

## Homeostasis as Regulated by Activated Macrophage. VII. Suppression of Serum Cholesterol Level by LPSw (a Lipopolysaccharide from Wheat Flour) in WHHL (Watanabe Heritable Hyperlipidemic) Rabbit

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The effect of LPSw (a lipopolysaccharide from wheat flour) on cholesterol catabolism was examined using WHHL (Watanabe heritable hyperlipidemic) rabbit, which is an experimental model of familial hyperlipidemia. The serum cholesterol level of the animal decreased by the addition of LPSw to drinking water. Following cessation of the addition of LPSw to the drinking water, the cholesterol level was decreased for 30 to 40 d and then gradually elevated. The serum level of apolipoprotein B, which is a constituent of apolipoprotein of low density lipoprotein (LDL), also decreased in accord with serum cholesterol at a nearly coincident rate. Conversely, the level of apolipoprotein A-I, which is a constituent of apolipoprotein of high density lipoprotein (HDL), did not change, nor did HDL-cholesterol. Furthermore, the atherosclerosis risk factor, expressed as the ratio of apolipoprotein B to apolipoprotein A-I, was decreased by LPSw administration.

**Keywords** lipopolysaccharide (LPS); hyperlipidemia; apolipoprotein; macrophage

### Introduction

Elevation of serum cholesterol levels is widely recognized as a major risk factor for the development of atherosclerosis. Familial hyperlipidemia is an intractable disease which is accompanied by a deficiency of low density lipoprotein (LDL) receptors resulting in high plasma cholesterol levels. To date, hypocholesterolemic drugs such as synthesis inhibitors, absorption inhibitors and promoters of cholesterol excretion have been used in its therapy.<sup>1)</sup> However, their therapeutic effect is insufficient and a new drug for hyperlipidemia has therefore been urgently desired.

Two pathways of LDL catabolism are known, one is mediated by parenchymal cells through the LDL receptor and the other is mediated by scavenger cells.<sup>2)</sup> Recent studies have demonstrated an interrelationship between the inflammatory event and plasma lipid concentration.<sup>3-6)</sup> It was also reported that plasma lipid concentrations in normal monkeys and cholesterol-supplemented rabbits were decreased by administration of bacterial substances such as inducers of activated macrophage and cytokine.<sup>3-5)</sup> However, these substances cannot be used clinically, due to their severe side effects.

We considered that LDL catabolism could be augmented through macrophage activation as phagocytic cells in patients with familial hyperlipidemia. Following the screening of many BRMs (biological response modifiers) for induction of macrophage activation especially at the primed stage ready for endogenous production of tumor necrosis factor (TNF),<sup>7-10)</sup> we found that LPSw, a Limulus positive substance obtained from a water extract of wheat flour, induced activated macrophage by oral or parenteral administration.<sup>11-13)</sup> We then investigated the effect of LPSw on WHHL rabbit which lacks LDL receptors and is an experimental model of familial hyperlipidemia.<sup>14,15)</sup> Administration of LPSw in drinking water of Watanabe heritable hyperlipidemic (WHHL) rabbits decreased serum cholesterol and apolipoprotein B levels.

### Materials and Methods

**Animals** WHHL rabbits weighing about 4 kg were kindly provided by

Dr. Watanabe.

**Preparation of LPSw** Details of LPSw preparation were given in the first report of this series.<sup>12)</sup> A crude sample of LPSw was used in this experiment, and the amounts reported here are those showing Limulus positive reaction.

**Suppressive Effect of Serum Cholesterol Level by LPSw** Experimental design was a cross-over study involving LPSw administration followed by cessation. LPSw was dissolved in pyrogen-free distilled water (1 µg/ml, as estimated by Limulus reaction), passed through a 0.45 µm filter, and administered to WHHL rabbits in their drinking water. Animals drank approximately 500 ml/d and were fed a CR-3 solid diet (Clea, Japan) of about 130 g/d.

**Cholesterol Assay** Serum cholesterol levels were determined using a V-Cholesterol kit (Nissui Seiyaku, Japan) and an HDL-cholesterol kit (Daiichi Pure Chemicals, Japan).

**Apolipoprotein Assay** Serum apolipoprotein A-I and B level was measured by Apo A-I plate and Apo B plate (Daiichi Pure Chemicals, Japan). Risk factor was calculated as follows<sup>16)</sup>:

$$\text{risk factor} = \frac{\text{apolipoprotein B}}{\text{apolipoprotein A-I}}$$

### Results

**Effect of LPSw on Serum Cholesterol in WHHL Rabbit** LPSw was dissolved in drinking water at 1 µg/ml and was offered to WHHL rabbits *ad libitum* to study its effect on suppression of serum cholesterol levels. As shown in Fig. 1A, the serum cholesterol levels began to decrease from the second to seventh day after administration of the LPSw, and reached a minimum level on about the 30th day. Following cessation of LPSw addition, cholesterol levels remained lowered for 30 to 40 d and then gradually recovered. However, the serum HDL-cholesterol levels did not change (Fig. 1B). No change in rabbit body weight was observed during the experimental period (Fig. 1A).

**Decrease of Apolipoprotein B on Administration of LPSw** To study mechanisms for the effect of LPSw on serum LDL, the levels of apolipoprotein B, a constituent of the main LDL apolipoprotein, was measured throughout the period of LPSw administration. As shown in Fig. 2A, serum apolipoprotein B levels decreased in accord with serum cholesterol at a nearly parallel rate. The levels of apolipoprotein A-I, which is a constituent of HDL

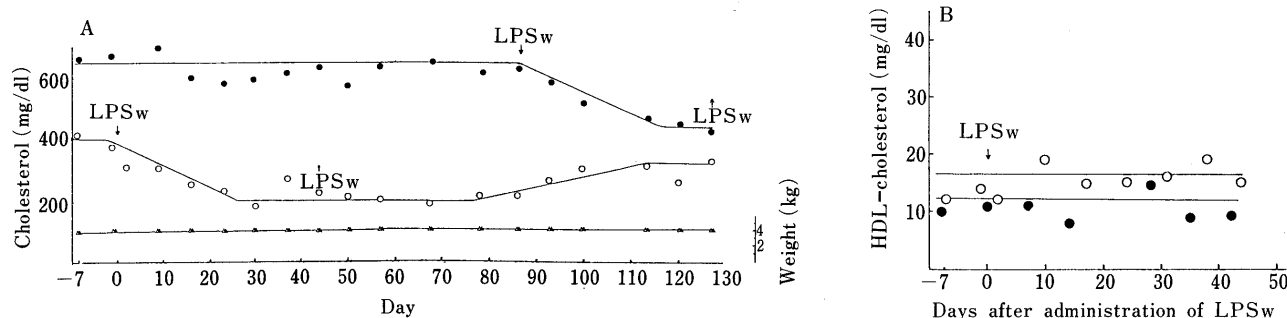


Fig. 1. Effect of LPSw on Cholesterol Level in Serum

LPSw in drinking water (1  $\mu$ g/ml, as estimated by Limulus reaction) was offered to two WHHL rabbits. The total cholesterol (A) and HDL-cholesterol (B) levels in serum were measured. Rabbits drank about 500 ml/d and were fed a CR-3 solid diet of about 130 g/d.  $\downarrow$ , start of addition of LPSw to drinking water;  $\uparrow$ , cessation of LPSw addition to drinking water.  $\circ$ — $\circ$ , Serum cholesterol;  $\triangle$ — $\triangle$ , weight of WHHL rabbit 1;  $\bullet$ — $\bullet$ , serum cholesterol;  $\blacktriangle$ — $\blacktriangle$ , weight of WHHL rabbit 2.

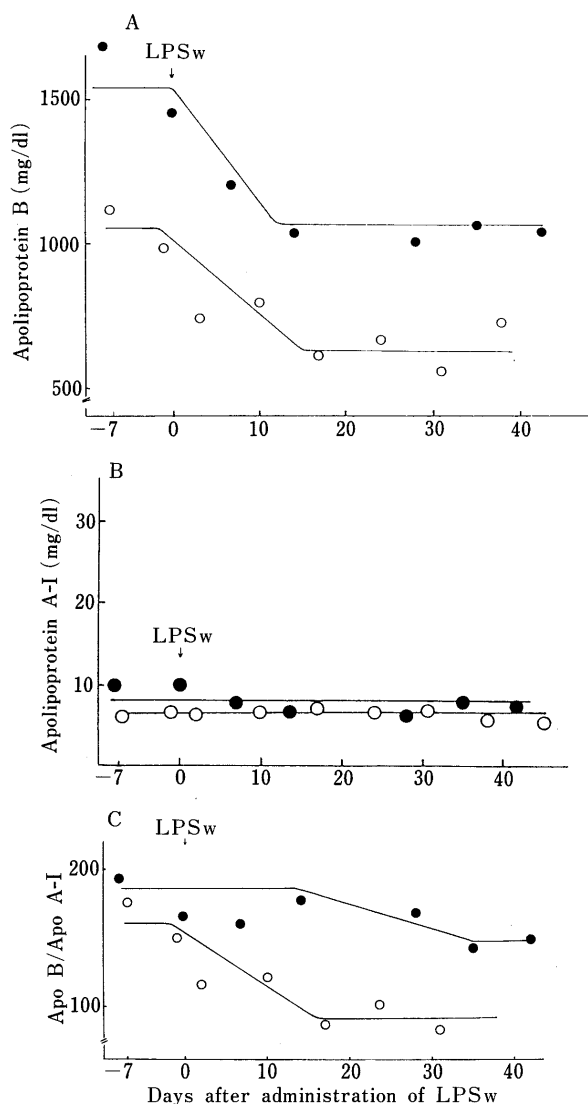


Fig. 2. Similarity of Decrease of Apolipoprotein in the Two WHHL Rabbits

LPSw in drinking water was offered to two WHHL rabbits and apolipoprotein B (A) and apolipoprotein A-I (B) in serum was measured. Risk factor of atherosclerosis which expresses ratio of Apo B/Apo A-I was calculated (C). For other details, see legend of Fig. 1.  $\downarrow$ , addition of LPSw to drinking water;  $\circ$ — $\circ$ , WHHL rabbit 1;  $\bullet$ — $\bullet$ , WHHL rabbit 2.

apolipoprotein, on the other hand, did not change, nor did HDL-cholesterol (Fig. 2B). The atherosclerosis risk factor which expresses the ratio of apolipoprotein B to

apolipoprotein A-I decreased remarkably (Fig. 2C).

### Discussion

LPSw suppresses elevated cholesterol levels in hyperlipidemic WHHL rabbits when offered *ad libitum* in drinking water at 1  $\mu$ g/ml. We found that total serum cholesterol levels began to decrease gradually from the 2nd to 7th day after LPSw administration and reached a minimum level by the 30th day (Fig. 1A); serum HDL-cholesterol levels did not change (Fig. 1B). Serum apolipoprotein B levels also decreased with serum cholesterol (Fig. 2). Furthermore the atherosclerosis risk factor which expresses the ratio of apolipoprotein B to apolipoprotein A-I decreased with administration of LPSw. These findings suggest that LDL-cholesterol level was reduced by the oral administration of LPSw.

It was recently reported that macrophage activating substance such as *E. coli* LPS and  $\beta$ -glucan induced suppression of serum cholesterol levels.<sup>3,17</sup> We reported that oral or parenterally administered LPSw induced activated macrophage ready for endogenous production of TNF.<sup>11,12</sup> Thus the decrease of serum cholesterol levels by LPSw may be attributable to augmentation of the phagocytic pathway in LDL catabolism through macrophage activation. It was also reported that injection of TNF in monkeys caused a reduction of serum cholesterol.<sup>4</sup> This suggests that TNF or precursor TNF in the primed macrophage<sup>18</sup> is probably involved in the reduction of cholesterol level during LPSw administration, because LPSw can induce the production of TNF or precursor TNF endogenously.<sup>11,12</sup> Macrophage activation, especially at the stage ready for TNF production (primed macrophages), may therefore have an important role in reversing hypercholesterolemia or hyperlipidemia.

LPSw is a structural component of wheat flour,<sup>11,12</sup> and Davidson *et al.* also reported the cholesterol-lowering effect of oatmeal and oat bran.<sup>17</sup> These findings suggest that a structural food component, especially plant lipopolysaccharide may have an important role in reversal of hyperlipidemia. As shown in our previous report,<sup>12</sup> those LPSs with a molecular size of less than 5 kDa should be biologically active even when administered by the oral or percutaneous route.<sup>19</sup>

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