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Apolipoprotein A-IV in the Fed and Fasting States

To the Editor:

Apolipoprotein A-IV (apo A-IV) is a 46-kDa plasma protein (1) with different isoforms (2). It is secreted with chylomicrons into the circulation (3). Within the plasma compartment, apo A-IV dissociates from chylomicrons (4) and associates with HDL, triglyceride-rich lipoproteins, and the lipoprotein-free fraction (5).

Apo A-IV is influenced by dietary intervention, and a low-fat, low-cholesterol diet (NCEP Step II diet) and is able to decrease apo A-IV secretion rates significantly (6). Apo A-IV is catabolized rapidly with a fractional

catabolic rate of ~2.4 pools/day (7). Apo A-IV is higher in patients with renal impairment (8). In ad-libitum-fed rats, serum apo A-IV concentrations showed a circadian rhythm associated with the feeding pattern (9). The role of apo A-IV as a risk indicator in coronary artery disease is currently unclear. Some studies have shown that apo A-IV concentrations are lower in patients with coronary artery disease (10), indicating that apo A-IV is an antiatherogenic protein. However, some questions remain unanswered because apo A-IV is also closely linked to several highly atherogenic situations in humans. For example, high triglyceride concentrations, renal impairment, or fat-enriched diets cause an increase in apo A-IV concentrations.

Because apo A-IV is linked to triglyceride concentrations, we were interested to study apo A-IV concentrations in the fasting and nonfasting state. We studied 28 normolipidemic healthy persons (13 men and 15 women; mean age, 30 years) with normal body weight for 24 h in the fasting and nonfasting states. Apo A-IV and lipoprotein concentrations were determined every 3 h for a total of 24 h by standard techniques as described recently (11). Each study sequence started at 0700 in the morning. For the fasting group, members were asked to skip their usual breakfast and remained fasting for at least 24 h. In contrast, for the nonfasting group, members were permitted to

follow their typical food intake during the study. During prolonged fasting, the apo A-IV concentration decreased to ~50% of the starting concentration, from a mean (SD) of 150 (37) to 84 (26) mg/L (Fig. 1). Triglycerides decreased slightly, reaching a plateau within 6–12 h [decrease from 910 (370) to 750 (220) mg/L]. In the nonfasting state, apo A-IV concentrations were rather stable for 24 h, whereas triglycerides showed an increase over the daytime [between 1080 (530) and 1340 (690) mg/L], which correlated with apo A-IV ($r = 0.238$; $P < 0.01$). We found no circadian rhythm of apo A-IV concentrations in our study participants; however, the decrease in apo A-IV plasma concentrations during extended fasting periods took surprisingly long. The usual 10- to 12-h fasting periods before blood drawing for lipid evaluations do not appear to be sufficient to reach baseline apo A-IV concentrations. Therefore, apo A-IV requires well-defined protocols when this protein is studied.

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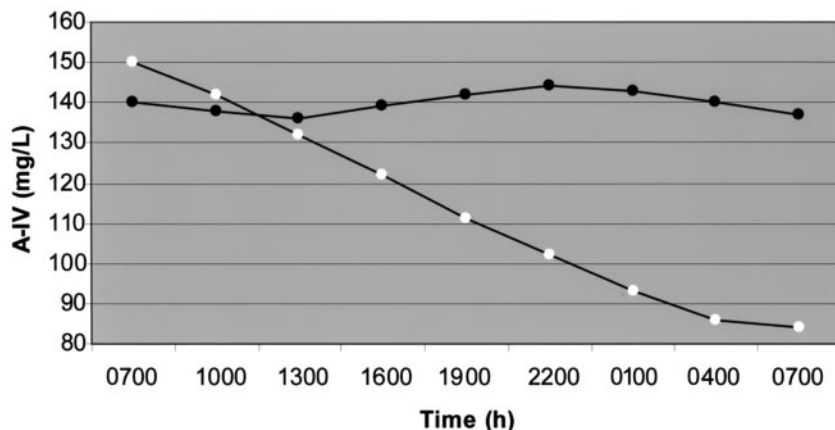


Fig. 1. Apo A-IV concentrations in the fed (●) and fasting state (○).

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Innotrac Aio! Second-Generation Cardiac Troponin I Assay: Imprecision Profile and Other Key Characteristics for Clinical Use

To the Editor:

Cardiac troponin has been designated as the preferred biomarker for diagnosis of myocardial infarction (MI) (1). Previously published data, however, confirm the large diversity among cardiac troponin assays with respect to important analytical characteristics, including assay standardization, antibody specificity, interferences, and assay imprecision, and underscore the need for improved cardiac troponin assays (2, 3). Results obtained with more recently released next-generation assays show that the newer assays indeed have substantially improved analytical performance (4, 5). The aim of this study was to evaluate one of these next-generation cardiac troponin

I (cTnI) assays, performed on the Aio!TM immunoanalyzer (Innotrac Diagnostics Oy), by defining key performance characteristics, including detection limit, linearity on dilution, imprecision, reference interval, and cutoff for MI diagnosis.

The assay is based on "all-in-one" dry chemistry technology, in which all of the reagents are precoated in assay cups, and time-resolved fluorometric detection, with a total analysis time of <20 min (6). An eight-point factory-constructed calibration curve is provided on a bar code with each reagent lot, and the instrument-specific calibration adjustment is performed by running the cups of the appropriate calibration pen. A purified preparation of human cardiac ternary troponin I-troponin T-troponin C complex (HyTest Ltd.) is used as calibration antigen. The antibody configuration of the assay, adding a monoclonal antibody with an epitope in the N-terminal region of cTnI (amino acid residues 20-35) and one with an epitope in the C-terminal region (amino acid residues 185-200) to the mid-fragment cTnI antibodies (epitopes in the region of amino acid residues 35-55 and 80-95), has recently been described, and the potential ramifications of this have been discussed (7, 8).

The Aio! analyzer was handled strictly according to the manufacturer's instructions. Unless otherwise stated, fresh serum was used as sample. The minimum detectable cTnI concentration was assessed by 20 replicate measurements of the cTnI-free diluent in a single run and defined as the cTnI value correspond-

ing to a signal 3 SD greater than the mean found for this sample (9). In the linearity study, five cTnI-rich serum specimens (native cTnI concentrations of 4.3, 9.2, 27.6, 46.9, and 79.5 $\mu\text{g/L}$) were serially diluted with serum pools having undetectable cTnI concentrations, i.e., lower than the detection limit of the Aio! assay, or with the instrument buffer solution. The undiluted sample and four separate dilutions (3:4, 1:2, 1:4, and 1:8) were assayed in duplicate in the same analytical run. The curve obtained was tested for linearity as suggested by Burnett (10). After demonstration of linearity, linear regression analysis of the data was performed, and correlation coefficients (r) were calculated. A recovery study was also performed. For the imprecision study, seven serum pools were prepared and stored at -80°C until used. Two replicates/specimens were analyzed per run, and one run per day for 20 days was performed, using two reagent lots and calibrations (3). Using ANOVA method, we calculated the total CV at different concentrations and used the CV values reported for the seven pools to construct the imprecision profile for the method (3).

To establish reference values, we a priori selected 150 apparently healthy individuals (75 women and 75 men; median age, 60 years; range, 23-89 years), using exclusion criteria as suggested in IFCC recommendations on the theory of reference values (11). Serum cTnI was measured, and the 99th percentile of the value distribution was calculated by non-parametric determination of percentiles. The procedures followed were

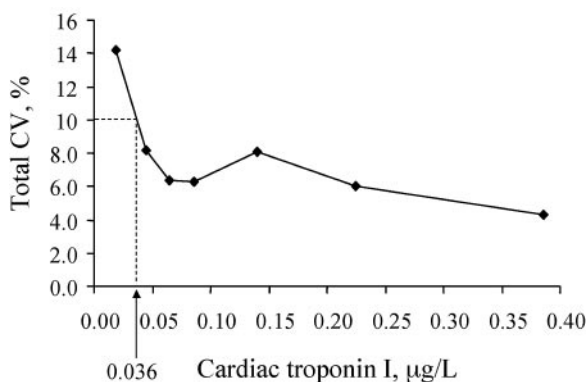


Fig. 1. Imprecision results for human serum pools measured with the Aio! second-generation cTnI assay.

The cTnI concentration corresponding to a 10% total CV is indicated by the arrow.