The Relationship between Lipoprotein Lipase Activity and Respiratory Quotient of Rats in Circadian Rhythms

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Plasma lipid levels and lipoprotein lipase (LPL) are known to follow circadian rhythms in rats. However, very little information is available on the variations in respiratory quotient (RQ) during the 24-h period in rats. The aims of this study were to provide an overall view of the effects of circadian rhythm on RQ and to determine the relationship of LPL and RQ with metabolic parameters in these animals. Male rats were fed ad libitum and were kept under a 12 : 12-h light–dark cycle. Rats were killed every 2 h over a 24-h period for measurement of metabolic parameters and tissue LPL activity. The RQ was measured every 4 h over the same 24-h period. The gastric contents increased during the dark phase and decreased during the light phase. For the metabolic parameters, circadian rhythms were detected for plasma glucose, triglycerides, high-density lipoprotein cholesterol and non esterified free fatty acids, but not for plasma total cholesterol or phospholipids. The RQ and adipose tissue LPL activity increased during the dark phase, while skeletal muscle LPL activity decreased during this phase. The RQ was inversely correlated with skeletal muscle LPL activity ($r=-0.880$) and positively correlated with adipose tissue LPL activity ($r=0.937$). These results appear to show that rats tend toward consumption of fat by accelerating fat oxidation, resulting in suppression of fat accumulation in the light phase, while tending toward fat accumulation by the suppression of fat oxidation in the dark phase.

Key words circadian rhythm; lipoprotein lipase; respiratory quotient; skeletal muscle; adipose tissue

Lipoprotein lipase (LPL) is a key enzyme to lipoprotein. LPL is synthesized in the parenchymal cells of extrahepatic tissues (*e.g.* skeletal muscle, adipose tissue, myocardium), and hydrolyzes triglycerides (TG) in circulating TG-rich lipoproteins, releasing non esterified free fatty acids (NEFA), which are taken up by underlying cells for oxidation or storage.[1—3] Tissue LPL activity is known to be regulated by feeding. Starvation is associated with a decrease in LPL activity in adipose tissue, but an increase in LPL activity in heart and skeletal muscle.[4,5] It has been suggested that the inverse changes in LPL activities in adipose tissue and muscle evoked by starvation divert NEFA derived from circulating TG from storage in adipose tissue to oxidation in muscle.[6]

Respiratory quotient (RQ) is the steady state ratio of carbon dioxide produced by tissue metabolism to oxygen consumed in the same metabolism for whole body. Twenty-four hour RQ, an index of the ratio of glucose/fat oxidation rate has been reported to vary widely between individuals under eucaloric conditions.[7] However, there is very little information concerning the relationship between RQ and circadian rhythm in human and animals. Therefore, we studied the effects of circadian rhythm on RQ and determined the relationship of LPL and RQ with metabolic parameters in rats.

MATERIALS AND METHODS

Materials Glycerol tri[1-^{14}C]oleate (2.2 Gbq/mmol) was obtained from Amersham International, Cardiff, United Kingdom and heparin from Novo, Bajsvaeld, Denmark. All other chemicals used were high grade commercially available products.

Animals and Environment Male Wistar rats, 6 weeks old were obtained from Charles River Japan, Inc (Yokohama, Japan). Room temperature was maintained at 23±2 °C with 55±10% relative humidity. The artificial light/dark cycle was 12 h with lights on at 7:00 a.m. and there were an average of 13—16 air changes per room per hour. Pelleted rat food (CRF1-R, Oriental Yeast Co., Ltd., Tokyo, Japan) were available ad libitum. All animals had access to tap water ad libitum.

Animal Experiment The rats were stratified by body weight and one group was 6 rats. Rats were killed by decapitation at 2 h intervals for 1 d and blood samples were collected for determination of plasma glucose and lipid. After blood sampling, soleus of skeletal muscle and epididymal adipose tissue were obtained for tissue LPL activity measurement at 4 h intervals. The stomach was also incised and the gastric contents were gathered and weighed. We performed another set of experiments to determine postheparin plasma (PHP). The rats were injected with heparin (100 U/kg body weight) *via* the tail vein at 2:00, 6:00, 10:00, 14:00, 18:00 and 22:00, and blood samples were collected 5 min later. Plasma samples were used to determine PHP-LPL activity.

To measure respiratory quotient (RQ), individual rats were placed in a glass metabolic chamber with sufficient food and water for 24 h, and the RQ was measured at 4 h intervals 4 h for 1 d.

All animal experiments were approved by the local animal ethics committee of Otsuka Pharmaceutical Factory, Inc.

Plasma Glucose and Lipid Measurement Plasma glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), TG, phospholipids (PL) and NEFA were determined by conventional enzymatic methods. Glucose CII-test Wako (Wako Pure Chemical Industries Ltd., Osaka, Japan) was used for glucose. The Cholesterol C-test Wako (Wako Pure Chemical Industries Ltd.) was used for TC, the Nescote HDL-C kit N (heparin calcium precipitation; Nippon Shoji Ltd., Osaka) for HDL-C, the Triglyceride G-test Wako for TG, the Phospholipid B-test Wako for PL, and the NEFA C-test Wako for NEFA (the latter three tests all from Wako Pure Chemical Industries, Ltd.)

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LPL Activity Measurement PHP-LPL: LPL activity in PHP was measured by an immunochemical method described previously[8] using glycerol tri[1-^{14}C]oleate as substrate and selective blocking of hepatic lipase with antiserum to rat hepatic lipase.

Tissue LPL Activity: Skeletal muscle LPL activity was as measured previously.[8] A specimen of skeletal muscle was homogenized in 50 mM NH_4OH–NH_4Cl buffer (pH 8.5) containing heparin for 60 min at 0 °C. The suspension was then centrifuged, and the supernatant was used to measure LPL activity. Adipose tissue LPL activity was measured as described earlier.[8] A specimen of epididymal adipose tissue weighing 100 mg was minced into small pieces and placed in Krebs–Ringer bicarbonate buffer (pH 7.4) in the presence of heparin for 60 min at 37 °C. The incubation medium was then assayed for LPL activity.

Respiratory Quotient Measurement Individual rats were placed in a glass metabolic chamber with sufficient food and water for 24 h. Air was drawn through the chamber at a rate of 1.5 l/min. Outflow air composition was measured using an automated open-circuit respirometer (Arco-1000, Arco System Ltd., Chiba, Japan).

Statistical Analysis The results are expressed as means±S.D.

RESULTS

Diurnal Variation of Gastric Contens Gastric contents increased from 20 to 8 h, while they decreased from 8 to 18 h. This shows that food intake increased in the dark phase and decreased in the light phase (Fig. 1A).

Diurnal Variation of Plasma Glucose and Lipids Levels Plasma glucose level increased from 18 to 22 h and decreased from 22 to 6 h. In light phase, there were 2 peaks of glucose levels, at 8 and 16 h (Fig. 1B). Plasma TG level increased from 22 to 6 h; in light phase, a high level was maintained but this decreased from 16 to 18 h (Fig. 1C). Plasma NEFA decreased from 20 to 6 h and increased from 8 to 12 h. In light phase, the NEFA level was higher than in the dark phase (Fig. 1D). Plasma TC in the two phases was unchanged (Fig. 1E). Plasma HDL-C decreased from 10 to 12 h (Fig. 1F), while plasma PL level was unchanged during 24 h (data not shown).

Diurnal Variation of RQ The RQ increased from 18 to 6 h in dark phase, but decreased from 8 to 14 h in light phase (Fig. 2).

Diurnal Variation of LPL Activity The LPL activity of adipose tissue in light phase was lower than in the dark phase (Fig. 2), while in skeletal muscle it was higher in the light than in the dark phase (Fig. 3). The PHP LPL activities were not particularly changed between the light and the dark phase (Fig. 4).

Relationship between RQ and LPL Activity The RQ was positively correlated with LPL activity of adipose tissues (r=0.937) and inversely correlated with LPL activity of skeletal muscle (r=−0.880). However, there was no relationship between RQ and PHP-LPL activity (r=0.172).

DISCUSSION

It is well known that laboratory rats fed ad libitum feed at night (dark phase).[9] The change of their gastric contents in this study showed that food intake increases during the dark phase and decreases during light phase.

Plasma glucose, TG and NEFA levels change with food intake. Plasma glucose levels tend to decrease with food intake, whereas food intake results in NEFA levels immediately decreasing from high levels caused by lack of food intake. This phenomenon may be caused by the elevation of insulin levels as a result of food intake.[10] However, plasma TG levels did not immediately increase. Despite food intake increasing from 20:00, TG levels started increasing from 24:00. This time lag may mean that very low density lipoprotein (VLDL) formation as a result of food intake requires time.

There are reports stating that plasma TC and HDL-C are not affected by food intake or circadian rhythms.[10,11] In this study, TC was not affected by circadian rhythms but HDL-C decreased from 10 to 12 h. This may mean that non HDL-C increased from 10 to 12 h. Therefore, we feel that the profile of lipoprotein by circadian rhythms needs to be clarified in the future.

Circadian rhythms were observed for RQ, skeletal muscle LPL and adipose tissue LPL activity. The change in RQ corresponds to the change in gastric contents. This means that RQ increases in accordance with start of food intake in dark phase and decreases with lack of food intake in light phase. The change in skeletal muscle LPL activity is inversely proportional to the change in RQ (r=−0.880). Whole-body RQ is the sum of a complex interaction between lipogenesis, fat oxidation, and glucose oxidation. However, in general, a decrease in RQ means an increase in fat oxidation.[12] It is well established that there is substrate competition between free fatty acids and glucose for oxidation, with elevated rates of fat oxidation resulting in reduced glucose oxidation.[13] Ferraro *et al.* reported that the relationship between 24-h RQ and skeletal muscle LPL activity is inverse in Pima Indian males and a decreased muscle LPL activity may be a predisposition for obesity.[7] Therefore, the circadian rhythms of RQ and skeletal muscle LPL mean that the increase in skeletal muscle LPL activity causes an increase in fat oxidation, resulting in a decrease in RQ in light phase. In this study, it was not clear why the increase in skeletal muscle LPL activity caused an increase in fat oxidation. However, Jensen *et al.* demonstrated that carcass lipid content (% body weight) decreased by muscle-specific overexpression of LPL in transgenic mice with high fat diet-induced obesity.[14] Weigle *et al.* reported that an administration of heparin, which activates LPL, results in an increase in the circulation of free fatty acid levels and causes significant increases in the uncoupling protein 3 (UCP3) mRNA in skeletal muscle.[15] UCP3, expressed abundantly in the skeletal muscle, is one of the carrier proteins dissipating the transmitochondrial electrochemical gradient as heat, thermogenesis.[16,17] In this study, circadian rhythms were determined for NEFA, with NEFA increasing in light phase. Therefore, we feel that additional experiments are necessary to understand the relationship between LPL, free fatty acid and UCP3 in skeletal muscle.

On the other hand, the change of adipose tissue LPL activity is similar to the change of gastric contents and also is positively correlated with RQ (r=0.937). This phenomenon may mean that rats have a tendency toward fat accumulation re-

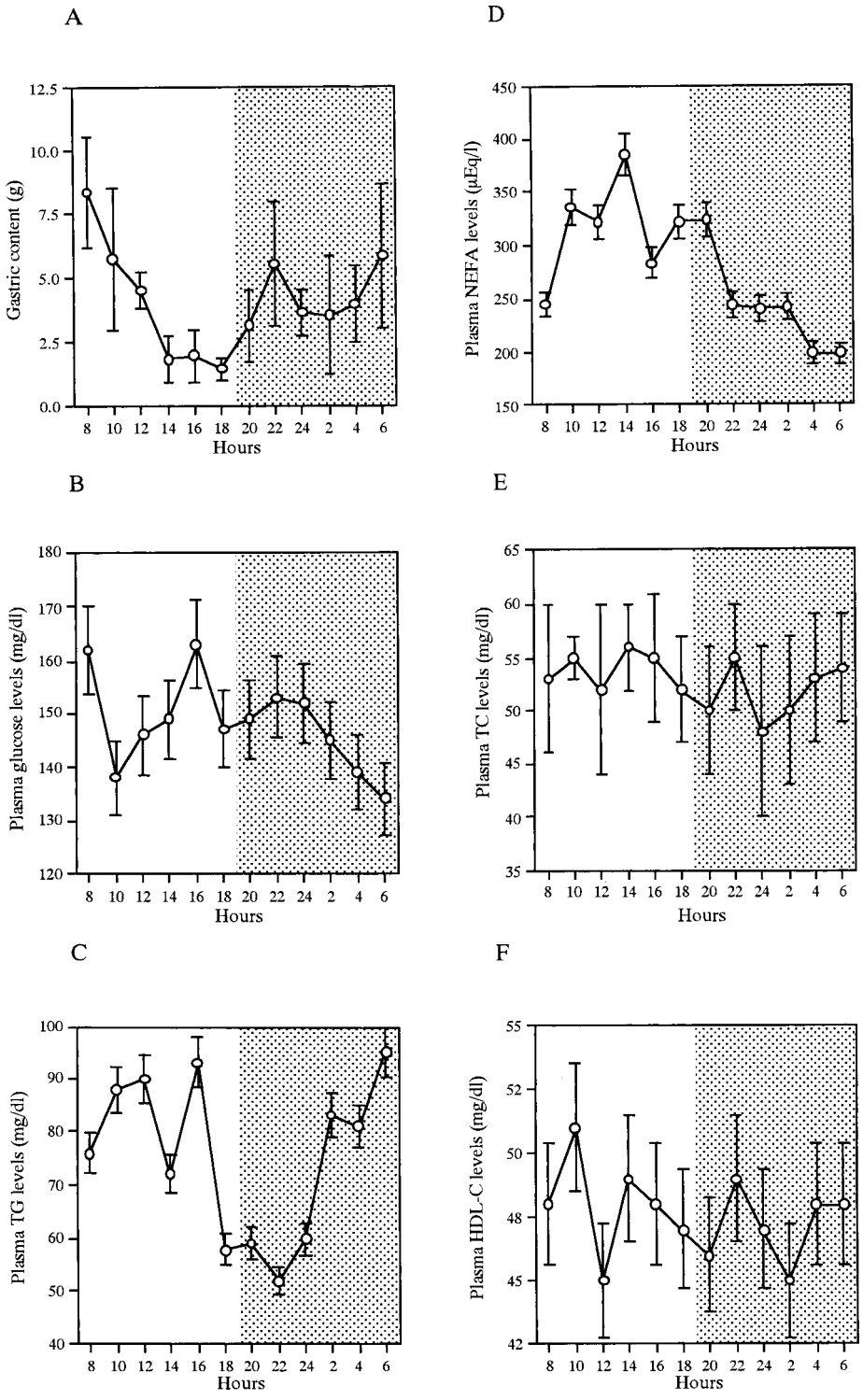

Fig. 1. Diurnal Variation of Gastric Contens (A), Plasma Glucose Levels (B), Plasma TG Levels (C), Plasma NEFA Levels (D), Plasma TC Levels (F), Plasma HDL-C Levels in 6-Week-Old Male Wistar Rats

White areas show light phase and shaded areas to dark phase.

sulting from an increase in LPL activity in adipose tissue and a tendency toward glucose oxidation. An increase in LPL activity in adipose tissue is known to causes fat accumulation.[18)]

Circadian rhythms were not observed for PHP-LPL activity, which is the sum of tissue LPL activity. In this study, circadian rhythms of skeletal LPL activity showed an inverse relationship with the circadian rhythms of adipose tissue LPL activity. Therefore, the circadian rhythms of PHP-LPL activity may not be detectable.

In summary, circadian rhythms were observed for plasma glucose, TG NEFA and HDL-C, but not for TC or PL. They were also determined for RQ, skeletal muscle LPL activity and adipose tissue LPL activity, but not for PHP-LPL activ-

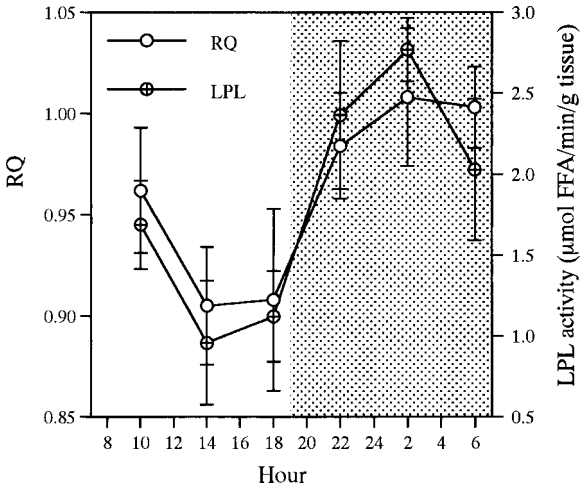

Fig. 2. Diurnal Variation of RQ and Adipose Tissue LPL Activity in 6-Week-Old Male Wistar Rats

White areas show light phase and shaded areas to dark phase.

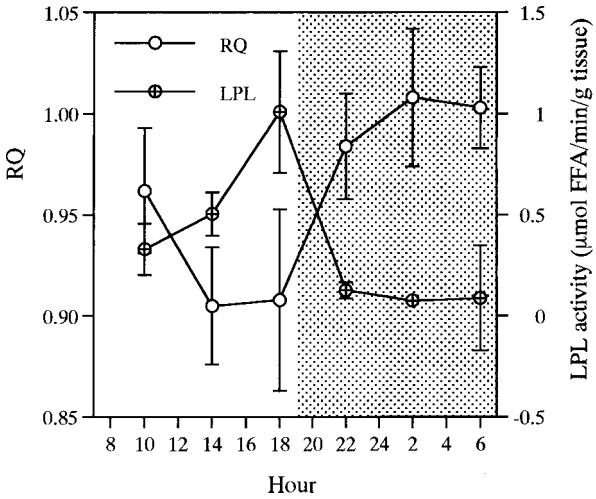

Fig. 3. Diurnal Variation of RQ and Skeletal Muscle LPL Activity in 6-Week-Old Male Wistar Rats

White areas correspond to light phase and shaded areas to dark phase.

ity. The circadian rhythms of skeletal LPL activity showed an inverse relationship with the adipose tissue LPL activity, and those of RQ showed an inverse relationship with skeletal muscle LPL activity. These data may indicate that in rats, there is an increase in fat oxidation caused by an increase in skeletal muscle LPL activity, resulting in a suppression of fat accumulation in light phase. On the other hand, in rats, there is a decrease in fat oxidation caused by an increase in adipose tissue LPL activity, resulting in accelerated fat accumulation in dark phase. It is thought that bodily fat may be adequately balanced in these animals by adjusting LPL activities

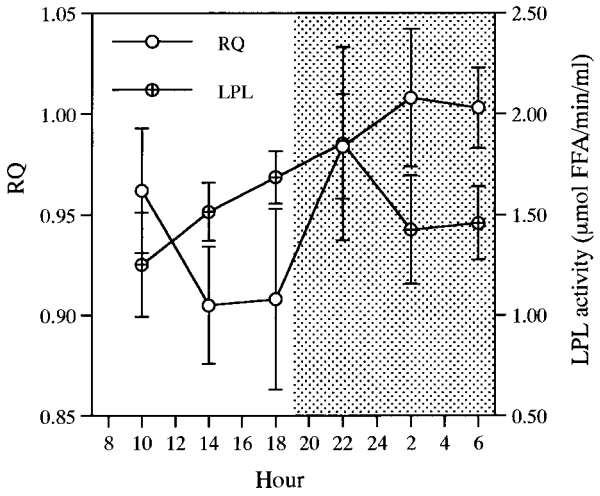

Fig. 4. Diurnal Variation of RQ and PHP-LPL Activity in 6-Week-Old Male Wistar Rats

White areas correspond to light phase and shaded areas to dark phase.

in skeletal muscle and adipose tissue according to daily circadian rhythms.

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