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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Running title: IGFBP-rP1 and vascular reactivity

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Type-2 diabetes mellitus (T2DM) is characterized by variable degrees of vascular dysfunction (1; 2). We have recently reported improved vasomotor responses in high-ferritin T2DM patients following blood letting (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 non-vascular 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 T2DM.

**RESEARCH DESIGN AND METHODS**

The study subjects [n=24 male patients; age (mean ± SD): 55.5 ± 8.1 y; body mass index (BMI) 29.1 ± 3.6 kg/m2; HbA1c 6.2 ± 0.9%], are part of a well characterized sample of high-ferritin T2DM patients subjected to blood letting and previously reported by us (3; 15). In the original cohort, twenty-eight 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 HbA1c. The two groups were also matched for pharmacological treatment and chronic diabetes complications The iron depletion intervention consisted of three blood extractions (each of 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 (supplementary Table 1). The clinical and biochemical characteristics of these subjects were comparable to those of the initial cohort (3).

Subjects were studied in the postabsorptive state. Anthropometry, blood pressure and insulin sensitivity ($K_{IT}$, 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, Japan) in response both to reactive hyperemia (flow-mediated endothelium dependent dilation, EDV) and to 400 µg of sublingual glyceryl trinitrate, a direct smooth muscle dilator (EIV), as previously described (3; 16).

Serum glucose, insulin, C-peptide, transferrin, transferrin saturation index, ferritin and HbA1c were measured as previously reported (3; 15). Whole-blood hemoglobin (Hb) and hematocrit were determined by routine laboratory tests (Coulter Electronics, Hialeah, FL). Serum IGFBP-rP1 was measured by an ELISA with CV <7% (13).

Statistical analyses were performed using SPSS 12.0 software. The study was powered to detect significant changes in serum IGFBP-rP1 (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 endothelial-independent vasodilation ($EIV; r=-0.08$, NS) in high-ferritin T2DM subjects, studied as a single group. IGFBP-rP1 was also correlated 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 HbA1c ($r=0.03$, NS)] in these subjects.

As expected, blood letting caused significant reductions in Hb, 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, but not in the observation group ($P<0.05$ and $P<0.01$, respectively; supplementary Table 2). Figure 1 depicts significant differences in the absolute changes at 4 mo for Hb, 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 Hb ($r=-0.59$, $P=0.009$) and of EDV ($r=0.58$, $P=0.005$) at follow-up.

The novel association between Hb 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 non-diabetic 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 Hb ($\beta=0.40$, $P=0.03$), 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, HbA1c, Hb or ferritin). Multivariate analyses documented also independent associations between the changes in IGFBP-rP1 and those in Hb and EDV on follow up (not shown).

CONCLUSION

Our results suggest for the first time that circulating IGFBP-rP1 is related to vascular function in high-ferritin T2DM. Blood letting 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 Hb, are possible regulators of the serum levels of IGFBP-rP1 in high-ferritin T2DM subjects (2; 18). While it is well known that cell-free Hb acts as a nitric oxide scavenger (19; 20), the role of cell-associated Hb (i.e. that carried by red blood cells) 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, are supportive of a deleterious effect on vascular function also for cell-associated Hb (22; 23).

Given the cross-sectional nature of our study, we cannot establish a cause-effect relationship in the sequence of
events that follow blood letting in high-ferritin T2DM, but it is herein suggested that phlebotomy causes increases in circulating IGFBP-rP1 by alleviating the deleterious effects of blood Hb on the vasculature and that increased IGFBP-rP1, in turns, may contribute to improve endothelium-dependent vasodilation after blood letting. Further research will determine whether there is a role for IGFBP-rP1 in the vascular dysfunction of diabetes mellitus.

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FIGURE LEGEND

Fig. 1: Changes in hemoglobin (Hb), IGFBP-rP1 and endothelium-dependent (EDV) and independent vasodilation (EIV) from baseline following 4 mo blood letting (intervention) or no treatment (observation). Changes are expressed as SD scores, calculated by dividing the absolute changes during the 4 mo by the corresponding baseline SD in the study subjects. Plots represent means ± 95% CI. *P<0.05, **P<0.01, ***P<0.001. Note: In the observation group, two outlier subjects with significant decreases in Hb values were not included in the analysis and the resulting n for this group was 12.