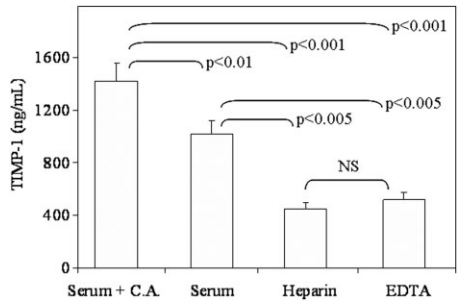

Fig. 1. Effects of blood sampling on the concentrations of TIMP-1. We analyzed 30 healthy volunteers aged 23-55 years (median 36 years). Samples were collected in plastic tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Serum: pure serum prepared in plastic tubes without additives; Serum + C.A.: serum prepared in plastic tubes containing silica particles as clot activators. Plasma was prepared in tubes coated with lithium heparin or potassium EDTA (Heparin and EDTA, respectively). After centrifugation at 1500*g* for 12 minutes at 4°C, the supernatants of samples were analyzed for TIMP-1 concentration by commercially available enzyme-linked immunosorbent assay kit (Biotrak ELISA kit, RPN 2611; Amersham Pharmacia). Columns, mean; Bars, standard errors. Statistical analyses were performed with Prism software (GraphPad Software, San Diego, CA). Differences were compared using a paired *t* test. In all instances, significance was set at $P < 0.05$. Significant differences between samples were indicated.

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Reply:

We thank Drs. Mannello and Jung for their comments regarding the influence of blood sample collection methods on the quantification of matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (TIMP) levels. The activity of these endopeptidases is regulated at the tissue level via various soluble cytokines and interaction with platelets and white blood cells during inflammation and repair. TIMPs form one-to-one noncovalent complexes with MMPs and are widely synthesized by many cells and tissues. Recently, the blood level of various MMPs and TIMPs have been proposed as useful biomarkers for hepatic fibrosis severity. In particular, the tissue and serum levels of TIMP-1 strongly correlate with liver fibrosis severity in hepatitis C virus (HCV) and other liver diseases.[1,2] However, the available TIMP-1 assays recognize both free TIMP-1 and that complexed with circulating MMPs in the blood. Recently, the importance of blood sample handling on the quantification of various MMP and TIMP levels in patients with cancer and atherosclerosis have been reported. For example, MMP-2 and TIMP-2 levels do not substantially differ in plasma versus serum samples whereas MMP-1, MMP-3, and MMP-9 levels are substantially lower in plasma compared to serum.[3,4] The consistently higher levels of TIMP-1 in serum versus plasma may, in part, be due to the release of TIMP-1 from circulating blood elements during the processing and handling of serum samples, as suggested by Manello and Jung in healthy volunteers.

Serum samples in our study were prospectively collected and processed according to a standard, written protocol at the four participating Hepatitis C Antiviral Long-Term Treatment Against Cirrhosis trial (HALT-C) sites. Frozen sera were then assayed for TIMP-1 using a commercial enzyme-linked immunosorbent assay kit. Statistically and clinically significant associations between the serum TIMP-1 levels and severity of hepatic fibrosis by both light microscopy and computerized morphometry were noted.[5] Two serum fibrosis marker panels have also demonstrated significant associations between serum TIMP-1 levels and liver fibrosis severity in patients with HCV, fatty liver, and other liver diseases.[6-8] Therefore, serum was selected as the analyte of choice when the HALT-C study was initiated in 2000, based on the information available at that time. Currently, we are unable to retrospectively determine if serum TIMP-1 levels are consistently higher than plasma TIMP-1 levels in the HALT-C patients and whether any potential differences would alter the utility of this marker in predicting liver fibrosis severity. Going forward, it will be worthwhile to compare the results of serum versus plasma TIMP-1 levels in future studies because the disease state itself may influence assay results in addition to specimen processing. Finally, the utility of TIMP-1 and other proposed noninvasive markers will require validation in longitudinal studies of liver disease progression and clinical outcomes.

ROBERT J. FONTANA, M.D., ON BEHALF OF THE HALT-C STUDY GROUP
Department of Internal Medicine
University of Michigan Medical Center, Ann Arbor, MI

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Reduced Cardio-Respiratory Fitness in Obesity With and Without Nonalcoholic Fatty Liver Disease

To the Editor:

We read with great interest the study by Krasnoff et al. in a recent issue of HEPATOLOGY.[1] The concept that health-related fitness and physical activity are beneficial in patients with nonalcoholic fatty liver disease (NAFLD) and, largely and generally, features of the metabolic syndromes, appears to be intuitive. Nonetheless, the authors address a matter which is very relevant but often disregarded in clinical practice. Therapeutic guidelines for individuals with NAFLD (as well as with simple obesity) suggest weight loss and increased physical activity, but the capacity for performing physical activity is never assessed before prescribing it.

The authors observed worse capacity for cardio-respiratory fitness associated with increasing NAFLD activity and disease severity as well as reduced muscle strength. Thus, it can be argued that caution should be adopted and strict monitoring ensured by a cardiologist when an individual with NAFLD is instructed to a lifestyle program of physical activity. In our experience, children with NAFLD show a significantly reduced capacity for performing physical activity compared with obese insulin-resistant children with no evidence of fatty liver disease. Therefore, a specialized cardiologist should enter the multidisciplinary team which takes care of children with NAFLD.[2]

As far as any causative relation between reduced cardio-respiratory fitness and NAFLD is concerned, several questions remain open for discussion, and they have not been addressed in the study by Krasnoff et al. The study design lacks two groups of normal-weight subjects serving as controls, one with both insulin resistance and NAFLD and the other one with insulin resistance alone, and thus does not allow us to rule out the confounding relations among body weight, insulin resistance, NAFLD, and reduced cardio-respiratory fitness. The question is the extent to which NAFLD impairs the capacity for exercise in respect to insulin resistance and/or obesity without NAFLD. Insulin resistance associates per se with an impaired activity of the sympathetic nervous system,[3] which augments the cardiovascular risk but also reduces the ability to perform physical exercise. Furthermore, the reduced lean mass ratio with body weight, which also characterizes obesity (lean mass, according to table 2 in the article, is approximately 60%-65%), the different proportion of small fibers,[4] and the increased deposition of triglycerides in the muscle tissue coupled with the inability to oxidize them correctly[5] can contribute to the phenomena observed in the study, independent of NAFLD.

In conclusion, we agree completely with the authors about the rationality of recommending physical exercise for patients with NAFLD, although we would emphasize the need to personalize any program of physical exercise along with monitoring the individual response in the clinical practice, and we encourage dissecting the single contributions of insulin resistance, impaired body composition, and NAFLD to reduced cardio-respiratory fitness in future research studies.

MELANIA MANCO, M.D., PH.D.[1]
VALERIO NOBILI, M.D.[2]
[1]Scientific Directorate and [2]Liver Unit
Bambino Gesù Hospital and Research Institute, Rome, Italy

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Reply:

We thank Drs. Manco and Nobili for their interest and thoughtful comments on our article examining the health-related fitness and physical activity participation of patients with a histological spectrum of nonalcoholic fatty liver disease (NAFLD).[1] It is noteworthy that they have observed similar findings of significantly reduced capacity to perform physical activity in children with NAFLD compared to obese insulin-resistant children without NAFLD.

We applaud the integrated care model they have implemented in their outpatient NAFLD clinic.[2,3] Indeed, a cardiologist attending to cardiovascular comorbidities (for example, hypertension) in persons with NAFLD would enhance patient care. However, par-