and others have shown IL-6 to be elevated with exercise in younger children and adolescents (3,4), and Perez Navero *et al.* (5) reported increased IL-6 following a soccer practice in 6- to 7-year-old boys.

One problem that might explain the unexpected negative results in boys lies, perhaps, with the sensitivity of their assay. This is a relevant concern with IL-6 because in healthy individuals at rest normal values in circulating blood are in the range of 1–20 or so pg/ml, whereas with infection or very strenuous exercise, these values can increase by several orders of magnitude.

Timmons *et al.* reported using the commercially available Human IL-6 ELISA system from Endogen (Woburn, MA). Although this assay is listed as having a range from 1–400 pg/ml, the lowest value of the standard curves provided by the company is 10.24 pg/ml. Extrapolating below this level without further dilution and creating an appropriate standard curve could lead to errors. Detecting changes in IL-6 at these low levels, even a doubling or tripling of circulating levels as may occur with light exercise, may not be feasible. Figure 3a in the Timmons article reveals that IL-6 levels were on average greater than 10 pg/ml only for the men post-exercise and in recovery, all of the samples obtained from the boys were, on average, below what appears to be the detectable range of the assay.

The problem of detecting low circulating levels of IL-6 has been observed by other investigators. For example, Ishiguro *et al.* (6) studying acute infections in children recently noted, "Concentrations of IL-6 were quantified by a commercially available ELISA system for infectious patients (Endogen, Woburn, MA, USA). IL-6 was not detectable in most control subjects by this system, and so we used a more sensitive ELISA system."

Timmons *et al.* also noted that, "In our experience, some samples from the boys (9 of 54 for IL-6. . .) and from the men (7 of 54 for IL-6. . .) were below the detection level of the respective kit and were, therefore, set to the lowest positive number on the standard curve derived from the plate on which the sample was determined."

In our own investigations of IL-6 responses to exercise in children in adolescents, we noted this sensitivity problem several years ago and when studying healthy subjects, we use an ELISA with a range of 0.56 to 10 pg/ml. The standard curves of this assay permit accurate measurements of IL-6 in a range likely to be encountered in healthy adults and children exposed to moderate exercise for relatively brief periods of time.

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Response

To the Editor: We would like to thank Cooper and colleagues for their letter regarding our recent article (1). In their letter, Cooper and colleagues raised a concern regarding our method of measuring plasma interleukin-6 (IL-6) and the sensitivity of the chosen assay. The authors are correct to point out that the ELISA kit from ENDOGEN suggests a standard curve between 10.24 and 400 pg/mL. We too were aware that plasma levels of IL-6 in humans tend to be low and, therefore, included a dilution to 5.12 pg/mL and excluded the highest 400 pg/mL concentration from each standard curve. With this approach our correlation coefficients for the standard curve regression equations ranged from 0.9954 to 0.9971 demonstrating excellent linearity and, therefore, providing confidence in extrapolating values between 0 and 5.12 pg/mL.

However, based on the concern of Cooper and colleagues and their assertion that exercise should increase circulating levels of IL-6 in children, we returned to our raw data and analyzed the absorbance units derived from the plate reader, before submitting to the standard curve equations. With this approach, our conclusions remained the same insofar as the absorbances after exercise ($p = 1.0$) and during recovery ($p = 0.9$) were not different than at rest for the boys, but were significantly higher after exercise ($p = 0.001$) and during recovery ($p = 0.0009$) than at rest in the men.

In their letter, Cooper and colleagues point out that IL-6 is reproducibly elevated with exercise in children and apparently dismiss our findings as "unexpected negative results." We would like to respectively address the evidence as provided by the authors. First, the study by Perez Navaro *et al*. (2) apparently measured IL-6 levels in saliva, which is not relevant to the discussion of circulating IL-6 levels. Second, Nemet *et al*. (3) investigated cytokine responses in boys ranging in age from 14 to 18.5 y (mean age of 16.5). It is very likely that most, if not all, of these boys were in advanced stages of puberty. If, as we hypothesized in our paper, maturity status plays a role in the magnitude of exercise-induced immune changes, one would expect this

group to respond similarly to adults. Therefore, it is interesting to note that their reported cytokine levels, ~1.4 pg/mL before exercise to ~11.0 pg/mL after exercise are strikingly similar to our own results from men (Figure 3a). Finally, the paper by Scheet *et al.* (4) reported an exercise-induced change in circulating IL-6 of ~1.6 pg/mL in children ~10 y-old. Unfortunately, it was not indicated whether changes in plasma volume had occurred or were accounted for, which would have further minimized the degree of change. Moreover, another paper (5) from Dr. Cooper's laboratory reported an exercise-induced change of ~1 pg/mL in 8- to 15-y-olds. Therefore, we do not regard our findings as "negative results," but rather consistent with the available data, regardless of measurement method, suggesting that young children may be relatively resistant to major cytokine changes with exercise.

In closing, much more research is required to delineate the cytokine, in particular IL-6, response to exercise in young children. This should prove to be a fruitful area of research in light of the emerging role for IL-6 in metabolic adaptations to exercise in adults. The influence of growth and maturation on differences or similarities in these responses may have important implications for several childhood diseases.

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