Insulin infusion increases levels of free IGF-I and IGFBP-3 proteolytic activity in patients after surgery

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DURING THE PAST YEARS, it has become clear that the catabolic change in metabolism after trauma is not solely regulated by the catabolic stress hormones but also by a complex set of changes in the anabolic regulators of metabolism. Thus marked reductions in the levels of insulin-like growth factor (IGF) I have been reported to occur after burn trauma (24), in critical illness (35), and after major surgical procedures (31). However, to establish the biological significance of IGF-I levels in serum, the simultaneous changes in the IGF-binding proteins (IGFBP) must be considered.

Levels of IGFBP-1 are increased after different types of trauma, such as burn injury (24) and surgery (30), and this increase may inhibit IGF-I activity by keeping the hormone bound (22, 23, 33). In healthy subjects, insulin is the main regulator of IGFBP-1 by inhibiting IGFBP-1 production (6). It is not known whether the increase in IGFBP-1 after trauma may be explained by an impaired sensitivity to the inhibitory effect of insulin on IGFBP-1 production or whether other factors, such as an increase in glucagon or cytokines, may further upregulate IGFBP-1 (15, 21, 36). In healthy subjects, most IGF-I is bound to IGFBP-3 and an acid-labile subunit in a ternary complex, and <1% is believed to be present in the free form. IGFBP-3 proteolytic activity (IGFBP-3-PA) reduces the affinity of IGF-I to IGFBP-3, and this has been shown to increase IGF-I bioactivity in vitro (5). IGFBP-3-PA has been shown to increase in a number of catabolic conditions, such as non-insulin-dependent diabetes mellitus (1), severe illness (8), and after surgery (7). In a recent study in surgical patients, no postoperative decrease was found in free IGF-I (fIGF-I), despite decreased levels of total IGF-I (tIGF-I) while IGFBP-3-PA increased, suggesting a role for IGFBP-3-PA in the maintenance of IGF-I after surgery (31). Moreover, we recently demonstrated that IGFBP-3-PA was increased in response to insulin infusion during a hyperinsulinemic, normoglycemic clamp performed on the day after abdominal surgery, suggesting that insulin may affect IGF-I bioavailability by induction of IGFBP-3-PA in postoperative patients (4).

The aims of the present study were to investigate how IGF-I bioavailability was affected by an insulin challenge after elective surgery and whether the changes were dependent on insulin sensitivity. Two different methods for measuring fIGF-I, ultracentrifugation and immunoradiometric assay (IRMA), were

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used, since these methods have been shown to provide slightly different results depending on whether changes in IGF-I availability were accomplished by changes in IGFBP-1 or IGFBP-3-PA (11, 14). The insulin challenge was performed as a hyperinsulinemic, normoglycemic clamp in patients before surgery and in the same patients with reduced insulin sensitivity on the day after surgery. The responses of tIGF-I, fIGF-I, fIGF-II, and IGFBP-1 were compared with previously reported changes in IGFBP-3-PA in an attempt to estimate changes in IGF bioavailability. We have demonstrated that, in the insulin-resistant state after surgery, insulin-induced IGFBP-3-PA is associated with an increase in levels of fIGF-I. In addition, we report that the inhibition of IGFBP-1 by insulin is largely intact in the insulin-resistant state after surgery.

MATERIALS AND METHODS

Patients. Eighteen patients scheduled for elective abdominal surgery were studied. Their surgical diagnoses (although not in an acute inflammatory stage of the disease) were inflammatory bowel disease (n = 8) and diverticular disease of the sigmoid colon (n = 6). The remaining subjects underwent surgery for hereditary colon polyposis (n = 3) and a hypernephroma of the right kidney (n = 1). The surgical and anesthetic procedures, as well as the perioperative care of the patients, have been presented elsewhere (4). We also previously reported data on insulin sensitivity and changes in IGFBP-3-PA and the circulating forms of IGFBP-3 (4). Briefly, eight patients (45 ± 5 yr old, 24 ± 1 kg/m² body mass index, 2 men and 6 women) were given 800 ml of an isosmolar carbohydrate-rich oral glucose load (12.5% carbohydrates; Numico, Zoetermeer, The Netherlands) on the evening before surgery and an additional 400 ml ±2 h before the induction of anesthesia (OGL group). Ten patients (45 ± 4 yr old, 24 ± 1 kg/m² body mass index, 4 men and 6 women) underwent surgery after fasting overnight (Fast group). All patients were subjected to a hyperinsulinemic, normoglycemic (4.5 mM), two-step clamp (insulin infusion rates 0.3 and 0.8 mU·kg⁻¹·min⁻¹ for 120 min at each level) twice after fasting overnight, once on the day before and once on the day after surgery. The glucose infusion rate required to maintain normoglycemia during the last 60 min of the clamp was used as a measure of insulin sensitivity (29). The study protocol was approved by the Institutional Ethical Committee at the Karolinska Hospital, and the subjects gave their informed consent before entering the study.

Sampling and analysis. Arterialized blood from a heated hand vein was collected as described elsewhere (34). Glucose was sampled at baseline and at least every 10 min during clamping and was measured immediately on collection using the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH) (17). Serum insulin was analyzed by radioimmunoassay (RIA) using an antibody developed in our laboratory (13). Serum IGFBP-1 (28) was analyzed using RIA methods. Serum tIGF-I was extracted with acid ethanol and then analyzed by RIA. Serum fIGF-I was extracted with acid ethanol and then analyzed by RIA technique developed in our own laboratory, with des-(1–3)-IGF-I as a radioligand to prevent interaction of IGFBPs (3). IGFBP-3-PA was determined as the ability of serum to degrade 125I-labeled human recombinant glycosylated IGFBP-3 as reported elsewhere (4), on the basis of the original report by Lamson et al. (20). Serum samples were allowed to clot, while plasma samples were centrifuged immediately at 4°C at 3,000 rpm for 10 min and stored at −20°C for subsequent batch analyses. tIGF-I, fIGF-I, fIGF-II, and IGFBP-3-PA were sampled immediately before and after 210 min of insulin infusion. Insulin and IGFBP-1 were sampled at baseline and every 30 min during the clamps (±0, 60, 90, 120, 180, 210, and 240 min). In comparisons with other parameters, we used insulin and IGFBP-1 sampled at the corresponding time of the study.

A commercially available kit (DSL, Webster, TX) was used to determine free dissociable IGF-I (fdIGF-I). The term “free dissociable” IGFs was chosen, since the theoretical fIGF-I fraction is much less than measurements of fIGF-I suggest, and some extraction of IGFs from binary complexes with IGFBPs is thought to take place (18). This assay uses immobilized anti-IGF-I-catch antibodies, which presumably recognize only unbound IGF-I.

Serum fIGF-I and fIGF-II were also determined by ultrafiltration and centrifugation as previously described (11). Amicon YMT 30 membranes and MPS-1 supporting devices were used (Amicon Division, W. R. Grace, Beverly, MA). Before centrifugation, serum samples were diluted in Krebs-Ringer buffer, which had been adjusted to pH 7.4 by airing with CO₂. From each dilution, an aliquot of 600 μl was applied to the membranes, incubated for 30 min at 37°C, and centrifuged at 1,500 rpm at 37°C (Hettich Zentrifugen, Tuttlingen, Germany). Ultrafiltrates were collected in 5-ml polyethylene tubes that were coated with human serum albumin before centrifugation. The lower detection limits in the ultrafiltrates were 30 and 110 ng/l for IGF-I and IGF-II, respectively. To compare concomitant changes in fIGF-I and fIGF-II, each free fraction was determined in triplicate in separate ultrafiltrates. Care was taken to analyze each subject within the same assay, and after ultrafiltration plus analysis, the intra-assay coefficients of variation averaged 16% for fIGF-I and 17% for fIGF-II.

Statistics. Values are means ± SE. Statistical significance was accepted at P < 0.05 using a two-way analysis of variance for repeated measurements and Student’s t-test for post hoc testing. Correlations were calculated using simple regression.

RESULTS

After surgery, glucose infusion rate during clamps decreased by 50–60% compared with the preoperative situation in the OGL and Fast groups (P = 0.016 and P < 0.0001, respectively), and infusion of insulin after surgery was associated with an increased IGFBP-3-PA, with no difference between the OGL and Fast groups (4). Before surgery, an 18% decrease in IGFBP-1 (P < 0.001) was demonstrated in response to insulin infusion (0.8 mU·kg⁻¹·min⁻¹; Fig. 1). No change was found in IGFBP-3-PA (Fig. 1). The levels of tIGF-I decreased in response to 210 min of insulin infusion (P < 0.05), while no change was found in fdIGF-I by IRMA or fdIGF-II and fdIGF-II by ultrafiltration.

After surgery, there was an increase in IGFBP-3-PA (P < 0.001) in response to 210 min of insulin infusion compared with baseline, as reported elsewhere (4). This was associated with a simultaneous 34% increase in fdIGF-I by IRMA (P < 0.005). Although the changes in fdIGF-I in response to insulin after surgery were not different regardless of whether fdIGF-I was analyzed using ultrafiltration or IRMA (P = 0.51), the apparent 54% increase in fdIGF-I, as determined by ultrafiltration, failed to reach statistical significance (P = 0.15).
No significant changes were found in tIGF-I ($P = 0.36$) or fdIGF-II ($P = 0.98$) in response to 210 min of insulin infusion after surgery.

The responses to insulin infusions before and after surgery were quite different for tIGF-I ($P < 0.0001$), fdIGF-I by ultrafiltration ($P < 0.01$), and fdIGF-I by IRMA ($P < 0.005$), while no significant difference was found for fdIGF-II by ultrafiltration (Fig. 1). The 17% decrease in IGFBP-1 ($P < 0.05$; Fig. 1) in response to insulin infusion after surgery was not different from the corresponding changes at the preoperative clamp ($P = 0.98$). During insulin infusion, the changes in IGFBP-1 over time were similar before and after surgery (Fig. 2). The changes in the reported parameters did not differ between the OGL and Fast groups at any time ($P > 0.4$). Therefore, the data are presented as means ± SE for all patients regardless of whether they received an oral glucose load before surgery.

After surgery, the individual increases in IGFBP-3-PA in response to insulin correlated with fdIGF-I measured by ultrafiltration ($r^2 = 0.23, P < 0.05$; Fig. 3) and with fdIGF-I by IRMA ($r^2 = 0.26, P < 0.05$; Fig. 3), but not significantly with fdIGF-II ($P = 0.19$). The increase in fdIGF-I by ultrafiltration in response to insulin after surgery correlated with the simultaneous changes in fdIGF-II ($r^2 = 0.66, P = 0.0001$) but not significantly with fdIGF-I by IRMA ($r^2 = 0.16, P = 0.098$). Preoperative insulin sensitivity did not correlate with any of the measured variables ($P > 0.3$). In contrast, postoperative insulin sensitivity correlated with the change in IGFBP-3-PA in response to insulin after surgery ($r^2 = -0.22, P < 0.05$; Fig. 4).

**DISCUSSION**

This is the first report suggesting that insulin may regulate fdIGF-I by modulating IGFBP-3-PA in the...
and insulin sensitivity. This may be explained simply by the small size of the study and the larger variability in the measurements of fdIGF-I compared with IGFBP-3-PA. However, circulating fIGF-I levels may not fully reflect the tissue levels of fIGF-I. Furthermore, IGFBP-3 and/or its fragments may have independent effects on glucose metabolism, none of which were determined in the present study. Furthermore, the present study does not address the question of whether, in addition to insulin resistance, there is resistance to IGF-I-induced glucose uptake in skeletal muscle after surgery, as reported in patients with type 2 diabetes (9).

Reduced tIGF-I after surgery has been suggested to be a result of impaired generation of IGF-I by growth hormone (GH) due to GH receptor “uncoupling” (29). Although increased IGFBP-3-PA results in increased concentrations of circulating fIGF-I, the ternary complex containing IGF, proteolyzed IGFBP-3, and acid-labile subunits has been reported to remain intact (32). Thus it appears that, despite increased IGFBP-3-PA, only a minor fraction of tIGF-I is allowed to dissociate from the main circulating IGF-I stores. It is therefore likely that a decreased IGF-I production rate, rather than increased IGFBP-3-PA, contributes to the substantial reduction of tIGF-I concentrations at baseline after surgery. This view is supported by the finding of increased tIGF-I concentrations in pregnant women, despite the complete fragmentation of IGFBP-3 in the ternary complex due to IGFBP-3 proteolysis (12, 16).

Table 1. Levels of glucose and hormones before and after abdominal surgery at baseline and during insulin infusions

<table>
<thead>
<tr>
<th>Blood glucose, mM</th>
<th>Baseline</th>
<th>Clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.6 ± 0.1</td>
<td>4.4 ± 0.0</td>
</tr>
<tr>
<td>Surgery</td>
<td>5.2 ± 0.2</td>
<td>4.5 ± 0.0&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum insulin, pM</td>
<td>82 ± 7</td>
<td>360 ± 17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>99 ± 9&lt;sup&gt;6&lt;/sup&gt;</td>
<td>321 ± 12&lt;sup&gt;c&lt;/sup&gt;,&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serum tIGF-I, mg/l</td>
<td>167 ± 13</td>
<td>149 ± 10&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surgery</td>
<td>121 ± 7&lt;sup&gt;6&lt;/sup&gt;</td>
<td>121 ± 6&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>fdIGF-If, mg/l</td>
<td>0.81 ± 0.12</td>
<td>0.88 ± 0.12</td>
</tr>
<tr>
<td>Control</td>
<td>0.58 ± 0.12</td>
<td>0.79 ± 0.13</td>
</tr>
<tr>
<td>fdIGF-I&lt;subdrs&lt;/sub&gt;, mg/l</td>
<td>1.56 ± 0.25</td>
<td>1.50 ± 0.33</td>
</tr>
<tr>
<td>Control</td>
<td>1.53 ± 0.18</td>
<td>1.98 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>fdIGF-II&lt;sub&gt;s&lt;/sub&gt;, mg/l</td>
<td>0.94 ± 0.06</td>
<td>1.02 ± 0.10</td>
</tr>
<tr>
<td>Control</td>
<td>0.65 ± 0.12</td>
<td>0.68 ± 0.10&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>IGFBP-3-PA, %</td>
<td>26.6 ± 0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>25.7 ± 1.0</td>
</tr>
<tr>
<td>Control</td>
<td>31.4 ± 1.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>34.8 ± 1.3&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>fdIGF-B&lt;sub&gt;s&lt;/sub&gt;, mg/l</td>
<td>35.4 ± 5.6</td>
<td>28.4 ± 4.4&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Surgery</td>
<td>49.5 ± 5.3</td>
<td>40.6 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SE; <sup>n</sup> = 18. IGF-I, insulin-like growth factor I; tIGF-I, total IGF-I; fdIGF-I, free dissociable IGF-I; IGFBP, IGF-binding protein; IGFBP-3-PA, IGFBP-3 proteolytic activity. Significantly different from baseline: <sup>c</sup>P < 0.05; <sup>f</sup>P < 0.01; <sup>b</sup>P < 0.001. Significantly different from control: <sup>6</sup>P < 0.05; <sup>e</sup>P < 0.01; <sup>a</sup>P < 0.001.
IGFBP-3 proteolysis may therefore be an efficient mechanism for increasing IGF-I bioavailability and, thereby, allowing anabolic processes such as glucose and amino acid uptake, as well as tissue repair, to occur without markedly increasing clearance of the IGF-I tissue stores. This is also supported by our present findings that IGF-I levels were unchanged during insulin infusion after surgery, despite an increase in IGFBP-3-PA. Such a mechanism would be sufficient to support anabolic events for a limited period of time until insulin sensitivity and IGF-I production are restored. Further studies are needed to determine whether the restoration of insulin sensitivity is also the signal that shuts off IGFBP-3 proteolysis.

Levels of IGFBP-1 increase even after a brief period of fasting (2). In the present study, the patients were semistarved before the postoperative measurement as a result of routine clinical treatment with hypocaloric nutrition (50–75 g glucose) during the first 24 h after the operation. Recently, we studied healthy subjects subjected to the same protocol used in this study before and after 24 h of combined treatment with hypocaloric nutrition (50 g glucose) and bed rest to determine the importance of these factors for the changes occurring after surgery (25). In that study, baseline levels of IGFBP-1 increased twofold after a 24-h period of bed rest and hypocaloric nutrition, possibly as a result of a simultaneous drop in insulin levels. In these subjects, IGFBP-1 was rapidly reduced in response to insulin infusion, indicating a normal response to insulin. In the same subjects, 24 h of hypocaloric nutrition and bed rest had no effect on IGFBP-3-PA (unpublished data). Because the change in IGFBP-3-PA appears to be one of the main determinants of IGF bioavailability at 1 day after surgery, hypocaloric nutrition and bed rest in connection with surgery are not likely to be important contributors to the above-mentioned postoperative changes in IGF-I bioavailability.

Although baseline levels of IGFBP-1 were elevated after surgery, IGFBP-1 was reduced similarly in response to insulin infusion before and after surgery. This indicates a normal sensitivity to insulin in the hepatic tissues. This is in line with our previous findings that hepatic insulin sensitivity is not affected after uncomplicated abdominal surgery (26). Increased baseline levels of IGFBP-1 postoperatively may be due to increased levels of glucagon, cytokines, or other factors that may stimulate IGFBP-1 production or reduce clearance (15, 21, 36).

In conclusion, increased IGFBP-3-PA in response to insulin infusions was associated with increased levels of fIGF-I after surgery, while no relationship was found between fIGF-I and IGFBP-1 before or after surgery. The insulin-induced increase in IGFBP-3-PA was more pronounced in more insulin-resistant patients, while the suppression of IGFBP-1 in response to an insulin infusion was not affected by surgery. These observations strongly suggest that insulin is involved in the modulation of IGF-I bioavailability through the induction of IGFBP-3-PA after surgery. This may be one important anabolic effect of insulin in postoperative patients.

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