To the Editor: We read with great interest the recent article by Timmons *et al.* (1) on stress/inflammatory responses to exercise in boys and men. While we applaud their efforts to directly compare subjects from these two age groups to research maturational mechanisms of immune responses, one aspect of their data caused us some concern. The authors report that the exercise bout had no effect on circulating interleukin-6 (IL-6) in the boys. This was troubling because of the many cytokines previously reported to be altered acutely by exercise, IL-6 has proved to be the most reproducibly elevated (2). Moreover, we

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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References

1. Timmons BW, Tarnopolsky MA, Bar-Or O 2004 Immune responses to strenuous exercise and carbohydrate intake in boys and men. Pediatr Res 56:227–234
2. Shephard RJ 2002 Cytokine responses to physical activity, with particular reference to IL-6: sources, actions, and clinical implications. Crit Rev Immunol 22:165–182
3. Scheett TP, Mills PJ, Ziegler MG, Stoppani J, Cooper DM 1999 Effect of exercise on cytokines and growth mediators in prepubertal children. Pediatr Res 46:429–434
4. Nemet D, Oh Y, Kim HS, Hill MA, Cooper DM 2002 The effect of intense exercise on inflammatory cyotkines and growth mediators in adolescent boys. Pediatrics 110:681–689
5. Perez Navero JL, Jaraba Caballero S, Ibarra de la Rosa I, Jaraba Caballero MP, Guillen del Castillo M, Montilla Lopez P, Tunez Finana I, Romanos Lezcano A 1999 [Effects of competitive physical exercise on neuroendocrine response and interleukin-6 liberation in children]. An Esp Pediatr 51:267–272
6. Ishiguro A, Suzuki Y, Mito M, Shimbo T, Matsubara K, Kato T, Miyazaki H 2002 Elevation of serum thrombopoietin precedes thrombocytosis in acute infections. Br J Haematol 116:612–618

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