55) A Selective Measurement of Lipoprotein Lipase and Hepatic Triglyceride Lipase in Post-Heparin Plasma in SHRSP—Preliminary Report. Hiroshi Ogawa, Tomoyo Nishikawa and Sukenari Sasagawa. Department of Hygiene, Kinki University School of Medicine, Osaka-fu 589.

Lipoprotein lipase (LPL) is mainly present in extrahepatic tissue where it catalyzes the hydrolysis of triglyceride-rich lipoproteins to their remnants. Hepatic triglyceride lipase (h-TGL) is primarily located on the sinusoidal surfaces of liver endothelium and appears to involve the conversion of intermediate lipoprotein (IDL) to low density lipoprotein (LDL), but the in vivo functions of h-TGL still remains to be established. LPL and h-TGL are rate-limiting enzymes of serum lipoprotein metabolism and are released into the circulation after intravenous injection of heparin. And, the total activity released is referred to as post-heparin plasma lipolytic activity (PHLA). The purpose of this study is 1) to establish a selective measurement of LPL and h-TGL from PHLA and 2) to estimate those activities in SHRSP in order to elucidate the possible disorder of lipid metabolism in SHRSP.

MATERIALS AND METHODS: Male WKY (300-400 g) fed a regular rat chow (CE-2, Clea Japan Inc.) were used for isolation of h-TGL. Their livers were removed and perfused with Krebs-Ringer bicarbonate buffer (pH 7.4) containing 20% glycerol and heparin (10 units/ml) after wash-out with the same buffer without heparin. The perfusate obtained was used as a starting material. Then, h-TGL was isolated by successive chromatographies of Heparin-Sepharose column, PD-10 column and FPLC system with Mono Q column. The details for this purification procedure will be described elsewhere. Antibody against h-TGL was elicited in male rabbit. By using the antiserum, h-TGL activity was selectively inactivated, and a selective measurement of LPL and h-TGL was accomplished. Post-heparin plasma (PH-plasma) samples were obtained from the jugular vein 3 min after heparin (300 units/kg) was injected into the same vein under ether anesthesia without fasting or after fasting overnight. Lipase assay was carried out by RIA method according to the method of Sholtz et al. (J.Lipid. Res., 17: 536-541, 1976) using tri-[1-¹⁴C] oleate as a substrate.

RESULTS AND DISCUSSION: The purified h-TGL appeared to be homogeneous by SDS gradient slab-PAGE and the apparent molecular weight was about 62,000. Next, antibody against rat h-TGL was successfully raised in rabbit. h-TGL in PH-plasma was immunoinactivated by the addition of anti h-TGL sera to PH-plasma, and the residual activity was estimated as LPL activity. h-TGL activity was estimated by subtracting LPL activity from PHLA.

Both PHLA and h-TGL activity in PH-plasma obtained after fasting overnight were higher than those without fasting in both strains. Therefore, it is postulated that feeding condition considerably affects PHLA and h-TGL activity. Accordingly, PH-plasma samples in the following experiments were obtained from nonfasting rats. PHLA, LPL and h-TGL activities were listed below.

Age (Week)	Strain [No.]	PHLA (μ mole free fatty acid/ml plasma/hr)	LPL (μ mole free fatty acid/ml plasma/hr)	H-TGL (μ mole free fatty acid/ml plasma/hr)
9	WKY [8]	72.0 $\pm$ 1.6	50.5 $\pm$ 1.8	23.1 $\pm$ 1.4
	SHRSP [6]	50.5 $\pm$ 3.4**	30.8 $\pm$ 3.5**	19.7 $\pm$ 2.2
18	WKY [8]	48.0 $\pm$ 2.7	28.1 $\pm$ 1.5	19.9 $\pm$ 2.4
	SHRSP [5]	43.4 $\pm$ 2.1	21.4 $\pm$ 0.1**	22.1 $\pm$ 2.1
33	WKY [5]	68.0 $\pm$ 3.1	44.1 $\pm$ 1.5	24.0 $\pm$ 3.3
	SHRSP [5]	44.7 $\pm$ 2.2**	24.4 $\pm$ 0.9**	20.3 $\pm$ 1.3
Mean $\pm$ SE, **: Significant difference against WKY (p<0.01)				

In conclusion, LPL activity in SHRSP was significantly lower than that in WKY, while no apparent difference in h-TGL activity was observed between SHRSP and WKY. LPL could be involved with abnormality of lipid metabolism in SHRSP. Age-related changes in PHLA, LPL and h-TGL activities are under investigation.