periodical washing during several hours, spontaneous activity was totally restored. Finally, nifedipine (3 μM) suppressed the activity.

Discussion. Spontaneous rhythmic activity has been reported in some preparations of human isolated saphenous vein[4], as well as in those of other human and animal vessels[2,3,5,9,10]. The appearance of such activity may be related to pathophysiological factors. Evidence has been presented that mechanical activity tends to develop in isolated vessels from hypertensive rats[11,12] or after experimental coronary occlusion[13]. On the other hand, the spontaneous rhythmic activity described in human isolated coronary arteries seems to be age-related, since it did not occur in preparations from young subjects[6-8]. Similarly, the study of a large number of saphenous veins enabled us to show that this phenomenon preferentially developed in vessels from old patients. As is the case in other isolated vessels[9,14], spontaneous activity of human saphenous vein was insensitive to adrenergic and histaminergic blockers, ruling out the involvement of catecholamines or histamine. By contrast, the inhibitory effect of aspirin and indomethacin strongly supports the idea that some cyclooxygenase products may be involved in the rhythmic contractions of human saphenous veins. It is known that various prostaglandins can induce rhythmic activity in some isolated vessels[14,15]. Human saphenous veins spontaneously release prostacyclin and thromboxane A_2[16]. Thromboxane A_2 is a potent vasoconstrictor and prostacyclin, in spite of its vasorelaxant effect on most vessels, appears to induce contraction of the human saphenous vein[17]. Lastly, the rhythmic contractile activity of human saphenous vein could be abolished by nifedipine; this observation is in line with the findings that spontaneous activity of other isolated vessels was inhibited by calcium entry blockers[5,8,13,14,18-20] or in Ca-free medium[5,11,12]. As a whole, our results suggest that, in preparations of human saphenous veins isolated from old subjects, some endogenous eicosanoid makes extracellular Ca^{++} available for spontaneous rhythmic contractions.

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VLDL substrate properties and efficiency of their metabolic transformation by LPL

A. D. Dergunov, V. V. Shuvaev and N. V. Perova

Department of Biochemistry, USSR Research Centre for Preventive Medicine, 10 Petroverigski Str., Moscow 101953 (USSR)
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Summary. A study was made of the regulation of the triglyceride hydrolysis catalysed by LPL from bovine milk, by the apoproteins from human plasma VLDL. Both isolated apolipoproteins, and those found on the surface of plasma VLDL particles, were investigated. A concentration-dependent activating action of apo C-II on the hydrolysis of emulsified triolein, and uncompetitive inhibition of VLDL triglyceride hydrolysis by apo C-III were found. It is suggested that VLDL lipolysis might be controlled in vivo trough the variation of the relative surface content of these enzymatic activity modulators.
Key words. Human plasma very low density lipoproteins; apolipoproteins C-II and C-III; lipoprotein lipase.

The involvement of lipoprotein lipase (LPL) in a directional transport of triglycerides (TG) from endogenous very low density lipoprotein (VLDL) and chylomicrons, as a part of a general energy storage pathway, suggests that regulation and control of such an ordered system must occur at the sites of hydrolysis of TG[1]. There is

experimental evidence that one of the minor VLDL apolipoproteins, apo C-II, may exert an activating action on enzymatic hydrolysis whereas another apolipoprotein, apo C-III, may inhibit the activity of LPL on triglyceride substrates[2]. However, the mechanism of their action and the interrelationship between these two metabolic regulators remain unclear. The involvement of VLDL C-apolipoproteins in the regulation of LPL activity during the hydrolysis of artificial and native substrates is described here.

Materials and methods. VLDL preparations were obtained by preparative ultracentrifugation of fresh human plasma[3]. LPL was purified from bovine milk by affinity chromatography on heparin-Sepharose[4]. Kinetic parameters were calculated from the results of the initial velocity studies[5], using linear double reciprocal plots drawn from the experimental data. Two types of substrate were used. The first was 'pseudo-substrate', i.e. triolein/Triton X-100 micelles, in the presence and absence of apo C-II isolated from VLDL apolipoproteins[5]. Measurements were done at a certain fixed concentration of apo C-II with varying concentrations (3–5 points) of triolein emulsified in 0.01% Triton X-100. The reaction mixture contained 0.1 M NaCl, 30 mg/ml human serum albumin, pH 8.3, 0.01% Triton X-100, 125 µM phenol red. The initial velocity of proton accumulation at 30 °C was recorded spectrophotometrically by means of this acid-base indicator at 558 nm[5].

The second substrate was VLDL particles with an altered C-II/C-III ratio. The latter were obtained after tween-20 treatment at non-solubilizing concentrations, with subsequent re-isolation of the particles by ultracentrifugation. Under such conditions the content of the main structural VLDL apolipoprotein, apo B, did not change, whereas apo E and apo C were dissociated from the particles[6]. When VLDL were used as a substrate for LPL, the measurements were made under the above conditions without Triton X-100. The relative content of apo C-II and apo C-III was determined after electrophoretic separation of apolipoproteins in urea at the basic pH values[7]. The protein concentration was determined as in Lowry[8] in the presence of sodium dodecyl sulfate. The TG content was assayed according to Gottfried and Rosenberg[9] or by an enzyme method using the CentrifiChem Test-Triglyceride kits supplied by Baker Instruments (USA).

Results and discusssion. In the presence of 8.5 µg/ml apo C-II, the apparent maximal velocity V_{max}^{app} of the hydrolysis of triolein by LPL in triolein/Triton X-100 micelles was increased 3-fold, as compared to the control value obtained in the absence of apo C-II, while the apparent Michaelis constant K_m^{app} was significantly decreased (table). When the activator concentration was increased up to 72 µg/ml, V_{max}^{app} did not change, and K_m^{app} continuously increased to the control value (table). Consequently, the conformational change in the LPL molecule induced by the activator at a low concentration is responsible for the increase of catalytic activity; this agrees with the results of Posner et al.[10]. When apo C-II was present at a high concentration, besides the saturation of the enzyme with the activator, enzyme/substrate binding worsened, which resulted in an increase of the apparent Michaelis constant. The latter supposition is based on the known experimental data on the reduction of LPL insertion into the phospholipid monolayer when its surface pressure increases[11]. Apo C-II is thought to induce the creation of an additional surface pressure in the VLDL surface monolayer.

The relationship between the content of apo C-II relative to the total apo C in re-isolated VLDL particles after tween-20 treatment, and the initial detergent/protein ratio was studied (fig. 1). The function was found to show a maximum, possibly due to different affinities of apo C-II and apo C-III to VLDL surface. The behaviour of V_{max}^{app} and K_m^{app} values was characterized by similar relationships with some quantitative differences (fig. 1). Figure 2 illustrates that the relative change of K_m^{app} for hydrolysis by LPL of the detergent-treated VLDL particles, as compared to that of the native ones, depends linearly on the relative change of V_{max}^{app}. These results can be related to the uncompetitive inhibition by apo C-III of the enzymatic hydrolysis of TG. This observation excluded the possibility of a direct interaction between the inhibitor and LPL, as was initially proposed by Wang et al.[12], and is in good accordance with the results of Matsuoka et al.[13], who have shown the absence of effective interac-

Relative change of kinetic parameters K_m^{app} and V_{max}^{app} for LPL hydrolysis of emulsified triolein in the presence of apo C-II

apo C-II, µg/ml	8.5	17	46	72
V_{max}^{app}(study)/V_{max}^{app}(control)	3.0	3.4	2.7	3.9
K_m^{app}(study)/K_m^{app}(control)	Very small	0.23	0.53	1.23

In the absence of the activator the apparent maximal velocity was equal to 57.5 ± 7.6 (mean ± SEM; n = 3) µmole H^+/min per mg of LPL protein; the apparent Michaelis constant was equal to 316.9 ± 26.0 µM.

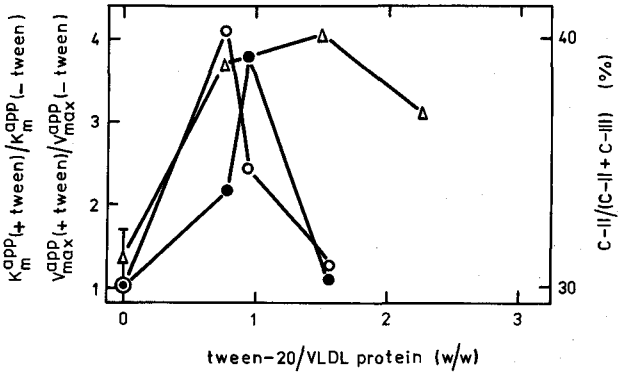

Figure 1. Effect of tween-20 on the relative content of apo C-II among the total apo C (△) and the kinetic parameters K_m^{app} (●) and V_{max}^{app} (○) for LPL hydrolysis of the VLDL particles re-isolated after detergent treatment. The results of a typical experiment are presented. For the native VLDL particles V_{max}^{app} was equal to 48.2 ± 3.3 (n = 3) µmole H^+/min per mg of LPL protein, K_m^{app} was equal to 0.108 ± 0.012 mg/ml.

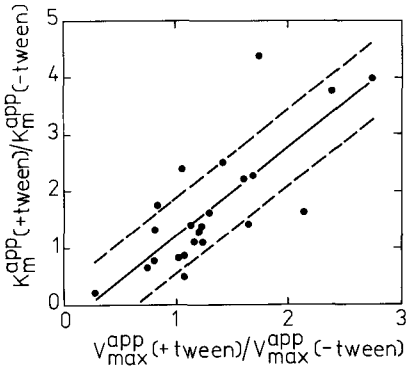

Figure 2. The relationship between the kinetic parameters K_m^{app} and V_{max}^{app} for LPL hydrolysis of the VLDL particles after tween-20 treatment (n = 23). The relation is subordinated to the equation y = 1.54x − 0.34 (r = 0.78). For the native VLDL V_{max}^{app} was equal to 50.5 ± 6.0 µmole H^+/min per mg of LPL protein, K_m^{app} was equal to 0.128 ± 0.036 mg/ml.

tion between apo C-III and the sorbent-immobilized enzyme. Thus, our results suggest an effective interaction between the inhibitor and the enzyme-substrate complex on the particle surface. We may conclude that the apo C-II activator action in the described model experiments is concentration-dependent, and that this behaviour is due to the effect of this apolipoprotein on the lipid phase state of the LPL hydrophobic substrate. This correlates with the observation [14] that in male VLDL, when there is a high HDL cholesterol concentration and a low TG plasma level – indicating that lipolysis is effective – the absolute and relative contents of apo C-II are reduced. In addition, it is suggested that lipolysis in vivo is influenced by the relative surface concentration of apo C-II and apo C-III, the modulators of LPL activity.

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Purification of α-ketoaldehyde dehydrogenase from the human liver and its possible significance in the control of glycation

M. Oimomi, F. Hata, N. Igaki, T. Nakamichi, S. Baba and H. Kato[a]

The Second Department of Internal Medicine, Kobe University School of Medicine, 5-1, 7-chome, Kusunoki-cho, Chuo-ku, Kobe 650 (Japan), and [a] Department of Agricultural Chemistry, The University of Tokyo, 1-1, 1-chome, Yayoi, Bunkyo-ku, Tokyo 113 (Japan)
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Summary. Alfa-ketoaldehyde dehydrogenase, which was extracted and purified from human livers, may act on carbonyl compounds, such as 3-deoxyglucosone, and be involved in the control of glycation (Maillard reaction) in the body.
Key words. α-Ketoaldehyde dehydrogenase; 3-deoxyglucosone; Maillard reaction; glycation and Diabetes mellitus.

Glycation is the nonspecific binding between an amino group of a protein and a reduced sugar. The process of glycation is sometimes referred to as the Maillard reaction [1]. The early-stage products of the Maillard reaction have been used clinically as an indicator of blood glucose control in diabetic patients [2]. This reaction involves repeated rearrangements and dehydrations after the initial early stage, and generates brown pigments, called late-stage products [3], which contain cross-links [4] and have the ability to fluoresce. Recently, it has been reported that these late-stage products exist in the living body [4–7], and they have been studied in connection with the etiology of diabetic complications and aging [8]. In view of the fact that 3-deoxyglucosone (3-DG) [9, 10] and other carbonyl compounds play an important role in the formation of late-stage products in the Maillard reaction, we attempt-