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THE EFFECT OF EXPERIMENTAL HEMORRHAGIC SHOCK ON SERUM HDL-LEVEL IN RABBITS*)

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ABSTRACT

In order to study the changes in the levels of serum HDL (high density lipoprotein) by hypovolemic shock due to hemorrhage, blood was withdrawn from five female rabbits through a catheter inserted into an aortic arch via carotid artery. Three to five milliliters of blood intermittently withdrawn, which accerelated the hemorrhagic shock condition, were served for the lipid and lipoprotein analysis. During the experimental period, the portal blood flow and mean blood pressure of aortic arch were measured and Indocyamine green test (R15) performed frequently in the animals.

(1) The serum concentration of HDL-cholesterol and HDL-phospholipids (d.$>$1.063 which involves VHDL (very high density lipoprotein)-phospholipids) gradually decreased and the total and LDL (low density lipoprotein)+VLDL (very low density lipoprotein)-cholesterol and phospholipids showed slight decreases immediately after the commencement of experiment and kept almost constant thereafter.

(2) The magnitude of decrease in lipids was higher in HDL_2 than HDL_3 fraction, and VHDL-phospholipids were not affected by hemorrhagic shock.

(3) The lipid and protein compositcon of HDL_2 revealed a singnificant decrease in percent cholesterol and phospholipids accompanied by a relative increase in prrcent protein and triglycerides, and that of HDL_3 showed no change in the serum 270 minutes after shock.

(4) Substantially high elevation of catecholamines, temporal increase in free fatty acid and slight increase in serum triglycerides were observed during the experimental period.

The hemorrhagic shock seems to disturb the regulation of serum HDL levels and its constituents.

INTRODUCTION

HDL is found by many investigators to reduce in patients with atherosclerosis and to be involved closely in the development of this disease. HDL is also found to be influenced by and to fluctuate in accordance with many factors such as smoking[1], exercise[2], alcohol[3], diseases (hepatic diseases[4] and diabetes mellitus[5]) and drugs (hypotensive drug[6], anti-epileptic drug[7], estrogen[8], CPIB[9], etc.).

In addition, the serum HDL-level can be

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considered as regulated by the hepatic and intestinal blood flow, becuase the majority of HDL is synthesized in these organs.

In the present study, the serum HDL concentration was serially determined on the basis of the measurements of portal blood flow during the experimental hemorrhagic shock induced in rabbits, in order to evaluate the effect of sudden change in blood flow and concomittant additional factors on the serum HDL-levels.

METHODS

Five 3-6 month old female rabbits, weighing 2200-3000 gr, were fed with a standard rabbit chaw (Oriental Food Co., Japan) for at least one week, after which 7 ml of blood were withdrawn from an ear artery at fast and used as control serum (before hemorrhagic shock) for a lipoprotein analysis. Immediately after the intravenous injection of 40 mg/kg pentobarbital-sodium, a 25 mm I. D. vinyl catheter was inserted through a carotid artery into the aortic arch. Abdomen was opened and the terminal of electromagnetic blood flowmeter (Nihon Koden Kogyo Co., Ltd., Japan) was placed around the portal vein. The outer end of vinyl tube inserted into a carotid artery was connected with a Stetham pressure transducer to measure the mean blood pressure. Both the portal blood flow and mean blood pressure were recorded by Siemens-Elma Mingography.

Withdrawal of 3-5 ml blood was repeated five times for 270 minutes until the termination of the experiment. At the final blood withdrawal, additional blood of 10 ml was collected for comparison with 10 ml blood initially drawn by ultracentrifugal analysis of lipoprotein. Serum total cholesterol was determined by the method described by Allain et al.[10] The influence of free glycerine was avoided by separation of triglyceride fraction by thin layer chromatography, that is, 0.5 ml serum was added with 20 volume of Folich's fluid[11] (chloroform : methanol, 2 : 1, v/v), filtered through No. 1 Toyo filter paper and after adding water, shaken vigorously in the separation. funnel. After removal of the combined layer of water and methanol, the solvent was concentrated and developed on a Kiesel gel thin layer chromatograph (Solvent system n-Hexane : dimethyl ether : glycial acetic acid, 114 : 45 : 2, v/v). Triglycerides identified with authentic tripalmitin was scraped, placed into a test tube and determined by a Fletcher's[12] method.

Phospholipids were determined by the modified method of choline determination reported by Takayama et al.[13]

HDL-cholesterol[14] and HDL-phospholipids[15] (which involved VHDL-phospholipids) were determined by an enzyme method after precipitation of LDL and VLDL fractions with polyanions[16]. The LDL+VLDL cholesterol (phospholipids) concentration was obtained by subtracting the HDL-cholesterol (phospholipids) concentration from the total cholesterol (phospholipids) concentration. Serum free fatty acids were determined by an ACS-ACO enzyme method[17]. Determination of total protein[18] in HDL subfractions and catecholamine[19], and Indocyamine green test (R15) were performed during the experiment. Isolation and lipid determination of HDL, HDL subfractions and VHDL (d.>1.210) were performed as desribed previously[20,21].

To prevent the effect of blood dilution on the serum lipid levels, the serum lipid concentrations were corrected with the aid of hematocrit before and during withdrawal of blood.

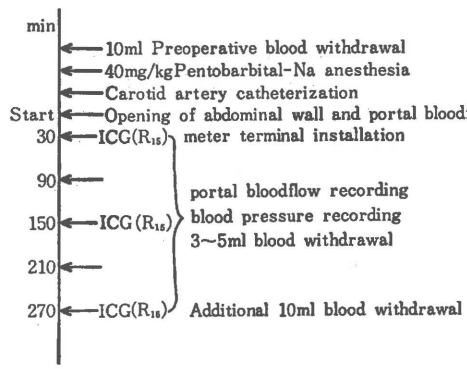


Fig. 1. Experimental procedure for hemorrhagic shock

RESULTS

(1) *Portal blood flow, mean blood pressure and ICG (R15) before and during hemorrhagic shock*:

As shown in Figure 2, the portal blood flow dropped immediately after blood withdrawwal and kept decreasing thereafter. The mean blood pressure also lowered with an elevation

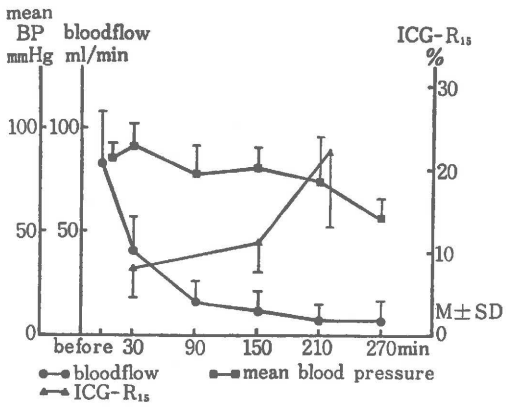


Fig. 2. Bloodflow in portal vein, Mean blood pressure in Aortic and ICG-R_{15} before and during hemorrhagic shock

of ICG (R15), showing the serial decrease in blood flow of the liver and other organs as a result of repeated blood withdrawal.

(2) *Total cholesterol, HDL-cholesterol and LDL+VLDL-cholesterol before and after hemorrhagic shock*:

As shown in Figure 3, total cholesterol considerably decreased after 30 minutes, then kept a moderate decrase until 150 minutes but revealed no change thereafter. HDL-cholesterol kept a gradual decrease until the end of the experiment. LDL+VLDL-cholesterol showed the parallel change with total cholesterol.

(3) *Total phospholipids, HDL-phospholipids (d.>1.063) and LDL+VLDL-phospholipids befor and after hemorrhagic shock*:

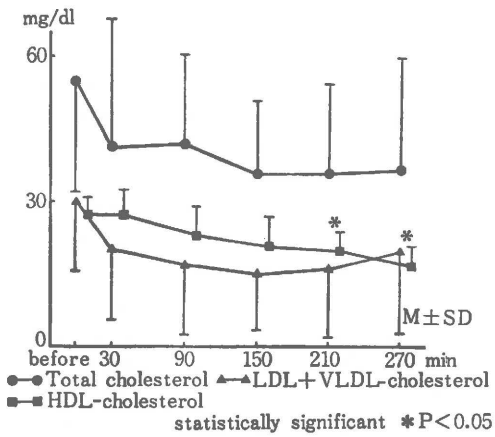


Fig. 3. Total cholesterol, LDL+VLDL-cholesterol and HDL-choletberol before and during hemorrhagic shock

As shown in Figure 4, the decreases in total phospholipids and HDL-phospholipids paralleled. LDL+VLDL-phospholipids showed an acute decrease after 30 minutes as seen in LDL+VLDL-cholesterol and kept on almost the constant level thereafter.

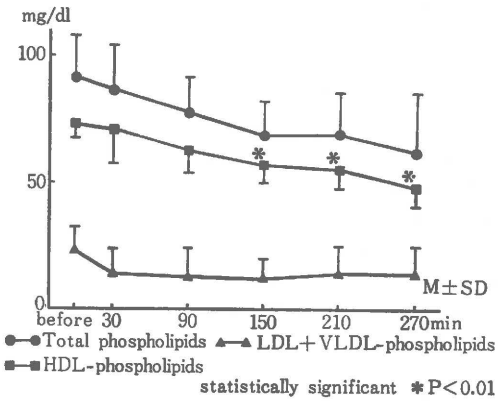


Fig. 4. Total phospholipids, LDL+VLDL-phospholipids and HDL-phospholipids before and during hemorrhagic shock

(4) *The effect of hemorrhagic shock on cholesterol and phospholids in HDL subfractions and VHDL fraction*:

Table 1 lists the changes in cholesterol and phospholipids in HDL_2, HDL_3 and VHDL fractions before and 270 minutes after serial withdrawal of blood.

Cholesterol and phospholipids in HDL_2 subfraction were significantly decreased, while it was phospholipids alone that was significantly decreased in HDL_3 subfraction. On the other hand, VHDL-phospholipids showed no remarkable change. The magnitude of decrease in the two lipids was predominantly higher in HDL_2 than HDL_3.

Table 1. Cholesterol and phospholipids in HDL subfractions, VHDL and HDL before and 270 minutes after hemorrhagic shock (mg/dl)

	HDL_2		HDL_3		VHDL	HDL	
	C	PL	C	PL	PL	C	PL
Before	5.9 ± 1.62	12.7 ± 4.61	25.2 ± 8.14	54.1 ±14.04	21.0 ±11.10	32.0 ±10.10	88.0 ±15.10
	P<0.01	P<0.05		P<0.05		P<0.05	P<0.05
After (270min)	2.4 ± 1.00	3.8 ± 1.01	17.2 ± 3.62	35.1 ± 8.25	25.3 ± 6.53	19.5 ± 2.89	64.5 ± 6.61

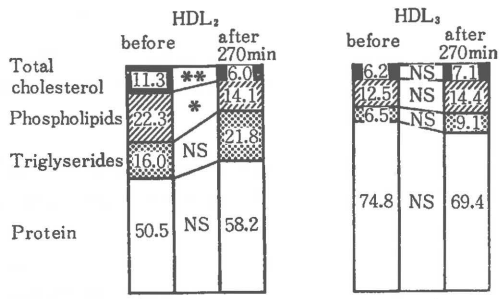
C : Cholesterol PL : Phospholipids

(5) *The effect of hemorrhagic shock on percent composition of lipids and total protein in HDL subfractions*:

The percent composition of lipids and total protein in HDL_2 and HDL_3 subfractions before and 270 minutes after serial withdrawal of blood is shown in Figure 5.

Approximately one half of HDL_2 was composed of protein with the other half consisting of phospholipids, triglycerides and cholesterol before blood withdrawal but serial withdrawal of blood caused an apparent decrease in the amounts of cholesterol and phospholipids of HDL_2 with the result of relative increase in total protein and triglycerides.

HDL_3 contained much larger amount of total protein than HDL_2 and serial blood withdrawal did not result in a change in the composition.



*P<0.01 **P<0.001 statistically significant between percent compositions before and 270minutes after hemorrhagic shock

Fig. 5. Composition of HDL subfractions before and 270 minutes after hemorrhagic shock

(6) *Serum triglycerides, free fatty acids and catecholamines before and during hemorrhagic shock*:

As shown in Figure 6, a slight increase occurred in serum triglycerides. There was an acute elevation of free fatty acids reaching more than double after 30 minutes with a slight decline after 150 minutes and maintaining a higher level until 270 minutes than what it had been before shock.

Catecholamines increased immediately and further rapid elevation was observed at 150 minutes. The increase continued until the end of the experiment. As shown in Figure 7, adrenaline showed a gradual and substantial increase. On the other hand, noradreanline showed a minumum increase until 210 minutes,

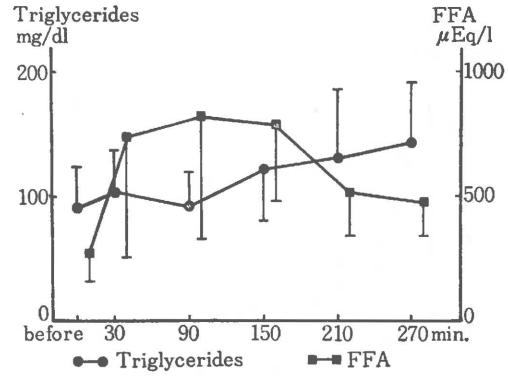


Fig. 6. Serum Triglycerides and FFA before and during hemorrhagic shock

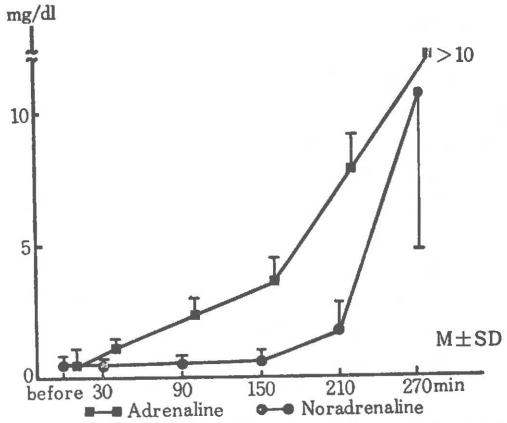


Fig. 7. Catecholamines (Adrenaline and Noradrenaline) before and during hemorrhagic shock

after when it turned up with a steep increase, attaining almost the same level as adrenaline at 270 minutes.

DISCUSSION

Eighteen patients suffering from ischemic cerebrogascular disorders were encountered, in whom the HDL-cholesterol level that had been examined several times prior to strokes had mostly proven already below the normal level before the stroke and had further proven by comparative examination much lower immediately (24 hours) after the stroke than before[22].

It was speculated, therefore, that the lowered serum HDL-cholesterol level accelerates the development of atherosclerosis in these patients and there exists a new different factor(s) that causes an additional decrease in HDL-cholesterol during the stroke. Various factors, i. e., nutrition, shock due to abnormal cardiopulmo-

nary systems, and consequent hepatic and intestinal circulatory disturbance, may contribute to fluctuations of the serum HDL levels during stroke. However, it is hardly conceivable that the nutritional factor can be involved in the HDL level, because blood was withdrawn from 18 patients within 24 hours after the stroke and the nutrititional factor exerts more influence on LDL and VLDL than on HDL[23-25]. It has been well known that many investigators are interested in the nutritional factor as an important sourse of hyper-LDL and VLDL emias.

In the present study the serial analyses were made of HDL and its subfractions including VHDL in rabbits before and after hemorrhagic shock as a factor that may contribute to the rapid fluctuation of HDL levels in a blood stream with serial measurement of the portal blood flow, mean blood pressure and ICG (R15). The portal blood flow rate seems to represent not only the fluctuation of hepatic blood flow but also of intestinal flow. The gradual decrease in serum cholesterol and phospholipids was observed, but LDL+VLDL-cholesterol and phospholipids were not so much affected as compared with HDL-cholesterol and phospholipids, indicating that the changes were limited to HDL fraction alone occurring in serum during hemorrhagic shock caused by repeated withdrawal of blood in rabbits.

HDL was also subdivided into HDL_2 and HDL_3, and when LDL and VLDL were precipitated by polyanions, supernatant-phospholipids (HDL-phospholipids) contained not only HDL_2 and HDL_3 but also VHDL phospholipids. The present study revealed in the hemorrhagic shock the largest decrease of cholesterol and phospholipids in HDL_2 and moderate in HDL_3.

There was no remarkable change in VHDL-phospholipids. Earlier studies showed a prominent decrease of cholesterol and phospholipids in HDL_3 as compared with HDL_2 in chronic hepatitis and liver cirrhosis, and also a decrease of VHDL-phospholipids in the advanced stage of these diseases[20]. Therefore, the lowered HDL caused by the decreased blood flow of the liver and other organs may have entirely different constituents of HDL and VHDL subfractions from those due to chronic hepatic diseases.

The lowered portal blood flow can be estimated to decrease the hepatic synthesis of HDL and its efflux into a blood stream, resulting in the reduction of serum HDL level. However, it is impossible to conclude in the present experiment that the lowered serum HDL is caused only by the reduced blood flow of liver and intestine. Other question may arise in relation to the metabolic turnover rate of HDL in man and rats. It was reported by Gitlin[26], Scanu[27] and Furman[28] that the half-life period of ^{125}I-iodinated human HDL was 3.5-4.8 days in human beings and those of apo A-I and apo A-II were only 13 and 9 hours respectively in rats. This indicates, therefore, the presence of different metabolic pathways between them in rats, while it was 5.8 days in man. Schaeffer et al.[30] described that the average retention period of HDL was 5.21, 3.41 and 0.52 days in healthy subjects, heterozygote and homozygote of Tangier's disease.

It can be concluded, therefore, that the metabolic turnover rate of HDL largely differs among species and varies depending on pathological states. Although the turnover rate in rabbits was not determined in this experiment, the decrease in HDL 270 minutes after hemorrhagic shock in rabbits was so short when compared with that in rats estimated from the turnover rate of apo A-II (9 hours). Further investigation is necessary to solve this problem.

Moreover, cholesterol and phospholipids in HDL_2 were differentially decreased with the result of relative increase in triglycerides and total protein after serial withdrawal of blood. This fact indicates that HDL_2 not only loses the number of lipoprotein particles but also incurs changes in its constituents and that the selective decrease in cholesterol and phospholipids brought on an additional decrease in the serum HDL-cholesterol and HDL-phospholipid levels, as compared with the decrase in HDL_2 lipoprotein particles alone. Contrary to HDL_2, HDL_3 displayed no constitutional change but a decrease only in the number of lipoprotein particles.

VHDL-phospholipids which were involved in HDL-phospholipids by the present analytical procedure, was reported to be lowered in patients with ischemic cerebrovascular diseases of steady state[21] but did not alter in rabbits under hemorrhagic shock. These results justi-

fies the speculation that the decreased blood flow of the liver and other organs contributes to the fluctuation of serum HDL level but the changes differ from those seen in other parenchymal disorders of the liver and cerebrovascular diseases in steady state in the lipoprotein constituents.

The present experiment was not designed to clarify whether the increase in catecholamine and free fatty acids, generally seen in shock, co-exists with the decrease in HDL or is related in some respects to the decrease in HDL in serum of these animals.

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