Circulating Leptin during Experimental Endotoxemia in Humans

To the Editor—Bornstein et al. [1] recently reported that plasma leptin levels did not change significantly from baseline values in human subjects injected with endotoxin. We also investigated whether leptin levels are different in the circulation of endotoxin-injected compared with saline-injected healthy human volunteers.

Non-obese, male volunteers between the ages of 18 and 30 years old were enrolled. An intravenous catheter was placed in the forearm of each subject after they fasted overnight in the hospital. In 3 volunteers, 3 ng/kg US standard reference endotoxin (lot EC-5, *Escherichia coli* O113; Bureau of Biologics, US Food and Drug Administration, Bethesda, MD) was given at 9:00 A.M. as an intravenous bolus injection; 3 subjects injected with saline under identical conditions served as controls. Each volunteer fasted until 3:00 P.M. At the time of injection and 0.5, 0.75, 1, 1.5, 2, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, 11, and 12 h thereafter, blood samples were obtained from the catheters, added to EDTA-containing tubes on ice, and centrifuged for 10 min at 450 *g*. The plasma was recentrifuged at 14,000 *g* to remove platelets. The platelet-poor plasma was stored at −70°C until assayed. Plasma samples were assayed for leptin by use of an ELISA (Margery Nicolson, Amgen, Thousand Oaks, CA). The limit of detection for the ELISA was 50 pg/mL.

As previously reported [2, 3], volunteers injected with 3 ng/kg endotoxin developed transient chills and generalized myalgias after 1–2 h. Fever reached peak levels after 4–5 h and then decreased over the next 6 h. Significant increases in circulating interleukin (IL)-1β, IL-1 receptor antagonist, IL-6, IL-8, tumor necrosis factor-α (TNF-α), and granulocyte colony-stimulating factor were detected. No changes were seen in the circulating cytokine levels in saline-injected volunteers. As shown in figure 1, circulating leptin levels were similar in the endotoxin- and saline-injected volunteers.

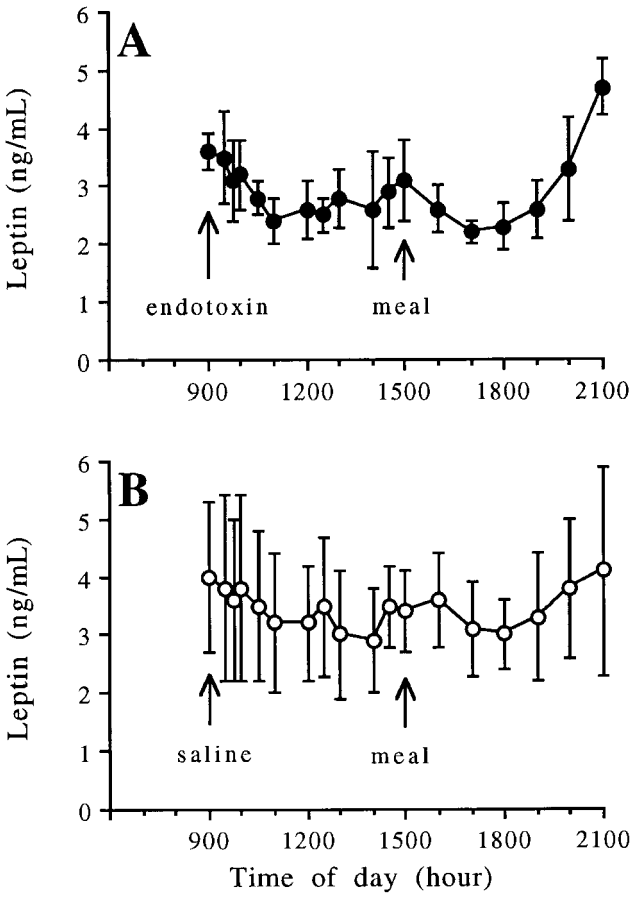

Figure 1. Changes in plasma leptin levels after intravenous injection of endotoxin. Healthy human volunteers were injected with 3 ng/kg endotoxin (*A, n* = 3) or saline (*B, n* = 3). At time of injection and at different times afterwards, plasma was isolated. Leptin levels were measured by ELISA. There were no significant changes in leptin levels over time in either group, nor were there significant differences between groups.

Using the same endotoxin preparation (albeit at a lower dose), our results corroborate and extend the findings of Bornstein and colleagues [1] that plasma leptin levels do not change significantly from baseline values in human subjects injected with endotoxin. Whereas their study measured circulating leptin for 6 h, we demonstrate that leptin levels do not increase in the 12 h after endotoxin injection. These results were unexpected when compared with previous findings. For example, circulating leptin levels are increased in patients with sepsis [4]. In hamsters and mice, endotoxin injection results in increased levels of plasma leptin and adipocyte leptin mRNA [5, 6]. Because leptin mRNA and circulating leptin are also higher in TNF-α–injected humans [7] and animals [5, 6], endotoxin-induced increases in transcription and translation have been attributed to this pro-inflammatory cytokine. Surprisingly, exposing 3T3-L1 murine adipocytes to TNF-α results in a decline in steady-state leptin mRNA [8, 9]. This observation suggests that in vivo TNF-α indirectly up-regulates leptin gene expression.

Meal shifts may have obscured an endotoxin-induced increase in circulating leptin by delaying the rise until >12 h after the endotoxin injection [10]. By omitting nocturnal blood samplings, we may have missed the changes. As discussed previously [1], the dose of endotoxin used in the human studies may have been insufficient to stimulate leptin synthesis or release. Perhaps endotoxin induces leptin in rodents but not in humans because of interspecies differences in endotoxin sensitivity. Prolonged endotoxemia superimposed on chronic illness may explain the elevations in plasma leptin observed in patients with sepsis but not in endotoxin-injected volunteers. In addition, renal or hepatic dysfunction may also inhibit the clearance of circulating leptin in sepsis.

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