Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat

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Benavides, Alex, Mariona Siches, and Miquel Llobèra. Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R811–R817, 1998.—Although intermediate metabolism is known to follow circadian rhythms, little information is available on the variations in lipoprotein lipase (LPL) and hepatic lipase (HL) activities during the 24-h period, and there is also a lack of adequate statistical analysis. Here, adult male rats were fed ad libitum and kept at 21°C under 12:12-h light-dark cycles. They were killed in batches every 3 h over a 24-h period. Lipase activities were determined in plasma and fresh homogenates of epididymal white adipose tissue (EWAT), interscapular brown adipose tissue (IBAT), heart, skeletal muscle, and liver. Plasma insulin, corticosterone, glucose, triacylglycerol (TAG), cholesterol, glycerol, β-hydroxybutyrate, and liver and muscle glycogen were determined. Cosinor analysis was used to evaluate the presence (significance of fit of cosine curve to data and variance explained by rhythm) and characteristics of possible circadian rhythms [acrophase (φ), mesor, and amplitude]. Statistically significant circadian rhythms were detected for 1) all metabolites studied, except TAG, cholesterol, and liver HL activity; 2) LPL and HL activity in plasma (both φ in light phase); and 3) LPL activity in all tissues studied (φ: heart in light phase; skeletal muscle, IBAT; and, EWAT in dark phase). Liver also showed a circadian rhythm of LPL activity, with φ near that in plasma. These findings demonstrate for the first time that, in physiological conditions, LPL activities in plasma and various tissues, including liver, and HL activity in plasma follow circadian rhythms. Their metabolic significance is discussed.

Lipoprotein lipase (LPL) and hepatic lipase (HL) are extracellular enzymes that are key to lipoprotein metabolism; their physiologically active forms are located on the luminal surfaces of the capillary endothelium. LPL is synthesized in the parenchymal cells of extrahepatic tissues, and it hydrolyzes triacylglycerols (TAG) in circulating TAG-rich lipoproteins, releasing nonesterified fatty acids (NEFA), which are taken up by underlying cells for oxidation or storage (4, 31, 32). Thus tissue-specific regulation of LPL activity controls the flow of circulating TAG between tissues. The functional LPL detected in the adult liver is mainly of extrahepatic origin. HL is synthesized in the liver but is also present in the adrenal gland and ovaries (17, 18). By hydrolyzing TAG and phospholipids, HL influences the physical characteristics of several lipoproteins and contributes to the reverse transport of cholesterol to the liver and steroidogenic tissues (30, 36). Both LPL and HL are under nutritional control (5, 9).

Intermediate metabolism is subject to rhythmic variations, which are probably based on the evolutionary adaptation of organisms to their environment. Several studies on circadian rhythms in intermediate metabolites (21), hormones (2, 27), enzymes (33), and basic metabolic pathways (41) have been reported. Nevertheless, the literature contains few experimental data on diurnal variations in LPL activity in tissues (3, 7, 8, 14, 24, 37) and lacks any specialized statistical analysis of these periodic variations. No general hypothesis about the diurnal variations of LPL activity in different tissues has been put forward. There are few data on changes in HL activity during the 24-h cycle.

The aims of the present study were to provide an overall view of the temporal organization of LPL and HL activities in various tissues in the adult male rat and to determine the relationship of the temporal organization of LPL and HL activities with both closely related metabolic parameters (plasma TAG, glyc erol, cholesterol, and ketones) and more general parameters (plasma glucose, insulin, and corticosterone and liver and muscle glycogen). The cosinor method (15) was used to evaluate the data and possible circadian oscillations.

MATERIALS AND METHODS

Animals and Tissue Collection

Male Wistar rats weighing 220–240 g were housed (3 animals/cage) and maintained under controlled conditions: 12:12-h light-dark cycle (lights on from 8 AM to 8 PM), a temperature of 21 ± 1°C, and humidity of 70 ± 2%. All rats were fed ad libitum on standard laboratory diet (12% water, 17% protein, 3% fat, 58.7% carbohydrate, 4.3% cellulose, and 5% vitamins and minerals, all by weight) and water. For a 2-wk acclimatization period, food consumption was monitored, and animals were weighed at different times of the day, with no apparent stress. To ensure the repetitivity of the circadian cycles, rats were killed in two groups. In each group, three animals were killed every 3 h from 8 AM to 8 AM the following day. After decapitation, blood was collected in heparinized tubes and maintained on ice until centrifuging. Epididymal white adipose tissue (EWAT), liver, heart, skeletal muscle (quadriceps), and interscapular brown adipose tissue (IBAT) were removed in sequence (only heart and liver were weighed), immediately frozen in liquid nitrogen and then kept at −80°C.

Experimental Protocols

Measurements of lipase activities. Tissues were homogenized (−200 mg/ml buffer) in HEPES-dithiothreitol-EDTA-saccharose buffer (pH 7.5) containing heparin (5 mU/ml). LPL was determined according to Ramírez et al. (34), slightly

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modified. Because HL slightly cross-reacts in the lipolytic activity assay used to measure LPL, in the liver and blood assays, the samples were previously incubated (1:1) with serum against rat HL for 90 min at 4°C (20). HL activity was determined by the method of Ehnholm and Kuusi (10) with the minor modifications recommended by Sabugal et al. (35).

Analytic techniques. Plasma samples were tested for the following metabolites: insulin and corticosterone by RIA, glucose by a commercially available kit from Boehringer (Mannheim, Germany), and TAG and cholesterol by commercially available kits from Reflab. For glycerol (12) and β-hydroxybutyrate (23) analysis, plasma was previously deproteinized (70:3 in perchloric acid 60%). Glycogen was determined in liver and muscle following the method of Good et al. (13), and the glucose produced was measured as described above. To distinguish whether the changes observed during the 24-h cycle were due to circadian variations in cell volume (perhaps caused by modifications in glycogen content) or to variations in tissue metabolite concentrations and enzyme activities, the DNA concentration in tissues was measured by Vytašek’s method (40). Finally, the results of the other determinations are expressed as a function of milligrams of DNA.

Statistics. Time was expressed in hours and minutes after time 0 (8 AM corresponds to 0:00), when the lights were turned on. All data were statistically evaluated for time effects by two-way ANOVA and for circadian rhythms by a computerized inferential statistical method, which involved fitting a 24-h cosine curve to data series by the least-squares nonlinear (cosinor) regression procedure (15). With this method, two statistical parameters were determined: 1) the probability or P value, which indicates the significance of the fit of the cosine curve to the data, and 2) the percentage of variance explained by the rhythm. Three parameters that characterize a rhythm were also determined (see Fig. 1): mesor, amplitude, and acrophase. In addition, confidence intervals for these parameters were calculated.

RESULTS

Food Consumption and Body Weight

It is well known that laboratory rats fed ad libitum feed intermittently. Food gathering mainly occurred at night, coupled to an inborn circadian activity rhythm (19). In this experiment, rats lost weight during the light phase (almost 5% initial body weight) and regained it during the dark phase (Fig. 2). Body weight variations followed a significant circadian rhythm (Table 1). In addition, by the end of each circadian period, rats had increased their weight by 4%, probably due to growth. According to the food intake pattern, the weight gain was not constant during the 24-h period: during the first 3 h after the onset of light, the animals lost 3% of their initial body weight; gradually, this loss became smaller, and during the activity hours, the increase became progressively higher with each subsequent 3-h interval. Notice that, during the last 3-h interval before the onset of darkness, the change of body weight switches from loss to gain and a significant (P < 0.05) amount of food was consumed. This “anticipatory activity” (19) probably enables wild animals to be prepared for changes in environmental conditions, such as food availability, but persists in laboratory animals with free access to food.

Plasma Metabolites

For plasma metabolite data, see Fig. 3 and Table 1. Although glycemia is considered a stable parameter, it showed a significant circadian rhythm: the lowest glucose values were found during the light phase, and levels increased concomitantly with the food consumed (Fig. 2) before the lights were turned off. Although...
slight (amplitude is 6% of mesor), the change in glucose level was probably the main factor responsible for the increase in circulating insulin at the end of the light phase. Plasma glycerol, an index of lipolysis in white adipose tissue, also had a significant circadian rhythm: the level rose during the light phase and fell at the beginning of the dark phase (when animals fed). Plasma β-hydroxybutyrate was analyzed as an index of liver ketogenesis. There was a peak at the onset of darkness; beginning of the dark phase (when animals fed). Plasma glucose, insulin, corticosterone, and body weight, EWAT LPL activity value doubled at the acrophase when the lights were turned on and in parallel with food intake and body weight, EWAT LPL activity value doubled at the acrophase, i.e., in the middle of the dark phase. Afterward, LPL activity fell, and as with glucose, light-phase values were lower than dark-phase values, as also observed by Goubern and Portet (14).

### Table 1. Cosinor test values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P</th>
<th>Variance Explained by Rhythm %</th>
<th>Mesor ± SE</th>
<th>Amplitude = CI (95%)</th>
<th>Acrophase = CI (95%), h:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, g</td>
<td>&lt;0.0001</td>
<td>76.8</td>
<td>206 ± 1</td>
<td>9.7 (8.5; 10.7)</td>
<td>22:24 (22:02; 23:10)</td>
</tr>
<tr>
<td>Plasma hormones, ng/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>&lt;0.0001</td>
<td>41.5</td>
<td>5.9 ± 0.5</td>
<td>2.1 (1.3; 2.8)</td>
<td>19:23 (18:03; 21:08)</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>&lt;0.0025</td>
<td>26.5</td>
<td>56.3 ± 12.5</td>
<td>32.9 (15.3; 50.6)</td>
<td>12:07 (9:16; 14:33)</td>
</tr>
<tr>
<td>Plasma metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>&lt;0.0037</td>
<td>22.9</td>
<td>8.2 ± 0.2</td>
<td>0.5 (0.2; 0.7)</td>
<td>17:14 (14:17; 20:10)</td>
</tr>
<tr>
<td>TAG, mM</td>
<td>&lt;0.1681</td>
<td>7.6</td>
<td>5.2 ± 0.2</td>
<td>0.2</td>
<td>20:24</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>&lt;0.6658</td>
<td>1.8</td>
<td>2.0 ± 0.1</td>
<td>0.1</td>
<td>11:13</td>
</tr>
<tr>
<td>Glycerol, µM</td>
<td>&lt;0.0008</td>
<td>27.3</td>
<td>66.9 ± 5.6</td>
<td>16.1 (8.2; 24.0)</td>
<td>8:10 (5:32; 10:23)</td>
</tr>
<tr>
<td>Ketones, µM</td>
<td>&lt;0.0223</td>
<td>15.5</td>
<td>18.6 ± 13</td>
<td>2.7 (0.8; 4.5)</td>
<td>11:35 (8:02; 15:34)</td>
</tr>
<tr>
<td>Glycogen, mg Glc/mg DNA</td>
<td>&lt;0.0001</td>
<td>68.5</td>
<td>1.3 ± 0.1</td>
<td>0.6 (0.5; 0.8)</td>
<td>21:01 (20:05; 21:33)</td>
</tr>
<tr>
<td>Muscle</td>
<td>&lt;0.0001</td>
<td>60.4</td>
<td>13.9 ± 0.8</td>
<td>4.9 (3.8; 6.2)</td>
<td>2:24 (1:24; 3:23)</td>
</tr>
<tr>
<td>Liver</td>
<td>&lt;0.0001</td>
<td>53.2</td>
<td>73.7 ± 5.6</td>
<td>28.1 (20.2; 36.1)</td>
<td>20:11 (19:05; 21:17)</td>
</tr>
<tr>
<td>LPL activity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EWAT, mU/mg DNA</td>
<td>&lt;0.0001</td>
<td>53.2</td>
<td>140.6 ± 8.4</td>
<td>24.9 (13.0; 36.7)</td>
<td>15:15 (13:05; 17:27)</td>
</tr>
<tr>
<td>IBAT, mU/mg DNA</td>
<td>&lt;0.0005</td>
<td>28.0</td>
<td>140.6 ± 8.4</td>
<td>24.9 (13.0; 36.7)</td>
<td>15:15 (13:05; 17:27)</td>
</tr>
<tr>
<td>Muscle, mU/mg DNA</td>
<td>&lt;0.0079</td>
<td>19.3</td>
<td>3.5 ± 0.4</td>
<td>0.9 (0.3; 1.4)</td>
<td>13:31 (10:20; 17:04)</td>
</tr>
<tr>
<td>Liver, mU/mg DNA</td>
<td>&lt;0.0002</td>
<td>30.8</td>
<td>8.5 ± 0.4</td>
<td>1.3 (0.7; 1.8)</td>
<td>8:32 (6:28; 11:01)</td>
</tr>
<tr>
<td>Plasma (mU/ml)</td>
<td>&lt;0.0001</td>
<td>44.3</td>
<td>1.0 ± 0.1</td>
<td>0.4 (0.3; 0.5)</td>
<td>9:26 (8:10; 11:07)</td>
</tr>
<tr>
<td>Heart, mU/mg DNA</td>
<td>&lt;0.0001</td>
<td>50.9</td>
<td>20.8 ± 2</td>
<td>9.8 (6.9; 12.7)</td>
<td>8:06 (6:34; 9:15)</td>
</tr>
<tr>
<td>HL activity</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Liver, mU/mg DNA</td>
<td>&lt;0.1325</td>
<td>8.6</td>
<td>27.3 ± 1.6</td>
<td>2.3</td>
<td>8:07</td>
</tr>
<tr>
<td>Plasma, mU/ml</td>
<td>&lt;0.0004</td>
<td>29.2</td>
<td>2.8 ± 0.2</td>
<td>0.5 (0.2; 0.7)</td>
<td>4:26 (2:16; 6:34)</td>
</tr>
</tbody>
</table>

Mesor values and amplitude are shown in corresponding parameter units. Acrophases are given in time scale (12:12-h light-dark cycle, light phase from time 0:00 corresponds to 8 AM). P value indicates significance of fitness of cosine curve to data (P < 0.05 was considered statistically significant). CI, confidence interval when rhythm is present (limits in parentheses); TAG, triacylglycerides; EWAT, epidymal white adipose tissue; IBAT, interscapular brown adipose tissue.

#### Glycogen

For glycogen, see Fig. 3 and Table 1. Both muscle and liver glycogen showed significant circadian rhythms. During the circadian period, liver glycogen stores increased 10 times more glycogen per gram of tissue than muscle, as befits its leading role in glucose homeostasis. However, as the greater amplitude of the mesor of the muscle rhythm (46%), compared with that of the liver (35%), showed, liver stores vary proportionally less than muscle stores. Throughout the circadian period, the muscle glycogen and insulin profiles were very similar, thus confirming the relationship between these two parameters. However, unlike muscle glycogen, liver glycogen changes, according to the glucose paradox theory (22), had a temporal pattern that differs from plasma glucose and insulin.

#### Hormones

For hormone data, see Fig. 3 and Table 1. Whereas insulin promotes glucose uptake through peripheral tissues, corticosterone restricts it and promotes liver gluconeogenesis (11). Our results confirmed the presence of significant circadian rhythms for both hormones (1, 2). Like glucose, insulin levels remained low during the light phase but rose before the onset of darkness, with the acrophase a little later than that of glucose. The amplitude of the insulin rhythm (36% of mesor) was much more evident than that of glucose, which showed the high sensitivity of insulin response to slight changes in glucose levels. It has been suggested that corticosterone levels are related to the animal’s activity rhythm (38). We observed that corticosterone began to increase in the middle of the light phase, before the start of feeding, and achieved the acrophase when the lights were turned off.

#### LPL Activity

For LPL activity, see Fig. 4 and Table 1. Cosinor analysis confirmed significant circadian rhythms of LPL activity in all tissues studied. In the 3 h before the lights were turned on and in parallel with food intake and body weight, EWAT LPL activity value doubled at the acrophase, i.e., in the middle of the dark phase. Afterward, LPL activity fell, and as with glucose, light-phase values were lower than dark-phase values, as also observed by Goubern and Portet (14). As previously described (8), heart LPL activity also changed during the circadian period. These changes followed a
significant circadian rhythm, with acrophase in the light phase 180° out of phase with that of EWAT. The mesor value in the heart was 30% that of EWAT, but its relative amplitude (47% of mesor) was higher (38%). Skeletal muscle LPL activity variations were not so clear and were quite different from those of heart LPL activity (24). Nevertheless, skeletal muscle LPL activity followed a circadian rhythm (resembling that of corticosterone), with a peak at the onset of darkness and a mesor value of 15% that of the heart. Goubern and Portet (14) reported that LPL activity in IBAT varied during the circadian period in cold conditions but not at 28°C. In our experimental conditions (21°C), there was a significant circadian rhythm with a mesor twice as high as in EWAT. IBAT LPL activity rose at the end of the light phase, reaching acrophase at the beginning of the dark phase.

LPL activity levels in plasma were very low but also followed a significant circadian rhythm, with the acrophase 3 h before the start of the dark phase, when EWAT LPL activity values were at their lowest. This was the first time plasma LPL activity rhythm had been characterized, since previous studies (26, 29) analyzed postheparinic plasma activity and only at two time points in the day. It is accepted that LPL, originating in extrahepatic tissues, travels through plasma to the liver to be degraded (39). Our results suggest that, in basal conditions, throughout the circadian period LPL activity in plasma comes mainly from adipose tissue because 1) the LPL activity profile of EWAT is the only one complementary to the plasma profile and 2) due to both high activity and total tissue mass (6), the contribution of adipose tissue to total body LPL activity is the highest of all the tissues studied (56–76% vs. 13–26% in skeletal muscle or 7–13% in brown adipose tissue). Therefore the increase in plasma LPL activity could be the consequence of an increase in the release of active LPL from EWAT, although it has never been demonstrated that this mechanism is regulated.
Although adult liver cannot synthesize LPL, it is accepted that active enzymes arriving on the surface of lipoproteins from extrahepatic tissues are captured on the hepatic capillary endothelium. The measurable LPL activity in liver belongs to the proportion of enzyme that is still functional and is briefly retained before its internalization and degradation (39). Thus the profile of liver LPL activity imitated that of plasma and, furthermore, their acrophases overlapped. Liver LPL activity variations also showed significant circadian rhythm.

HL Activity

For HL activity data, see Fig. 4 and Table 1. Liver HL variations did not follow a significant circadian rhythm, but when results are expressed in terms of units of liver weight, a significant circadian rhythm was observed (data not shown), probably due to changes in the size of liver cells in line with variations in glycogen stores. Given that we measured the TAG acylhydrolase activity of HL and that its main activity is phosphohydrolysis, it is remarkable that the mean value of liver HL activity was as high as the mesor of heart LPL. It has been noted that liver is the only tissue in which HL is synthesized, released to the plasma in an inactive form, and captured and reactivated in adrenal glands and ovaries by a mechanism which is still unknown (16). In contrast to liver HL activity, plasma HL activity followed a significant circadian rhythm. Levels remained low during most of the period and only increased during the dark phase of the last interval. The mesor value was almost three times that of LPL.

Temporal Relationships

Figure 5 shows a phase map to clarify the temporal metabolic relationships between the parameters studied. At the start of feeding (at end of light phase, see Fig. 2), levels of circulating glucose rose and led to insulin secretion. The increase in insulin levels stimulated LPL activity in white adipose tissue and the uptake and storage of glucose (as glycogen) in skeletal muscle. However, glycogen stored in liver did not rise until 6–7 h after the acrophase of insulin, in line with the glucose paradox (22). All the subsequent acrophases, in the second half of the light phase, corresponded to parameters concerning the mobilization and use of lipid storage: heart LPL, glycerol, liver and plasma LPL, and ketone bodies. With the start of the active dark phase, the corticosterone acrophase, which started the cycle again with new feeding, was identified.

DISCUSSION

Throughout the circadian period, circulating levels of TAG and cholesterol vary very little, probably due to the equilibrium between ingestion and biosynthesis on one hand and their use by different tissues and excretion on the other. It is possible that lipid-enriched feeding could cause rhythmic variations, as occurs in other cases (25, 28), with acrophases during the feeding phase.

It is difficult to explain the absence of a relationship between the HL activity of liver and plasma. The presence of the rhythm of HL in plasma but not in liver could be understood as a consequence of circadian variation in the enzyme uptake by extrahepatic tissues. Specialized studies (16) on inactivation mechanisms, transport, and possible reactivation and uptake by extrahepatic tissues have not yielded enough information on this question.

During the circadian period, two metabolic situations can be differentiated: short-term fasting and refeeding. During the first half of the light phase, although the animal remained inactive and did not feed, plasma levels of glucose fell. This slight change generated a decrease in circulating levels of insulin and probably a rise in glucagon. In response to hormonal changes, two mechanisms that tend to maintain glycemia switch on: 1) mobilization of glucidic stores, as observed in muscle and liver glycogen, and 2) saving of circulating glucose; the decrease in insulin levels reduces glucose uptake by insulin-dependent tissues (11). Furthermore, the increase in endogenous TAG lipolysis, suggested by the increase in glycerol levels and the decrease in the LPL activity of EWAT, supplies tissues with NEFA, which are used as alternative energy substrates of glucose (11). The increase in LPL activity provides a complementary source of NEFA from circulating TAG for the heart to maintain its continuous, high-energy expenditure. At the same time, plasma and liver LPL rise, which suggests an increase in LPL release from extrahepatic tissues.

In the second half of the light phase, the rise in corticosterone plasma levels restricted even more the use of carbohydrates by peripheral tissues. A concomitant rise in muscle LPL levels allowed this tissue to compete for circulating TAG uptake at a moment when significant.
its glycogen stores were exhausted. Circulating NEFA coming from EWAT lipolysis are transformed in the liver into ketone bodies (such as \( \beta \)-hydroxybutyrate). The plasma concentration of \( \beta \)-hydroxybutyrate rose slightly at the end of the light phase, at which time it is used as an alternative substrate to glucose (11).

A few hours before the onset of the dark phase, the animal awakens, begins physical activity, and eats a little. This small amount of food triggers a set of important metabolic changes: glucose and insulin levels, muscle glycogen, and EWAT LPL activity rise as plasma and liver LPL activity begin to fall. Other processes maintain a tendency typical of slight fasting: corticosterone, glycerol and ketone circulating levels, and muscle LPL activity still increase, liver glycogen still decreases, and the heart maintains a high LPL activity. Thus, before the onset of darkness, feeding metabolic processes coexist with fasting processes. In EWAT, an increase in LPL activity takes place when lipolysis has hardly increased (slightly raised plasma levels of glycerol and ketones); in skeletal muscle, the uptake of glucose and the content of glycogen increase while LPL is still rising. However, in the skeletal muscle, the use of NEFA from circulating TAG due to the raised LPL activity enables the newly uptaken glucose to be used mainly in glycogen synthesis instead of being oxidized. These not strictly opposed variations in catabolic and anabolic processes clarify different degrees of responsiveness to hormones. Finally, with the onset of darkness, the metabolic processes associated with feeding take over with an important increase in feeding and circulating glucose and insulin. Thereby, skeletal muscle LPL activity decreases and the glycogen store recovers. In the liver, glycogen levels increase and ketogenesis diminishes. In EWAT, LPL activity increases and lipolysis decreases, as can be seen from the reduction in plasma glycerol levels.

Perspectives

This paper has sought to offer an integrated view of the temporal organization of LPL and HL activities in the global metabolic environment. LPL activity displays circadian rhythms in various tissues. These rhythms seem to be related to those in intermediate metabolism. The degree of responsiveness of these rhythms to external cues (zeitgebers) as they synchronize with environmental cycles (e.g., light, temperature, food intake, and physical activity) and/or metabolic changes and whether these rhythms originate at the pre- or posttranslational level remain to be determined. The present authors are now studying whether LPL activity rhythms are endogenous, generated by an internal structure, “oscillator,” controlled by a time-encoding part of the central nervous system, “pacemaker,” and are therefore also expressed in constant external conditions.

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