from cultures of CHO-K1 cells stably expressing hLOX-1 (3, 5), but the amount of hsLOX-1 obtained was too small for ELISA development.

hsLOX-1 should be similar to the ECD of hLOX-1 (hLOX-1-ECD), considering a structure of bovine sLOX-1 (3). We produced recombinant hLOX-1-ECD [rhLOX-1-ECD; hLOX-1 (84–273)] in Escherichia coli and purified it as described previously (5); we then used the soluble fraction of rhLOX-1-ECD instead of hsLOX-1 as the assay calibrator and immunogen. We estimated the amount of rhLOX-1-ECD by ultraviolet spectrophotometry, using the formula: \[ E_{280} = 10.0. \]

To produce the K266 and K267 antisera, we immunized 2 female rabbits (KBL:JW; Kitayama Labs) with an emulsion of rhLOX-1-ECD solution in Freund’s complete adjuvant [0.4 mg (0.5 mL) per dose]. Each rabbit received a dose once every 3 weeks for a total of 6 doses.

We used K266 IgG as the immobilized (capture) antibody and horse-radish peroxidase (HRP)-labeled K267 Fab’ as the detection antibody. K267 Fab’ was prepared and labeled with HRP by use of succinimidyl 4-[(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce), as described previously (6). Absorbance ratios at 280 and 403 nm indicated a 1:1 molecular binding ratio of HRP to K267 Fab’. We added the preservative Proclin 150 (final concentration, 1 mL/L; Supelco) to the HRP-labeled antibody, which was stored at −80 °C.

To immobilize the K266 antibody, we added 100 μL of K266 IgG solution (0.01 g/L in 0.1 mol/L phosphate buffer, pH 7.0) to the wells of microplates. The microplates were kept at room temperature for 16 h and then washed twice with 200 μL of assay buffer (0.1 mol/L phosphate buffer (pH 7.0) containing 5 g/L bovine serum albumin (Sigma), 1 g/L CHAPS (Dojindo), and Proclin 150). The plate was then blocked with the assay buffer (200 μL) for 2 h at room temperature.

Calibrator samples (1–100 μg/L rhLOX-1-ECD in commercially available normal human plasma; George King Biomedical) or unknown samples of human serum/plasma (10 μL) were added to 100 μL of assay buffer in duplicate microplate wells containing immobilized antibody. The microplates were then incubated for 2 h at room temperature and washed twice. The HRP-labeled antibody solution (100 μL; diluted to ~900 μg/L in the assay buffer) was then added to the microplate wells and incubated for 16 h at room temperature. We then washed the microplate wells twice and added the substrate solution (100 μL) containing 3,3’,5,5’-tetramethylbenzidine (TMB+; Dako). We allowed the enzyme reaction to take place for 30 min in the dark and then stopped the reaction with 0.5 mol/L sulfuric acid (100 μL). The absorbance at 450 nm was measured by a plate reader (ARVOsx; Wallac/Perkin-Elmer).

We evaluated the effects of endogenous sLOX-1 by assaying 10-μL samples of individual plasmas from 6 healthy volunteers and the commercially available plasma; the differences between the results were negligible. ELISA binding was not changed in plasma samples containing heparin, EDTA, or citrate, and we observed no differences between plasma and serum. Various substances (final concentrations: bilirubin F, 34–170 mg/L; bilirubin C, 42–210 mg/L; hemolytic hemoglobin, 1–5 g/L; chyle, 392–1960 FTU; Interference check-A; International Reagent Corp.) in the calibrator samples (50 μg/L) did not interfere with the ELISA. The presence of oxidized or native LDL (3.2–50 000 μg/L), which may bind sLOX-1 in the calibrator solutions (10 μg/L), had no effect (4).

The ELISA calibration curve indicated a good response to rhLOX-1-ECD (1–100 μg/L) as well as to hsLOX-1 (Fig. 1), suggesting that this ELISA can measure natural forms of human sLOX-1. Intra-/interassay imprecision (as CV) was 2.0%–12%, and measured values were within ~2.5% to +7.0% of the expected values for the range 1–100 μg/L. The limit of quantification was 1.0 μg/L. Plasma (serum) concentrations >0.5 μg/L were detectable; however, de-
tion of concentrations <1 μg/L was unreliable.

rhLOX-1-ECD in plasma at concentrations of 1.79, 8.64, and 41.4 μg/L was stable at −40 °C for 14 weeks and during 3 freeze–thaw cycles.

Our results indicate that the proposed ELISA measured sLOX-1 specifically and sensitively in human serum/plasma and can be used as a diagnostic test for ACS at the earliest stage.

References

To the Editor:
B-Type natriuretic peptide (BNP) and its co-released peptide N-terminal propeptide (NT-proBNP) are both secreted mainly by the left cardiac ventricle as a consequence of pressure overload and wall stretch. This situation often occurs in obesity, in which the amount of intraabdominal fat may worsen the severity of morphologic and dynamic cardiac abnormalities detectable by echocardiography (1).

Many studies have confirmed NT-proBNP as a sensitive marker for left ventricular hypertrophy and/or asymptomatic left ventricular dysfunction (2,3), and it is particularly reliable because of its high negative predictive value (4). To our knowledge, however, recent findings on the relationship between NT-proBNP and morphologic and dynamic cardiac abnormalities in obesity are still inconsistent and controversial. Rivera et al. (5) reported lower NT-proBNP concentrations in obese patients with heart failure compared with nonobese patients. Conversely, Hermann-Arnhof et al. (6) found that NT-proBNP concentrations were increased in obese individuals and were comparable to the values for New York Heart Association class I patients. Therefore, to gain further information on the utility of NT-proBNP as an indicator of possible preclinical cardiac disease in normotensive, severely obese individuals, we measured NT-proBNP concentrations in 27 severely obese women with no complications [mean (SD) body mass index, 43.5 (4.8) kg/m² (median, 41.7 kg/m²); mean (SD) age, 33.3 (8.3) years (median, 31 years)] and 15 normal-weight patients. All participants were premenopausal, normotensive, normoglycemic, drug-free young women with normal renal function, who were not dyspneic; this excluded the possibility that high NT-proBNP

Fig. 1. ELISA calibration curves.
(Left) ELISA calibration curve for rhLOX-1-ECD in the assay buffer (○) and comparison with the dilution curve for sLOX-1 obtained from the conditioned media of hLOX-1-CHO cells (□). Although hsLOX-1 released from hLOX-1-CHO cells was not used as the assay calibrator, its response in the ELISA at various dilutions matched the ELISA calibration curve obtained by use of rhLOX-1-ECD. (Right) calibration curve for rhLOX-1-ECD in normal human plasma (○) and comparison with the dilution curve for a patient serum sample with a high sLOX-1 concentration (□). The responses of serial dilutions of patient serum were comparable to the ELISA calibration curve, thus demonstrating that this ELISA measures sLOX-1 in human blood.