Insulin-Like Growth Factor Binding Protein–Related Protein 1 (IGFBP-rP1/MAC25) Is Linked to Endothelial-Dependent Vasodilation in High-Ferritin Type 2 Diabetes

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Type 2 diabetes is characterized by variable degrees of vascular dysfunction (1,2). We have recently reported improved vasomotor responses in high-ferritin type 2 diabetic patients following bloodletting (3), an intervention that is believed to reduce the deleterious effects of circulating iron on vascular function (4,5).

Insulin-like growth factor binding protein–related protein 1 (IGFBP-rP1) is a 30 kDa modular glycoprotein known to be secreted by vascular and nonvascular cells (6–8). Besides its involvement in developmental processes and tumor growth (9,10), IGFBP-rP1 may play a role in the vasculature, given its abundance as an endothelial marker (11–13), its stimulatory actions on prostacyclin synthesis (7), and its vasodilatory effects on the retina of diabetic rats (14).

To our knowledge, no clinical studies have examined the role of serum IGFBP-rP1 as a vascular factor. We hypothesized that circulating IGFBP-rP1 is linked to the vasomotor responses that follow iron depletion in high-ferritin type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The study subjects (n = 24 male patients, mean ± SD age 55.5 ± 8.1 years, BMI 29.1 ± 3.6 kg/m^2, and A1C 6.2 ± 0.9%), are part of a well-characterized sample of high-ferritin type 2 diabetic patients subjected to bloodletting as previously reported by us (3,15). In the original cohort, 28 diabetic patients with elevated serum ferritin concentrations were randomized to either iron depletion (intervention group; n = 13) or to observation (n = 15) according to a randomization table that included age, BMI, and A1C. The two groups were also matched for pharmacological treatment and chronic diabetes complications. The iron depletion intervention consisted of three blood extractions (each 500 ml) at 2-week intervals. The patients were studied at baseline and 3 months after the last phlebotomy. In the present study, available serum samples were used for measuring IGFBP-rP1 in 10 patients in the intervention group and 14 patients in the observation group (online appendix Table 1 [available at http://dx.doi.org/10.2337/dc06-1905]). The clinical and biochemical characteristics of these subjects were comparable with those of the initial cohort (3).

Subjects were studied in the postabsorptive state. Anthropometry, blood pressure, and insulin sensitivity (K_{ITT}, from insulin tolerance tests) were assessed as previously reported (15). Brachial artery vascular reactivity was assessed by a high-resolution external ultrasound (Toshiba SSH-140A) in response both to reactive hyperemia (flow-mediated endothelium-dependent vasodilation [EDV]) and to 400 μg sublingual glyceryl trinitrate, a direct smooth muscle dilator (endothelial-independent vasodilation [EIV]), as previously described (3,16).

Serum glucose, insulin, C-peptide, transferrin, transferrin saturation index, ferritin, and A1C were measured as previously reported (3,15). Whole-blood hemoglobin and hematocrit were determined by routine laboratory tests (Coulter Electronics, Hialeah, FL). Serum IGFBP-rP1 was measured by an enzyme-linked immunosorbent assay with a coefficient of variation <7% (13).

Statistical analyses were performed using SPSS 12.0 software. The study was powered to detect significant changes in serum IGFBP-rP1 of at least 1 SD (paired-samples t test) following phlebotomy.

The experimental protocol was approved by the ethics committee of the Hospital of Girona. Informed written consent was obtained from the study subjects.

RESULTS

— At baseline, circulating IGFBP-rP1 was directly correlated with EDV (r = 0.48, P = 0.018) but not with EIV (r = −0.08, P = NS) in high-ferritin type 2 diabetic subjects studied as a single group. IGFBP-rP1 was also correlated

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Abbreviations: EDV, endothelium-dependent vasodilation; EIV, endothelial-independent vasodilation; IGFBP-rP1, insulin-like growth factor binding protein–related protein 1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

with BMI ($r = 0.57$, $P = 0.003$) and metabolic parameters (C-peptide [$r = 0.58$, $P = 0.004$] and K_{ITT} [$r = -0.46$, $P = 0.034$] but not A1C [$r = 0.03$, P = NS]) in these subjects.

As expected, bloodletting caused significant reductions in hemoglobin, ferritin, and transferrin saturation index in the intervention group ($P < 0.05$ to $P < 0.001$). In parallel, both serum IGFBP-rP1 and EIV significantly increased in the intervention group but not in the observation group ($P < 0.05$ and $P < 0.01$, respectively; online appendix Table 2). Figure 1 depicts significant differences in the absolute changes at 4 months for hemoglobin, IGFBP-rP1, and both EDV and EIV between the two randomization groups ($P < 0.05$ to $P = 0.001$). Of note, the changes in IGFBP-rP1 correlated with those of hemoglobin ($r = -0.59$, $P = 0.009$) and of EDV ($r = 0.58$, $P = 0.005$) at follow-up.

The novel association between hemoglobin and IGFBP-rP1 was also documented for baseline values in these subjects ($r = 0.61$, $P = 0.002$), as well as in a cross-sectional analysis of a sample of nondiabetic men previously reported by us ($n = 113$; $r = 0.28$, $P = 0.003$ [17]).

On multiple regression analyses, both BMI ($\beta = 0.47$, $P = 0.01$) and hemoglobin ($\beta = 0.40$, $P = 0.03$), but not other metabolic parameters or ferritin, were independently associated with baseline serum IGFBP-rP1, explaining 34 and 12% of its variance, respectively. Only age ($\beta = -0.51$, $P = 0.004$) and IGFBP-rP1 ($\beta = 0.51$, $P = 0.004$) were significantly associated with baseline EDV, explaining 24 and 20% of its variance, respectively, after adjusting for effect modifiers (basal artery diameter, smoking, BMI, C-peptide, insulin sensitivity, A1C, hemoglobin, or ferritin). Multivariate analyses documented also independent associations between the changes in IGFBP-rP1 and those in hemoglobin and EDV on follow-up (data not shown).

CONCLUSIONS

— Our results suggest for the first time that circulating IGFBP-rP1 is related to vascular function in high-ferritin type 2 diabetic patients. Bloodletting is associated with improved vasomotor responses (3) and with positive changes in circulating IGFBP-rP1.

Our data concur with previous reports showing stimulatory effects of IGFBP-rP1 on prostacyclin synthesis (7) and on blood flow in the rat retina (14) and suggest that factors associated with endothelial stress, namely obesity and blood hemoglobin, are possible regulators of the serum levels of IGFBP-rP1 in high-ferritin type 2 diabetic subjects (2,18). While it is well known that cell-free hemoglobin acts as a nitric oxide scavenger (19,20), the role of cell-associated hemoglobin (i.e., that carried by erythrocytes) is more complex, as it may act as a nitric oxide generator by virtue of its nitrite reductase activity (21). The current clinical evidence, however, is supportive of a deleterious effect on vascular function also for cell-associated hemoglobin (22,23).

Given the cross-sectional nature of our study, we cannot establish a cause-effect relationship in the sequence of events that follow bloodletting in high-ferritin type 2 diabetic patients, but it is herein suggested that phlebotomy causes increases in circulating IGFBP-rP1 by alleviating the deleterious effects of blood hemoglobin on the vasculature and that increased IGFBP-rP1, in turn, may contribute to improve EDV after bloodletting. Further research will determine whether there is a role for IGFBP-rP1 in the vascular dysfunction of type 2 diabetes.

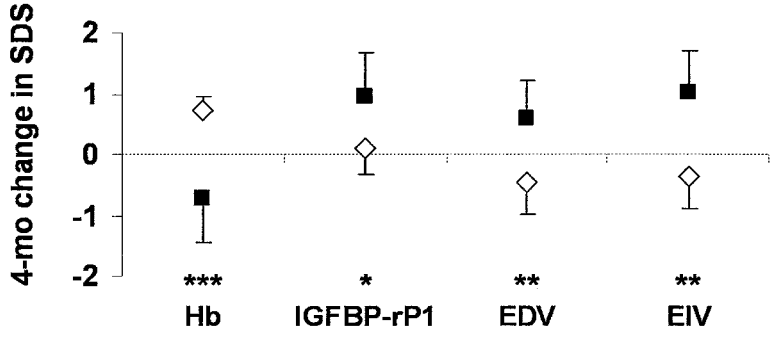

Figure 1— *Changes in hemoglobin (Hb), IGFBP-rP1, EDV, and EIV from baseline following 4 months of bloodletting (■; intervention,* n = *10) or no treatment (◇; observation,* n = *12). (In the observation group, two outlier subjects with significant decreases in hemoglobin values were not included in the analysis; thus, the resulting* n *for this group was 12.) Changes are expressed as SD scores (SDS), calculated by dividing the absolute changes during the 4 months by the corresponding baseline SD in the study subjects. Plots represent means ± 95% CI.* *P < *0.05,* **P < *0.01,* ***P < *0.001.*

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