

Starvation and diet composition affect mRNA levels of the high density-lipoprotein- β glucan binding protein in the shrimp *Litopenaeus vannamei*

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Abstract

A high density lipoprotein-beta glucan binding protein (HDL-BGBP) is synthesized in the hepatopancreas of the white shrimp *Litopenaeus vannamei* and secreted to the hemolymph. Recently, we reported the HDL-BGBP full length cDNA sequence and found that the predicted polypeptide is larger than the mature protein and also, that it contains a long 5'- and 3'-UTRs that may be involved in transcript level regulation. To test whether starvation and feeding may play a role in regulating HDL-BGBP mRNA levels, two different stimuli were evaluated: starvation and composition of diets. After 24 h, the steady state HDL-BGBP mRNA levels of starved shrimp decreased, suggesting that synthesis of the lipoprotein is less required in the absence of food. When shrimp were fed with diets containing different concentrations of protein and lipids, changes in HDL-BGBP mRNA levels were also detected. Shrimp fed the lower concentration of protein and lipid feed accumulated higher levels of HDL-BGBP mRNA. These results indicate that feeding influences HDL-BGBP transcript levels in the hepatopancreas.

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1. Introduction

The HDL-BGBP from the white shrimp *Litopenaeus vannamei* has two main physiological roles: 1) transport of lipids (Yepiz-Plascencia et al., 1995; Ravid et al., 1999), and, 2) a particle recognition protein (PRP), a component of the immune system (Vargas-Albores et al., 1996). In the crayfish *Pacifastacus leniusculus*, this plasma protein was first identified as a β -glucan binding protein (BGBP) that forms a complex with β -glucans, inducing spreading and degranulation of hemocytes and activation of the prophenoloxidase (proPo) system (Barracco et al., 1991) and afterwards, it was identified as a lipoprotein (Hall et al.,

1995). The HDL-BGBP is synthesized in the hepatopancreas of *L. vannamei*, as was shown in juvenile shrimp using an in vitro cell-free translation system and immunodetection (Yepiz-Plascencia et al., 2000b) and RT-PCR (Romo-Figueroa et al., 2004). No transcript was detected in hemocytes, indicating that the hepatopancreas is the source of the plasma circulating lipoprotein, as was previously shown for *P. leniusculus* (Cerenius et al., 1994).

We recently reported the full length cDNA sequence of the HDL-BGBP from *L. vannamei* (GenBank accession no. AY249858). It is a longer than anticipated 6 Kb transcript (6379 bp) coding for 1454 amino acid residues and a predicted 141 kDa precursor protein, with no clear secretion signal peptide sequence. It also contains two potential subtilisin-like processing sites near the mature N- and C-termini, suggesting a highly processed mature protein

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(Romo-Figueroa et al., 2004) that is also glycosylated (Yepiz-Plascencia et al., 1995). Furthermore, the full length cDNA contains long 5' and 3'-untranslated regions with as yet, unknown functions.

Although insect hemolymph lipoproteins have been extensively studied (Van der Horst, 1990; Ryan and Van der Horst, 2000; Arrese et al., 2001), the lipid transport mechanism, and the regulation of gene expression of the crustacean lipoproteins are still unknown. Nevertheless, the dual role, nutrition and defense, of HDL-BGBP makes it an interesting molecule to investigate gene expression. Bifunctional hemolymph proteins as HDL-BGBP and VHDL-CP have been reported from shrimp (Yepiz-Plascencia et al., 1998; Yepiz-Plascencia et al., 2002), and crayfish species (Komatsu et al., 1993; Hall et al., 1995).

Starvation effects have been used to understand mechanisms leading to acute-responses generated by imbalance in energy homeostasis. Starvation changes gene expression of several genes in vertebrates. For example, after 48 h of starvation in rats, the expression of more than 54 genes increased, including proteins involved in energy and protein metabolism, stress response, signal transduction and nutrient transporters and receptors. Interestingly one of these is the apolipoprotein B-100, a component of the plasma LDL (Zhang et al., 2001). Starvation also causes acute responses in crustaceans, affecting metabolism, growth, and the biochemical composition of the hepatopancreas in shrimp species (Cuzon et al., 1980; Barclay et al., 1983; Muhlia-Almazán et al., 2003; Sánchez-Paz et al., 2003). Crustaceans are constantly challenged by several stressors, including endogenous (as molting) or exogenous agents (temperature and salinity changes, pathogens, predators, food, etc.). A timely response to these threats is critical for survival. The present study was designed to evaluate the effect of starvation and diet composition on the HDL-BGBP mRNA levels.

2. Materials and methods

Two separate bioassays were conducted to evaluate the effect of: i) starvation, and ii) diets containing different levels of protein and lipids. Juvenile *L. vannamei* shrimp, weighing 9.0 ± 1.0 g, were obtained from culture ponds at CIBNOR (Mexico) and maintained under controlled laboratory conditions. During a 15-days acclimatization period, shrimp were placed in 120 L plastic tanks in filtered marine water at 28 °C, and 34 ppt salinity, and were fed twice daily

with commercial feed (Silvercup™, El Pedregal, Mexico, under license of Sterling H. Nelson and Sons, Inc.). Uneaten food and solid excreta were discarded daily. All shrimp used in the assays were previously selected at intermolt stage by setogenesis, by observing the changes in the seta of the inner margin of uropods (Chan et al., 1988).

2.1. Starvation assay

This assay was designed to evaluate the effect of starvation on the HDL-BGBP mRNA steady state levels in the hepatopancreas of shrimp. At the end of the acclimatization, after being selected by molting stage, shrimp were fed commercial food (Silver cup containing 46% protein and 7.2% lipids, according to the producers) and then sampled at 2, 24, 72 and 120 h after the last feeding. Seven replicates of each time point were collected. Shrimp were decapitated; the hepatopancreas was dissected, individually weighed and stored at -80 °C until used.

2.2. Diets with different content assay

After acclimatization, three groups of shrimp were randomly chosen and separated in plastic tanks. Three isocaloric diets were prepared: diet 1, 2, and 3, with different protein and lipid content as shown in Table 1 (being the protein content, the indicator of quality of a diet, as commonly assumed in marine cultured animals). The diets were designed and formulated using the Mixit-winx computer software (Agricultural Software Consultants, Inc., USA). A proximal analysis was done by triplicate for each diet. Statistical analyses between the three diets replicates found no statistical differences between the three energy values of the experimental diets. Shrimp of each tank (9), were fed with one of the experimental diets, twice a day ad libitum during 3 weeks. Once shrimp were acclimated to a diet, six replicates of each experimental trial were collected after ~2 h of feeding, selected at inter-molt stage, individually weighed, decapitated and the hepatopancreas was extracted and frozen at -80 °C until use.

2.3. HDL-BGBP mRNA levels by semi-quantitative RT-PCR

Total RNA was isolated from the hepatopancreas of each shrimp individually using TRIzol® reagent (GIBCO-BRL, New York). Purity and concentration of the isolated RNA were examined by A_{260}/A_{280} ratios and analyzed on a 1.2% agarose–formaldehyde gel and ethidium bromide staining

Table 1
Experimental diets composition

| Feed label | Wet (%) | Protein content (%) | Lipids (%) | Ash (%) | Crude fiber (%) | NFE** | Energy (Cal/g) |
|------------|-----------|---------------------|------------|-----------|-----------------|-------|----------------|
| 1 | 14.3±0.2 | 15.2±0.20 | 3.06±0.08 | 5.82±0.09 | 2.77±0.07 | 73.11 | 4562±13 |
| 2 | 12.7±0.05 | 31.8±0.02 | 4.49±0.07 | 6.41±0.1 | 5.79±0.09 | 51.49 | 4461±8.8 |
| 3 | 17.2±0.08 | 49.8±0.20 | 5.65±0.21 | 10.6±0.21 | 6.26±0.24 | 27.59 | 4602±24 |

*Protein main source was fish meal (70.3% protein). **Nitrogen free extracts, considered mainly as carbohydrates. Each value represents mean±S.D ($n=3$).

(Sambrook et al., 1989). Intact total RNA was used for reverse transcription; and the cDNA was synthesized from each individual sample using the Superscript™ first strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using 5 µg of total RNA, 1 µL (10 mM) dNTP mix, 1 µL (20 µM) reverse HDL-BGBP or L8 (ribosomal protein gene used as house-keeping for normalization control) primers and DEPC water added to adjust to a final volume of 10 µL. The reaction mix was incubated for 5 min at 70 °C and then placed on ice for 1 min. Then, 2 µL of (10X) RT buffer (200 mM Tris–HCl (pH 8.4), 500 mM KCl), 4 µL (25 mM) of MgCl₂, 2 µL (0.1 M) DTT, 1 µL of RNase inhibitor, and 1 µL (50 units) of Superscript II™ RT were added to a final reaction volume of 20 µL and incubated for 50 min at 42 °C.

HDL-BGBP primers for PCR amplification were designed and reported for *L. vannamei* by Romo-Figueroa et al. (2004). Primer sequences used were HDL6forward 5'-TCAATATGGAAATGAGCACG-3' and HDL13reverse 5'-GAAGCTGTAATCCACATC-3' that match positions 3005 to 3024 and 3754 to 3772, respectively, and produce a 730 bp gene fragment (Romo-Figueroa et al., 2004) (GenBank accession no. AY249858). Two primers were designed for the ribosomal protein L8 mRNA for *L. vannamei* (Yepiz-Plascencia et al., to be published elsewhere). Primer sequences were L8Fwd 5' GAAGAGGATC-GGTGTTCAAGTC-3' and L8Rev 5'-CTCCTGAAG-GAAGCTTTACACG-3', producing a 430 bp gene fragment. PCR amplifications were performed in a 50-µL final reaction mixture containing 27 µL of H₂O, 5 µL of 10X PCR buffer (100 mM Tris–HCl (pH 8.3), 500 mM KCl), 6 µL of 50 mM MgCl₂, 4 µL of 2.5 mM dNTP mix, 2.5 µL of 20 µM each primer, 2.5 µL of the obtained cDNA from each sample, and 1 U of Taq DNA polymerase (Invitrogen). The amplifications were done using the PTC 200 DNA Engine Thermal Cycler, (MJ Research) under the following conditions: 3 min, 94 °C; 1 min, 52 °C; 3 min, 72 °C (one cycle); 1 min, 94 °C; 1 min, 55 °C, and 3 min, 72 °C (28 cycles); and an over-extension step for 10 min at 72 °C. The number of PCR cycles was evaluated to reach an exponential phase of the reaction and kept constant to obtain detectable PCR product for both genes (Dimopoulos et al., 1998; Xu et al., 1999).

The resulting PCR products (20 µL) for HDL-BGBP and L8 were analyzed in a 1.0% agarose gel and stained with ethidium bromide (Sambrook et al., 1989). Gel images were obtained and analyzed using a Digital Camera (Kodak, Rochester, NY, USA) and the intensity of the bands was evaluated by densitometry using Quantity One software (BioRad, CA, USA).

2.4. Calculation of the steady state mRNA levels

Aliquots of 2 µL of Low DNA mass ladder (Invitrogen) with known DNA concentration (ng) per band were loaded onto the 1.0% agarose gel. Intensity data (pixels/mm) from

the bands were obtained by image analysis and a standard curve was used to obtain a linear equation to calculate the DNA concentrations (ng) of the PCR products. The intensity of each experimental sample was also obtained by image analysis and the concentration of HDL-BGBP and L8 PCR products (ng) was calculated from the intensity values. The ribosomal protein L8 mRNA level was used to normalize the HDL-BGBP mRNA.

2.5. Statistical analyses

Statistical analyses to determine differences between treatments were performed by non-parametric tests, including Kruskal–Wallis ANOVA by Ranks Test, and Kolmogorov–Smirnov Test (Zar, 1984). Statistical significance was considered when $p \leq 0.05$, using Statistica v. 6.0 software.

3. Results and discussion

3.1. Expression of HDL-BGBP

Amplification of internal fragments of HDL-BGBP and L8 by RT-PCR produced the expected specific fragments of 730 and 430 bp, respectively; (Fig. 1, panel A). The RT-PCR reactions were done independently for every hepatopancreas and run in a single agarose gel for every assay. To determine the number of PCR cycles for semi-quantitative analysis, the reactions were run for 26 to 34 cycles, obtaining more PCR products until cycle 32 (Fig. 1, panel B). To detect the HDL-BGBP band, a minimum of 28 PCR cycles were necessary and thus, all the samples were evaluated using 28 cycles. Two standard curves were constructed from the known concentration of DNA marker bands intensities and adjusted to a linear model to calculate the concentration (ng) of the HDL-BGBP and L8 mRNAs. The steady state HDL-BGBP mRNA levels for each treatment were calculated by normalizing against ribosomal protein L8 mRNA, that was used as a constitutive gene, since actin mRNA is affected by other physiological conditions in crustaceans (Roux et al., 2002; Sánchez-Paz et al., 2003; Muhlia-Almazán et al., 2003).

3.2. Starvation down regulates the HDL-BGBP mRNA steady state level

HDL-BGBP and L8 mRNA levels were analyzed independently (Fig. 2A). We found HDL-BGBP mRNA levels to be statistically different between 2 h starved and control shrimp and compared with those starved 24, 72, and 120 h ($p \leq 0.05$). No statistical differences were detected for starvation times when L8 levels were analyzed ($p \geq 0.05$; Fig. 2A). Normalized (HDL-BGBP)/L8 data also resulted in statistical different values for the 2, 24, 72, and 120 h of starvation groups, respectively ($p \leq 0.05$; Fig. 2B; relative scale). A maximum value of HDL-BGBP mRNA was

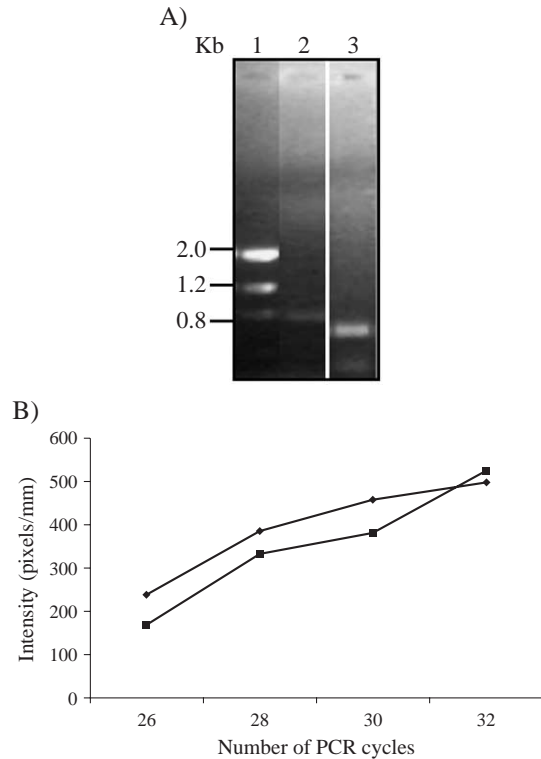


Fig. 1. HDL-BGBP and L8 RT-PCR products from hepatopancreas of the shrimp *Litopenaeus vannamei*. Panel A, HDL-BGBP (730 bp) and L8 (430 bp) RT-PCR products from hepatopancreas of 120 h starved *Litopenaeus vannamei*. Lane 1, Molecular mass and size standards; lane 2, HDL-BGBP product (730 bp); lane 3, L8 product (430 bp). Panel B: amount of RT-PCR products obtained at different PCR cycles (HDL-BGBP, squares; L8 triangles).

observed 2 h after ingestion, then after 24 h a down-regulation with a reduction of 5.7-fold on the mRNA level in comparison to 2 h starvation was detected. This was followed by a small recovery at 72 and 120 h; nevertheless, the values are still 3.8 and 3.6-fold less, respectively, than the mRNA level at 2 h. This suggests that the synthesis of the lipoprotein is reduced when the shrimp face no food

available. In contrast, the levels of L8 were kept fairly constant, although, statistically significant, indicating a fast response on the expression of HDL-BGBP; perhaps resembling the starvation that shrimp naturally face during molting.

Molting is a cyclical physiological process that is required to grow and during this process, shrimp naturally starve for short periods of time (3–4 days maximum) due to the lack of hard structures necessary to handle their food (Chan et al., 1988). Therefore, shrimp and others crustaceans, probably have developed mechanisms to cope with temporal fasting, by using and/or mobilizing efficiently energy reserves. Shrimp hepatopancreas is the major organ for the synthesis of digestive enzymes and plasma proteins, probably including all the lipoproteins, although this has only been shown for the HDL-BGBP (Romo-Figueroa et al., 2004). The effect of starvation is larger and faster on the HDL-BGBP expression than in trypsin (Sánchez-Paz et al., 2003) related probably to the secretion process and functions of these two proteins. Trypsin mRNA levels is increased slightly (1.2-fold) after 24 h starvation, followed by a 1.5-fold decreased after 72 h and then remained unchanged until 120 h (Sánchez-Paz et al., 2003).

Contrasting results in response to starvation have been reported for different animals. In rats, starvation promoted a general up-regulation of hepatic mRNAs (Zhang et al., 2001). The mRNA levels of genes coding for proteins involved in protein metabolism, stress response proteins, and also an apolipoprotein B-100, have been reported to increase up to 7.1-fold in starved rats. In contrast, in invertebrate lipoproteins mRNAs, the changes appear to vary even among closely related species. In the mosquito *Aedes aegypti* blood feeding induces lipophorin gene expression, (Van Heusden et al., 1998), while in *Anopheles gambiae*, the lipophorin mRNA is not significantly changed after the blood meal (Nirmala et al., 2005), appearing, according to the authors, as a constitutively expressed gene.

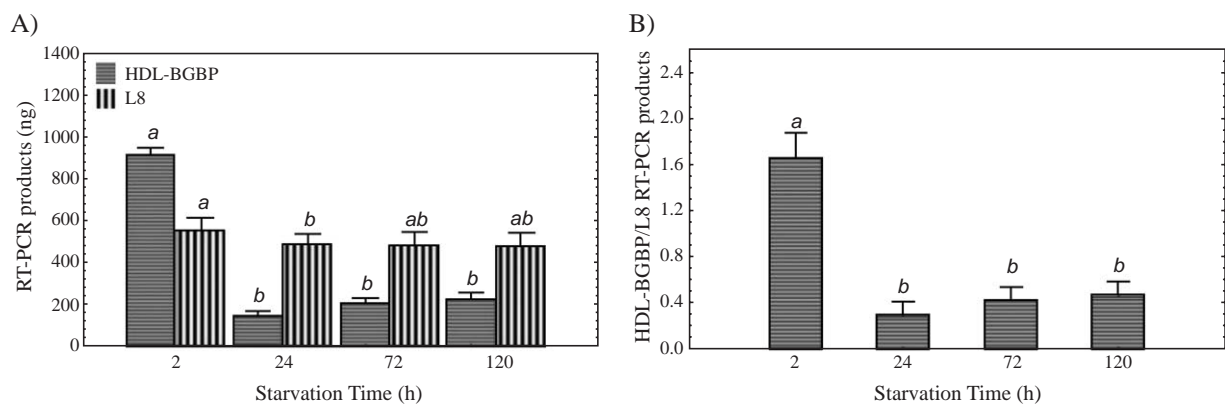


Fig. 2. HDL mRNA levels in response to starvation. Panel A, independently evaluated HDL and L8 mRNA levels. Panel B, normalized data of ((HDL-BGBP)/L8) mRNA. Different italic letters indicate statistical differences between groups ($p < 0.05$). Each value represents mean \pm S.D. ($n = 7$).

Starvation changes the expression of several genes in *Drosophila* larvae. In particular, proteins involved in lipid synthesis or degradation are affected in opposite forms. A fatty acid synthase and a phosphatidylcholine transfer protein are 4-fold down-regulated after 4 h of starvation, while a triacylglycerol lipase is 10-fold induced. These levels appear to be dynamic, since after 12 h starvation, the fatty acid synthase mRNA levels are 2-fold up-regulated, indicating mobilization of energy reserves (Zinke et al., 2002). Under short term starvation, the shrimp HDL-BGBP mRNA levels decreased, while the ribosomal L8 levels are kept fairly constant, indicating a gene specific down-regulation.

3.3. The HDL-BGBP mRNA levels are modulated by diet composition

Three different diets were prepared in the laboratory and tested. The composition of the formulated feeds is shown in Table 1 and they were designed to test mainly, the effect of protein concentration. The protein content between diets varied from 15% to 49%, whereas the lipid content was 3% to 5%, so a small increase was observed also in lipid concentration from diet 1 to diet 3. No statistical differences were observed between energy values of formulated diets.

HDL-BGBP and L8 mRNA levels were separately analyzed (Fig. 3A). HDL-BGBP levels were not statistically different between shrimp fed the three different diets ($p \geq 0.05$), while statistical differences were detected in response to the diets in the L8 mRNA levels ($p \leq 0.05$; Fig. 3A). The increase for L8 mRNA levels probably reflect the larger availability of protein affecting general transcription; however, when the HDL-BGBP/L8 normalized levels are considered, the values are statistically different, with relative values of 1.49, 0.84 y 0.71 for diets 1, 2, and 3, respectively ($p \leq 0.05$), closely resembling the data from the starvation experiment after 2 h. The highest mRNA level was detected in shrimps fed with diet 1 (15% protein, 3% lipid);

contrasting to diets 2 and 3 that had 1.77 and 2.09-fold less mRNA levels, respectively (Fig. 3B).

Food proteins and lipids are major energy sources for shrimp, since both are necessary in several metabolic processes for survival, growth, molting and reproduction (Rosas et al., 1995). Ingested food is digested by enzymes produced by the hepatopancreas, absorbed through the gut and transported to appropriate cells for storage or utilization (Dall et al., 1990; Lhoste et al., 1994; Rosas et al., 1995). Proteases produced by shrimp hepatopancreas appear to respond to the type and amount (%) of dietary protein. Trypsin activity and mRNA changes in response to feeding diets containing different content of fish protein, indicating specific and complex responses to the diet (Le Moullac et al., 1997; Muhlia-Almazán et al., 2003) and this appear to occur also for the HDL-BGBP mRNA.

Since the differences in lipid concentration among the diets is very small, this is probably not affecting the response largely, however, an appropriate relationship between protein and lipid may be necessary to have a proportionate lipoprotein synthesized or conversely, this may be less dramatic once a threshold is reached and may depend on the particular species and/or tissue. In the red sea bream visceral adipose tissue, the amount of lipoprotein lipase (LPL) mRNA was significantly decreased by starvation, irrespective of the amount of lipid in the diet used before starvation (Liang et al., 2002). In contrast, in the liver of fish fed low-lipid diet, the expression of LDL was drastically stimulated by starvation, but no effect was seen after starvation on LPL expression in fish fed high-lipid diet (Liang et al., 2002). According to Shiau (1998), shrimp may not have a definite amount of lipid requirement; although cholesterol and unsaturated fatty acids are essentials, recommended lipid concentration for commercial shrimp feeds range from 6–7.5%. So, the three experimental foods we tested are low-lipid content diets ranging from 3% to 5% but not far from the usual concentrations used in commercial feeds; thus, the effect observed is larger in L8, that is not a lipoprotein, than for the normalized HDL-BGBP/L8

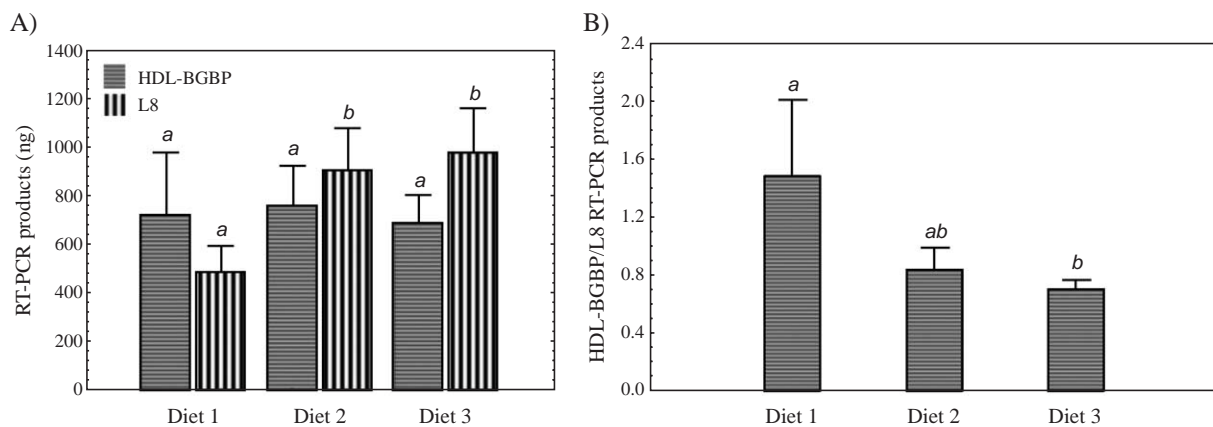


Fig. 3. HDL mRNA levels in response to diet composition. Panel A, independently evaluated HDL and L8 mRNA levels. Panel B, normalized data of ((HDL-BGBP)/L8) mRNA. Different italic letters indicate statistical differences between groups ($p < 0.05$). Each value represents mean \pm S.D. ($n = 6$).

levels, indicating perhaps, the need for larger dietary protein and lipid concentration to have a pronounced effect on the HDL-BGBP levels.

3.4. Are shrimp plasma lipoproteins concentrations regulated by starvation and/or feeding?

As mentioned before, hepatopancreas is the major lipid storage organ for crustacean (Dall et al., 1990), and it is also the organ where lipoproteins are synthesized and secreted to the hemolymph (Yepiz-Plascencia et al., 2000a). After food ingestion, lipids must be absorbed, transferred to hemolymph and directed to the appropriate storage. After 2 h of food ingestion, when lipids in hemolymph are presumably increased, the HDL-BGBP mRNA levels are also high, indicating synthesis of the HDL-BGBP. After 24 h of starvation, HDL-BGBP mRNA decreased significantly, suggesting that during food scantiness, protein and lipids concentrations drop and as a consequence, the HDL-BGBP synthesis is also reduced. A small increase was observed after 72 and 120 h of starvation, but the mRNA amounts are still lower than at the beginning of starvation (Fig. 2A and B). This small increase of HDL-BGBP mRNA at 72 and 120 h of starvation, suggests that hepatopancreas reserves, which are mainly lipids, are perhaps mobilized and HDL-BGBP is synthesized to transport lipids from the hepatopancreas to other organs. However, in long-term starvation, it is possible that the HDL-BGBP mRNA levels are again reduced, since the lipid and protein reserves have already been used up and perhaps, only an essential basal amount of the HDL-BGBP is maintained in the hemolymph.

Our results showed that starvation induced changes in HDL-BGBP expression of 5.7-fold between the maximum and the minimum levels at 2 and 24 h. Also, we observed statistical differences when shrimp were fed with the three experimental diets, with a maximum difference value of 2.09-fold, between the lower and higher minimum levels. However, the larger differences observed in the HDL-BGBP expression promoted by the lipid and protein content of diets (2.09-fold), were 2.7 times less significant than the bigger differences promoted by starvation (5.7-fold). Approximately the same values were found for the HDL-BGBP mRNA levels in shrimp fed with a commercial food (46% protein, and 7.2% lipid content) after 2 h post-feeding for the starvation assay, and for shrimp fed with the experimental diet 1 (15% protein, and 3.05% lipid contents). This may indicate that both, protein and lipid affect the HDL-BGBP mRNA levels, since there is no direct response only to high protein levels.

No information is currently available on the relationship between nutrition and lipoprotein gene expression in shrimp. However, completely reciprocal regulation (down-regulation in adipose tissue and up-regulation in muscle under starvation) occurs in lipoprotein lipase in the two tissues of human and rats (Enerback and Gimble, 1993; Zechner, 1997).

So far, only two lipoproteins (HDL and VHDL) have been detected in penaeid shrimp hemolymph (Lubzens et al., 1997; Yepiz-Plascencia et al., 2000a, 2002) and similarly in other crustaceans (Hall et al., 1995; Komatsu and Ando, 1998). Most of the hemolymph lipids appear to be protein-bound as part of these two lipoproteins and although there are no reports about starvation effects on crustacean hemolymph lipoproteins, long term starvation in *L. vannamei* (up to 21 days) causes 3-fold reduction of plasma acylglycerides (Pascual et al., in press). If this reflects a decrease on the overall plasma lipids, is still unknown. Nevertheless, this appears to agree with a 1.8-fold reduction in total plasma lipids under short term starvation (120 h) (Sánchez-Paz and Yepiz-Plascencia, personal communication) and possible, directly related to HDL-BGBP mRNA level reduction.

On the other hand, other processes may change significantly the half life or steady state levels of the lipoprotein and/or its mRNA. In innate defense and acting as a PRP the HDL-BGBP forms a beta-glucan-BGBP complex that binds to a putative hemocyte membrane receptor and triggers degranulation, spreading and activation of the prophenoloxidase (Barracco et al., 1991). Perhaps, during this process, the HDL-BGBP is taken up by the hemocyte cell to activate a signal cascade and the protein is degraded. Supporting this hypothesis, a recent report indicates that the HDL-BGBP is up-regulated in *Penaeus japonicus* after white spot syndrome virus (WSSV) infection (Pan et al., 2005). Since Walker et al. (2003) detected the first appearance of the lipoprotein in stage 5 embryos in the blue crab *Callinectes sapidus*, and that its amount increased during development, they proposed that the function as PRP in the immune system was more important during development, while the lipid transport function is more central in juveniles and adult crustaceans. Further work may resolve the developmental role of the HDL-BGBP as PRP.

In summary, we report that HDL-BGBP expression is influenced by dietary components and feeding regime, suggesting that it is an inducible gene. Future studies could be directed to test whether different mechanisms respond to dietary changes and its defense function, since defense and nutrition are basic aspects for proper growth and health. How the HDL-BGBP loads lipids from the hepatopancreas reserves, or if lipids are taken up directly from the gut, remains unknown.

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