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## Review

# Lipid storage and mobilization in insects: current status and future directions

Estela L. Arrese <sup>1</sup>, Lilian E. Canavoso, Zeina E. Jouni, James E. Pennington,  
Kozo Tsuchida <sup>2</sup>, Michael A. Wells <sup>\*</sup>

*Department of Biochemistry and Center for Insect Science, Biological Sciences West, The University of Arizona, Tucson, AZ 85721-0088, USA*

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## Abstract

In this paper we review the current status of research on fatty acid absorption and conversion to diacylglycerol in the midgut. We further discuss how diacylglycerol may leave the midgut and associate with lipophorin in hemolymph. We review the present understanding of the role of the lipid transfer particle and lipophorin receptors in lipid delivery between lipophorin and tissues. Finally, we discuss recent studies on the mobilization of diacylglycerol from the fat body in response to adipokinetic hormone. Several suggestions for exciting areas of future research are described. © 2001 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

The pace of gene and genome sequencing seems to increase almost daily. As we try to cope with exponentially expanding databases, there is, perhaps, a tendency to lose sight of the fact that biochemistry and physiology provide the context within which we can interpret the functions of all these genes and their protein products. We have written this review in the hope that it will stimulate insect scientists to explore new ways to combine the power of molecular biology with biochemical and physiological studies that will ultimately lead to a more complete understanding of the regulation of lipid storage and mobilization.

For reasons of space and interest, we have chosen to focus this review on the metabolic pathways by which dietary fatty acids are converted to stored triacylglycerol (TAG) in the fat body and then mobilized to support metabolic needs. If the readers think that this material is already well understood and holds no exciting ques-

tions for future research, consider the life history of the tobacco hornworm, *Manduca sexta*. Larvae feed constantly (*Manduca* means glutton in Latin) and this behavior is only interrupted during the molt (Ziegler, 1985). As a result, the content of fat body TAG increases from a few milligrams at hatching up to 300 mg at the end of the larval stage (Fernando-Warnakulasuriya et al., 1988). During the pupal period, these lipid stores are used to support metamorphosis and the adult moth utilizes the stores to support the energy demands imposed by reproduction and flight (Ziegler, 1991). In short, the larva accumulates reserves while the adult consumes them. Thus, during development the fat body changes from a lipid-storing tissue to a lipid-mobilizing tissue (Tsuchida and Wells, 1988); however, at present, there is no information about how this profound transition in the metabolic conditions of the fat body is regulated, but clearly it must be regulated.

Perhaps the general reader will also be surprised to learn that we know little of the details about the processes by which dietary fatty acids are digested and absorbed within the midgut and then transported to the fat body for storage. Equally sparse is our understanding of the details about how fatty acids are mobilized from the fat body and transported to tissues for utilization. Although many laboratories, including our own, have contributed to a broad picture of these processes, close

<sup>\*</sup> Corresponding author. Tel.: +1-520-621-3847; fax: +1-520-621-9288.

*E-mail address:* mawells@u.arizona.edu (M.A. Wells).

<sup>1</sup> Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK, USA.

<sup>2</sup> National Institute for Infectious Diseases, Tokyo, Japan.

examination, as we shall see, reveals many critical gaps. Thus, the purpose of this review is to describe this broad picture and point out where, in our opinion, the gaps lie and to suggest how those gaps might be filled.

## 2. Unique properties of lipid transport in insects

Before proceeding to the heart of this review, it is worth reminding the reader about several unique properties of lipid transport in insects that will be a common thread for much of what follows. Lipophorin is the major lipoprotein found in insect hemolymph and serves as a reusable, non-internalized shuttle (Chino and Kitazawa, 1981; Chino, 1985; Tsuchida and Wells, 1988). Lipophorins are named according to their density: low-density lipophorin (LDLp), high-density lipophorin (HDLp), and very high-density lipophorin (VHDLp) (Beenackers et al., 1985). Lipophorin is a nearly spherical particle with a surface composed of phospholipids and proteins. In contrast to vertebrate lipoproteins, diacylglycerol (DAG) is the predominant core neutral lipid of lipophorin with lesser amounts of sterols, hydrocarbons, carotenoids and other acylglycerols—a comprehensive tabulation of lipophorin properties has been published (Soulages and Wells, 1994). Most of the fatty acids, whether in transit to storage or utilization sites, are transported as DAG in lipophorin. One hallmark of lipid transport in insects is the tissue specificity of lipid delivery. Thus, the same lipophorin molecule can selectively deliver DAG to the fat body, and hydrocarbon and carotenoids to the cuticle in larval *M. sexta*, while in *Bombyx mori* lipophorin also delivers carotenoids to the middle silk gland.

## 3. Digestion, absorption and transport of lipids from the midgut

One of the major functions of the midgut is to digest dietary lipids, and absorb and process the digestion products for export into the hemolymph. TAG is a major lipid component of the diet and the major form for fatty acid storage (Beenackers et al., 1985; Downer, 1985; Turunen and Crailsheim, 1996). Lipid export from enterocytes in insects differs from that in vertebrates in two important ways: (1) DAG is the main lipid exported and (2) DAG release does not involve the biosynthesis of a lipoprotein particle in the midgut, but rather the DAG is released directly into existing lipophorin in the hemolymph (Chino, 1985; Prasad et al., 1986; Shapiro et al., 1988; Ryan, 1990; Soulages and Wells, 1994). Understanding the regulation of the export of DAG from the midgut involves integrating several processes: (1) the mechanisms of lipid digestion and absorption within the midgut lumen; (2) the pathway(s) for DAG synthesis in

the enterocyte; and (3) the mechanism of transfer of DAG from the enterocyte to lipophorin in the hemolymph.

The digestive process has been sufficiently characterized to suggest two models of lipolysis in the midgut lumen, the complete hydrolysis of TAG to fatty acids and glycerol (Weintraub and Tietz, 1973; Weintraub and Tietz, 1978; Tsuchida and Wells, 1988) and the formation of fatty acids and monoacylglycerol (MAG) (Hoffman and Downer, 1979; Male and Storey, 1981). Triacylglycerol lipase has been studied only in crude preparations from a few species. The enzyme released fatty acids from the 1- and 3-positions and showed a preference for unsaturated fatty acid (Bollade et al., 1970; Weintraub and Tietz, 1973; Hoffman and Downer, 1979). When 2-MAG is the product of hydrolysis, it may be absorbed as in *Periplaneta americana* (Bollade et al., 1970), or further hydrolyzed as seen in *Locusta migratoria* (Weintraub and Tietz, 1973). The complete hydrolysis of TAG to fatty acids and glycerol, involving migration of the fatty acid from 2-MAG to 1-MAG, was suggested to occur in midgut of *M. sexta* (Tsuchida and Wells, 1988). It is known that absorption of fatty acids in insect midgut is efficient (Tsuchida and Wells, 1988; Law et al., 1992; Soulages and Wells, 1994), but nothing is known about the possible role of fatty acid transporters in the luminal membrane of the enterocyte (Hui and Bernlohr, 1997; Stahl et al., 1999).

Lipid transport within the midgut epithelial cell may involve specific intracellular proteins. Two fatty acid-binding proteins were isolated from the midgut of *M. sexta* (Smith et al., 1992) and a lutein-binding protein was isolated from midgut of silkworm *B. mori* (Jouni and Wells, 1996). However, the role(s) of these proteins remains unclear in both insect and vertebrate systems. It is possible that these proteins prevent accumulation of toxic levels of free fatty acids in the cell or that the fatty acid bound to the protein is directed into specific metabolic pathways.

In vivo and in vitro studies using several insect orders demonstrated that DAG is the main lipid appearing in the hemolymph after glyceride digestion (Weintraub and Tietz 1973, 1978; Turunen, 1975; Reisser-Bollade, 1976; Turunen and Chipendale, 1977; Chino and Downer, 1979; Tsuchida and Wells, 1988). In the midgut of *M. sexta*, Tsuchida and Wells (1988) showed that nearly 90% of the fed labeled triolein was absorbed after 4 h and, of that absorbed, more than 70% was found in fat body as TAG. In the hemolymph, more than 90% of the label was in DAG and all the hemolymph-DAG was present in lipophorin.

Within the cells, the pathway for DAG formation could involve the stereospecific acylation of 2-MAG to form sn-1,2-DAG or de novo synthesis of DAG from sn-glycerol-3-phosphate via phosphatidic acid, using the fatty acids produced by TAG hydrolysis. In the intestine

of mammals the monoacylglycerol and phosphatidic acid pathways contribute about 80% and 20%, respectively, to chylomicron-TAG synthesis (Yang and Kuksis, 1991). In *Pieris brassicae* larvae, dietary glycerol was used in the midgut cells in the synthesis of sn-1,2-DAG, indicating the presence of the phosphatidic acid pathway (Turunen, 1993). In *M. sexta* larvae, we have observed that DAG is synthesized via the phosphatidic acid pathway and that there is no contribution from the MAG pathway (L.E. Canavoso and M.A. Wells, 2000). Although it is possible that the newly synthesized DAG could be released immediately to hemolymph lipophorin, we observed significant labeling of the midgut TAG pool. These results are consistent with the suggestion that absorbed fatty acids are first converted to TAG, which serves as a reservoir from which DAG is released at a rate consistent with its movement from the cell into lipophorin. This mechanism assures maximal absorption of fatty acid from the lumen, while maintaining a low intracellular concentration of DAG, which can be toxic at high concentrations. TAG accumulation was also reported in the midgut of *Aeshna cyanea* (Komnick et al., 1984). In *M. sexta*, the conversion of TAG to sn-1,2-DAG is accomplished by a cytosolic lipase, which shows preferential hydrolysis of the sn-3 fatty acid from TAG to form sn-1,2 DAG (E.R. Rubiolo and M.A. Wells, unpublished).

The roles of lipophorin receptors and lipid transfer particles (LTPs) appear to be critical in understanding the lipid transfer process in midgut and its regulation. Both aspects will be discussed in detail in other sections of this review.

### 3.1. Future directions

There are still many unanswered questions regarding lipid digestion and absorption. Fig. 1 shows a possible

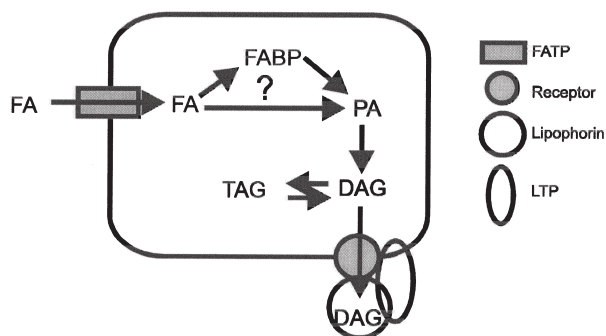


Fig. 1. A scheme showing the possible steps involved in fatty acid (FA) absorption into the midgut enterocyte, its transformation into diacylglycerol (DAG) and export of DAG from the cell into the hemolymph. The scheme emphasizes the need for more studies on fatty acid uptake into the enterocyte and the potential role of the fatty acid transport protein (FATP), the role of intracellular binding proteins such as the fatty acid binding protein (FABP), the pathway for DAG biosynthesis, and the mechanism of DAG export from the enterocyte.

scheme that illustrates several areas for future studies. Among these are:

- understanding the mechanism of fatty acid uptake into the midgut cell;
- delineating the role of intracellular lipid-binding proteins, such as fatty acid-binding protein and lutein-binding protein;
- elucidating the mechanism by which DAG is exported into hemolymph lipophorin.

## 4. Lipophorin receptors

Despite the fact that lipophorin receptor activity has been characterized in several organisms, little is known about the function of the receptors in the transfer of lipid between lipophorin and cells. A membrane protein with lipophorin binding activity has been identified in the larval fat body of *M. sexta* (Tsuchida and Wells, 1990). The purification of this receptor activity from solubilized membranes yielded a 120-kDa protein with a  $K_d$  for HDLp of  $4.1 \pm 0.19 \times 10^{-8}$  M. Binding of HDLp to this receptor showed a dependence on  $Ca^{2+}$  and inhibition by suramin. Lipophorin binding to the midgut of *M. sexta* larvae was characterized in a midgut membrane preparation (Gondim and Wells, 2000). Specific binding of lipophorin to the midgut membrane was a saturable process with  $K_d = 1.5 \pm 0.2 \times 10^{-7}$  M and, in contrast to the receptor from the fat body, binding did not depend on calcium. The lipid content of the lipophorin did not significantly affect the affinity of the membrane preparation for lipoprotein. Immunocytochemical studies in *A. cyanea* revealed that lipophorin binds to the surface of midgut and fat body cells (Bauerfeind and Komnick, 1992a,b). However, it is not yet known whether a membrane-associated protein mediates this binding.

A characteristic feature of insect lipophorin is that it acts as a reusable shuttle. The turnover of the DAG component occurs at a much higher rate than that of the protein component (Downer, 1985). Therefore, because lipophorin is able to deliver its neutral lipid contents to tissues without degradation of its protein subunits (Tsuchida and Wells, 1988), a system involving the endocytosis and degradation of HDLp, similar to that occurring in vertebrates, seems to be unimportant in insects. More likely, the mechanism of lipid transfer from lipophorin to cells involves the binding of HDLp to the receptor and transfer of lipid either through passive diffusion or by the action of active transporters. Alternatively, lipophorin could be internalized into the cell, and, after release of the lipids, the delipidated lipophorin particle could return to the hemolymph and reload with lipid. This internalization/secretion cycle does not readily account for tissue-specific lipid transfer and delipid-

ated lipophorin has not been reported in hemolymph, although it might occur at low levels if reloading is rapid.

Endocytosis of lipophorin by fat body cells has been shown in *L. migratoria* (Dantuma et al. 1997, 1998), and *A. cyanea* (Bauerfeind and Komnick, 1992b). In *A. cyanea*, lipophorin was shown to be bound to the larval fat body cell surface as well as being located intracellularly. The intracellular localization revealed the presence of lipophorin in the secretory pathway (endoplasmic reticulum, Golgi apparatus and *trans* Golgi network), as would be expected for a secreted protein. Interestingly, lipophorin was also found in endosomes, which would be consistent with an endocytotic uptake of lipophorin from the hemolymph. In a similar study, the authors were unable to find lipophorin associated with endosomes in the midgut (Bauerfeind and Komnick, 1992b). Bauerfeind and Komnick postulated that endocytosis of lipophorin in the fat body may be involved in the removal of old or damaged lipophorin. The authors also postulated that a mechanism of HDLp endocytosis into the fat body, lipid delivery, and recycling of the delipidated particle into the hemolymph might exist. Other than the existence of endosomal associated lipophorin, no evidence for such a unique and complex mechanism exists in *A. cyanea*.

In *L. migratoria*, a single fat body binding site for lipophorin was characterized (Dantuma et al., 1996). This binding activity differs from that of *M. sexta* in that it does not require divalent cations and seems to have a broader specificity as it can bind human low-density lipoprotein (LDL). In a later study (Dantuma et al., 1997), the lipophorin binding activity in *L. migratoria* fat body was found to be involved in the endocytosis of lipophorin. The authors found that lipophorin, even though it is endocytosed, does not appear to accumulate in the cell and an insignificant amount of lipophorin is degraded. On the other hand, the lipid moiety of HDLp is able to accumulate intracellularly. Furthermore, by using ammonium chloride, an endocytosis inhibitor, the authors demonstrated that the inhibition of endocytosis did not significantly affect the rate of exchange of DAG and cholesterol between the fat body and HDLp *in vitro*. Based on these observations, the authors concluded that the endocytosis of lipophorin is not necessary for the transfer of DAG and cholesterol. The authors also postulated that the majority of the endocytosed lipophorin is not lysosomally degraded and may be resecreted after endocytosis. The physiological relevance of the endocytosis and subsequent secretion of lipophorin is still unclear, but it is clear that it does not play a significant role in the transfer of the major neutral lipids.

A putative cDNA for the receptor involved in the endocytosis of lipophorin has been cloned from the fat body of *L. migratoria* (Dantuma et al., 1999). By performing polymerase chain reaction (PCR) using

degenerate primers designed against the conserved regions of the LDL receptor family, the authors were able to obtain a product that was used to probe a cDNA library. The cDNA coded for an 883-residue integral membrane protein belonging to the LDL receptor family. This receptor is expressed in several tissues, including oocytes, brain, and midgut, in addition to fat body. When expressed in vertebrate cells, the receptor mediated the endocytosis of *L. migratoria* HDLp from the media.

A homologue of the vertebrate macrophage-specific scavenger receptor has been cloned and characterized from *Drosophila melanogaster* L2 cells (Pearson et al., 1995). Macrophage scavenger receptors have broad ligand specificity and are able to bind and assist in the endocytosis of molecules as part of a clearance mechanism to remove damaged or foreign molecules (Abrams et al., 1992). Using a technique known as expression cloning, in which an expression library transfected in COS-M6 cells was probed with fluorescent labeled acetylated human LDL, the authors isolated a cDNA for this receptor, named dSR-C1. The cDNA codes for a 609-residue membrane protein, but little is known what role, if any, this receptor might play in lipid metabolism in insects. In addition, little is known about the binding characteristics of this receptor for endogenous insect lipoproteins.

#### 4.1. Future directions

More research is needed to determine the mechanism of lipid transfer between lipophorin and tissues, and the function of lipophorin receptors in this transfer. What is clearly needed is more information about lipophorin receptors, including:

- the types of receptors present—docking and/or endocytotic;
- sequence and structure of the various receptors. This information can possibly be obtained using an expression cloning technique such as the one used by Pearson et al. (1995). There are several significant benefits of this approach:
  - it would retrieve only clones that are functional;
  - it would retrieve every type of lipophorin binding protein that can bind labeled lipophorin;
  - it does not depend on any assumptions about the nature of the receptors, as is required when cloning using PCR and sequences of receptors characterized from vertebrates.

This approach could provide a good indication of the types of receptors in insect tissues and the various functions that they may perform. The cDNAs derived from such an approach could then be further characterized after expressing them in a cell culture system (Dantuma et al., 1999). These systems would reveal how lipophorin

receptors function (docking or endocytosis) and if other factors, such as lipid specific active transporters or LTPs, are necessary to facilitate the transfer of lipid.

## 5. Lipid transfer particle

LTP was first purified from the hemolymph of larval *M. sexta* (Ryan et al., 1986a,b). LTP is a very high-density lipoprotein with a molecular mass of more than 670 kDa. It contains 14% lipids and three glycosylated apolipoproteins, apoLTP-I, -II and -III of 350, 85, and 60 kDa respectively. LTP has also been identified and purified from the hemolymph of other species, including *L. migratoria* (Hirayama and Chino, 1990), *P. americana* (Takeuchi and Chino, 1993), *Musca domestica* (Capurro and De Bianchi, 1990) and *B. mori* (Tsuchida et al., 1997). In *M. sexta*, LTP is synthesized in the fat body and secreted into the hemolymph (Van Heusden et al., 1996). LTP is also present in oocytes where it plays a role in the conversion of adult HDLp to egg VHDLP (Liu and Ryan, 1991). Whether LTP is synthesized in oocytes or is transported into oocytes has not been determined.

Electron microscopy showed that LTP consists of a spherical head and an elongated, hinged, cylindrical tail (Ryan et al., 1990a). The location of the three apolipoproteins within the particle structure, their stoichiometry and their specific functions are not yet known, although existing data demonstrate that the three apoproteins are required for LTP activity (Van Heusden et al., 1996).

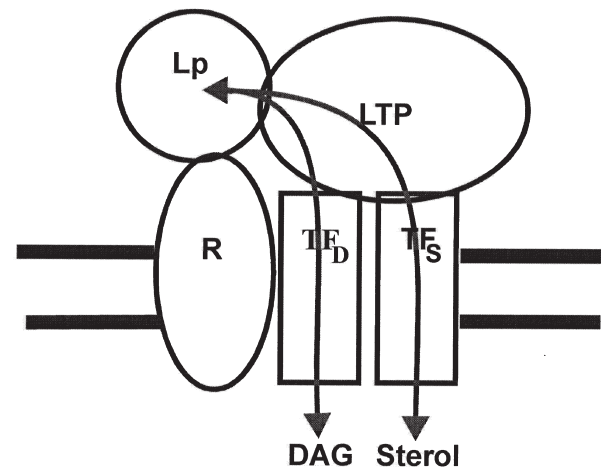
The physiological function of LTP is not completely elucidated but in vitro studies have shown that LTP catalyzes the exchange and/or transfer of DAG: (1) between lipophorins and human lipoproteins (Blacklock and Ryan, 1994); (2) from fat body to HDLp resulting in the formation of LDLp (Van Heusden and Law, 1989); (3) from the insect midgut to HDLp (L.E. Canavoso and M.A. Wells, unpublished); and (4) from HDLp or LDLp to vitellogenin, a major female specific lipoprotein in insect hemolymph (Tsuchida et al., 1997). LTP also facilitates the transfer of other lipids from HDLp to LDLp, including phospholipids (Tsuchida et al., 1997), carotenoids (Tsuchida et al., 1998) and hydrocarbons (Takeuchi and Chino, 1993).

It is interesting to focus on the role of LTP in DAG transfer between cells and lipophorin. In *M. sexta*, in vitro assays using either TAG-labeled adult fat bodies (Van Heusden and Law, 1989) or DAG-labeled larval midgut sacs (L.E. Canavoso and M.A. Wells, unpublished) showed similar features: (a) the release of labeled DAG from the tissues was inhibited by treating fat bodies or midgut sacs with anti-LTP, and (b) in both systems, the DAG transfer activity could be restored by adding LTP to the media. While these results indicate that LTP plays an essential role in promoting DAG

transfer from the cells to lipophorin and supports the model depicted in Fig. 2, the mechanism is unknown. One possibility is that LTP plays a role in the formation of the lipophorin–receptor complex. Alternatively, LTP might act to enhance the function of the tissue-specific lipid transfer factors, which in turn could determine the direction of the lipid transfer. In vitro experiments using anti-receptor antibodies could provide a useful approach to address this point.

In vitro, it has been demonstrated that LTP can promote the net transfer of lipids between lipophorins obtained from different life stages of *M. sexta*, e.g. larval HDLp and adult LDLp (Ryan et al., 1987), and of *M. domestica* (Capurro and De Bianchi, 1990). The ability of *M. sexta* LTP to convert adult LDLp particles into two new lipoproteins species with different sizes, densities, and apolipoprotein contents has been demonstrated in vitro (Ryan et al., 1990b) and LTP converts a partially delipidated lipophorin into HDLp and VHDLP (Z.E. Jouni and M.A. Wells, unpublished). However, the ability of LTP to promote net lipid transfer was absent when lipophorins were obtained from the same life stages, e.g. adult *L. migratoria* (Hirayama and Chino, 1990), *P. americana* (Takeuchi and Chino, 1993) and *M. sexta* (Tsuchida et al., 1997). This later result is consistent with the fact that HDLp and LDLp coexist in adult hemolymph, which would not be possible if LTP transferred lipids between the two lipophorins in vivo.

## Hemolymph



## Intracellular

Fig. 2. A model of a lipid transfer complex involved in selective delivery of lipid to tissues. Lipophorin (Lp) is bound to the cell via a membrane receptor (R). While bound, lipophorin lipids can be transferred to or from the cell via lipid transfer particles (LTPs) and specific transfer factors (TFs) that are integral membrane proteins. Illustrated here are TFs for diacylglycerol (DAG) (TF<sub>D</sub>) and sterol (TF<sub>S</sub>).

Thus, the physiological significance of net lipid transfer between lipophorin species remains unclear.

### 5.1. Future directions

There are many unanswered questions about the physiological role of LTP, including the following.

- The mechanism by which LTP mediates the transfer of lipids is clearly an important area for future study.
- LTP biosynthesis and assembly in the fat body have not been studied. Nor has the role of the various apolipoproteins in LTP function been studied in detail. Being a very large protein, LTP imposes difficulties in exploring and answering many questions, because cloning and sequencing the cDNA may be a formidable task if apoLTP-I, -II, and -III are made from a single gene, as in the case of apolipophorin-I and -II.
- The role of LTP in specific lipid transfer is also unknown. A possible lipid transfer complex is depicted in Fig. 2, in which LTP is proposed to mediate the transfer of any lipid with specificity being determined by the properties of putative lipid transfer factors in the cell membrane. Although no such factors have yet been characterized, their existence is supported by genetic evidence from the white cocoon mutants in *B. mori*, in which the normal carotenoid pigment of the cocoon, lutein, is absent (Tazima, 1978). There are two important points: (1) lutein is a lipid and (2) in one mutant, the I mutant, lutein transfer from the midgut to lipophorin is inhibited—lutein accumulates within midgut epithelia cells. Importantly, in the I mutant the transfer of other lipids, DAG and sterol, from the midgut to lipophorin is normal. Thus, only the transfer of lutein is affected. One possible explanation for these observations is that the I mutation is in the lutein-transfer factor. The presence, or absence, of such transfer factors is then proposed to account for the specificity of cellular lipid uptake. Much work will be required to prove such a mechanism.

## 6. Lipid mobilization

Chino and Gilbert (1964) first demonstrated that the fat body mobilizes lipids as DAG, and this has been confirmed in many insect species (Beenackers et al., 1985). Adipokinetic hormones (AKHs) are a large family of 8–10-amino-acid peptides secreted by the neurosecretory cells of the corpora cardiaca that control lipid mobilization from the fat body (Beenackers et al., 1985; Goldsworthy and Mordue, 1989; Goldsworthy et al., 1997). Thus, the injection of AKH into adult insects such as *Acheta domesticus*, *L. migratoria* and *M. sexta* stimu-

lates the formation of DAG which accumulates in the hemolymph associated with lipophorin (Gäde and Beenackers, 1977; Shapiro and Law, 1983; Woodring et al., 1989; Strobel et al., 1990). For example, after hormonal stimulation of adult *M. sexta*, the content of hemolymph DAG doubles and DAG comprises about 95% of the total hemolymph lipids, whereas FFA constitutes less than 1% (Arrese and Wells, 1997).

In *L. migratoria* (Tietz et al., 1975) and *M. sexta* (Arrese and Wells, 1997) the fat body synthesizes primarily sn-1,2-DAG and only trace amounts of the sn-1,3- and sn-2,3-isomers have been detected. DAG is secreted into the hemolymph and loaded into pre-existing HDLp. As a result, lipophorin becomes considerably lower in density and an LDLp is formed. This particle, in addition to apoLp-I and apoLp-II, also contains apoLp-III, which binds to surface patches enriched in DAG and stabilizes the particle (Soulages and Wells, 1994). LDLp transports DAG to the sites of utilization, the flight muscle and ovaries, where it is hydrolyzed to FFA by a lipophorin-lipase (Van Antwerpen and Law, 1992; Van Heusden and Law, 1989; Van Antwerpen et al., 1998).

Most of the current information about lipid mobilization in insects comes from studies carried out using *L. migratoria* and *M. sexta*. Since Tietz et al. (1975) described the presence of a microsomal monoacylglycerol acyltransferase (MGAT) activity in the fat body of *L. migratoria*, it had been widely speculated that the synthesis of DAG involves the hydrolysis of TAG into 2-monoacylglycerol (2-MAG) followed by its reacylation to DAG catalyzed by MGAT. However, more recent studies in *M. sexta* support the conclusion that the stored TAG is converted directly to DAG (Arrese and Wells, 1997). In other words, the direct stereospecific hydrolysis of TAG into sn-1,2-DAG is the pathway for the synthesis of DAG that is released into the hemolymph as result of AKH action. This conclusion is based on the following results.

- Although microsomal MGAT activity was found in *M. sexta* fat body, AKH did not stimulate its activity (Arrese et al., 1996a). Given the rapid response in DAG production as a result of AKH injection, it seems highly unlikely that AKH activates MGAT gene expression.
- The content of MAG in the microsomes or in the whole fat body remains unchanged after activation of DAG synthesis by AKH. Thus, AKH does not modify the synthesis of sn-1,2-DAG through the activation of MGAT.
- Precursor-product analysis showed that the only significant change among the fat body lipid components induced by AKH was an accumulation of sn-1,2-DAG. Thus, the size of the DAG pool doubles 60 min after AKH stimulation, whereas the fatty acid, MAG

and phosphatidic acid pool sizes remained unchanged (Arrese and Wells, 1997).

- It is important to note that the metabolic fate of the fatty acid removed from the sn-3 position of TAG is unknown.

Altogether, these observations support the conclusion that AKH-stimulated synthesis of sn-1,2-DAG in the fat body involves the stereospecific hydrolysis of the TAG stores into DAG, a step that must be catalyzed by a TAG-lipase. At present, the only fat body TAG-lipase that has been purified is that from the fat body of adult *M. sexta* (Arrese and Wells, 1994). The enzyme is a single polypeptide of 76 kDa that has several properties in common with the vertebrate hormone-sensitive lipase (HSL), which catalyzes the rate-limiting step in mobilization of adipose tissue fatty acids. Like HSL, the *M. sexta* fat body TAG-lipase is a phosphorylatable enzyme. In adult *M. sexta*, the activation of the fat body TAG-lipase precedes the appearance of DAG in the hemolymph, suggesting that AKH stimulates DAG secretion by activating the fat body TAG-lipase (Arrese et al., 1996b).

The sequence of events leading to the stimulation of lipolysis induced by AKH is still controversial. The present data support a model in which the initial event involves the binding of AKH to its receptor (Ziegler et al., 1995), which induces a rapid and sustained increase in  $\text{Ca}^{2+}$  influx and an activation of adenylate cyclase. These processes give rise to two intracellular messengers,  $\text{Ca}^{2+}$  and cAMP (Gäde and Holwerda, 1976; Spencer and Candy, 1976; Lum and Chino, 1990; Wang et al., 1990; Arrese et al., 1999). In addition to the evidence in favor of  $\text{Ca}^{2+}$  and cAMP as second messengers in the action of AKH-mobilizing lipids, recent reports showed that AKH increases levels of inositol (1,4,5)-triphosphate ( $\text{InP}_3$ ) in the fat body of two locusts, *Schistocerca gregaria* (Stagg and Candy, 1996) and *L. migratoria* (Van Marrewijk et al., 1996; Vroemen et al., 1998). Interestingly, the report on *S. gregaria* showed a difference in sensitivity between cAMP and the  $\text{InP}_3$  responses. The latter exhibited an  $\text{EC}_{50}$  value for AKH that was 100-fold higher than the corresponding value for cAMP. In any case, these findings raise the question of whether the increase in  $\text{Ca}^{2+}$  influx mentioned above is due to a direct stimulation by AKH on calcium entry or a secondary consequence resulting from the depletion of internal stores (e.g.  $\text{InP}_3$ -sensitive stores) initiated by AKH.

How is the presence of these second messengers related? Two possibilities can be envisaged: (1) the accumulation of one of the messengers induces the appearance of the other; or (2) the AKH receptor activates multiple signal transduction pathways leading to the accumulation of both second messengers. In vertebrate tissues, different patterns of cross-talk between

the  $\text{Ca}^{2+}$  and cAMP signal transduction systems have been established. For example, intracellular free calcium can affect cAMP levels by modulation of adenylate cyclase activity or phosphodiesterase activities (Beavo and Reifsnnyder, 1990; Choi et al., 1993). On the other hand, A-kinase or cAMP can affect intracellular  $\text{Ca}^{2+}$  levels by regulating  $\text{Ca}^{2+}$  channel activity (Hell et al., 1994). Thus, unlike the vertebrate system in which lipolysis is regulated solely by the cAMP system, in the insect fat body both cAMP and calcium are second messengers in the hormone-stimulated production of sn-1,2-DAG, suggesting that a novel signal transduction mechanism for activating lipolysis could be present in insects.

Recently, it has been shown that AKH rapidly induces an increase in the A-kinase activity of fat body cells from the adult *M. sexta* and that this increase in A-kinase activity precedes activation of the fat body TAG-lipase and the mobilization of DAG into the hemolymph (Arrese et al., 1999). The substrates for the *M. sexta* fat body A-kinase are under investigation, but we know that A-kinase purified from the *M. sexta* fat body phosphorylates the fat body TAG-lipase as well as other proteins associated with the fat droplets (E.L. Arrese and M.A. Wells, unpublished). A possible scenario is that activation of A-kinase by cAMP results in the phosphorylation of the TAG-lipase, which, similar to the vertebrate system (Egan et al., 1992), leads to its activation and/or translocation to the substrate stores in the fat droplets.

In addition to understanding the pathway for sn-1,2-DAG synthesis and its regulation, a complete understanding of lipid mobilization in the insect fat body requires an understanding of the mechanism of transport of DAG from its site of synthesis at the fat droplet to the membrane for export into the hemolymph. As mentioned above, TAG is stored in the fat body cell as cytoplasmic droplets. The intracellular location of the fat body TAG-lipase seems to be the cytosol from where this enzyme has been purified (Arrese and Wells, 1994). The activation of lipolysis evidently involves the translocation of the lipase from the cytosol to its substrate located in the fat droplet. After stimulation of lipid mobilization, the content of DAG in the fat body gradually increases and simultaneously a large mobilization of DAG into the hemolymph is observed (Arrese and Wells, 1997). It has been observed that DAG localized in the cytosol, and to a lesser extent in the fat droplets, is preferentially mobilized into the hemolymph (E.L. Arrese et al., unpublished). This result suggests that the mechanism of lipid mobilization could involve two steps: (1) stored TAG is hydrolyzed into DAG; and (2) DAG is moved from the fat droplet to the membrane by a cytosolic carrier for delivery to the hemolymph. The cytosolic carrier could be a specialized protein that binds DAG and also interacts with the plasma membrane. Binding of DAG to such a protein may be important in preventing toxic

levels of free DAG from accumulating in the cell (Weisiger, 1996). In contrast to the transport of DAG in the hemolymph by lipophorin, which has been the focus of extensive studies, nothing is known about the intracellular transport of DAG. This is also an unsolved question for mammalian cells in which DAG, acting as second messengers, undergoes intracellular translocation.

Starvation is another condition that leads to increased hemolymph lipid levels in both the adult *L. migratoria* and larval *M. sexta* (Jutsum et al., 1975; Ziegler, 1991). Because starved cardioectomized insects mobilize lipids as well as the controls, it has been concluded that the mechanism by which starvation induces lipid mobilization in these systems cannot be attributed to AKH but to some other factor (Cheesman and Goldsworthy, 1979; Ziegler, 1991). The brain could secrete this factor and one possible candidate is octopamine, the monohydroxyphenolic analog of noradrenaline, which has been shown to stimulate lipid mobilization in *L. migratoria* (Orchard, 1987) and *A. domesticus* (Fields and Woodring, 1991). Octopamine also shows a moderate response, compared to AKH, in stimulating lipid mobilization in adult *M. sexta* (E.L. Arrese and M.A. Wells, unpublished results). Davenport and Evans (1984) showed that the level of octopamine in the hemolymph of *L. migratoria* that has been deprived of food increased and that the increased levels persisted for as long as the insects were unable to feed. Therefore, it is possible that octopamine could be involved in the regulation of lipid mobilization induced by starvation, as has been suggested by Downer (1985).

### 6.1. Future directions

Fig. 3 shows a proposed model for mobilization of DAG from the fat body in response to AKH. There are several points in this model that need to be clarified by future experiments.

- How do cAMP and  $Ca^{2+}$  interact to transduce the AKH effect? Not shown in the model but also requiring clarification is the possible role of  $IP_3$ .
- How does phosphorylation of the TAG-lipase lead to activation? How does the lipase express its stereospecificity?
- How is DAG transported from the fat droplet to the plasma membrane? Is a DAG-binding protein involved?
- How does DAG leave the fat body cell and enter HDLp to produce LDLp?

## 7. Summary

In this brief review we have presented a few of what we consider to be interesting areas related to lipid stor-

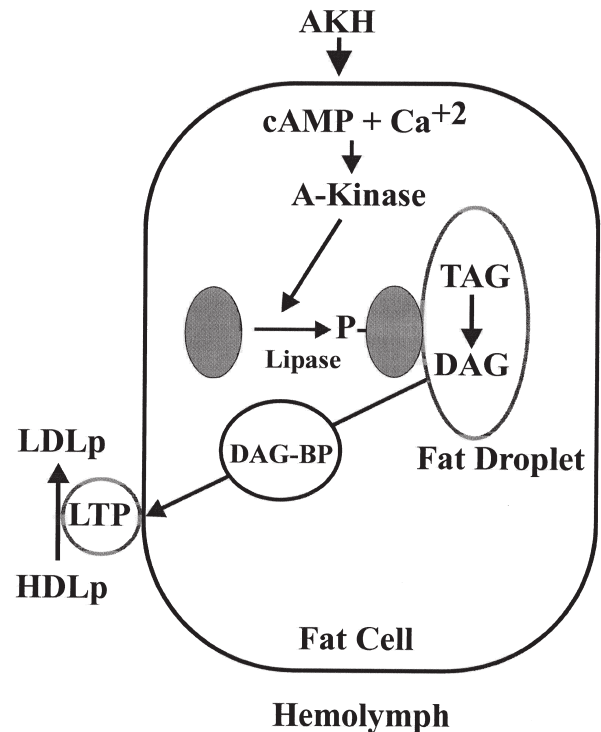


Fig. 3. A model for adipokinetic hormone (AKH)-stimulated diacylglycerol (DAG) production and secretion in the fat body. AKH increases the intracellular concentrations of cAMP and  $Ca^{2+}$ . cAMP, with or without  $Ca^{2+}$ , then activates the cAMP-dependent protein kinase (A-Kinase), which in turn phosphorylates the lipase, causing it to translocate to the surface of the fat droplet. On the surface of the fat droplet the lipase produces DAG, which is then transported to the plasma membrane via a DAG-binding protein (DAG-BP). Once in the membrane the DAG leaves the cell and is added to high-density lipophorin (HDLp) to produce low-density lipophorin (LDLp).

age and mobilization in insects. It is our hope that others will be stimulated to use biochemical, molecular biological and physiological approaches to prove, or disprove, the models we have presented. Regardless of the outcome of those experiments, if some of you are encouraged to enter this fascinating field, then our purpose will have been well served.

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