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Characterization of cholesterol transport from midgut to fat body in *Manduca sexta* larvae[☆]

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Abstract

Using in vitro methods, we investigated the transfer of cholesterol from larval *Manduca sexta* midgut to the hemolymph lipoprotein, lipophorin, and the transfer of cholesterol from lipophorin to larval fat body. In the midgut, transfer of free cholesterol shows saturation kinetics, but the apparent K_m is higher than the measured K_d for the midgut lipophorin-receptor complex. In addition, the transfer is unaffected by suramin, which binds to the receptor and inhibits lipophorin binding, and by antibodies to the lipid transfer particle, which is required for export of diacylglycerol from the midgut to lipophorin. In the fat body, transfer of free cholesterol also shows saturation kinetics, and the apparent K_m is higher than the measured K_d for the fat body lipophorin-receptor complex. Suramin and anti-lipid transfer particle antibodies exert only a small (20%) inhibitory effect. In both tissues it seems that the most likely mode of cholesterol transfer is via aqueous diffusion, which is also an important mechanism in vertebrate cells. Based on these results, we propose that cholesterol homeostasis in larval *M. sexta* is maintained by a mass action mechanism in which cholesterol is freely transferred between lipophorin and tissues depending on the needs of the tissues. This simple mechanism is ideally suited to insects, which can neither make cholesterol nor internalize lipophorin, the two mechanisms that vertebrate cells use to control their cholesterol content. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Manduca sexta*; Cholesterol transport; Fat body; Midgut; HDLp

1. Introduction

Cholesterol is an important component of the membranes and is the precursor of the molting hormones, ecdysones, which are essential for controlling the molecular and cellular events that lead to molting and metamorphosis (Sehnal, 1989; Gilbert et al., 1988, 1996). In *Manduca sexta* larvae absorption of this sterol is relatively slow (Jouni et al., 2002c) compared to digestion and absorption of triacylglycerol (Tsuchida and Wells, 1988). Cholesterol is absorbed from the midgut lumen mainly in the free form and transported via high-density

lipophorin (HDLp) exclusively in the free form (Jouni et al., 2002c). HDLp, the sole carrier of cholesterol in hemolymph, distributes its sterol to different tissues, including the fat body where cholesterol is stored both in the free and the esterified forms (Jouni et al., 2002c).

The mechanisms by which cholesterol is released from the midgut and taken up by the fat body of *M. sexta* larvae and adults are unknown. In larvae, an aqueous diffusion mechanism has been shown to be the major route by which cholesterol is transferred from the fat body to HDLp, whereas the contributions of the lipid transport particle (LTP) and a receptor-mediated pathway are minor in this cholesterol transfer process (Jouni et al., 2002b).

In mammalian systems the generally accepted mechanisms for cholesterol transfer between membranes and extracellular phospholipid containing acceptors, such as high-density lipoprotein (HDL), are aqueous diffusion (Johnson et al., 1991) and scavenger receptor class B type I (SR-BI) (Rothblat et al., 1999). Aqueous diffusion

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Abbreviations: HDLp, high-density lipophorin; [³H]-C-HDLp, [³H]-cholesterol-labeled HDLp; LTP, lipid transfer particles; MG, midgut; FB, fat body

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involves cholesterol movement from the donor cell membranes to acceptors through the unstirred water layer (Johnson et al., 1991). This mechanism occurs passively without the direct input of metabolic energy and requires the desorption of cholesterol molecules from plasma membrane into the aqueous phase followed by the incorporation of the desorbed cholesterol molecule into the acceptor particle (Johnson et al., 1991). The presence of a scavenger receptor SR-BI in insects has not been reported.

In this paper, we have characterized, *in vitro*, the physiological pathways for the transfer of cholesterol from the midgut to HDLp and from HDLp to the fat body of feeding *M. sexta* larvae.

2. Materials and methods

2.1. Materials

Glutathione, dithiothreitol, benzamidine hydrochloride, phenylmethylsulfonyl fluoride, suramin, cholesterol oxidase, and Grace's medium were purchased from Sigma (St Louis, MO). (1,2n)-[³H]-cholesterol (specific activity 52 Ci/mmol) and Affi-gel Protein A were purchased from Amersham Pharmacia Inc. (Piscataway, NJ). Falcon multi-well tissue culture plates and cell strainers were obtained from Becton Dickinson (Franklin Lakes, NJ). Centriprep Centrifugal Filter Devices were from Millipore-Amicon (Bedford, MA). All other chemicals were analytical grade.

2.2. Insects

M. sexta were reared according to Fernando-Warnakulasuriya et al. (1988) on a high wheat germ diet, in an incubator at 26 °C, with a 18 h light/dark cycle. All insects were synchronized at the end of the fourth larval instar by the appearance of head capsule apolysis.

2.3. HDLp isolation

Hemolymph from third day fifth instar larva fed 4 μCi [³H]-cholesterol 24 h earlier was collected in ice-cold bleeding solution (30 mM KH₂PO₄ pH 6.5, containing 2 mM Na₂EDTA, 10 mM glutathione, and 3 mM NaN₃) via an incision in the second pair of prolegs. Hemocytes were separated by centrifugation for 10 min at 2000g. The supernatant was adjusted to a density of 1.31 g/ml with KBr then 20 ml of this solution was overlaid with 20 ml of 0.9% NaCl, followed by ultracentrifugation in a Beckman VTi 50 rotor at 50,000 rpm for 16 h (Shapiro et al., 1984) and densities were determined along the gradient. Fractions containing [³H]-cholesterol-labeled HDLp ([³H]-C-HDLp) were combined and dialyzed against a lepidopteran saline (5 mM KH₂PO₄,

100 mM KCl, 4 mM NaCl, 15 mM MgCl₂, 2 mM CaCl₂, pH 6.5). The peak of radioactivity (92%) was associated with HDLp peak (*d*=1.14–1.16 g/ml), as confirmed by immunoblotting. The specific activity of [³H]-C-HDLp was 2×10⁵ cpm/mg lipophorin. Unlabeled HDLp was prepared following the same procedure. The purity of the HDLp preparations was confirmed by SDS-PAGE analysis (Laemmli, 1970). Gels were stained with Coomassie Brilliant Blue R-250.

2.4. Measurement of cholesterol transfer from midgut to HDLp

Third day fifth instar larvae (3.1–3.2 g) were fasted for 10 min, and then fed a small piece of diet containing 1 μCi [³H]-cholesterol. Following consumption of the diet, the insects were placed on unlabeled diet for 1 h; subsequently, the midgut was dissected under cold PBS buffer (50 mM sodium phosphate, 150 mM NaCl, pH 6.5) containing 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 0.1 mM glutathione, 0.1 mM diisopropylfluorophosphate, and 0.5 mM benzamidine. Midgut contents were removed, and the flat sheet midgut tissue was then washed twice with Grace's medium. [³H]-cholesterol-labeled midgut (120 mg) was incubated in Grace's medium containing different concentrations of HDLp. At different times, 50 μl aliquots of the incubation medium were mixed with 100 μl of PBS containing 2 mg/ml of BSA and centrifuged at 10,000g for 10 min to remove any detached cells (Jouni et al., 2002b). At the end of the incubation, the tissues were separated from the incubation media by filtration using a nylon mesh cell strainer, washed thoroughly with PBS and lysed in 1 ml of 0.1 N NaOH. Aliquots were used for radioactivity counting using a scintillation counter.

2.5. Measurements of cholesterol transfer from [³H]-C-HDLp to fat body

To study cholesterol transfer from [³H]-C-HDLp to fat body, 120 mg/ml of tissue, isolated from third to fourth day fifth instar larvae, was incubated in Grace's medium containing different concentrations of [³H]-C-HDLp. At the end of the incubation period (2 h), the fat body was extensively washed with Grace's medium and lysed in 1 ml of 0.1 N NaOH. Aliquots were used for radioactivity counting.

2.6. Effect of suramin and endocytic inhibitors on cholesterol transfer

To determine whether cholesterol transfer occurs via a receptor-mediated process, the effect of suramin, which has been shown to inhibit the fat body HDLp receptor (Tsuchida and Wells, 1990), was determined by preincubating the tissues (fat body or midgut) with 5 mM

suramin for 30 min, after which the tissues were washed with Grace's medium and then transfer studies were conducted as described above.

To study the effect of endocytic inhibitors on the transfer of cholesterol to and from HDLp, cholesterol-labeled midgut was incubated in insect Grace's medium containing HDLp (1.5 mg/ml) and either ammonium chloride (10 mM) or chloroquine (0.1 mM). Similarly, transfer of cholesterol was determined by incubating the fat body in Grace's medium containing [³H]-C-HDLp and the corresponding endocytic inhibitors. Control studies were carried out in the absence of the endocytic inhibitors. After 2 h, midgut and fat body tissues were removed from the medium, washed and processed as described above.

2.7. Effect of anti-LTP antibody on cholesterol transfer

Antiserum against purified LTP was obtained from a New Zealand white rabbit as described by Ryan et al. (1988). The IgG fraction was then purified using Affigel Protein A and stored at -80 °C. To elucidate the role of LTP in the transfer of cholesterol from midgut to HDLp, labeled-midguts were preincubated with anti-LTP antibodies (2 mg IgG/ml) for 30 min, washed extensively, then incubated in the presence or absence of HDLp (1.5 mg/ml) and transfer studies were conducted for 2 h. The effect of anti-LTP antibodies on the transfer of cholesterol from [³H]-C-HDLp (1.5 mg/ml) to fat body of *M. sexta* larvae were also determined following the same procedure.

2.8. Effect of EDTA on cholesterol transfer

To determine the effects of EDTA on cholesterol transfer from [³H]-cholesterol-labeled midgut to HDLp or from [³H]-C-HDLp to fat body, tissues were incubated with 1.5 mg/ml of HDLp in the presence or absence of 10 mM EDTA. The amount of radioactivity transferred was measured as described above.

2.9. Treatment of labeled-fat body with cholesterol oxidase

In order to measure the amount of cholesterol in the outer leaflet of the plasma membrane, transfer studies were carried out by incubating the fat body in Grace's medium containing 1.5 mg/ml [³H]-C-HDLp. After 2 h, the tissues were washed three times with Grace's medium. One set of fat body was incubated in 1 ml of lepidopteran saline containing 200 µg BSA and 4 units of cholesterol oxidase at 34 °C, a procedure modified from that of Fukasawa et al. (2000); the other set was incubated with only 200 µg/ml BSA (control). After 30 min tissues were washed extensively with the same

buffer, homogenized in 1 ml NaOH, and 100 µl were used for radioactivity determination. Another 100 µl of the homogenized fat body and a 200 µl aliquot of the incubation media containing HDLp were extracted with methanol:chloroform (1:2) (Folch et al., 1957). Solvents were evaporated under nitrogen, lipids were redissolved in hexane and fractionated by TLC using hexane:diethyl ether:acetic acid—120:20:1 (Jouni et al., 2002b). Spots corresponding to cholesterol and cholestenone were assayed for radioactivity by liquid scintillation counting.

2.10. Other assays

Protein concentrations were determined using the modified Lowry method with bovine serum albumin as a standard (Markwell et al., 1978). For statistical analysis, student's unpaired *t*-tests were used to determine the significance of differences between means.

2.11. Analysis of data

Transfer studies are reported as cpm of [³H]-cholesterol transferred to HDLp and fat body, or are calculated as the percentage of initial [³H]-cholesterol in the midgut or [³H]-C-HDLp added to the fat body. For the concentration dependence transfer studies, data were fit to a nonlinear regression equation (Graph Pad Software, Inc.).

3. Results and discussion

Transport of cholesterol from sites of absorption to sites of storage/utilization is important to the insects, because insects are unable to synthesize cholesterol and require a dietary sterol source. We have shown recently that the midgut is the major site of cholesterol absorption and release into the hemolymph and that HDLp is the sole cholesterol carrier-protein in hemolymph. (Jouni et al., 2002c). In this paper, we have investigated possible mechanisms by which cholesterol is transported from midgut to fat body of feeding *M. sexta* larvae. In planning these experiments, we were influenced by recent studies showing that diacylglycerol transport is both receptor- and LTP-mediated in both the midgut (Canavoso and Wells, 2001) and fat body (Canavoso et al., 2002). However, as we show, the process of cholesterol transport is fundamentally different than diacylglycerol transport.

3.1. Transfer of cholesterol from midgut to HDLp and HDLp to fat body

Fig. 1 shows the transfer of cholesterol from midgut to HDLp (A) and from HDLp to fat body (B). In both cases, cholesterol transfer showed a hyperbolic depen-

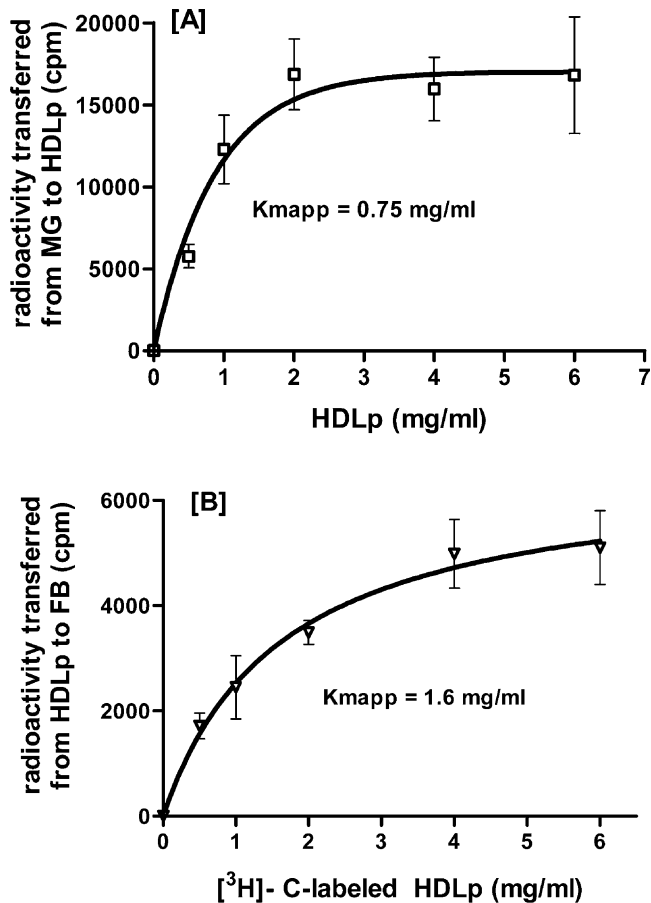


Fig. 1. Concentration dependence of cholesterol transfer. (A) [³H]-Cholesterol-labeled midgut was incubated with increasing concentrations of HDLp ranging from 0.5 to 6 mg/ml. Cholesterol transferred to HDLp was determined at the end of the incubation time (2 h). Data are reported as cpm of radioactivity recovered in HDLp. (B) Fat body was incubated with increasing concentrations of [³H]-C-HDLp (0.5–6 mg/ml). At the end of the incubation time of 2 h, tissues were washed, and radioactivity was determined. Data are reported as cpm of radioactivity recovered in fat body. Values represent averages of three to four determinations \pm SEM.

dence on HDLp concentration. The observed saturation processes for the release of cholesterol from midgut to HDLp and its uptake by fat body would be consistent with either of the following processes: (1) the binding of HDLp to a specific receptor on plasma membranes with subsequent transfer of cholesterol to HDLp from midgut and from HDLp to fat body; and/or (2) a process in which transfer of cholesterol takes place via a simple aqueous diffusion mechanism (Davidson et al., 1995). This aqueous diffusion process from the midgut cell membrane to HDLp or from HDLp to the fat body cell membrane does not require HDLp binding to the membrane. The apparent K_m ($0.75 \pm 0.3 \text{ mg/ml}$) derived from the kinetic experiments with midgut is about 10-fold higher than the K_d determined for the dissociation of the HDLp-midgut receptor complex (Gondim and Wells, 2000). Similarly, the apparent K_m ($1.6 \pm 0.2 \text{ mg/ml}$)

derived from the kinetic experiments with the fat body is nearly 100-fold higher than the K_d for the HDLp fat body-receptor complex (Tsuchida and Wells, 1990). Because the involvement of a receptor-mediated transfer process was not supported by the data in Fig. 1 several other experiments were conducted.

3.2. Effect of anti-LTP, suramin, endocytic inhibitors and EDTA on cholesterol transfer from midgut to HDLp

Treatment of midgut with suramin (Fig. 2A), or with the two endocytosis inhibitors; ammonium chloride and chloroquine (Fig. 3A) did not significantly inhibit the transfer of cholesterol from midgut to lipophorin. Although LTP plays a central role in the transfer of DAG

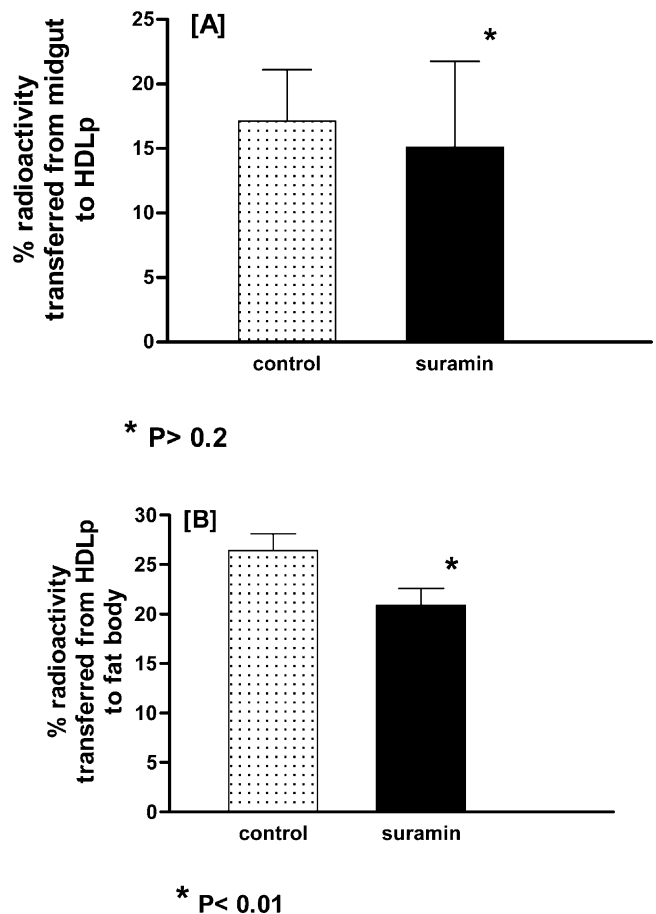


Fig. 2. Effect of suramin on cholesterol transfer. (A) [³H]-Cholesterol-labeled midgut was preincubated for 30 min with 5 mM suramin, extensively washed then transfer studies were carried out in the presence of 1.5 mg/ml HDLp. (B) Fat body was preincubated with 5 mM suramin for 30 min, extensively washed then transferred to Grace's medium containing 1.5 mg/ml [³H]-C-HDLp for 2 h. Transfer studies were carried out as described in Section 2. Values represent averages \pm SEM of three to four determinations. Suramin significantly inhibited cholesterol transfer from HDLp to fat body by 25% ($P < 0.01$), but did not significantly inhibit cholesterol transfer from midgut to HDLp.

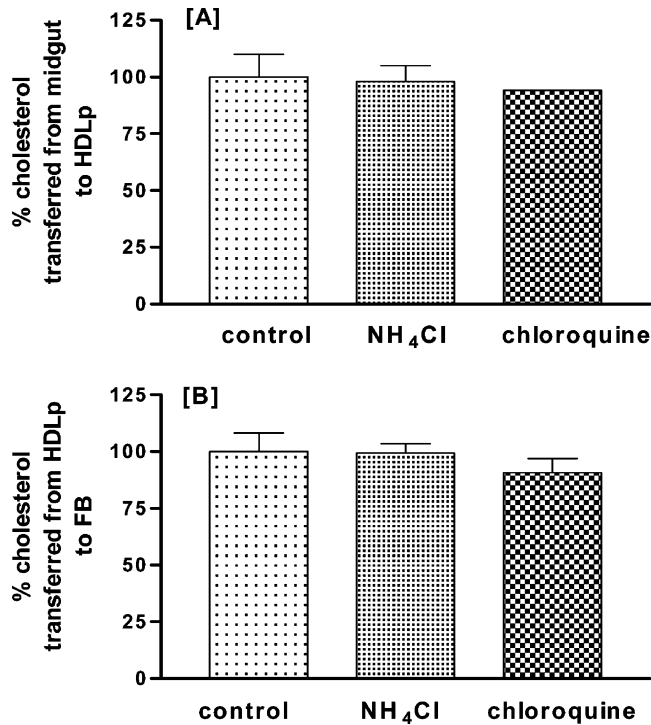


Fig. 3. Effect of endocytic inhibitors on cholesterol transfer. (A) [³H]-Cholesterol labeled midgut was incubated with lipophorin (1.5 mg/ml) in the presence of two endocytic inhibitors; ammonium chloride (10 mM) or chloroquine (0.1 mM) for 30 min. Transfer studies were carried out as described in Section 2. (B) [³H]-C-HDLp was incubated with larval fat body was in the presence of ammonium chloride or chloroquine for 30 min and transfer studies were carried out as described in the Section 2. For control studies, transfer studies were determined in the absence of endocytic inhibitors. Values represent averages±SEM of three to four determinations.

from midgut to HDLp (Canavoso and Wells, 2001), pretreatment of the midgut with anti-LTP IgG (Fig. 4A) exerted no significant inhibitory effect on the transfer of cholesterol from midgut. Similarly, EDTA (Fig. 5A) did not significantly inhibit the transfer of cholesterol from midgut to lipophorin. These data, coupled with the high apparent *K_m* value are consistent with the suggestion that cholesterol transfer from midgut to HDLp proceeds by an aqueous diffusion pathway. The same conclusion was reached for the transfer of cholesterol from fat body to HDLp (Jouni et al., 2002b).

3.3. Effect of anti-LTP, suramin, endocytic inhibitors and EDTA on cholesterol transfer from HDLp to fat body

LTP also plays a critical role in the transfer of DAG from HDLp to fat body (Canavoso et al., 2002). Treatment of fat body with suramin (Fig. 2B), anti-LTP IgG (Fig. 4B) or EDTA (Fig. 5B) did lead to significant inhibition of the transfer of cholesterol from HDLp and to fat body by 20, 27 and 48%, respectively. In addition, ammonium chloride and chloroquine exerted no inhibi-

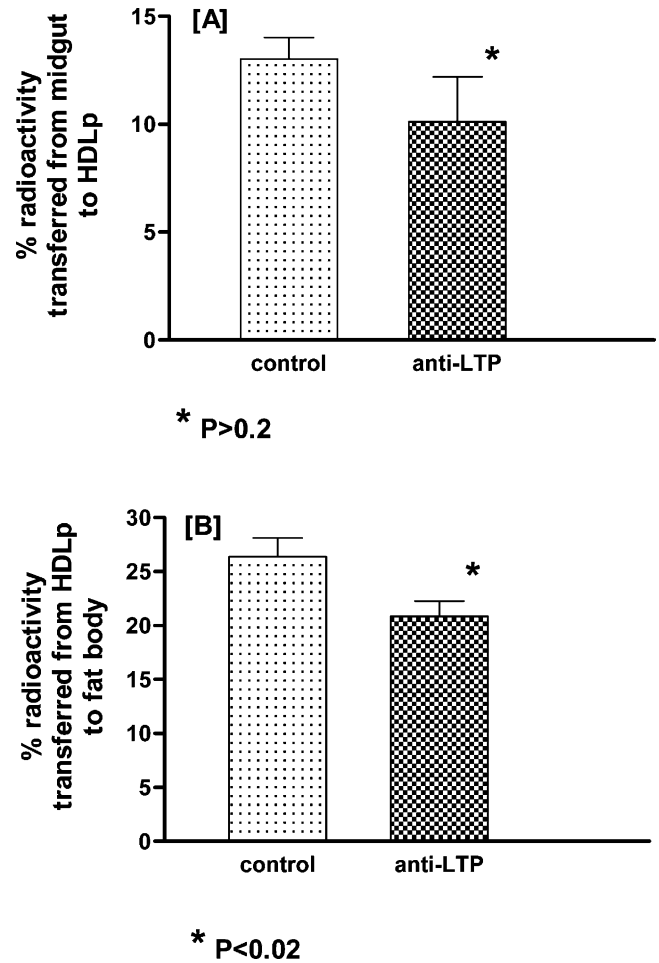


Fig. 4. Effect of anti-LTP antibody on cholesterol transfer. (A) [³H]-Cholesterol-labeled midgut was preincubated for 30 min with 2 mg/ml anti-LTP-IgG, extensively washed, and then transfer studies were determined in the presence of 1.5 mg/ml HDLp. (B) Fat body was preincubated with 2 mg/ml anti-LTP-IgG for 30 min, extensively washed, and then transferred to Grace's medium containing 1.5 mg/ml [³H]-C-HDLp for 2 h. Transfer studies were carried out as described in Section 2. Values represent averages±SEM of three to four determinations. Anti-LTP antibody significantly inhibited cholesterol transfer from HDLp to fat body by 20% ($P < 0.02$), but did not significantly inhibit cholesterol transfer from midgut to HDLp.

tory effect on the transfer of cholesterol to the fat body (<5%; Fig. 3B). While these results could be consistent with some involvement of the HDLp receptor and LTP in the transfer of cholesterol from HDLp to fat body, it seems likely that this is a minor pathway. Not only are the effects of the two most important inhibitors, suramin and anti-LTP antibody small, but the apparent *K_m* is two orders of magnitude higher than the measured *K_d* value for the HDLp–receptor complex.

Vertebrate cells use an endocytotic mechanism to acquire lipoprotein cholesterol, but the endocytotic inhibitors ammonium chloride and chloroquine did not inhibit cholesterol transfer from the midgut to HDLp or from HDLp to fat body, thus eliminating this mechanism. The aqueous diffusion of cholesterol from lipo-

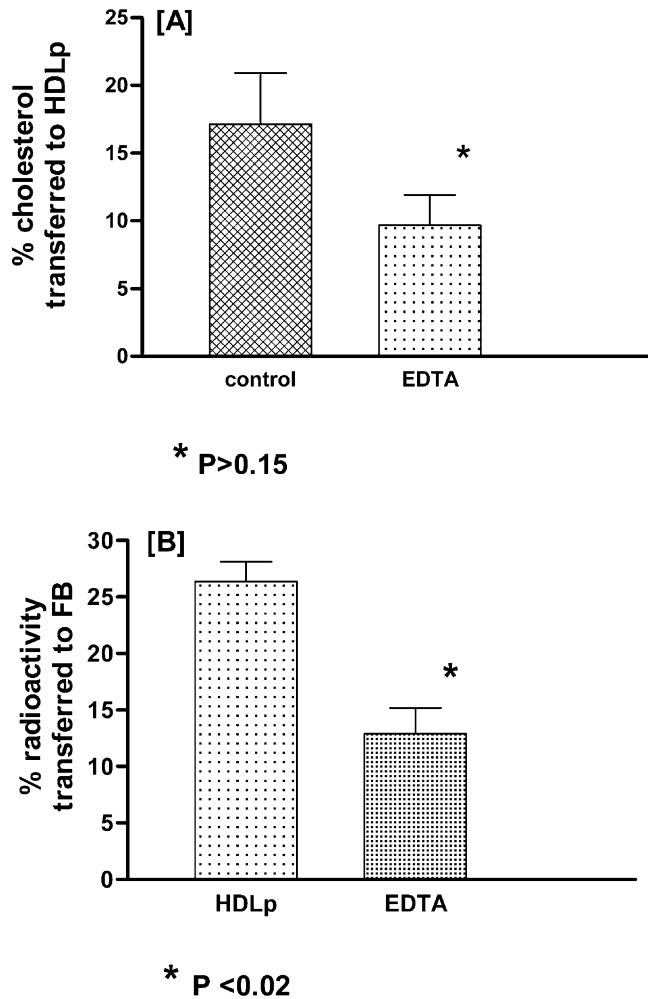


Fig. 5. Effect of EDTA on cholesterol transfer. (A) [^3H]-Cholesterol-labeled midgut with or without (control) 10 mM EDTA were incubated with 1.5 mg/ml of HDLp. (B) Fat body was incubated in Grace's medium containing 1.5 mg/ml [^3H]-C-HDLp with or without (control) EDTA. Transfer studies were carried out as described in the Section 2. Values represent averages \pm SEM of three to four determinations. EDTA significantly inhibited cholesterol transfer from HDLp to fat body by 48% ($P < 0.02$), but did not significantly inhibit cholesterol transfer from midgut to HDLp.

proteins to cells has not received much attention, but one might expect it to behave similarly to the reverse reaction (Rothblat et al., 1999). The ability of cholesterol to desorb from a membrane is strongly influenced by the properties of the membrane and it is reasonable to assume that addition of cholesterol to a membrane, as occurs in the transfer of cholesterol from HDLp to fat body, would also be sensitive to membrane properties. We know that both suramin and anti-LTP will bind to the fat body cell membrane because they inhibit DAG transfer from HDLp to fat body. If the presence of a charged molecule (suramin) or a bulky antibody affected the properties of the membrane, then their small effect on cholesterol transfer could be explained. Likewise, EDTA may extract Ca^{+2} from the membrane, altering its

charge properties and the ability of cholesterol to be added to the membrane.

3.4. Cholesterol transferred from HDLp to fat body resides in the external leaflet of the membrane

Cholesterol oxidase catalyzes the conversion of cholesterol to cholestenone and has been used to study the distribution and transport of cholesterol in many systems. Under physiological conditions, the enzyme is able to act on cholesterol only in the outer leaflet of the plasma membrane (Johnson et al., 1991). After fat body was incubated with [^3H]-C-HDLp for 2 h and extensively washed, one sample was incubated with cholesterol oxidase for 30 min and the control was not. Cholesterol oxidase treatment did not cause any loss of radioactivity that was already transferred to fat body (data not shown). The enzyme oxidized about 25% of the total radioactive cholesterol transferred to fat body (Fig. 6), showing that a significant amount of the transferred cholesterol still remained in the outer leaflet of the membrane, even though most of the transferred cholesterol had moved to an inaccessible site, presumably inside the cell. In addition, longer incubations (1 h) with cholesterol oxidase did not significantly affect the amount of cholesterol converted to cholestenone. These data demonstrate the presence of more than one cholesterol pool in the fat body, and show that cholesterol in both pools originated from HDLp. The possibility of non-specific binding of cholesterol-labeled HDLp to the fat body membrane, which is accessible to the action of cholesterol oxidase, cannot be ruled out at this time. However, recent work from our laboratory has demonstrated that 2-hydroxypropyl- β -cyclodextrin and methyl- β -cyclodextrin promote cholesterol efflux from two pools of larval *M. sexta* fat body (Jouni et al., 2002a).

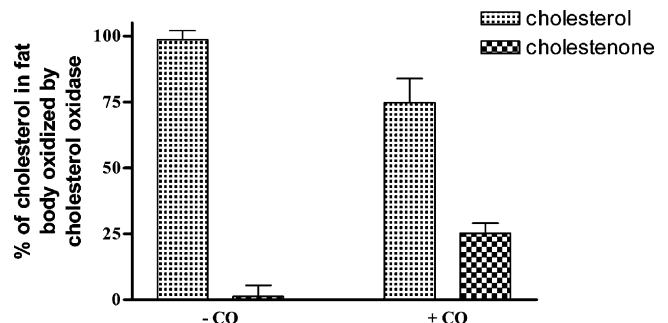


Fig. 6. Transferred cholesterol remains in the outer leaflet of the fat body membrane. Transfer studies were carried out by incubating fat body in Grace's medium containing 1.5 mg/ml [^3H]-C-HDLp. After 2 h, tissues were washed with Grace's medium. One set of tissues was treated with 4 units of cholesterol oxidase for 30 min in a buffer containing 200 $\mu\text{g}/\text{ml}$ BSA; the other set was incubated with only 200 $\mu\text{g}/\text{ml}$ BSA (control) for 30 min. Lipids were extracted from both samples and fractionated on TLC and the spots corresponding to cholesterol and cholestenone were counted. Values represent averages \pm SEM of three to four determinations.

The two cholesterol pools are kinetically distinct and are in continuous communication (Jouni et al., 2002a). Even if we assume that the cholesterol oxidase accessible pool represents a low-affinity/non-specific binding of HDLp to the fat body and subtract its value from the observed transfer measurements, no relative changes to the calculated data would be observed.

3.5. HDLp transfers cholesterol to fat body preferentially

Fig. 7 shows the amount of radioactive cholesterol transferred from [³H]-C-HDLp to either fat body or midgut when 120 mg of either tissue was incubated with 1.5 mg/ml of [³H]-C-HDLp. The fat body took up about 20-fold more cholesterol than did the midgut ($P < 0.001$). In the case of DAG transfer, it was also shown that HDLp did not transfer appreciable amount of DAG to the midgut (Canavoso and Wells, 2001). Thus, although cholesterol moves freely from midgut to HDLp, it appears that there is some type of barrier to the movement of cholesterol from HDLp back to midgut. This is in contrast to the situation in fat body where cholesterol moves freely in both directions. Perhaps the cholesterol content of the basolateral membrane is relatively high, which would favor cholesterol export from the midgut and inhibit cholesterol import into the midgut.

3.6. Model for cholesterol transport in larval hemolymph

The preponderance of data presented in this paper support the suggestion that cholesterol transfer from midgut to HDLp and from HDLp to fat body occurs by an aqueous diffusion mechanism and that there seems to be little, if any, significant contribution by pathways using the HDLp receptor and/or LTP. These results are in stark contrast to the results of studies on DAG transfer (Canavoso and Wells, 2001; Canavoso et al., 2002), where both transfer processes are mediated by LTP. The clear implication of these results is that cholesterol transfer throughout the caterpillar is essentially accomplished by a mass action mechanism. If a tissue requires cholesterol, it would be transferred from HDLp consequently the midgut or the fat body would replenish HDLp cholesterol.

In addition to the data presented here, the kinetics of cholesterol clearance from the hemolymph are consistent with such a mass action model (Jouni et al., 2002c). In that work it was shown that feeding a single bolus of radioactive cholesterol to larvae resulted in labeling of the hemolymph and that the label was cleared from the hemolymph with a half-life of several days. In contrast, when radioactive fatty acid was fed to larvae it was cleared from the hemolymph with a half-life of 2–3 h (Tsuchida and Wells, 1988; Canavoso and Wells, 2000).

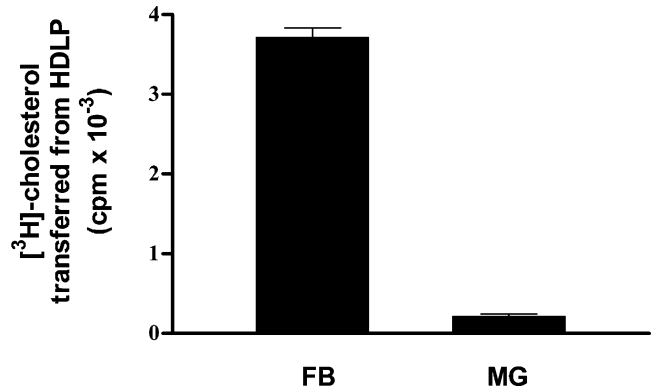


Fig. 7. Cholesterol transfer from [³H]-C-HDLp to fat body or midgut. Fat body and midgut (120 mg each) of third day fifth instar larvae were incubated in Grace's medium containing 1.5 mg/ml [³H]-C-HDLp. After 2 h, the tissues were washed, homogenized and samples were used to measure the radioactivity transferred from HDLp. Values represent averages ± SEM for three determinations. Significantly more cholesterol was transferred from HDLp to fat body than from HDLp to midgut ($P < 0.01$).

The fact that the amount of radioactive cholesterol in the hemolymph remains more or less constant for such a long period of time would be consistent with a mass action model of cholesterol transfer.

Such a model would allow the insect to satisfy all its tissues needs for cholesterol in the absence of either de novo synthesis or an endocytotic uptake mechanism, which are the main mechanisms that vertebrates use to control cell cholesterol content. At some stages cholesterol does seem to be stored in fat body as cholesterol ester (Jouni et al., 2002c), but this must be in equilibrium with free cholesterol in the cell and hence with hemolymph cholesterol. More work will be required to prove this mechanism, but, if true, it is a simple and elegant solution to the problem of how to move cholesterol around the body and takes advantage of cholesterol's ability to move between lipoproteins and cell membranes by aqueous diffusion.

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