Rapid method for determining cholesteryl ester transitions of apoB-containing lipoproteins

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Summary A wide variety of cholesteryl ester-rich apoB-containing lipoproteins undergo an order-disorder transition in the cholesteryl ester core at approximately normal body temperature. The transition occurs over several °C with the mid-point being as high as 57°C in some cholesterol-fed animals. The transition mid-point of normal human low density lipoprotein (LDL) appears to vary from as low as 26°C to about body temperature. However, to screen a large population of patients at risk for atherosclerotic cardiovascular disease (ACD), a rapid method for determining the transition temperature of LDL is needed. Since apoB-containing lipoproteins (VLDL and LDL) are readily precipitated from plasma by dextran sulfate and magnesium sulfate, we have studied the thermal properties of this precipitate using differential scanning calorimetry (DSC). The VLDL-LDL precipitate undergoes a reversible thermal transition similar in transition temperature and enthalpy to the cholesteryl ester transition of isolated pure LDL. The transition is seen with the precipitate from VLDL-free plasma, but no transition is seen when VLDL and LDL have been removed. Cholesteryl ester-rich apoB containing lipoproteins were isolated from a variety of sources (man, cholesterol-fed monkeys, and rabbits) and their transition temperatures compared with the apoB-containing lipoprotein precipitates from the same source. The mid-point of individual transitions varied over a wide range (17–57°C) and the correlation between the pure lipoprotein and the plasma precipitate was strong ($r = 0.98$, $P < 0.001$). Thus, DSC of the plasma apoB precipitate may be used as a rapid method of determining the cholesteryl ester transition of LDL and other apoB-containing lipoproteins.—**Waugh, D. A., and D. M. Small.** Rapid method for determining cholesteryl ester transitions of apoB-containing lipoproteins. *J. Lipid Res.* 1982. **23:** 201–204.

Supplementary key words differential scanning calorimetry • VLDL • LDL • dextran sulfate-Mg^{2+} precipitation of apoB-containing lipoproteins • lipid transition • cholesteryl esters • lipoprotein transitions

Serum low density lipoproteins are the major carriers of cholesterol in human plasma. Most of the cholesterol is esterified and resides in the core of the particle with the other neutral lipid, triglyceride (1). When isolated LDL is heated from 0°C to 45°C it undergoes a reversible endothermic transition which has been ascribed to an order → disorder phase transition of the cholesteryl ester (CE) (2). X-ray diffraction studies suggest that the core cholesteryl ester below the transition is organized in a smectic arrangement, whereas above the transition it is in a more disordered state (3).

Recent experiments in subhuman primates have shown strong positive correlations between the severity of atherosclerosis, LDL molecular weight, and CE transition temperature of LDL (4). Therefore, the transition temperature may be physiologically important. If it were above 37°C, the cholesteryl ester of the LDL would be in a more ordered state at body temperature, which could effect its metabolism.

In normal humans and Type II patients fed polyunsaturated diets, the mean transition of isolated LDL temperature was 30.3 ± 2.3°C. However, some subjects had transitions as low as 26°C while others were as high as 38°C (1, 2). Thus, some subjects and perhaps patients at risk for ACD may have transition temperatures above body temperature. Therefore, we wished to screen a large number of normal subjects and high risk patients for the LDL transition temperature. Since determination of LDL transition requires tedious procedures to isolate and purify intact LDL and concentrate it for calorimetry, we tested the hypothesis that apoB-containing lipoproteins, rapidly precipitated from plasma as polyanion-metal complexes (5), would show the LDL transition. Thus, we have compared the thermal properties of the pellet formed when the apoB-containing lipoproteins are precipitated from plasma by dextran sulfate and magnesium sulfate (6) with ultracentrifugally isolated LDL from the same source. We have chosen a variety of LDL or cholesteryl ester-rich lipoproteins from both human and animal sources so that the peak transition temperature ranged from 17–57°C. We find the precipitate undergoes a reversible CE transition which closely resembles that for LDL in temperature and enthalpy and believe that this technique may be used to screen large populations for LDL transition temperatures.

METHODS

Plasma was obtained from five fasted healthy young men on a normal American diet, one woman on a high salmon oil diet,[1] nine non-human primates (*Cercopithecus aethiops*, n = 6, *Macaca fascicularis*, n = 3) fed either monkey chow or a high cholesterol diet, and three rabbits fed a high cholesterol diet. (The non-human primate samples were kindly supplied by Dr. L. Rudel). In humans and rabbits, lipoproteins were separated by sequential ultracentrifugation (7) (VLDL was isolated at d 1.006 g/ml and LDL in density range 1.006–1.063 g/ml). Fractions were dialyzed where necessary to remove KBr and were concentrated by vacuum dialysis in

Abbreviations: LDL, low density lipoprotein; VLDL, very low density lipoprotein; DSC, differential scanning calorimetry; CE, cholesteryl ester; ACD, atherosclerotic cardiovascular disease.

[1] This plasma was kindly supplied by Dr. W. Connor.

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preparation for calorimetry as described (2). Lipoproteins from non-human primates were isolated by ultracentrifugation and agarose column chromatography as described (4).

ApoB-containing lipoproteins were precipitated from plasma using dextran sulfate and magnesium sulfate (6). Fifty μl of dextran sulfate solution (20 g/l), and 100 μl of 1.1 M magnesium sulfate were added to 1 ml of plasma. After mixing and centrifuging at 1000 rpm for 10 min, the supernatant was removed and the pellet was transferred to a 75-μl DSC pan (Perkin-Elmer, Norwalk, CT) with a long Pasteur pipet. The same combination of reagents was added to 1 ml of subnatant after VLDL or VLDL and LDL had been removed by ultracentrifugation at 1.006 or 1.063 g/ml, respectively. A precipitate occurred with the former, but not after both apoB-containing lipoproteins had been removed.

Calorimetric studies were performed on a Perkin-Elmer DSC-2 differential scanning calorimeter at a full range sensitivity of 0.2 M Cal/sec. Samples were hermetically sealed in stainless steel DSC pans (Perkin-Elmer, Norwalk, CT) and heated at 5°/min. A variety of heating and cooling runs were performed between 0°–45° and 0°–100°C and enthalpies (ΔH) were calculated from the areas under the peaks as measured by planimetry, and compared to an indium standard as described previously (1, 2).

To express ΔH in terms of cholesteryl ester content, following calorimetry DSC pans were opened and the lipids were dissolved in at least 40 volumes of chloroform–methanol 2:1 (v:v) and extracted by the procedure of Folch, Lees, and Sloane Stanley (8). The cholesteryl ester composition was determined by thin-layer chromatography (9). Repeated runs on the same LDL sample gave a variation in peak temperature of less than 0.3°C.

RESULTS

Fig. 1 shows representative heating and cooling curves of intact LDL and precipitate from the same plasma sample. LDL undergoes a reversible transition over a range of about 12°C. Qualitatively similar behavior was seen with the precipitate, including a small degree of undercooling when cooling from 45°C to 0°C. The width and shape of the transition of pure lipoprotein and precipitate was similar. The mean enthalpy of transition of the precipitate was 0.68 ± 0.07 cal/g CE (mean ± standard deviation) which is not different from the enthalpy found by us earlier for LDL (0.69 ± 0.06 cal/g CE) (2). On heating the precipitate to 100°C, an irreversible transition occurs. After cooling to 0°C and reheating, a reversible transition occurs between 20°C and 37°C that has a larger enthalpy (0.85 ± 0.04 cal/gm CE), similar to that described previously (1, 2).

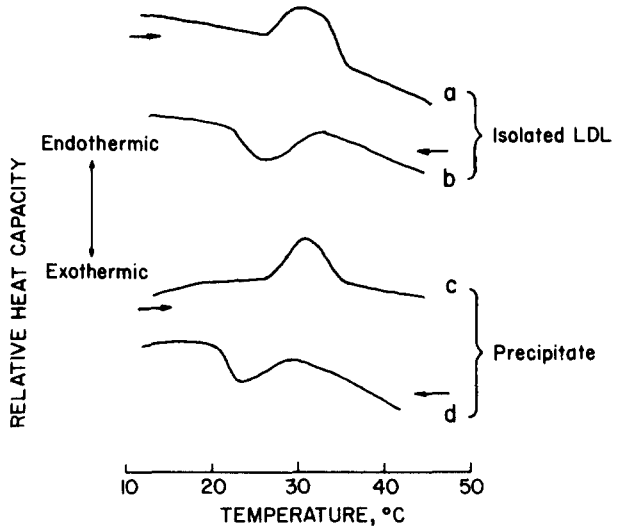

Fig. 1. Differential scanning calorimetry curves of solutions of pure LDL and whole plasma precipitates from the same subject. a), Heating curve LDL from 0° to 45°C; b), cooling of LDL from 45°C to 0°C; c), heating of whole plasma precipitate 0°–45°C; and d), cooling whole plasma precipitate 45°–0°C.

To identify the source of the transition, precipitates were prepared from whole plasma, VLDL-free plasma, and VLDL, LDL-free plasma. **Fig. 2** shows that a comparison of heating curves obtained from LDL, precipitate from whole plasma, and precipitate from the VLDL-free plasma are similar. When dextran sulfate and magnesium sulfate are added to apoB lipoprotein-deficient plasma, no precipitate is formed and no transition occurs in this precipitate-free mixture.

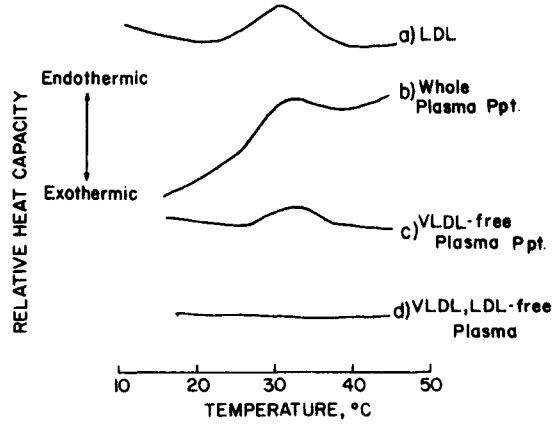

Fig. 2. Differential scanning calorimetry curves of intact LDL, and plasma precipitates, from the same subject. a), Intact LDL; b), whole plasma precipitate; c), VLDL-free plasma precipitate; and d), apoB lipoprotein-free plasma plus dextran and magnesium sulfate (no precipitate formed).

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By using plasma from several sources, we were able to obtain a wide range of lipoprotein transition temperatures. LDL from the human subjects had transition temperatures between 17.7°C and 31.0°C and in cholesterol-fed monkeys between 41.5°C and 57.0°C. Rabbits fed a high cholesterol diet develop hypercholesterolemia and a cholesteryl ester-enriched VLDL (β VLDL) that undergoes thermotropic transitions (11). Since β VLDL is an apoB-containing lipoprotein, it precipitates with dextran sulfate and magnesium sulfate. The isolated rabbit VLDL had transition temperatures at or slightly above body temperature and were identical to those for the precipitate formed from the same plasma.

When the lipoprotein transition temperatures were plotted against precipitate transitions from the same subject a very strong correlation was found ($r = 0.98$, $P < 0.001$). The line of best fit lies very close to the line of identity (**Fig. 3**).

DISCUSSION

Low density lipoprotein undergoes a reversible thermotropic transition at approximately body temperature and an irreversible transition at high temperatures. We have previously shown that the lower transition is due to a reorganization of the cholesteryl ester within the core of the particle and the high transition due to particle disruption (1–3). The pellet of precipitated apoB lipoproteins (VLDL and LDL) appear to undergo very similar transitions. Since normal human VLDL does not undergo any thermal transitions between 10 and 50°C (10), the transition seen in the precipitate appears to arise from the precipitated LDL. Several other lines of evidence support this. First, there is a strong correlation between LDL transition and precipitate transition temperatures, including high melting LDL of cholesterol-fed monkeys. Second, when VLDL is removed from plasma, the transition persists in the VLDL-free precipitate, but not when both VLDL and LDL are removed. Finally, the enthalpy of transition of the precipitate is the same as that of LDL reported in the literature both before and after denaturation (2). Thus, the transition seen in the precipitate most probably arises from LDL.

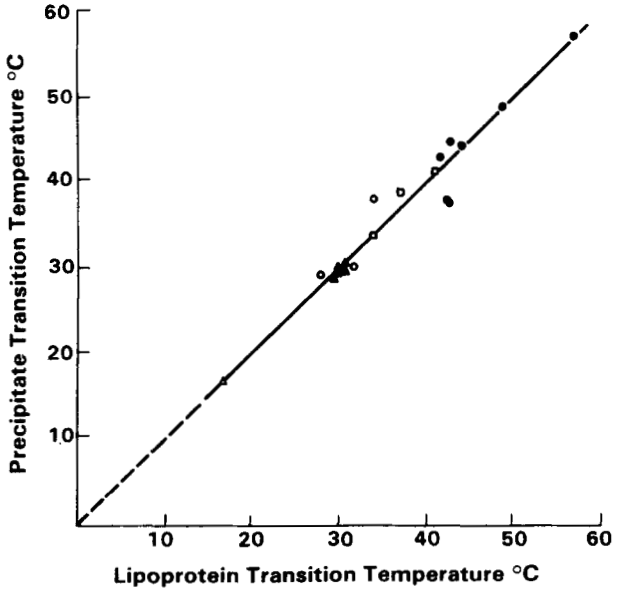

Fig. 3. Lipoprotein transition temperatures plotted against plasma apoB lipoprotein precipitate transition temperatures. $r = 0.98$; $P < 0.001$. ●, Cholesterol-fed monkeys; ○, chow-fed monkeys; △, humans; and □, cholesterol-fed rabbits.

The main advantages of using the precipitate to identify the LDL transition are the ease and speed with which the determination can be made. Previously, several days were required to isolate and concentrate the LDL sample before calorimetric experiments could be performed. Using the precipitation method, isolation and concentration occur simultaneously and the total determination takes less than 1 hr. Thus, it should prove a valuable tool for screening large numbers of subjects. It should be noted that rabbits fed a high cholesterol diet carry almost all their plasma cholesterol in cholesteryl ester-rich β-VLDL (11). This lipoprotein contains apoB and is thus precipitable by dextran sulfate and magnesium sulfate. The precipitate transitions are identical to those for the isolated β-VLDL and thus probably reflect β-VLDL transitions. The technique, therefore, would have application for determining transition temperatures of any apoB-containing lipoprotein that normally undergoes CE transitions.

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