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Transport and Utilization of Lipids in Insect Flight Muscles*

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ABSTRACT. In migrating lepidopteran and orthopteran insects, lipid is the preferred fuel for sustained flight activity. Diacylglycerol is delivered by lipophorin to the flight muscle and hydrolyzed to free fatty acid and glycerol. After penetrating the plasma membrane by an unknown mechanism, fatty acids are bound by the intracellular fatty acid binding protein (FABP) and transported through the cytosol. After their conversion to acyl-CoA esters, the fatty acids enter the mitochondrial matrix via the carnitine shuttle for subsequent β-oxidation. This article reviews the current knowledge of lipid metabolism in insect flight muscle, with particular emphasis on the structure and function of FABP and its expression during locust development and flight. comp biochem physiol 117B;4:475–482, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. Fatty acid oxidation, insect flight metabolism, locust, fatty acid transport, lipase, FABP

OVERVIEW OVER LIPID METABOLISM IN FLIGHT MUSCLE

Insects that engage in migratory flight activity usually depend on lipids as an energy source for the flight muscle. Lipid utilization during sustained flight has been extensively studied in lepidopteran and orthopteran species. The processes involved in lipid release from the fat body and their transport through the aqueous hemolymph have been described in detail in the reviews by Goldsworthy *et al.*, van der Horst and van Marrewijk and Chino elsewhere in this issue. Upon stimulation by adipokinetic hormone, diacylglycerol (DAG) is released from fat body into the hemolymph, where it associates with the core high-density lipophorin (HDLp) particle and free apolipophorin III (apoLp-III) to form a DAG-enriched low-density lipoprotein molecule. Low-density lipophorin (LDLp) delivers DAG to the flight muscle. DAG is hydrolyzed outside of the muscle cell, giving rise to free fatty acid, glycerol, the DAG-depleted HDLp particle and free apoLp-III. Except for the fatty acid, all these components are hydrophilic and remain initially in the hemolymph. Fatty acids, however, are imported directly into the muscle cell. Once inside, the hydrophobic fatty acid molecules must move through the aqueous cytosol. They are activated by coenzyme A and then converted to their carnitine esters that are transported through the outer and inner mitochondrial membranes. In the mitochondrial matrix, fatty acids are again transformed to their CoA-esters that enter the β-oxidation pathway.

This brief description highlights both the parallels and differences in lipid metabolizing pathways in insects and vertebrates. Extended activity in vertebrate red muscle is fueled by β-oxidation of fatty acid as well. In the circulatory system, proteins are also responsible for the transport of the hydrophobic lipids, either triacylglycerol or free fatty acid. Triglycerides are complexed in a large lipoprotein (very-low-density lipoprotein, VLDL), whereas free fatty acids are transported by serum albumin, an abundant blood protein that has several high affinity and many additional low affinity binding sites for fatty acids (28). Free fatty acid is the only lipid that can enter the muscle cell; most muscles therefore rely on albumin to deliver the needed fatty acid, but heart muscle in particular can also obtain its fatty acid from the VHDL particle. To release fatty acid from the protein, triacylglycerol must be first hydrolyzed by the action of a membrane-bound lipoprotein lipase [for a review, see (35)].

The fate of fatty acids within the muscle cell is similar in both insects and vertebrates. One important difference, however, is that the metabolic activity and hence the rate of β-oxidation is much higher in insect than in vertebrate muscles. The exact values depend on the method used to determine the metabolic rates, and values reported from different laboratories cannot always be easily compared. In locusts, Crabtree and Newsholme (9) found maximal rates of fatty acid utilization of 0.9 μmol palmitate/min/g muscle weight as opposed to 0.3 μmol for rat heart. From other studies (40), it is clear that heart muscle has the highest

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Abbreviations–ACBP, acyl-CoA-binding protein; AKH, adipokinetic hormone; apoLp, apolipophorin; DAG, diacylglycerol; FABP, fatty acid binding protein; HDLp, high-density lipophorin; LDLp, low-density lipophorin; VLDL, very-low-density lipoprotein.

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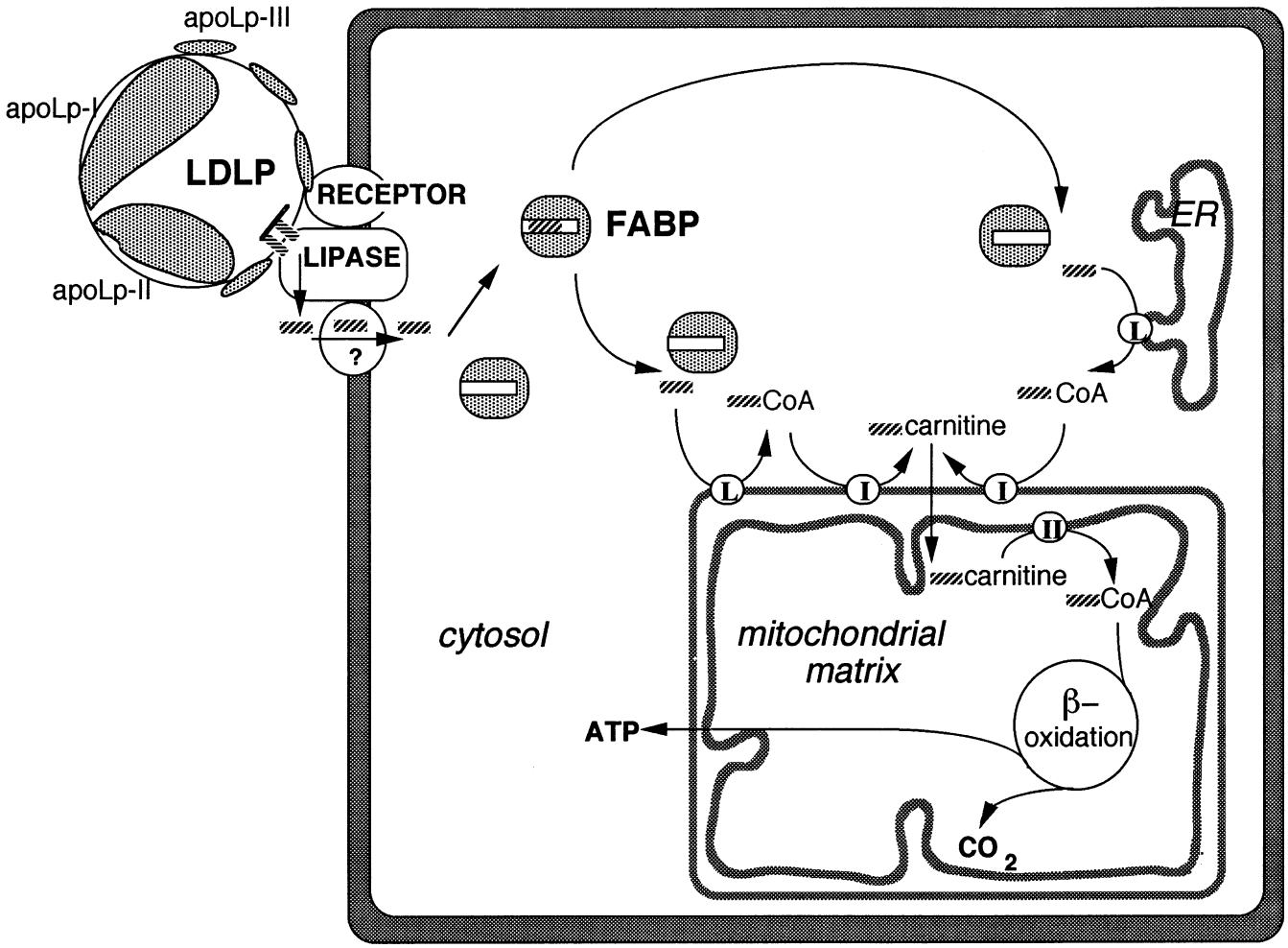

FIG. 1. Schematic overview of fatty acid utilization in flight muscle. I, carnitine acyltransferase I; II, carnitine acyltransferase II; L, fatty acyl-CoA ligase. The hatched bar represents a long-chain fatty acid molecule.

fatty acid oxidizing capacity of mammalian muscles. Flight muscles of migratory birds [pigeon, (9)] achieve higher rates than mammals but lower than locusts (0.6 μmol/min/g). In contrast to mammalian heart, the metabolic activity of flight muscle varies dramatically; during locust flight, metabolic rates are 20 times as high as in resting animals (18).

Insect muscles do not store lipids, and hence all fatty acid needed to sustain flight must be obtained from mobilized fat body reserves. Although it is obvious that the lipophorin shuttle is a very efficient transport mechanism, the rate of fatty acid uptake into and the transport through the flight muscle cell must be very effective as well to sustain the high metabolic rates encountered during flight. The recent progress in the latter two areas, summarized in Fig. 1, is the main topic of this review.

HYDROLYSIS OF DAG BY LIPOPHORIN LIPASE AT FLIGHT MUSCLE MEMBRANES

In immunocytochemical studies, van Antwerpen *et al.* (32) have shown that LDLp is present only in the extracellular matrix of locust flight muscles, even after flight activity. This direct visualization provided unambiguous evidence that lipid uptake into fat body does not occur via endocytosis of the lipophorin particle. Instead, DAG is hydrolyzed at the outside of the flight muscle cell. Because all DAG present in the hemolymph is contained in lipophorin particles, the hydrolysis should be catalyzed by a lipoprotein lipase present at the hemolymph side of the flight muscle membrane. Early evidence for such an enzyme was obtained by Wheeler *et al.* (43), who found that muscle cell homogenates can hydrolyze lipophorin-bound DAG to glycerol and free fatty acids. In these experiments, LDLp was a much better substrate than HDLp. The enzymatic activity is associated with the muscle cell membranes, as demonstrated by Wheeler and Goldsworthy (42). It appears that the enzyme is directly anchored to the flight muscle membrane (37). Attempts to purify the lipase were not successful in *Locusta*. Similar lipolytic activity has been found in flight muscle homogenates of a lepidopteran species, the hawk moth *Manduca sexta*. This enzyme has been partially purified by van Heusden (36). The lepidopteran lipase requires the

presence of a detergent in all its purification steps, which is consistent with its membrane localization. Lipoprotein lipase is a 37-kDa protein that appears to be present in very small quantities. The enzyme seems to belong to the class of serine esterases because it is inhibited by the common serine esterase inhibitors diisopropyl fluorophosphate, and phenylmethylsulfonyl fluoride.

In addition to a high lipolytic activity, efficient hydrolysis of LDLp-bound DAG would also require recognition and binding of the LDLp particle by or in close proximity to the lipoprotein lipase. Such interactions have been shown first in locust by Hayakawa (17), who found that flight muscle membranes bind HDLp with high affinity (K_d = 1.4 10^{-7}) and postulated the presence of a lipophorin receptor. Van Antwerpen *et al.* (33) confirmed these finding and showed that bound HDLp can be replaced *in vitro* by LDLp; the efficient exchange of DAG-depleted and DAG-enriched lipophorin species is an essential element of the lipophorin shuttle mechanism. Through ligand blotting techniques, a number of flight muscle membrane proteins were shown to bind to HDLp or LDLp, most prominently a 30-kDa protein. At present, it is unclear whether this or another lipophorin-binding protein is involved in the lipid delivery to the flight muscle and whether different proteins are responsible for lipoprotein binding and lipolytic activity. The lipophorin receptor may serve as docking site to fix the lipophorin particle to the flight muscle membrane in the vicinity of the lipase. It is equally possible that the lipoprotein lipase itself binds its lipophorin substrate; in this case, the 30-kDa protein found in the locust may be the equivalent to the 37-kDA lipoprotein lipase found in *Manduca*. Other studies have suggested that apoLp-III, which is much more abundant in LDLp, may contain an important recognition site at the LDLp particle (44). The low lipase activity observed in resting locusts has been explained by an inhibition of LDLp recognition sites by the excess of free apoLp-III. Although proposed long before the structural details of the apoLp-III–lipophorin interactions were known, this hypothesis is certainly not inconsistent with current knowledge, as reviewed by Ryan (25). Locust apolipophorin III is composed of five amphipathic helices. When free in solution, the hydrophobic sites stick to each other, and only hydrophilic regions are exposed to the aqueous environment. Upon binding to DAG molecules on the surface of LDLp, the protein undergoes an dramatic conformation change, which could also result in an altered recognition site. However, more research is required to decide whether apoLp-III is indeed involved in the recognition of the LDLp particle and to elucidate the mode of action of the lipophorin lipase.

UPTAKE OF FATTY ACIDS

Little is known about the mechanisms by which the free fatty acids enter the flight muscle cell. Because they are produced by the lipoprotein lipase at the plasma side of the muscle cell, fatty acids must cross the sarcolemma before they can enter the cytosol. Traditionally, it has been assumed that the hydrophobic fatty acids can freely diffuse through cell membranes; however, their carboxyl-group is fully ionic at physiological pH values (~pH 7), and this may limit their solubility in the membrane. In effect, fatty acids must change their orientation when crossing a membrane, and it is questionable whether diffusion is fast enough to ensure the rapid uptake of fatty acids required during flight. In mammalian cells, evidence is accumulating for integral membrane proteins that appear to transport fatty acid through the plasma membrane of various cell types, including cardiac myocytes. The best studied protein is a 43-kDa plasma membrane fatty acid binding protein ($FABP_{PM}$) (30), which it is not related to the cytosolic fatty acid binding proteins described below. Antibodies raised against this protein blocked the uptake of fatty acids, and hence it has been speculated that the protein may either directly transport fatty acid through the membrane or bind fatty acid molecules at the extracellular side and aid their partitioning into the membrane. Another possible mediator of fatty acid uptake is a recently described 88-kDa fatty acid translocase (38); in rat heart and skeletal muscle, this protein is coexpressed with the cytosolic FABP thought to be involved in the intracellular transport of fatty acids. If indeed a membrane transporter is required for the import of fatty acids into mammalian muscle cells, one must assume that a similar mechanism exists in flying insects as well where much larger quantities of fatty acids are metabolized, but this protein has not yet been discovered in insect cells.

INTRACELLULAR TRANSPORT REQUIREMENTS

Once fatty acids have crossed the plasma membrane, they need to enter the hydrophilic cytosol and move to the mitochondria where β-oxidation takes place. Considering their hydrophobic hydrocarbon tail, fatty acids should mostly remain in the plasma membrane, unless an intracellular mechanism exists to increase their partition into the cytosol. It is now generally assumed that intracellular fatty acid transport is mediated by a small fatty acid binding protein (M_r 15,000) that has been found in many different cell types and species (39). The fatty acid binding protein found in heart and skeletal muscle is encoded by a single gene related to but clearly distinct from FABPs found in other tissues.

In insects, FABP has been studied in the flight muscle of two locust species, *Schistocerca gregaria* (14) and *Locusta migratoria* (34). Both locust species metabolize fatty acids at extremely high levels during migratory flight, and FABP may be required for their rapid transport. Locust muscle FABPs are very similar in their characteristics to mammalian FABPs (13,21). They are small, acidic proteins (M_r 15,000; p*I* 5.2–5.8) with a single binding site for fatty acids. They are major components of flight muscle cytosol, amounting to as much as 20% of all cytosolic proteins.

FABP has not been isolated from lepidopteran flight muscle; however, van der Horst *et al.* (34) found evidence for a low-molecular-weight fatty acid binding protein in the flight muscle of the death's head hawk moth *Acherontia atropos*. When separating muscle cytosol after incubation with radiolabeled fatty acid, most of the fatty acid appeared in a high-molecular-weight fraction, probably containing contaminant HDLp. Because only small amounts of radioactivity were found with the 15-kDa fraction, it was speculated that this insect contains only small amounts of FABP. Alternatively, different binding characteristics could explain these results. Although physiological reasoning would suggest that migratory moths must also possess FABP or an alternative fatty acid transport mechanism, this can be confirmed only after the isolation, characterization and quantification of FABP from Lepidoptera.

FABP from *L. migratoria* was found to interact with both saturated and unsaturated fatty acids; the binding constant was initially determined as 5 μM (34) but recently re-evaluated as 0.5 μM, a similar value as found for human M-FABP (21). The binding of fatty acid requires not only the hydrophobic hydrocarbon tail but also the charged carboxyl-group. Binding was observed only at pH values above 4; fatty acid dissociates from FABP at lower pH where fatty acid is no longer charged (14).

FABP Structure

The primary structure of the muscle FABP from both locust species is known from amino terminal sequencing and cDNA cloning (21,23). The sequences are virtually identical, with only two substitutions over the entire 133 amino acid sequence. Locust muscle FABP is highly homologous to mammalian muscle FABP, with 42% identity and a total of 82% similarity. This sequence homology is remarkable, especially when considering the evolutionary distance between locust and mammals; it is much higher than the similarity found between the different families of mammalian FABPs or between locust muscle FABP and the FABPs from *Manduca sexta* midgut (23), the only other insect FABP known (26). This may indicate that only small variations in the FABP structure are permissible for a correct functioning in muscle tissues.

Indeed, the tertiary structure of locust FABP is very similar to that of human muscle FABP. Locust FABP was crystallized and its structure solved at 2.1 Å resolution (16). The protein displays the characteristic β-barrel motif common to all FABP. Two perpendicularly oriented β-sheets form a barrel-like structure; within this barrel, the fatty acid binding site is located. The β-barrel itself is open to one side, but this opening is closed off by a helix-turn-helix motif (Fig. 2). It is believed that, through dynamic changes, this helical structure provides the entrance and exit port for fatty acids (1). Because locust fatty acid binding protein crystallized without its ligand fatty acid, its exact location in the protein is not known. However, from the structural similarities to mammalian heart FABP (45) and biochemical binding experiments, it is likely that the carboxyl-group of the fatty acid binds to an arginine deep within the binding pocket, whereas the hydrocarbon tail interacts with various hydrophobic amino acids that line the binding site. The fatty acid tail, however, may be bent differently, because some amino acid side chains within the binding pocket prevent an orientation identical to that found in mammalian heart FABP. Nevertheless, the overall structure of the locust M-FABP is highly similar to human heart FABP, also in many surface areas; this conservation may indicate that many structural details are essential for the function of FABP in muscle tissue.

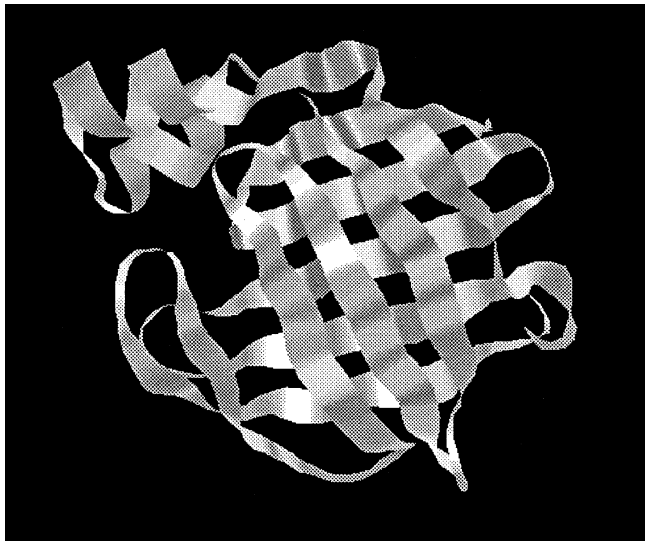

FIG. 2. Schematic drawing of the three-dimensional structure of locust FABP. The figure was drawn using the Brookhaven Protein Data Bank file 1FTB (16).

Function of FABP

Although for many FABP-types the exact function has not been elucidated, it is generally accepted that muscle FABP serves several purposes. FABP will increase the solubility of fatty acids and thus lead to a more rapid transport through the cytosol. Moreover, it may serve as intracellular acceptor for free fatty acids that have passed through the plasma membrane. Without such an acceptor, fatty acids would mostly remain in the membrane and not dissolve in the aqueous cytosol. Finally, the binding protein may serve as a buffer for free fatty acids, both to ensure the presence of fuel molecules before muscle activity and to prevent the buildup of high concentrations of unbound fatty acids afterwards. With their hydrophilic carboxy-groups and the hydrophobic tails, free fatty acids are amphiphilic molecules that, by detergent-like interactions, could destroy membrane structures within a muscle cell. Considering the high

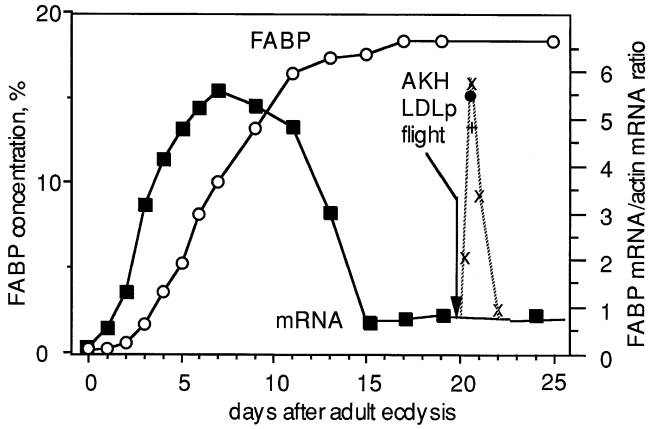

FIG. 3. Intracellular FABP and mRNA concentration in adult locusts. FABP concentration (○), expressed as percent of total cytosolic protein, was determined in resting locusts by Haunerland *et al.* (15). mRNA levels are expressed as the ratio between FABP mRNA and the constant actin mRNA. ■, mRNA ratio in resting adults (15); mRNA levels after 4 hr of flight (+), injection of AKH (×) or injection of LDLp (●) were determined by Chen and Haunerland (6). The arrow indicates the time of injection or flight initiation.

metabolic rate and fatty acid flux sustained by locust flight muscles, one would expect an even greater need than in mammalian muscle for any of these three functions. Indeed, locust flight muscle contains extraordinarily high amounts of FABP (15). In mature adult locust, FABP comprises almost 18% of the total soluble muscle proteins, a value more than three times as high as found maximally in mammalian muscle. Apparently, there is a direct relationship between the fatty acid metabolizing capacity of a muscle and its FABP content; muscles that metabolize only minimal amounts of fatty acids, such as fast-twitch skeletal muscles, possess only small amounts of FABP, whereas fatty acid dependent tissues (e.g., the heart or the flight muscle) need much larger amounts of the protein (13,40).

Control of FABP Synthesis and Expression

Although FABP is the most prominent flight muscle protein in mature adult locusts, it is completely absent in immature locusts and in newly emerged adults. FABP expression commences immediately after adult ecdysis and continues at high rates for almost 2 weeks, as demonstrated in Northern blots (Fig. 3) (15). During this period, the FABP concentration gradually increases from 0 to approximately 13 mg/g wet tissue, or 18% of the total cytosolic proteins, as determined in an ELISA. The increase in the FABP concentration is concomitant to the flight ability of the insect. Locusts cannot fly for longer time periods during the first 10 days of adult life, and one reason for this may be the lack of an efficient intracellular fatty acid transport mechanism. Once FABP reaches a significant concentration, it becomes possible to use large amounts of fatty acids and hence to fly for long time periods.

Locust flight muscle continuously grows and develops during the last nymphal stage; final differentiation is concluded 4 days after adult ecdysis. Afterward, the muscle continues to grow for 10 more days but remains structurally unchanged (5,41). Between the penultimate day of the last instar and the fifth day after ecdysis, the citrate synthase activity and palmitate oxidation capacity found in locust flight muscle increase 3- to 4-fold (34); at the same time, the mitochondrial volume increases by 30%, suggesting that the higher mitochondrial density contributes to the elevation in fatty acid oxidation and citric acid cycle activity. In addition, the concentration of most catabolic enzymes, including those involved in β-oxidation, increase at the beginning of the adult stage. Unlike FABP, however, all these proteins are already present in the muscle before metamorphosis.

Flight muscle FABP is a truly adult specific protein in contrast to all other proteins involved in lipid metabolism. Neither FABP nor its mRNA were ever detected in any amounts in nymphs. To analyze whether the expression of FABP is initiated before metamorphosis, a series of experiments involving insect growth regulators were performed (7). Treatment of last larval nymphs with azadirachtin, an ecdysone antagonist, prevented the final molt, and locusts remained for up to 90 days in their last nymphal stage. Although the mesothorax muscles continued to grow and develop, FABP mRNA was never detectable. Similarly, when insects were treated with juvenile hormone analogues during their last nymphal stage, they molted into an additional, supernumerary nymphal stage, with clearly enlarged mesothorax muscles. However, even these muscles never expressed FABP. In contrast, if metamorphosis was induced prematurely, after the fourth nymphal instar, by treatment with ethoxyprecocene, FABP was expressed immediately after the final molt, and its concentration rose similarly as in normal adults. Although immature locusts never synthesized FABP, its expression was always strong in adults. It does not seem to be induced by an extracellular factor, such as a hormone; juvenile hormone treatment shortly before and after metamorphosis did not prevent FABP expression, and ligation of freshly emerged adults around the neck and abdomen had no influence on the FABP expression either. These results indicate that the expression of the FABP gene is directly linked to metamorphosis. As for other adult-specific genes, the molecular mechanism that prevents FABP expression is not known. Possibly, the FABP gene is not accessible for RNA polymerase in nymphs, and gross structural changes or the removal of nymph-specific DNA-binding proteins make the gene and its promoter accessible. Alternatively, adult specific cofactors could be required for the transcription, but this is less likely, considering the complete absence of FABP mRNA in nymphs. Because most adult proteins known in locusts are expressed at least in

minute amounts in immature insects, FABP may be an excellent model to study the control of gene expression in truly adult specific proteins.

As mentioned before, the level of FABP expression is very high in young adult locusts. However, after 10 days, the FABP concentration within the muscle cell approaches its maximum and the amount of FABP mRNA rapidly declines (Fig. 3) (15). Two weeks after adult ecdysis, the FABP levels have reached their maximum and remain high for the entire adult life. FABP mRNA, however, is present only at very small levels (less than 10% of the maximal values seen in young adults), indicating that FABP is a relatively stable protein and that only small amounts of the protein must be replenished.

Physiological Regulation

Flight activity was shown to further increase FABP expression in mature adults (6). When 20-day-old locusts were exercised in tethered flight, the levels of FABP mRNA increased more than 12-fold. This increase occurred with a delay, approximately 16 hr after the initiation of flight. A similar effect was observed after flight periods lasting 2, 4, 8 or 12 hr. The elevated levels persisted for almost 2 days before the mRNA concentration returned to the low base value. Flight times shorter than 1 hr, however, had no influence on FABP expression. The latter flights are fueled by carbohydrates (see Goldsworthy *et al.*, this volume), whereas longer flight periods depend on the β-oxidation of lipids and hence an efficient fatty acid transport. It was shown that a similar, strong accumulation of FABP mRNA could be induced by adipokinetic hormone (AKH) alone, which mobilizes fatty acid from the fat body and stimulates their transport to the flight muscle. Again, this effect was seen 16 hr after the injection of AKH. It appears that the increased fatty acid delivery is responsible for the stimulation of FABP gene expression, and not AKH directly, because FABP mRNA increases to similar levels when isolated LDLp was injected into insects instead of the hormone (Fig. 3). With the assumption that the level of fatty acid uptake into the flight muscle depends mostly on the supply of LDLp, it seems that an increase in intracellular fatty acids triggers the expression of their own binding protein. In mature locusts that have never flown, almost all FABP contained a bound fatty acid molecule (14). It is plausible that FABP expression is stimulated when the concentration of free fatty acid in the cell exceeds the amount of available binding sites, as encountered during extended flight or after lipophorin or AKH injection. Fatty acids or one of their metabolites may act as a cofactor in gene control, for example, by activating a transcription factor in the nucleus. Such a mechanism is only possible if fatty acid and FABP can exchange freely between cytosol and nucleus through nuclear pores that generally permit the free access of small molecules and proteins of less than 30 kDa. Through immunohistochemical studies, it was demonstrated that FABP is indeed present in the nucleus in similar concentration as in the cytosol in all developmental stages (15). Hence, it appears that FABP indirectly participates in the control of its own gene; if enough fatty acid-free FABP is present in the nucleus, the expression of the FABP gene is suppressed. However, once free fatty acid exceeds the available binding sites, FABP expression is induced.

From a physiological point of view, this mechanism would be very advantageous. Not only does an increased FABP concentration support the delivery of fatty acids to the flight muscle, but, more importantly, it will also increase the cell's buffering capacity for fatty acids and thus prevent a buildup of unbound fatty acids. This feedback may therefore be a mechanism to protect the cells from damage due to large concentrations of free fatty acids.

FABP Gene

To study these phenomena on a molecular level, it will be necessary to conduct experiments with the FABP gene. Only recently has the mammalian muscle FABP gene been cloned from mouse (31). New data from our laboratory confirm a similar gene structure for the locust FABP gene. The locust gene has only two introns instead of three, but these introns are located in exactly the same positions as in the mouse gene. The locust gene is nevertheless much larger (13 kb) because the two remaining introns are much larger than in mouse. For further studies on the gene expression of FABP, it is now necessary to sequence the locust FABP gene and search for possible conserved regulatory sequences upstream of the gene.

ACTIVATION OF FATTY ACIDS AND IMPORT INTO MITOCHONDRIA

Before their import into mitochondria and subsequent β-oxidation, fatty acids must be activated by conversion into their CoA-esters. Microsomal fatty acyl-CoA ligase (fatty acid thiokinase) activity has been detected in lepidopteran flight muscle (10). *In vitro* evidence from mammalian muscle suggests that the presence of FABP increases the rate of acyl-CoA formation (11), possibly because of the increased fatty acid availability at the endoplasmic reticulum membrane or at the mitochondrial membrane, an alternative location of fatty acyl-CoA ligase. Once converted into their CoA derivatives, fatty acids must still be transported into the mitochondrial matrix. It is possible that the translocation of the still hydrophobic fatty acyl-CoA esters is also mediated by a transport protein. A conserved superfamily of acyl-CoA binding proteins (ACBP) has been found in various mammalian tissues (19) and also in *Manduca sexta* (27) midgut and numerous tissues of *Drosophila melanogaster*

(20). The tissue distribution in *Drosophila* points toward its involvement in energy metabolism in lipid utilizing tissues. In mammals, ACBP has been shown to donate acyl-CoA for mitochondrial β-oxidation (24). Although these findings suggest its presence in the flight muscle of insects that use lipid as fuel for migratory flight, ACBP has not yet been studied in these tissues.

Because the mitochondrial membrane is normally impermeable for fatty acyl-CoA esters, their import requires a specific shuttle mechanisms. In the cytosol, the acyl chain is transferred to carnitine by carnitine acyltransferase I, an enzyme that is located at the outer mitochondrial membrane. Carnitine esters are then shuttled into the mitochondrial matrix in exchange for free carnitine. A second carnitine acyltransferase, bound to the inner mitochondrial membrane, transfers the fatty acyl-chain back onto CoA. Although these enzymes have been studied only in mammals, there is ample evidence that suggests that insect muscle also uses a similar shuttle mechanism. Palmitoyl transferase activity was demonstrated in flight muscles of the migratory locust (3) and of the tobacco hornworn (12) but not in insects that use carbohydrate as energy source (8). It is interesting, however, that two other lipid utilizing moths species (*Prodenia eridanina* and *Trichoplusia ni*) do not use this common import mechanism; their flight muscle mitochondria, although capable of oxidizing free fatty acid, cannot oxidize carnitine esters and do not seem to possess palmitoylCoA transferase activity (29). In these species, fatty acids possibly enter the mitochondria as CoA esters, as suggested by Beenakkers *et al.* (4), or as free fatty acids that are converted by a matrical fatty acyl-CoA synthase (22).

MITOCHONDRIAL β-OXIDATION

The oxidation of fatty acids in the mitochondrial matrix of insect flight muscles follows the common β-oxidation pathway. The acyl-CoA ester is oxidized by fatty acyl-CoA dehydrogenase, hydrated by enoylCoA hydratase, further oxidized to the 3-ketoacyl-CoA derivative by 3-hydroxyacyl-CoA dehydrogenase and finally thiolytically cleaved, by β-ketothiolase, to acetylCoA and a two carbon shorter acyl-CoA compound. In lipid mobilizing flight muscles, at least two of these enzymes have been found in much larger concentrations than in flight muscles that depend on carbohydrate as fuel (2). Although the flight muscles of migratory insects are much more active than mammalian muscles, there is no evidence to suggest that insect mitochondria are biochemically different. ATP production by mitochondria is inhibited in resting muscle by the accumulation of citrate, both in muscle that rely on carbohydrate and in lipid-dependent insect flight muscle (12). The higher metabolic rates during flight appear to be possible because of a higher mitochondrial density, better supply of oxygen by the trachea and the increased fatty acid availability. As summarized in this article, much progress has been made with respect to fatty acid delivery, uptake and transport in flight muscle, but more research is needed to unravel the detailed mechanisms involved.

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