Annu. Rev. Nutr. 2001. 21:23–46

FAT METABOLISM IN INSECTS

Lilián E Canavoso, Zeina E Jouni, K Joy Karnas, James E Pennington, and Michael A Wells

Department of Biochemistry and Molecular Biophysics, and Center for Insect Science, University of Arizona, Tucson, Arizona 85721; e-mail: lcanavoso@email.arizona.edu, eljouniz@email.arizona.edu, karnas@email.arizona.edu, penningj@email.arizona.edu, mawells@email.arizona.edu

Key Words lipophorin, fat body, midgut, hemolymph, flight muscle, oocyte

■ **Abstract** The study of fat metabolism in insects has received considerable attention over the years. Although by no means complete, there is a growing body of information about dietary lipid requirements, and the absolute requirement for sterol is of particular note. In this review we (*a*) summarize the state of understanding of the dietary requirements for the major lipids and (*b*) describe in detail the insect lipid transport system. Insects digest and absorb lipids similarly to vertebrates, but with some important differences. The hallmark of fat metabolism in insects centers on the lipid transport system. The major lipid transported is diacylglycerol, and it is carried by a high-density lipoprotein called lipophorin. Lipophorin is a reusable shuttle that picks up lipid from the gut and delivers it to tissues for storage or utilization without using the endocytic processes common to vertebrate cells. The mechanisms by which this occurs are not completely understood and offer fruitful areas for future research.

CONTENTS

0199-9885/01/0715-0023$14.00

INTRODUCTION

Insects are the most abundant form of animal life on the planet, with perhaps a million species and at least a billion individuals for each of the approximately 5 billion humans. Insects have evolved to utilize every conceivable type of available nutrient and are obviously well established and successful. Humans most often think of insects as pests that transmit diseases or consume agricultural products. In fact, life as we know it would not be possible without insects. Insects pollinate plants, produce useful products such as honey and silk, and play a critical role in removing dead material from the biosphere—without insects we would soon be buried in debris. Except in Eurocentric cultures, insects are also an important supplement to human nutrition (43).

Insects and vertebrates share many common metabolic pathways. In many areas of research, insects are useful models that can facilitate our general understanding of biology. This review, however, focuses on several insect-specific processes in fat metabolism. Understanding fat metabolism in insects increases our knowledge of insect metabolism and expands our appreciation of the ways in which different organisms have solved common biochemical problems. In many ways, fat metabolism in insects is less complex than in vertebrates, so insects can serve as a viable model system for understanding fundamental aspects of fat metabolism.

Most of the results reported in the literature and reviewed here are based on the study of only a few insect species, usually those that are easy to rear in the laboratory. Thus, caution should be exercised in drawing general conclusions, particularly when examining dietary lipid requirements. To obtain unequivocal results, such studies often require raising insects for several generations on artificial diets. As is understandable, this arduous task has not been undertaken often. In this review, we take a broad view, which we hope will inform those readers who are interested in nutrition but who have just a passing curiosity about insects. Because of the breadth of the topic and space limitations, we rely heavily on review articles rather than original literature. We apologize to our insect science colleagues for this necessity.

We first summarize the state of understanding of the requirements for the major lipids in the diet and then describe in detail the insect lipid transport system.

LIPID NUTRITIONAL REQUIREMENTS

Three classical approaches have been used to assess the essentiality of lipid nutrients (35): (*a*) the deletion method, which measures the effect of eliminating one specific component from a chemically defined diet on which the insect can develop under sterile conditions; (*b*) substitution of an essential nutrient by analogues; and (*c*) the use of radiolabeled precursors to measure endogenous biosynthesis (54). The complexity of establishing nutritional requirements for insects can be appreciated by considering the case of aphids. Aphids derive their food from plant phloem, a carbohydrate-rich and amino acid–poor food. Aphids derive their essential amino acids from intracellular symbionts (46). It has been reported that aphids can be reared on fat-free diets for many generations (46). Some interpret this to mean that aphids either do not require essential fatty acids and sterols or that bacterial symbionts provide them. The lack of a requirement for essential fatty acids is due to the fact that aphids, unlike vertebrates, are able to make linoleic acid (44). Linoleic acid biosynthesis has been found in 8 of 32 insect species tested, representing 4 of 13 orders, meaning that most insects probably cannot make it. When some aphids, containing their obligate symbionts, were grown on media containing labeled mevalonate, no incorporation of label was found in any sterols (20). In this case, neither the aphid nor its symbionts can make sterol, so the question of the origin of this aphid's sterol remains unanswered.

Sterols

Dietary Requirements All insects require sterol in their diets (36, 129). The dietary need for sterols was first established in the blowfly *Lucilla sericata* (61), and has been extended to all orders studied, including Hymenoptera (ants, wasps, bees), Coleoptera (beetles), Diptera (flies), Hemiptera (bugs), Lepidoptera (moths and butterflies), Orthoptera (grasshoppers and crickets), and Homoptera (aphids and cicadas) (117). In a few specific cases, such as cigarette beetles, *Laspoderma serrlione*, microbial symbionts provide sufficient sterol for the insects (36).

Cholesterol is the major sterol found in insects. It serves as a structural component of cell membranes and as the precursor of the insect molting hormones, ecdysteroids (58, 117). Cholesterol has been shown to support normal development in most insects (36, 54). Most species have adapted to transform a wide range of dietary sterols into cholesterol (117, 118). However, there are some exceptions to this generalization. Both the fruit fly *Drosophila pachea* (60) and the beetle *Xyleborus ferrugineous* (32) require dietary Δ^7-sterols (where Δ refers to the position of the double bond). On the other hand, grasshoppers develop most efficiently to the adult stage on diets containing Δ^5-sterols (e.g. cholesterol and sitosterol) and fail to develop on diets that contain Δ^7 and/or Δ^{22}-sterols (e.g. stigmasterol) (15). It is interesting that these Δ^7- and/or Δ^{22}-sterols can prevent development in the desert locusts *Schistocerca americana*, even when a suitable sterol (e.g. sitosterol)

is present (14). In the corn earworm *Heliothis zea*, cholesterol also supports normal growth of larvae, which die when reared on ergosterol or lanosterol (83). A diet containing a high concentration of cholesterol (5%) was mildly toxic to larvae of the tobacco hornworm *Manduca sexta*, whereas the presence of comparable amounts of sitosterol in the diet was toxic (ZE Jouni, MA Wells, unpublished data). The replacement of cholesterol or campesterol by β-sitosterol in the diet of the housefly *Musca domestica* produced adults that failed to produce viable eggs (67).

Metabolic Transformations Most omnivorous and hematophagous (blood feeding) insects are able to obtain sufficient cholesterol from their diets (118). However, cholesterol itself is not a normal dietary sterol for phytophagous (plant feeding) insects that consume a wide variety of phytosterols, C_{28} or C_{29} sterols with a double bond at position 5 or 7 and a methyl or ethyl group at position C_{24}. In plants, phytosterols are usually esterified to fatty acids. Utilization of sterol esters depends on the rate of hydrolysis in the intestinal lumen, as demonstrated in the beetle *Trogoderma granarium* (82). As in vertebrates, cholesterol and other lipids present in the diet may affect phytosterol absorption. Absorbed phytosterols may be unchanged or converted to cholesterol or other sterols in the midgut cells. Then they are released to lipophorin, the major lipoprotein circulating in the hemolymph (blood), and distributed to different tissues for utilization or storage in free or esterified forms.

Dealkylation at C_{24} is the most common metabolic pathway for the conversion of C_{28} and C_{29} dietary sterols into cholesterol. Dealkylation, prevalent in most phytophagous and omnivorous insects, is preceded by oxidation and epoxidation steps (118). Demosterol has been identified as the dealkylation intermediate of phytosterols in several insect species (126).

Cholesterol is not always the end product of the dealkylation reactions. 7-Dehydrocholesterol was produced in the confused flour beetle *Tribolium confusum* (121) and the phytophagous sawfly *Xiphydria maculata* (117). In the Mexican bean beetle *Epilachna varivestis*, saturation of the Δ^5-bond precedes C_{24} dealkylation of phytosterols, resulting in the saturated sterol, cholestanol, instead of cholesterol (117).

Synthesis of Insect Steroid Hormones The major steroid hormones in insects are the molting hormones, ecdysone or 20-hydroxyecdysone. These steroids are made from cholesterol in the prothoracic glands and other steroidogenic organs, such as ovaries, testes, and epidermis. In this pathway, an endoplasmic reticulum (ER) cytochrome P-450 catalyzes the conversion of cholesterol to 7-dehydrocholesterol (58, 152), followed by rapid conversion to ecdysone by the action of a 3-ketosteroid reductase, a hemolymph enzyme (98). The metabolism of ecdysone to 20-hydroxyecdysone occurs in several tissues, e.g. fat body, Malpighian tubules (insect kidney), and midgut (80). This step is catalyzed by ecdysone 20-monooxygenase (126, 156). Both the honeybee *Apis mellifera* (119) and the solitary bee *Megachile rotundata* (120) lack the ability to dealkylate phytosterols. Hence,

both have 24-methylcholesterol as their major sterol and the C_{28} ecdysteroid, makisterone A, as their molting hormone.

A number of other steroid hormones, including estrogens, androgens, and the mineralcorticoids, are produced by insects (58). The function of these steroid hormones in insects is unknown.

Essential Fatty Acids

Nutritional Requirements In addition to sterols, most insects have a dietary requirement for polyunsaturated fatty acids, and many studies have shown that either linoleic or linolenic acids adequately satisfy this nutritional need (36). This is an area that generated some controversy in the past because the concentration of linoleic and linolenic acid in tissue phospholipids is quite low, and many early analyses failed to detect them. Even lower concentrations of arachidonic acid and other polyunsaturated fatty acids went undetected until special efforts were made to measure them (113).

The requirement for essential fatty acids may differ substantially between species. One clear-cut example of essential fatty acid deficiency in insects is found in Lepidoptera and Hymenoptera, where there is a failure of the adult insect to form properly during metamorphosis; however, there are no effects during larval development (36). This deficiency is alleviated by linolenic acid, not linoleic acid. The function of linolenic acid in metamorphosis has yet to be established. Some members of the Orthoptera order express fatty acid deficiency by a markedly retarded nymph growth and the emergence of deformed adults (36). In contrast, the cockroach *Blatella germanica* shows no ill effect from deficiency imposed through larval development, but females develop deformed oothecae (a structure that covers the egg after laying), or a second generation of weak, short-lived nymphs (57). Coleoptera show a requirement for essential polyunsaturated fatty acids mainly by slow larval growth and decreased adult fecundity (36).

With the exception of mosquitoes, polyunsaturated fatty acids have not been found to be essential for any of the several species of Diptera (79). Larval mosquitoes require arachidonic acid or related 20- and 22-carbon polyunsaturated fatty acids to survive to adulthood. Neither linoleic nor linolenic acid satisfies that requirement (37).

Prostaglandins Prostaglandins (PGs) have been extensively studied in vertebrates, where they cause myriad physiological and pharmacological reactions. By comparison, relatively few studies have been carried out using insects (62, 115). One well-defined system is the requirement of PGE_2 for egg-laying behavior in the cricket *Acheta domesticus* (45). PGs have been shown to mediate egg-laying behavior in a few other insects, but in most they have no obvious effects. PGs may play other undetermined roles in reproduction because they are found in the reproductive tracts of insect species in which they do not affect egg laying. Another well-studied system involves control of fluid secretion by the salivary glands of the

blowfly *Calliphora erythrocephala*, where PGE_1 inhibits adenyl cyclase, a critical component of the fluid secretion signaling system (38, 39).

Other possible roles of PGs have been inferred based on the use of PG biosynthesis inhibitors. Among these are reports that PG may regulate fluid secretion in the Malpighian tubules of the mosquito *Aedes aegypti* (88) and the forest ant *Formica polyctena* (146), and that it may modulate insect immunity (114).

Carotenoids

Carotenoids are lipid-soluble pigments made up of two diterpenoid units joined tail to tail. There are two types of carotenoids: the carotenes (hydrocarbons), which are unsubstituted terpenes, and the xanthophylls, which are oxidized derivatives of carotenes (70). Only plants and microorganisms synthesize carotenoids, so insects, like all animals, must obtain them from their diets. In some insects, absorption of carotenoids is selective, with some preferentially absorbing carotenes and others preferentially absorbing xanthophylls (50). Carotenoids contribute to the body colors of insects. Although the functions of carotenoids in coloration are not well understood, color has evolved as part of a strategy to avoid predators. It acts as a camouflage or as an advertisement of distastefulness (48).

The participation of carotenoids in vision is well characterized in both vertebrates and invertebrates. In insects, retinal and 3-hydroxyretinal are used as chromophores of visual pigments (101). The name xanthopsin is used for the visual pigments based on 3-hydroxyretinal, which is derived from such xanthophylls as lutein and zeaxanthin (149–151). The visual pigment in Diptera is 11-*cis* 3-hydroxyretinal (100, 149, 150). In *Drosophila melanogaster*, both retinoids and carotenoids serve as precursors of the chromophore. When carotenoids are the precursors, the insect can form 11-*cis* 3-hydroxyretinal in the dark. When retinoids are the precursors, the presence of light is obligatory for the isomerization of all-*trans* to 11-*cis* 3-hydroxyretinal (101).

Fat-Soluble Vitamins

The few studies reported to date indicate that with the exception of vitamin D, the fat-soluble vitamins A, E, and K have some beneficial effect on the physiology of insects (36, 78). A deficiency in vitamin A or carotenes retards growth and causes anomalous color and behavior in the second generation of the migratory locust *Locusta migratoria* and the desert locust *Schistocerca gregaria* (36). A specific nutritional requirement for vitamin E (α-tocopherol) has been demonstrated in a few species, mainly in connection with adult reproductive function. Vitamin E improves fecundity in moths and beetles. Its effect in *A. domesticus* is most clear in males, where larvae reared on diets without tocopherol failed to develop viable sperm (36, 78). Because of its function as an antioxidant, vitamin E is included in the formulation of most synthetic diets that incorporate polyunsaturated fatty acids (54). Vitamin K is required in vertebrates for its role in the synthesis of the clotting factor prothrombin and consequently is not expected to be necessary

for insects. When vitamin K was tested in crickets as a substitute for vitamin E, it showed a growth-stimulatory effect, but it had no effect on male sterility (54, 77).

LIPOPHORIN

Properties

Most of the lipid in hemolymph is associated with a single lipoprotein particle, and although there is considerable variation in lipid composition between insects, the common name lipophorin is used for all insect lipoproteins (29). Under most physiological conditions, lipophorin exists as high-density lipophorin (HDLp) (D ~ 1.15 g/ml). Every HDLp particle contains one molecule each of two apolipoproteins: apolipophorin-I (apoLp-I) ($M_r \sim 250$ kDa) and apolipophorin-II (apoLp-II) ($M_r \sim 70$ kDa) (31, 95). Several studies suggest that apoLp-I is located on the surface of HDLp and that apoLp-II is sequestered away from the surface (108). A third exchangeable apolipoprotein, apolipophorin-III (apoLp-III) ($M_r \sim 18$ kDa), is found free in the hemolymph or associated with low-density lipophorin (LDLp) (D ~ 1.03 g/ml), which are discussed below.

Lipophorin contains a phospholipid-protein surface and a neutral lipid core (66). The core lipid composition varies with physiological status and across insect species (Table 1). The neutral lipid most commonly carried by lipophorin is *sn*-1,2-diacylglycerol (DAG) (31, 108). However, some Diptera belonging to the family Culicomorpha (mosquitoes, black flies, midges) transport neutral lipid in the form of triacylglycerol (TAG) (53; JE Pennington, MA Wells, unpublished data). Small amounts of sterols, free fatty acids, carotenoids, and monoacylglycerols are also transported by lipophorin, but sterol-esters are never found in any significant amount (108).

TABLE 1 Representative lipid compositions of lipophorins from several insect species[a]

Insect lipophorin (Reference)	Density (g/ml)	%PL	%DAG	%ST	%TAG	%HC
Manduca sexta HDLp (89)	1.151	16.7	15.7	1.2	1.1	2.8
M. sexta LDLp (94)	1.030	14.0	25.0	1.3	2.5	3.5
Aedes aegypti HDLp (53)	1.113	15.7	4.0	6.9	15.7	ND
Locusta migratoria HDLp (31)	1.120	14.8	13.4	3.2	0.7	8.7
L. migratoria LDLp (28)	1.065	10.9	26.1	2.4	0.5	6.4
Periplaneta americana HDLp (30)	1.120	22.8	8.0	2.7	1.0	15.0

[a]Lipid percentages are expressed as a percentage of total weight of the particle: HDLp, high-density lipophorin; LDLp, low-density lipophorin; PL, phospholipid; DAG, diacylglycerol; ST, sterol; TAG, triacylglycerol; HC, hydrocarbon; ND, not determined.

Other neutral lipids transported by lipophorin include long-chain hydrocarbons that make up a considerable portion of the neutral lipid content in some insects (30). Hydrocarbons (*a*) are synthesized in cells associated with the epidermis, (*b*) serve as sex attractants, and (*c*) are essential components of the insect cuticle, preventing loss of water and subsequent dehydration (18, 99). Methyl-branched hydrocarbons are a unique feature of insects and these compounds are made from the corresponding fatty acids by decarboxylation. The methyl branches are introduced using methylmalonyl-CoA in place of malonyl-CoA at specific points during fatty acid biosynthesis.

Biosynthesis

Apolipoproteins Labeling studies have demonstrated that apoLp-I and -II are synthesized in the fat body as a single precursor protein (140, 143, 153). The precursor protein is encoded by a single ~10-kb mRNA that has been sequenced fully for *M. sexta* (116) and *D. melanogaster* (72) and sequenced partially for *L. migratoria* (140) and *A. aegypti* (143). The cDNA sequences showed proapolipophorin is arranged with apoLp-II at its N-terminus and apoLp-I at its C-terminus (72, 116). A single consensus convertase cleavage site, RXRR, is present in between apoLp-II and apoLp-I in the precursor protein (72, 116). Thus, the 1:1 apolipoprotein stoichiometry in all lipophorins is accomplished by the proteolytic cleavage of proapolipophorin into its subunits.

Comparison of the entire deduced amino acid sequences of *M. sexta* and *D. melanogaster* precursor proteins shows an identity of approximately 21% (143). A higher percent identity (40%–70%) is found for some regions, which suggests that there are portions of these proteins that are more highly conserved (72, 143). Comparisons of sequences from *L. migratoria*, *M. sexta*, and *D. melanogaster* proapolipophorin with human apolipoprotein B (apoB), invertebrate and vertebrate vitellogenins, and the large subunit of mammalian microsomal triglyceride transfer protein revealed contiguous conserved sequence motifs, which the authors interpreted to mean that the genes coding for these proteins are members of the same multigene superfamily (9).

Lipidation Lipidation refers to those processes whereby the apolipoproteins and the transported lipid are packaged together to form a soluble lipoprotein particle. Most of the work in the field of lipoprotein biosynthesis has involved studies on the lipidation of apoB. Its association with lipids to form very-low-density lipoproteins (VLDL) is a multistep process that begins with cotranslational translocation of apoB across the ER (92, 103). Lipid availability and the proper folding of apoB, particularly the formation of disulfide bonds, are required for lipidation (19, 127). Small amounts of core lipids are added to the membrane-bound apoB, leading to the formation of a high-density apoB-containing particle. One hypothesis for how this occurs is that apoB is actually inserted into the inner leaflet of the ER

during translocation (34, 87, 110). It has been suggested (1, 33) that in apoB, pause transfer sequences cause a pause in translocation (1, 33), which allows addition of lipids to the protein.

The exact mechanism for lipidation of apolipophorins is unknown; however, the insolubility of apoLp-II (108), as well as the rapid (approximately 35 min) synthesis, processing, assembly, and secretion of apoLp-I and apoLp-II as HDLp (154), suggests that lipidation occurs during or closely following the translation of the precursor protein. The extent to which the nascent particle is lipidated prior to its release from the fat body is a controversial topic and may differ between insect species. The nascent HDLp described for *L. migratoria*, *Diatraea grandiosella* (the Colorado potato beetle), and *M. domestica* is similar in lipid composition and lipid load to that of circulating HDLp, which suggests it is fully loaded with lipid prior to its secretion from the fat body (25, 148, 154). Alternatively, in larval *M. sexta*, HDLp is secreted as a very high-density phospholipid-protein complex that is loaded with core lipid at the midgut (73, 89).

Two major differences exist between insect and human lipogenesis. First, the assembly of human lipoproteins requires the presence of the microsomal triglyceride transfer protein. This protein, not believed to be present in insects, is located in the lumen of the ER and is required for the proper assembly of apoB-containing lipoproteins (91). A second major difference is that VLDL production is regulated by lipid availability and not apoB translation. In lipid-poor conditions, cytosolic degradation of apoB occurs (157). In lipid-rich conditions, more apoB is translocated into the ER, where a second proteolytic pathway degrades any apoB that is not efficiently transferred to the Golgi. This second proteolysis step is designed presumably to eliminate any misfolded apoB (16). In contrast, the rate of lipophorin biosynthesis in larval *M. sexta* is independent of the amount of lipid in the diet. There is a direct correlation between the lipid content of lipophorin and the amount of lipid in the diet (51).

Role as a Reusable Shuttle in Lipid Transport

There are several types of lipoproteins in vertebrate systems, most delivering their neutral lipid load to target tissues by a combination of lipoprotein lipase-mediated lipolysis (chylomicrons and VLDL) or endocytosis and degradation of the whole particle (LDL and chylomicron remnants). The insect system is more versatile than the vertebrate system in that in different insects, the same basic lipophorin particle can carry a wide variety of lipids. Additionally, lipophorin has the unique ability to selectively deliver specific lipids to specific tissues, e.g. hydrocarbons to the cuticle, carotenoids to the cuticle or the silk gland, etc (3, 73). The insect system is more efficient because lipid is delivered to tissues, for the most part, without internalization and destruction of lipophorin (3, 26, 96, 108). This observation led to the idea that lipophorin functions as a reusable shuttle moving lipid from sites of absorption or storage to sites of utilization (3, 26, 66, 108, 138).

LIPOLYSIS, ABSORPTION, AND EXPORT

Lipolysis

Midgut cells produce lipases that hydrolyze dietary TAGs, forming monoacylglycerols and free fatty acids. However, in some species, especially Lepidoptera, which have a high pH in the midgut lumen, acyl migration and subsequent hydrolysis leads to free glycerol and fatty acids (2, 3, 13, 47, 130, 133). Digestion of phospholipids and glycolipids has been examined in Lepidoptera, Coleoptera, and Diptera (133). Phospholipases A_1 and A_2 were found in the midgut of some Lepidoptera and are probably widespread among insects (125). Phospholipases C and/or D have also been found in the midgut of some insects (131). Galactolipids are hydrolyzed by α- and β-galactosidases yielding galactose and DAG (74, 81, 123, 124). As noted above, utilization of sterol esters may be dependent on the rate of the hydrolysis in the midgut lumen (71, 133).

Absorption

Insects do not have bile salts and have developed other strategies to facilitate lipid solubilization. These strategies include the use of lumenal glycolipids and the formation of fatty acyl–amino acid complexes, fatty acid micelles in the highly alkaline environment of Lepidopteran gut, and lysophospholipid micelles (133).

Absorbed fatty acids and partial acylglycerols, if present, are converted into intestinal DAG, TAG, and phospholipids. Synthesis could involve acylation of 2-monoacylglycerol (the monoacylglycerol pathway) or the de novo pathway that involves acylation of *sn*-glycerol-3-phosphate (the phosphatidic acid pathway) (22, 47, 77, 133). The relative contribution of the two pathways has only been established in larval *M. sexta*, where it was shown that the fatty acids arising from complete lumenal hydrolysis of TAG are transformed into DAG using the phosphatidic pathway (22). In the same insect, DAG is rapidly converted to TAG, which serves as a reservoir for absorbed fatty acids, or the DAG can be exported to the hemolymph (22). This mechanism assures maximal absorption of fatty acids from the midgut lumen while maintaining a low intracellular concentration of both fatty acids and DAG, which can be toxic at high concentrations. It has been proposed (22) that the uptake of fatty acids by the midgut cell is the rate-limiting step, which suggests the presence of a fatty acid transporter in the lumenal membrane (63, 112). Within midgut cells, fatty acids and other absorbed lipids are targeted to their metabolic pathways by unknown mechanisms. It is possible that cytosolic lipid-binding proteins are involved. Two fatty acid–binding proteins have been isolated from the midgut of *M. sexta* (104) and a lutein-binding protein was isolated from the midgut of the silkworm *Bombyx mori* (65).

Phospholipid and glycolipid absorption have received less attention and have been studied only in Lepidoptera (131). Lysophosphatidylcholine is readily absorbed by the midgut cells, where it is reacylated to phosphatidylcholine (133).

Sugars and DAG from glycolipid hydrolysis are also absorbed, and then DAG is exported to the hemolymph or converted to TAG (133). The midgut is the main site for absorption of cholesterol, although in some omnivorous and carnivorous insects, its absorption has been reported to take place in the foregut or the crop (64). Free cholesterol and cholesterol ester may be absorbed, but free cholesterol is subjected to some intracellular esterification (71, 132). In *M. sexta*, dietary free cholesterol is absorbed in a concentration-dependent manner and is stored in the midgut mainly in the free form (ZE Jouni, MA Wells, unpublished data). In phytophagous insects, the midgut is also the major tissue where nonmetabolized plant sterols are accumulated (15).

Export

Unlike in vertebrates, which synthesize intestinal lipoproteins to carry dietary lipids through the blood, lipid export from the enterocytes in insects does not involve de novo synthesis of a lipoprotein particle. Rather, the lipids are added directly to the existing lipophorin in the hemolymph (26, 89, 93, 102, 108). Lipophorin cycles between the midgut, where it picks up lipids, and the fat body, where the lipids are delivered and stored. It is important to note that lipophorin does not enter the midgut cell or the fat body cell during this process (108, 128).

In vivo and in vitro studies using several insect orders showed DAG is the main lipid in the hemolymph after lipid digestion (10, 22, 90, 128, 155). In *M. sexta* it was demonstrated that dietary lipid is the source of lipophorin DAG because insects raised on a fat-free diet contained a circulating lipophorin essentially depleted of DAG (51). In the same insect, nearly 90% of fatty acid–labeled triolein was absorbed after 4 h, and of that absorbed, more than 70% was found in fat body as TAG (128). In the hemolymph, more than 90% of the label was in DAG and all the DAG was associated with lipophorin. Also, in *M. sexta* larvae, a kinetic model for DAG export showed that its release from the midgut occurs at a rate consistent with the intracellular lipolysis of the TAG pool (22; ER Rubiolo, MA Wells, unpublished data).

Little is known about the mechanisms involved in the transfer of lipids from the midgut to lipophorin in the hemolymph. Two factors proposed to be critical in this transfer are a lipophorin receptor and the lipid transfer particle (LTP). It is generally accepted that lipophorin interacts with tissues through specific binding sites (3, 8, 129, 136) and that the transfer process occurs at the surface of the cell without particle internalization (3, 10, 108, 128). Recently, lipophorin binding to the midgut of *M. sexta* larvae was characterized using membrane preparations (56). In *Aeschna cyanea* larvae, biochemical and immunocytochemical approaches showed that lipid loading and unloading of lipophorin both occur at the midgut without lipophorin internalization (10).

LTP is a very-high-density lipoprotein (VHDL) isolated from the hemolymph of several species (17, 94, 96, 97). The physiological function of LTP is not completely understood, but studies indicate that it serves to redistribute lipids between lipophorins and between lipophorins and membranes (3, 96). In vitro studies show

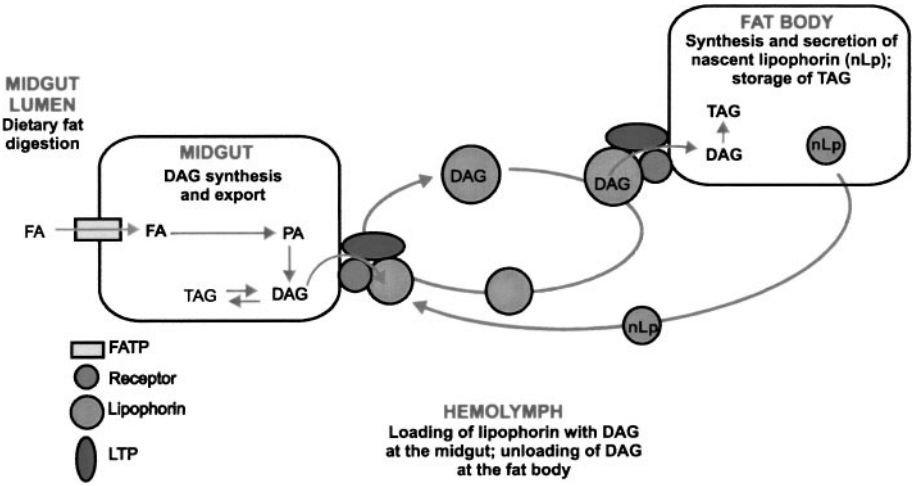

Figure 1 A scheme showing the absorption of fatty acid (FA) into the midgut enterocyte, the synthesis of diacylglycerol (DAG) in the midgut cell, and the transport of DAG to the fat body for storage. It has been proposed that a fatty acid transporter (FATP) is involved in the absorption of FA. The synthesis of DAG in the midgut cell involves the phosphatidic acid (PA) pathway. The export of DAG from the midgut cell requires a lipophorin receptor and lipid transfer particle (LTP). Central to this scheme is the biosynthesis of lipophorin in the fat body and its function as a reusable shuttle. TAG, triacylglycerol.

that DAG transfer from labeled *M. sexta* midgut sacs to lipophorin is completely blocked by pretreatment with anti-LTP antibody and is restored when LTP is added to the incubation medium (LE Canavoso, MA Wells, unpublished data). Therefore, it seems likely that LTP is necessary to facilitate DAG transfer from the midgut. A model for fatty acid absorption, DAG synthesis, and export to lipophorin is presented in Figure 1 (3).

FAT STORAGE AND MOBILIZATION

Storage in Fat Body

In insects, the majority of stored lipids are found in the fat body, an organ analogous to vertebrate adipose tissue and liver. It is also the site of synthesis of hemolymph proteins, the major organ involved in metabolism and the major storage site for glycogen (13, 23). More than 90% of the lipid stored in the fat body is TAG (7, 13, 21, 47). TAG storage is mainly the result of transfer of dietary fat from the midgut to the fat body during the feeding period (Figure 1). In addition, lipid storage can result from de novo lipid synthesis in the fat body from carbohydrates (13, 47).

During the transfer of lipids from lipophorin to the fat body, it has been proposed that lipophorin binds to a receptor (3, 96). A candidate receptor is a HDLp-binding

protein that has been characterized in the fat body of larval *M. sexta* (129). This protein requires Ca^{2+} for HDLp binding (129). In contrast, another HDLp-binding protein found in both intact fat body tissue and isolated membranes of nymph and adult *L. migratoria* did not require divalent cations. The *L. migratoria* protein has a broader specificity than the *M. sexta* protein, as it can bind human lipoproteins. Additionally, it was found that unlike the *M. sexta* protein (129), the *L. migratoria* protein is involved in the endocytic internalization of lipophorin (40–42). Endocytosis of HDLp by fat body cells has also been shown in *A. cyanea* (11). The function of this receptor-mediated endocytosis remains unclear because inhibition of endocytosis did not reduce the transfer of the two main lipophorin lipid cargos, diacylglycerol and cholesterol, to the fat body (3, 41, 96). The role of LTP in the transfer of lipid from lipophorin to the fat body is also unclear. Anti-LTP antibodies reversibly reduce DAG transfer by only 50% in in vivo and in vitro experiments with larval *M. sexta* (LE Canavoso, MA Wells, unpublished data), which suggests more than one pathway for DAG transfer from lipophorin to fat body.

Mobilization from Fat Body

Hormones Lipids are mobilized from the fat body as DAG, not free fatty acids as in vertebrates. Mobilization is induced by two types of hormones: adipokinetic hormone (AKH) (13) and octopamine (49). AKHs are a large family of 8– to 10–amino acid peptides secreted into hemolymph by the neurosecretory cells of the corpora cardiaca (55). Injection of AKH into adult *L. migratoria* (76) and *M. sexta* (5) stimulates the formation of lipophorin-associated *sn*-1,2-DAG. The most likely mechanism for the production of *sn*-1,2-DAG is the direct stereospecific hydrolysis of TAG catalyzed by a TAG-lipase (6, 7). A TAG-lipase has been purified from the fat body of adult *M. sexta* (6). Like the vertebrate hormone-sensitive lipase, which catalyzes the rate-limiting step in mobilization of adipose tissue fatty acid, the *M. sexta* lipase is a phosphorylatable enzyme. In adult *M. sexta*, the activation of the fat body TAG-lipase precedes the appearance of the *sn*-1,2-DAG in the hemolymph, which suggests that AKH stimulates DAG secretion by activating the fat body TAG-lipase (5).

AKH exerts its effects on lipid mobilization via signal transduction. Binding of AKH to its receptor results in the induction of several events that activate key enzymes in lipid and carbohydrate mobilization in the fat body. Receptor binding both activates adenylate cyclase and mediates a rapid and sustained increase in Ca^{2+} influx, giving rise to two intracellular messengers, Ca^{2+} and cAMP (4, 139). In the fat body of two locusts, *S. gregaria* (111) and *L. migratoria* (147), AKH increases the levels of Ca^{2+}, cAMP, and inositol (1,4,5)-triphosphate. Beyond activation of TAG-lipase by phosphorylation (5), other roles of these intracellular messages have not been characterized. Figure 2 presents a model for activation of DAG production in response to AKH.

Octopamine, an analog of the vertebrate catecholamine noradrenaline, acts as a neurotransmitter, modulating the release of AKH from the corpus cardiacum (86). In addition, it acts as a neurohormone with direct energy store mobilizing

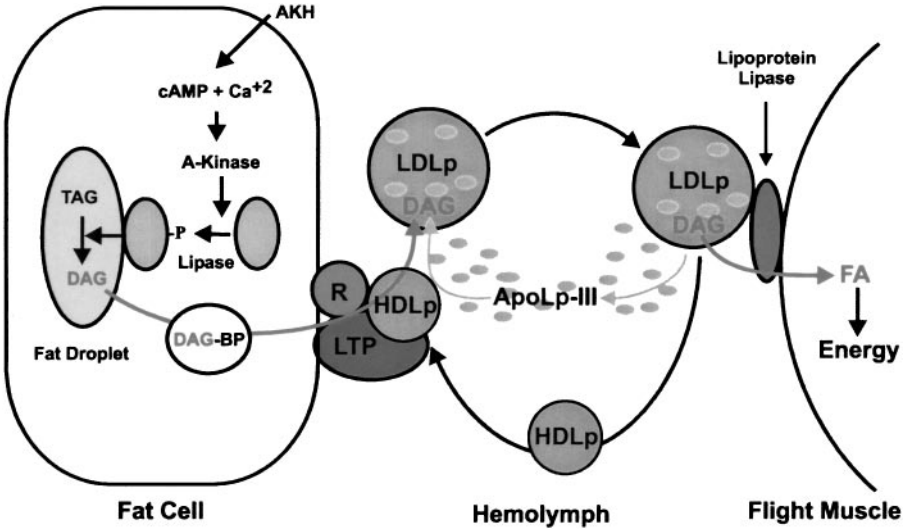

Figure 2 A model for low-density lipophorin (LDLp) production by the fat body and delivery of lipid to the flight muscle. Adipokinetic hormone (AKH) stimulates diacylglycerol (DAG) production and secretion from the fat body. DAG is produced by the action of a lipase acting on the stored triacylglycerol (TAG) and is 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) with the assistance of lipid transfer particle (LTP) to produce LDLp. LDLp is stabilized by binding apolipophorin-III (apoLp-III). LDLp moves to the muscle cell where the DAG is hydrolyzed by a lipophorin lipase. After delipidation, apoLp-III dissociates and LDLp is converted back to HDLp. HDLp then cycles back to the fat body to pick additional DAG and apoLp-III. FA, Fatty acid; R, receptor.

activity (52, 84, 85). In *L. migratoria* and *A. domesticus*, an increase in hemolymph octopamine levels is a general response to different forms of stress, such as handling or starvation (47).

Low-Density Lipophorin Synthesis Once DAG has been produced from the TAG stores in the fat body, it must be exported to the hemolymph for transport to tissues. As is the case in the midgut, DAG export from the fat body does not involve synthesis of new lipophorin particles; instead, DAG is added to preexisting HDLp in the hemolymph (27, 59, 108). The mechanism by which DAG traverses the fat body membrane and is added to HDLp is not completely understood, but LTP is required (142). As DAG is added to HDLp, the particle increases in diameter, leading to a particle whose surface is destabilized by DAG. ApoLp-III binds to the DAG-destabilized surface of lipophorin, stabilizing it and allowing the uptake of additional DAG (105–107). The end product of DAG and apoLp-III addition to HDLp is LDLp, whose mass is about twice that of HDLp. This increase in mass is due to the addition of approximately equal amounts of apoLp-III and DAG (Figure 2).

Why do insects go to the trouble of making DAG from stored TAG and repackaging it into LDLp for transport to tissues when the release of free fatty acids would seem a simpler solution? The reason probably lies in the fact that insects have an open circulatory system. If free fatty acids were released from the fat body they would not be carried away by blood flow. They would be rapidly taken back into the fat body and made into TAG (109). The synthesis of LDLp provides a lipid carrier that can be directed to specific tissues, such as flight muscle and oocytes (see below).

LIPID DELIVERY TO TISSUES

Flight Muscle

Insect flight muscle is the most metabolically active tissue known. On activation, oxygen consumption of flight muscle increases 50- to 100-fold, whereas in vertebrate muscle, the increase is only 7- to 14-fold. At maximum output, a locust flight muscle hydrolyzes ATP at a rate of 18 μmol/s, which means the entire energy-rich phosphate pool (ATP and phosphoarginine) of the muscle can sustain flight for 1 s. Hence, there must be prodigious ATP synthesis during flight, which can last several hours (12, 24, 27). Flight muscle has the capacity to use several fuel sources. Trehalose, proline, and ketone bodies have been described as sources of energy for flight muscle, but probably the most efficient source of energy is fatty acid (24).

Insect flight muscle is incapable of storing significant amounts of lipid, and the source of fatty acid is the DAG of LDLp. In keeping with the reusable shuttle model, LDLp is not internalized and degraded at any point in lipid delivery to the flight muscle (145). Based on immunofluorescence and immunogold studies, LDLp was found to be associated with the extracellular matrix and basement membranes of both resting and flying flight muscles (135). A muscle-specific lipophorin lipase hydrolyzes the DAG carried by LDLp, releasing free fatty acid and glycerol, which are taken up by the flight muscle. Membrane-associated flight muscle lipophorin lipases have been described in *L. migratoria* and *M. sexta* (141, 144, 145, 156). As DAG is removed from LDLp, apoLp-III dissociates from the particle and HDLp is regenerated in the hemolymph. Both HDLp and apoLp-III can then be used to reform LDLp at the fat body (Figure 2).

Oocyte

In *M. sexta*, the most thoroughly studied system for lipid delivery to the oocyte, up to 40% of the dry weight of the mature egg is lipid (68). Biosynthesis accounts for about 1% of the lipid in the egg, and another 5% of the lipid is delivered by the yolk protein vitellogenin (68). LDLp and HDLp deliver the rest of the lipid to the oocyte. Of the total lipid, 90% is delivered by a lipophorin lipase-mediated process similar to that described for flight muscle (68, 137). The oocyte lipophorin lipase

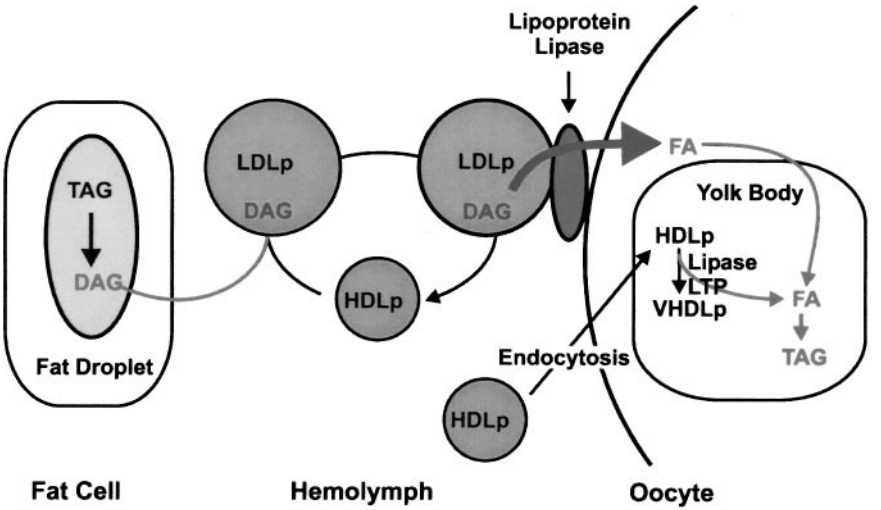

Figure 3 Lipid delivery to the oocyte. Lipid is delivered to the oocyte primarily by low-density lipophorin (LDLp) and requires the activity of a lipophorin lipase. In this case, lipophorin shuttles between the oocyte and the fat body. A minor pathway involves endocytosis of high-density lipophorin (HDLp), which is delipidated intracellularly and stored in the egg as VHDLp. TAG, triacylglycerol; DAG, *sn*-1,2-diacylglycerol; FA, fatty acids; LTP, lipid transfer particle; VHDLp, very-high-density lipophorin.

displays specificity for LDLp over HDLp, and a putative lipase responsible for this activity has been partially purified from follicle cell membranes (68, 137). The final mechanism involved in the delivery of lipid to the egg is selective endocytosis of HDLp. This is the only significant exception to the reusable shuttle model described so far. The lipid of HDLp is processed by a lipase and LTP, producing a very-high-density lipophorin (VHDLp) (D = 1.238 g/ml), which is stored in yolk bodies (69, 75, 134). During this processing, the protein subunits of HDLp are not degraded. A model for lipid delivery to the oocyte is shown in Figure 3.

CONCLUSION

We have presented a broad overview of fat metabolism in insects. In doing so, we have tried to summarize the current state of knowledge and point out some of the many fruitful areas for future research. Indeed, there are many exciting questions begging to be investigated—from a thorough understanding of the requirements of lipids in insect nutrition to the elucidation of the detailed mechanisms of lipophorin biosynthesis and the mechanisms whereby lipophorin serves as a reusable shuttle. We hope some of the readers will become as fascinated as we are by this amazing group of animals and join the fun.

ACKNOWLEDGMENTS

We thank Drs. G Bloomquist, C Schal, and D Stanley for helpful suggestions and Dr. G Bindford and A Stonehouse for providing editorial feedback. Work in our laboratory is supported by NIH grant GM 50008.

LITERATURE CITED

1. Andrews DW, Johnson AE. 1996. The translocon: more than a hole in the ER membrane? *Trends Biochem. Sci.* 21:365–69
2. Applebaum SW. 1985. Biochemistry of digestion. See Ref. 70a, 10:279–311
3. Aresse EL, Canavoso LE, Jouni ZE, Pennington JE, Tsuchida K, Wells MA. 2001. Lipoprotein metabolism in insects: current status and future directions. *Insect Biochem. Mol. Biol.* 31:7–17
4. Arrese EL, Flowers MT, Gazard JL, Wells MA. 1999. Calcium and cAMP are second messengers in the adipokinetic hormone-induced lipolysis of triacylglycerols in *Manduca sexta* fat body. *J. Lipid Res.* 40:555–64
5. Arrese EL, Rojas-Rivas BI, Wells MA. 1996. The use of decapitated insects to study lipid mobilization in adult *Manduca sexta*, effects of adipokinetic hormone and trehalose on fat body lipase activity. *Insect Biochem. Mol. Biol.* 26:775–82
6. Arrese EL, Wells MA. 1994. Purification and properties of a phosphorylatable triacylglycerol lipase from the fat body of an insect, *Manduca sexta*. *J. Lipid Res.* 35:1652–59
7. 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
8. Atella GC, Gondim KC, Masuda H. 1995. Loading of lipophorin particles with phospholipids at the midgut of *Rhodnius prolixus*. *Arch. Insect Biochem. Physiol.* 30:337–50
9. Babin PJ, Bogerd J, Kooiman FP, Van Marrewijk WJ, Van der Horst DJ. 1999. Apolipophorin II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. *J. Mol. Evol.* 49:150–60
10. Bauerfeind R, Komnick H. 1992. Lipid-loading and unloading of lipophorin in the midgut epithelium of dragonfly larvae, *Aeshna cyanea*. *J. Insect Physiol.* 38:147–60
11. Bauerfeind R, Komnick H. 1992. Immunocytochemical localization of lipophorin in the fat body of dragonfly larvae, *Aeshna cyanea*. *J. Insect Physiol.* 38:185–98
12. Beenakkers AMT, Van der Horst DJ, Van Marrewijk JA. 1984. Insect flight muscle metabolism. *Insect Biochem.* 14:243–60
13. Beenakkers AMT, Van der Horst DJ, Van Marrewijk WJA. 1985. Insect lipids and their role in physiological processes. *Prog. Lipid Res.* 24:19–67
14. Behmer ST, Elias DO. 1999. The nutritional significance of sterol metabolic constraints in the generalist grasshopper *Schistocerca americana*. *J. Insect Physiol.* 45:339–48
15. Behmer ST, Elias DO, Grebenok RJ. 1999. Phytosterol metabolism and absorption in the generalist grasshopper, *Schistocerca americana* (Orthoptera: Acriidae). *Arch. Insect Biochem. Physiol.* 42:13–25
16. Benoist F, Grand-Perret T. 1997. Co-translational degradation of apolipoprotein B100 by the proteasome is prevented by microsomal triglyceride transfer protein. *J. Biol. Chem.* 272:20435–42
17. Blacklock BJ, Ryan RO. 1994. Hemo-

lymph lipid transport. *Insect Biochem. Mol. Biol.* 24:855–73
18. Blomquist GJ, Tillman JA, Mpuru S, Seybold SJ. 1998. The cuticle and cuticular hydrocarbons of insects: structure, function and biochemistry. In *Pheromone Communication in Social Insects: Ants, Wasps, Bees and Termites*, ed. RK Vander Meer, MD Breed, ML Winston, KE Espelid, pp. 34–54. Boulder, CO: Westview
19. Burch WL, Herscovitz H. 2000. Disulfide bonds are required for folding and secretion of apolipoprotein B regardless of its lipidation state. *J. Biol. Chem.* 275:16267–74
20. Campbell BC, Nes WD. 1983. A reappraisal of sterol biosynthesis and metabolism in aphids. *J. Insect Physiol.* 29:149–56
21. Canavoso LE, Bertello LE, de Lederkremer RM, Rubiolo ER. 1998. Effect of fasting on the composition of the fat body lipid of *Dipetalogaster maximus, Triatoma infestans* and *Panstrongylus megistus* (Hemiptera: Reduviidae). *J. Comp. Physiol. B* 168:549–54
22. Canavoso LE, Wells MA. 2000. Metabolic pathways for diacylglycerol biosynthesis and release in the midgut of larval *Manduca sexta*. *Insect Biochem. Mol. Biol.* 30:1173–80
23. Candy DJ. 1985. Intermediary metabolism. See Ref. 70a, 10:1–41
24. Candy DJ, Becker A, Wegener G. 1997. Coordination and integration of metabolism in insect flight. *Comp. Biochem. Physiol.* 117B:475–82
25. Capurro M deL, de Bianchi AG. 1990. Larval *Musca domestica* lipophorin biosynthesis. *Comp. Biochem. Physiol.* 97B:655–59
26. Chino H. 1985. Lipid transport: biochemistry of hemolymph lipophorin. See Ref. 70a, 10:115–35
27. Chino H. 1997. Physiological significance of lipid transport by lipophorin for long-distance flight in insects. *Comp. Biochem. Physiol.* 117B:475–82
28. Chino H, Downer RG, Takahashi K. 1986. Effect of adipokinetic hormone on the structure and properties of lipophorin in locusts. *J. Lipid Res.* 27:21–29
29. Chino H, Downer RG, Wyatt GR, Gilbert LI. 1981. Lipophorins, a major class of lipoprotein in insect hemolymph. *Insect Biochem.* 11:491
30. Chino H, Katase H, Downer RGH, Takahashi K. 1981. Diacylglycerol-carrying lipoprotein of hemolymph of the American cockroach: purification, characterization, and function. *J. Lipid. Res.* 22:7–15
31. Chino H, Kitazawa K. 1981. Diacylglycerol-carrying lipoprotein of hemolymph of the locust and some insects. *J. Lipid Res.* 22:1042–52
32. Chu HM, Norris DM, Kok LT. 1970. Pupation requirement of the beetle, *Xyleborus ferrugineus*: sterols other than cholesterol. *J. Insect Physiol.* 16:1379–87
33. Chuck SL, Lingappa VR. 1992. Pause transfer: a topogenic sequence in apolipoprotein B mediates stopping and restarting of translocation. *Cell* 68:9–21
34. Chuck SL, Yao Z, Blackhart BD, McCarthy BJ, Lingappa VR. 1990. New variation on the translocation of proteins during early biogenesis of apolipoprotein B. *Nature* 346:282–85
35. Dadd RH. 1977. Qualitative requirements and utilization of nutrients: insects. In *CRC Handbook Series in Nutrition and Food*, ed. M Rechcigl Jr, pp. 305–46. Cleveland, OH: CRC
36. Dadd RH. 1985. Nutrition: organisms. See Ref. 70a, 4:313–90
37. Dadd RH, Kleinjan JE, Stanley-Samuelson DW. 1987. Polyunsaturated fatty acids on mosquitoes reared with single dietary polyunsaturated. *Insect Biochem.* 17:7–16
38. Dalton T. 1977. Threshold and receptor reserve in the action of 5-hydroxytryptamine on the salivary glands of *Calliphora erythrocephala*. *J. Insect Physiol.* 23:625–31
39. Dalton T. 1977. The effect of prostaglandin E_1 on cyclic AMP production in the

salivary glands of *Calliphora erythrocephala*. *Experientia* 33:1320–30
40. Dantuma NP, Pijnenburg MAP, Diederen JHB, Van der Horst DJ. 1997. Developmental down-regulation of receptor-mediated endocytosis of an insect lipoprotein. *J. Lipid Res.* 38:254–65
41. Dantuma NP, Potters M, De Winther MPJ, Tensen CP, Kooiman FP, et al. 1999. An insect homolog of the vertebrate very low density lipoprotein receptor mediates endocytosis of lipophorins. *J. Lipid Res.* 40:973–78
42. Dantuma NP, Van Marrewijk WJA, Wynne HJ, Van der Horst DJ. 1996. Interaction of an insect lipoprotein with its binding site at the fat body. *J. Lipid Res.* 37:1345–55
43. DeFoliart GR. 1999. Insects as food: why the western attitude is important. *Annu. Rev. Entomol.* 44:21–50
44. de Renobles M, Cripps C, Stanley-Samuelson DW, Jurenka RA, Blomquist GJ. 1987. Biosynthesis of linoleic acid in insects. *Trends Biochem. Sci.* 12:346–66
45. Destephano DB, Brady UE, Lovins RE. 1974. Synthesis of prostaglandin by reproductive tissue of the male house cricket, *Acheta domesticus*. *Prostaglandins* 6:71–79
46. Douglas AE. 1998. Nutritional interactions in insect-microbial symbioses: aphids and their symbiotic bacteria *Buchnera*. *Annu. Rev. Entomol.* 43:17–37
47. Downer RGH. 1985. Lipid metabolism. See Ref. 70a, 10:77–113
48. Edmunds M. 1974. *Defence in Animals: A Survey of Anti-Predator Defences*. New York: Longman. 357 pp.
49. Evans PD. 1985. Octopamine. See Ref. 70a, 11:499–530
50. Feltwell J. 1978. The distribution of carotenoids in insects. In *Biochemical Aspects of Plant and Animal Coevolution*, ed. JB Harborne, pp. 227–307. London: Academic
51. Fernando-Warnakulasuriya GJP, Tsuchida K, Wells MA. 1988. Effect of dietary lipid content on lipid transport and storage during larval development of *Manduca sexta*. *Insect Biochem.* 18:211–14
52. Fields PE, Woodring JP. 1991. Octopamine mobilization of lipids and carbohydrates in the house cricket, *Acheta domesticus*. *J. Insect Physiol.* 37:193–99
53. Ford PS, Van Heusden MC. 1994. Triglyceride-rich lipophorin in *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 31:435–41
54. Friend WG, Dadd RH. 1982. Insect nutrition. In *Advances in Nutritional Research*, ed. HH Draper, pp. 205–47. New York: Plenum
55. Goldsworthy GJ, Mordue W. 1989. Adipokinetic hormones, functions and structures. *Biol. Bull.* 177:218–24
56. Gondim KC, Wells MA. 2000. Characterization of lipophorin binding to the midgut of larval *Manduca sexta*. *Insect Biochem. Mol. Biol.* 30:405–13
57. Gordon HT. 1959. Minimum nutritional requirements of the German roach, *Blattella germanica*. *Ann. NY Acad. Sci.* 77:290–351
58. Grieneisen ML. 1994. Recent advances in our knowledge of ecdysteroid biosynthesis in insects and crustaceans. *Insect Biochem. Mol. Biol.* 24:115–32
59. Haunerland NH. 1997. Transport and utilization of lipids in insect flight muscles. *Comp. Biochem. Physiol.* 117B:475–82
60. Heed WB, Kircher HW. 1965. Unique sterol in the ecology and nutrition of *Drosophila pachea*. *Science* 149:758–61
61. Hobson RP. 1935. On a fat soluble growth factor requirement by blowfly larvae. II. Identity of growth factor with cholesterol. *Biochem. J.* 29:2023–36
62. Howard RW, Stanley-Samuelson DW. 1999. The tie that binds: eicosanoids in invertebrate biology. *Ann. Entomol. Soc. Am.* 92:880–90
63. Hui TY, Bernlohr DA. 1997. Fatty acid transporters in animal cells. *Front. Biosci.* 2:222–31

64. Joshi M, Agarwall HC. 1977. Site of cholesterol absorption in some insects. *J. Insect Physiol.* 23:403–4
65. Jouni ZE, Wells MA. 1996. Purification and partial characterization of a lutein-binding protein from the midgut of the silkworm, *Bombyx mori*. *J. Biol. Chem.* 271:14722–26
66. Kanost MR, Kawooya JK, Law JH, Ryan RO, Van Heusden MC, Ziegler R. 1990. Insect haemolymph proteins. *Adv. Insect Physiol.* 22:299–396
67. Kaplanis NJ, Robbins WE, Monroe RE, Shortino TJ, Thompson MJ. 1965. The utilization and the fate of β-sitosterol in the larva of the housefly, *Musca domestica L*. *J. Insect Physiol.* 11:251–58
68. Kawooya JK, Law JH. 1988. Role of lipophorin in lipid transport to the insect egg. *J. Biol. Chem.* 263:8748–53
69. Kawooya JK, Osir EO, Law JH. 1988. Uptake of the major hemolymph lipoprotein and its transformation in the insect egg. *J. Biol. Chem.* 263:8740–47
70. Kayser H. 1985. Pigments. See Ref. 70a, 10:367–415
70a. Kerkurt GA, Gilbert LI, eds. 1985. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Oxford, UK: Pergamon
71. Komnick H, Giesa U. 1994. Intestinal absorption of cholesterol, transport in the haemolymph, and incorporation into the fat body and Malpighian tubules of the larval dragonfly *Aeshna cyanea*. *Comp. Biochem. Physiol.* 107A:553–57
72. Kutty RK, Kutty G, Kambadur R, Duncan T, Koonin EV, et al. 1996. Molecular characterization and developmental expression of a retinoid- and fatty acid-binding glycoprotein from *Drosophila*. *J. Biol. Chem.* 271:20641–49
73. Law JH, Wells MA. 1989. Insects as biochemical models. *J. Biol. Chem.* 264:16335–38
73a. Lehane MJ, Billingsley PF, eds. 1996. *Biology of Insect Midgut*. London: Chapman & Hall
74. Leroy B, Chararas C, Chipoulet JM. 1984. Etude des activites osidasques du tube digestif des adultes et des larves de la bruchhe du haricot, *Acanthoscelides obtectus* (Coleoptera: Bruucidae). *Entomol. Exp. Appl.* 35:269–73
75. Liu H, Ryan RO. 1991. Role of lipid transfer particle in transformation of lipophorin in *Manduca sexta* oocytes. *Biochim. Biophys. Acta* 1085:112–18
76. Lok CM, Van der Horst DJ. 1980. Chiral 1,2-diacylglycerols in the haemolymph of the locust, *Locusta migratoria*. *Biochim. Biophys. Acta* 618:80–87
77. McFarlane JE. 1976. Vitamin K: growth factor for the house cricket (Orthoptera: Gryllidae). *Can. Entomol.* 108:391–94
78. McFarlane JE. 1983. Lipid factors in insect growth and reproduction. In *Metabolic Aspects of Lipid Nutrition in Insects*, ed. TE Mittler, RH Dadd, pp. 149–57. Boulder, CO: Westview
79. Merritt RW, Dadd RH, Walker ED. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37:349–76
80. Mitchell MJ, Smith SL. 1986. Characterization of ecdysone 20-monoonxygenase activity in wandering stage larvae of *Drosophila melanogaster*. Evidence of mitochondrial and microsomal cytochrome P-450 dependent systems. *Insect Biochem.* 16:525–37
81. Morgan MRJ. 1975. A qualitative survey of the carbohydrates of the alimentary tract of the migratory locust, *Locusta migratoria migratorioides*. *J. Insect Physiol.* 21:1045–53
82. Nair A, Agarwall H. 1977. Sterols and sterol esters in nutrition of the beetle *Trogoderma granarium* Everts. *Indian J. Exp. Biol.* 15:576–78
83. Ness WD, Lopez M, Zhou W, Guo D, Dowd PF, Northon RA. 1997. Sterol

utilization and metabolism in *Heliothis zea*. *Lipids* 32:1317–23
84. Orchard I. 1987. Adipokinetic hormones—an update. *J. Insect Physiol.* 33:451–63
85. Orchard I, Loughton BG. 1985. Neurosecretion. See Ref. 70a, 7:61–67
86. Passier PC, Vullings HG, Diederen JH, Van der Horst DJ. 1995. Modulatory effects of biogenic amines on adipokinetic hormone secretion from locust corpora cardiaca *in vitro*. *Gen. Comp. Endocrinol.* 97:231–38
87. Pease RJ, Harrison GB, Scott J. 1991. Co-translocational insertion of apolipoprotein B into the inner leaflet of the endoplasmic reticulum. *Nature* 353:448–50
88. Petzel DH, Stanley-Samuelson DW. 1992. Inhibition of eicosanoids biosynthesis modulates basal fluid secretion in the Malpighian tubules of the yellow fever mosquito (*Aedes aegypti*). *J. Insect Physiol.* 38:1–8
89. Prasad SV, Fernando-Warnakulasuriya GJ, Sumida M, Law JH, Wells MA. 1986. Lipoprotein biosynthesis in the larvae of the tobacco hornworm, *Manduca sexta*. *J. Biol. Chem.* 261:17174–76
90. Rimoldi OM, Peluffo RO, Gonzalez SM, Brenner RR. 1985. Lipid digestion, absorption and transport in *Triatoma infestans*. *Comp. Biochem. Physiol.* 82B:187–90
91. Rusinol AE, Jamil H, Vance JE. 1997. *In vitro* reconstitution of assembly of apolipoprotein B48-containing lipoproteins. *J. Biol. Chem.* 272:8019–25
92. Rustaeus S, Lindberg K, Stillemark P, Claesson C, Asp L, et al. 1999. Assembly of very low density lipoprotein: a two-step process of apolipoprotein B core lipidation. *J. Nutr.* 129:463–66S
93. Ryan RO. 1990. Dynamics of insect lipophorin metabolism. *J. Lipid Res.* 31:1725–39
94. Ryan RO, Prasad SV, Henriksen EJ, Wells MA, Law JH. 1986. Lipoprotein interconversions in an insect, *Manduca sexta*. Evidence for a lipid transfer factor in the hemolymph. *J. Biol. Chem.* 261:563–68
95. Ryan RO, Schmidt JO, Law JH. 1984. Chemical and immunological properties of lipophorins from seven insect orders. *Arch. Insect Biochem. Physiol.* 1:373–83
96. Ryan RO, Van der Horst DJ. 2000. Lipid transport biochemistry and its role in energy metabolism. *Annu. Rev. Entomol.* 45:233–60
97. Ryan RO, Wells MA, Law JH. 1986. Lipid transfer protein from *Manduca sexta* hemolymph. *Biochem. Biophys. Res. Commun.* 136:260–65
98. Sakura S, Warren TJ, Gilbert LI. 1989. Mediation of ecdysone synthesis in *Manduca sexta* by a hemolymph enzyme. *Arch. Insect Biochem. Physiol.* 10:179–97
99. Schal C, Sevala VL, Young HP, Bachmann JAS. 1998. Sites of synthesis and transport pathways of insect hydrocarbons: cuticle and ovary as target tissues. *Am. Zool.* 38:382–93
100. Seki T, Fujishita S, Ito M, Matsuoka N, Tsukida K. 1986. A fly, *Drosophila melanogaster*, forms 11-cis 3-hydroxyretinal in the dark. *Vis. Res.* 26:255–58
101. Seki T, Isono K, Ozaki K, Tsukahara K, Shibata-Katsuta Y, et al. 1998. The metabolic pathway of visual pigment chromophore formation in *Drosophila melanogaster*. All-*trans* (3S)-3-hydroxyretinal is formed from all retinal via (3R)-3-hydroxyretinal in the dark. *Eur. J. Biochem.* 257:522–27
102. Shapiro JP, Law JH, Wells MA. 1988. Lipid transport in insects. *Annu. Rev. Entomol.* 33:297–318
103. Shelness GS, Ingram MF, Huang XF, DeLozier JA. 1999. Apolipoprotein B in the rough endoplasmic reticulum: translation, translocation and the initiation of lipoprotein assembly. *J. Nutr.* 129:456–62S
104. Smith AF, Tsuchida K, Hanneman E, Suzuki TC, Wells MA. 1992. Isolation,

characterization, and cDNA sequence of two fatty acid-binding proteins from the midgut of *Manduca sexta*. *J. Biol. Chem.* 267:380–84

105. Soulages JL, Salamon Z, Wells MA, Tollin G. 1995. Low concentrations of diacylglycerol promote the binding of apolipophorin-III to a phospholipid surface: a surface plasmon resonance spectroscopy study. *Proc. Natl. Acad. Sci. USA* 92:5650–54
106. Soulages JL, Van Antwerpen R, Wells MA. 1996. Role of diacylglycerol and apolipophorin-III in regulating the physiochemical properties of the lipophorin surface: metabolic implications. *Biochemistry* 35:5191–98
107. Soulages JL, Wells MA. 1994. Effect of diacylglycerol content on some physicochemical properties of the insect lipoprotein, lipophorin. Correlation with the binding of apolipophorin-III. *Biochemistry* 33:2356–62
108. Soulages JL, Wells MA. 1994. Lipophorin, the structure of an insect lipoprotein and its role in lipid transport in insects. *Adv. Prot. Chem.* 45:371–415
109. Soulages JL, Wells MA. 1994. Metabolic fate and turnover rate of hemolymph free fatty acids in adult *Manduca sexta*. *Insect Biochem. Mol. Biol.* 24:79–86
110. Spring DJ, Chen-Liu LW, Chatterton JE, Elovson J, Schumaker VN. 1992. Lipoprotein assembly: apolipoprotein B size determines lipoprotein core circumference. *J. Biol. Chem.* 267:14839–45
111. Stagg LE, Candy DJ. 1996. The effect of adipokinetic hormones on the levels of inositol phosphates and cyclic AMP in the fat body of the desert locust *Schistocerca gregaria*. *Insect Biochem. Mol. Biol.* 26:537–44
112. Stahl A, Hirsch DJ, Gimeno RE, Punreddy S, Ge P, et al. 1999. Identification of the major intestinal fatty acid transport protein. *Mol. Cell* 4:299–308
113. Stanley-Samuelson DW. 1994. Prostaglandins and related eicosanoids in insects. *Adv. Insect Physiol.* 24:115–212
114. Stanley-Samuelson DW, Jensen E, Nickerson KW, Tiebel K, Ogg CL, Howard RW. 1992. Insect immune response to bacterial infections is mediated by eicosanoids. *Proc. Natl. Acad. Sci. USA* 88:1064–68
115. Stanley-Samuelson DW, Pedibholta VK. 1996. What can we learn from prostaglandins and related eicosanoids in insects? *Insect Biochem. Mol. Biol.* 26: 223–34
116. Sundermeyer K, Hendricks JK, Prasad SV, Wells MA. 1996. The precursor protein of the structural apolipoproteins of lipophorin: cDNA and deduced amino acid sequence. *Insect Biochem. Mol. Biol.* 26:735–38
117. Svoboda JA. 1999. Variability of metabolism and function of sterols in insects. *Crit. Rev. Biochem. Mol. Biol.* 34:49–57
118. Svoboda JA, Feldlaufer MF. 1991. Neutral sterol metabolism in insects. *Lipids* 26:614–28
119. Svoboda JA, Herbert EW Jr, Thompson MJ, Shimanuki H. 1981. The fate of radiolabeled C_{28} and C_{29} phytosterols in the honey bee. *J. Insect Physiol.* 27:183–88
120. Svoboda JA, Lusby WR. 1986. Sterols of phytophagous and omnivorous species of Hymenoptera. *Arch. Insect Biochem. Physiol.* 3:13–18
121. Svoboda JA, Robbins WE, Cohen CE, Shortino TJ. 1972. Phytosterol utilization and metabolism in insects: recent studies with *Tribolium confusum*. In *Insect and Mite Nutrition*, ed. JG Rodriguez, pp. 505–16. Amsterdam: North-Holland
122. Svoboda JA, Thompson MJ. 1985. Steroids. See Ref. 70a, 10:137–75
123. Terra WR. 1990. Evolution of digestive systems of insects. *Annu. Rev. Entomol.* 35:181–200
124. Terra WR, Ferreira C, de Bianchi AG. 1979. Distribution of digestive enzymes

among the endo- and ectoperitrophic spaces and midgut cells of *Rhynchosciara* and its physiological significance. *J. Insect Physiol.* 25:487–94

125. Terra WR, Ferreira C, Jordao BP, Dillon RJ. 1996. Digestive enzymes. See Ref. 73a, pp. 153–94
126. Thompson MJ, Weirich GF, Svoboda JA. 1990. Metabolism of insect molting hormone: bioconversion and titer regulation. In *Morphogenetic Hormones of Arthropods: Discovery, Synthesis, Metabolism, Evolution, Modes of Action, and Technique*, ed. AP Gupta, pp. 325–60. New Brunswick, NJ: Rutgers Univ. Press
127. Tran K, Boren J, Macri J, Wang Y, McLeod R, et al. 1988. Functional analysis of disulfide linkages clustered within the amino terminus of human apolipoprotein B. *J. Biol. Chem.* 273:7244–51
128. Tsuchida K, Wells MA. 1988. Digestion, absorption, transport and storage of fat during the last larval stadium of *Manduca sexta*. Changes in the role of lipophorin in the delivery of dietary lipid to the fat body. *Insect Biochem.* 18:263–68
129. Tsuchida K, Wells MA. 1990. Isolation and characterization of a lipoprotein receptor from the fat body of an insect, *Manduca sexta*. *J. Biol. Chem.* 265:5761–67
130. Turunen S. 1985. Absorption. See Ref. 70a, 4:241–78
131. Turunen S. 1993. Metabolic pathways in the midgut epithelium of *Pieris brassicae* during carbohydrate and assimilation. *Insect Biochem. Mol. Biol.* 23:681–89
132. Turunen S, Chippendale GM. 1977. Lipid absorption and transport: sectional analysis of the larval midgut of the corn borer, *Diatraea grandiosella*. *Insect Biochem.* 7:203–8
133. Turunen S, Crailsheim K. 1996. Lipid and sugar absorption. See Ref. 73a, pp. 293–320
134. Van Antwerpen R, Law JH. 1992. Lipophorin lipase from the yolk of *Manduca sexta* eggs: identification and partial characterization. *Arch. Insect Biochem. Physiol.* 20:1–12
135. Van Antwerpen R, Linnemans WAM, Van der Horst DJ, Beenakkers AMT. 1988. Immunocytochemical localization of lipoproteins in the flight muscles of the migratory locust (*Locusta migratoria*) at rest and during flight. *Cell Tissue Res.* 252:661–68
136. Van Antwerpen R, Linnemans WAM, Van der Horst DJ, Beenakkers AMT. 1989. Binding of lipophorin to the fat body of the migratory locust. *Insect Biochem.* 19:809–14
137. Van Antwerpen R, Salvador K, Tolman K, Gentry C. 1998. Uptake of lipids by developing oocytes of the hawkmoth *Manduca sexta*. The possible role of lipoprotein lipase. *Insect Biochem. Mol. Biol.* 28:399–408
138. Van der Horst DJ. 1990. Lipid transport function of lipoproteins in flying insects. *Biochim. Biophys. Acta* 1047:195–211
139. Van der Horst DJ, Van Marrewijk WJA, Vullings HGB, Diederen JHB. 1999. Metabolic neurohormones: release, signal transduction and physiological responses of adipokinetic hormones in insects. *Eur. J. Entomol.* 96:299–308
140. Van der Horst DJ, Weers PMM, Van Marrewijk WJA. 1993. Lipoproteins and lipid transport. In *Insect Lipids: Chemistry, Biochemistry, and Biology*, ed. DW Stanley-Samuelson, DR Nelson, pp. 1–24. Lincoln: Univ. Nebr. Press
141. Van Heusden MC. 1993. Characterization and identification of a lipoprotein lipase from *Manduca sexta* flight muscle. *Insect Biochem. Mol. Biol.* 23:785–92
142. Van Heusden MC, Law JH. 1989. An insect lipid transfer particle promotes lipid loading from fat body to lipoprotein. *J. Biol. Chem.* 264:17287–92
143. Van Heusden MC, Thompson F, Dennis J. 1998. Biosynthesis of *Aedes aegypti* lipophorin and gene expression of

its apolipoproteins. *Insect Biochem. Mol. Biol.* 28:733–38
144. Van Heusden MC, Van der Horst DJ, Van Doorn JM, Beenakkers AMT. 1987. Partial purification of locust flight muscle lipoprotein lipase (LpL): apparent differences from mammalian LpL. *Comp. Biochem. Physiol.* 88B:523–27
145. Van Heusden MC, Van der Horst DJ, Voshol J, Beenakkers AMT. 1987. The recycling of protein components of the flight-specific lipophorin in *Locusta migratoria*. *Insect Biochem.* 17:771–76
146. Van Kerkove E, Pirotte P, Petzel DH, Stanley-Samuelson DW. 1995. Eicosanoid biosynthesis inhibitors modulate basal fluid secretion rates in the Malpighian tubule of the ant, *Formica polyctena*. *J. Insect Physiol.* 41:435–41
147. Van Marrewijk WJA, Van den Broek ATM, Gielbert ML, Van der Horst DJ. 1996. Insect adipokinetic hormone stimulates inositol phosphate metabolism, roles for both Ins,1,4,5.P_3 and Ins,1,3,4,5.P_4 in signal transduction? *Mol. Cell Endocrinol.* 122:141–50
148. Venkatesh K, Lenz CJ, Bergman DK, Chippendale GM. 1987. Synthesis and release of lipophorin in larvae of the southwestern corn borer, *Diatraea grandiosella*: an *in vitro* study. *Insect Biochem.* 17:1173–80
149. Vogt K. 1983. Is the fly visual pigment a rhodopsin? *Z. Natureforsch. Sect. C* 38: 329–33
150. Vogt K. 1984. Chromophores of insect visual pigments. *Photobiochem. Phytobiophys. Suppl.* 7:273–96
151. Vogt K. 1988. Naming visual pigments. *J. Photochem. Photobiol. B* 2:133–34
152. Warren JT, Sakurai S, Rountree DB, Gilbert LI. 1988. Synthesis and secretion of ecdysteroids by the prothoracic glands of *Manduca sexta*. *J. Insect Physiol.* 34:571–76
153. Weer PMM, Van der Horst DJ, Van Marrewijk WJA, Van den Eijnde M, Van Door JM, Beenakker AMT. 1992. Biosynthesis and secretion of insect lipoprotein. *J. Lipid Res.* 33:485–91
154. Weintrab H, Tietz A. 1973. Triglyceride digestion and absorption in the locust, *Locusta migratoria*. *Biochim. Biophys. Acta* 306:31–41
155. Weer PMM, Van Marrewijk WJA, Beenakker AMT, Van der Horst DJ. 1993. Biosynthesis of locust lipophorin. Apolipophorins I and II originate from a common precursor. *J. Biol. Chem.* 268:4300–3
156. Wheeler CH, Goldsworthy GJ. 1985. Specificity and localization of lipoprotein lipase in the flight muscles of *Locusta migratoria*. *Biol. Chem. Hoppe-Seyler* 366:1071–77
157. Wu X, Sakata N, Lele KM, Zhou M, Jiang H, Ginsberg HN. 1997. A two-site model for apoB degradation in HepG2 cells. *J. Biol. Chem.* 272:11575–80