7% in LDLp and phospholipids in HDLp were found in 37.0% vs 17.4 % in LDLp. Determinations were done in duplicate and both lipophorins showed ApoLp-I, ApoLp-II and ApoLp-III .

This ability of producing a new particle with a high content of lipids and low density has been shown, even in insects which have lost their flying ability by artificial selection, such as *Bombyx mori* (K Miara & I Shimizu 1989 *Comp Biochem Physiol* 89(B): 94-103). The results point out that *T.infestans*, a Chagas infection vector, possesses the biochemical elements which allow the potential transformation of high density lipophorins into another one of low density.

Although flying ability depends upon many factors such as wings, flying muscles, acylglyceride levels in fat body, and presence of lipid transfer proteins (LTP), it is obvious that *T.infestans* would be able to change HDLp to LDLp according to our results, since the *in vitro* system was able to display a hyperlipidic response on stimulation with AKH.

Further surveys will be necessary before drawing final conclusions regarding this prominent spreading mechanism of vectors in the chagasic endemia.