Basal insulin, glucagon, and growth hormone replacement

Suzanne M. Breckenridge,1 Bharathi Raju,1 Ana Maria Arbelaez,1 Bruce W. Patterson,2 Benjamin A. Cooperberg,1 and Philip E. Cryer1

Divisions of 1Endocrinology, Metabolism, and Lipid Research and 2Geriatrics and Nutritional Science, Washington University School of Medicine, St. Louis, Missouri

Submitted 25 May 2007; accepted in final form 14 August 2007

Breckenridge SM, Raju B, Arbelaez AM, Patterson BW, Cooperberg BA, Cryer PE. Basal insulin, glucagon, and growth hormone replacement. Am J Physiol Endocrinol Metab 293: E1303–E1310, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00325.2007.—Conclusions drawn from the pancreatic (or islet) clamp technique (suppression of endogenous insulin, glucagon, and growth hormone secretion with somatostatin and replacement of basal hormone levels by intravenous infusion) are critically dependent on the biological appropriateness of the selected doses of the replaced hormones. To assess the appropriateness of representative doses we infused saline alone, insulin (initially 0.20 mU·kg−1·min−1 alone, glucagon (1.0 ng·kg−1·min−1) alone, and growth hormone (3.0 ng·kg−1·min−1) alone intravenously for 4 h in 13 healthy individuals. That dose of insulin raised plasma insulin concentrations approximately threefold, suppressed glucose production, and drove plasma glucose concentrations down to subphysiological levels (65 ± 3 mg/dl, P < 0.0001 vs. saline), resulting in nearly complete suppression of insulin secretion (P < 0.0001) and stimulation of glucagon (P = 0.0059) and epinephrine (P = 0.0009) secretion. An insulin dose of 0.15 mU·kg−1·min−1 caused similar effects, but a dose of 0.10 mU·kg−1·min−1 did not. The glucagon and growth hormone infusions did not alter plasma glucose levels or those of glucoregulatory factors. Thus, insulin “replacement” doses of 0.20 and even 0.15 mU·kg−1·min−1 are excessive, and conclusions drawn from the pancreatic clamp technique using such doses may need to be reassessed.

clorectide; pancreatic clamp

THE PANCREATIC (OR ISLET) CLAMP TECHNIQUE INVOLVES THE INFUSION OF somatostatin (or the somatostatin analog octreotide) to suppress endogenous insulin, glucagon, and growth hormone secretion and replacement of basal levels of these hormones by intravenous infusion (1, 3, 12, 23, 24, 25, 27, 33). It has been used to assess the metabolic roles of these hormones and led, for example, to the conclusion that glucagon normally supports the postabsorptive plasma glucose concentration, since plasma glucose levels decreased when somatostatin and insulin were infused and glucagon was not replaced (27). Although there is a body of evidence that supports that view (26), much of that evidence is open to alternative interpretations. Clearly, such conclusions are critically dependent on the biological appropriateness of the selected doses of the replaced hormones.

The glycemic response to infusion of somatostatin is biphasic with an initial decrease in glucose production (19, 28, 31) and the plasma glucose concentration (20, 28, 31) followed by an increase in glucose production (19, 28, 31) and the plasma glucose concentration (20, 28, 31) in healthy humans. Like native somatostatin, infusion of the more potent somatostatin analog octreotide suppresses plasma insulin, glucagon, and growth hormone concentrations in humans (16).

Insulin has been infused peripherally in doses of 0.14 (25), 0.15 (19), 0.20 (1, 27), and 0.24 (24) mU·kg−1·min−1 in humans [and intraportally in a dose of 0.25 mU·kg−1·min−1 in dogs (12)] to replace basal insulin levels during the infusion of somatostatin. In the human studies peripheral insulin concentrations were intended to be raised approximately twofold on the basis of the rationale that, given ~50% extraction of insulin by the liver, it was necessary to double peripheral insulin concentrations to replace basal hepatic portal venous insulin levels, which were thought to be the sole determinant of endogenous glucose production and thus the postabsorptive plasma glucose concentration. However, that rationale could be questioned given more recent evidence that some of the effect of insulin to reduce endogenous glucose production is indirect, i.e., initially extrahepatic (and extrarenal), on adipose tissue to reduce nonesterified fatty acid levels (2), on muscle to reduce gluconeogenic precursor flux to the liver (and kidneys), on pancreatic α-cells to reduce glucagon secretion, and on the brain to increase parasympathetic firing to the liver (4) and thus the result of peripheral insulin actions. The relative contributions of the direct and indirect actions continue to be debated (6, 11). Clearly, it is important that insulin not be substantially overreplaced during pancreatic clamps. To our knowledge, the effects of putative basal insulin replacement doses alone (in the absence of somatostatin) in healthy humans has not been reported. Lewis et al. (18) found that an intravenous insulin dose of 0.7 units/h (0.17 mU·kg−1·min−1 if one assumes an average body weight of 70 kg) did not lower plasma glucose concentrations substantially in four patients with type 1 diabetes.

Glucagon has been infused peripherally in doses of 0.40 (23), 0.65 (1, 25), 0.75 (33), and 1.00 (3, 24) ng·kg−1·min−1 in humans [and intraportally in a dose of 0.50 ng·kg−1·min−1 in dogs (12)] to replace basal glucagon levels during the infusion of somatostatin. The use of the higher doses, infused peripherally, in the human studies was based on the assumption of ~25% extraction of glucagon by the liver and the premise that only portal levels are relevant to the actions of glucagon on glucose production. Clearly, it is important that glucagon not be substantially overreplaced during pancreatic clamps.

Growth hormone has been infused peripherally in doses of 2.0 (25), 3.0 (1), and 4.7 (24) ng·kg−1·min−1 in humans to replace basal growth hormone levels during the infusion of somatostatin. Growth hormone levels were held constant with the 3.0 ng·kg−1·min−1 dose (1); they appeared to be somewhat lower than baseline with the 2.0 ng·kg−1·min−1 dose.
(25). Since the plasma glucose-raising action of growth hormone is delayed for several hours (22), the need to replace growth hormone could be questioned. However, growth hormone has an initial insulin-like effect (22).

To assess the appropriateness of representative doses of insulin, glucagon, and growth hormone to produce putative replacement of basal hormone levels, we infused saline alone, insulin (initially 0.20 mU·kg⁻¹·min⁻¹) alone, glucagon (1.0 ng·kg⁻¹·min⁻¹) alone, or growth hormone (3.0 ng·kg⁻¹·min⁻¹) alone intravenously, in random sequence, for 4 h without somatostatin in healthy adults. We reasoned that if these were truly basal replacement doses they would have little effect on glucose kinetics and plasma glucose concentrations.

METHODS

Subjects. Thirteen healthy individuals [7 women/6 men, mean (±SD) age 30 ± 8 yr, mean (±SD) body mass index 24 ± 3 kg/m²] gave their written, informed consent to participate in this study, which was approved by the Washington University Medical Center Human Research Protection Office and conducted in the outpatient facility of the Washington University General Clinical Research Center. Inclusion criteria included negative medical histories and normal physical examinations as well as normal fasting plasma glucose concentrations, hematocrits, and electrocardiograms.

Experimental design. All studies were performed in the morning after an overnight fast. Subjects were in the supine position throughout. To permit estimation of glucose kinetics, a primed (22.5 mg/kg after an overnight fast. Subjects were in the supine position through hematocrits, and electrocardiograms.

Glucose tracer methodology. Plasma proteins were precipitated with ice-cold acetone and lipids extracted with hexane. The aqueous phase was dried by Speed-Vac centrifugation (Savant Instruments, Farmingdale, NY). Samples were derivatized with 10% heptafluorobutyric (HFB) anhydride in ethyl acetate (30 min at 70°C). The single isotope derivative (radioenzymatic) method (30). Serum non-esterified fatty acids (15) and blood lactate (21) were measured with a Biochemical analytical methods. Plasma glucose concentrations were measured using a glucose oxidase method (Yellow Springs Analyzer 2; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin (17), C-peptide (17), glucagon (7), pancreatic polypeptide (10), growth hormone (29), and cortisol (8) were measured with radioimmunoassays. The insulin, C-peptide, glucagon, and pancreatic polypeptide assays were performed with materials purchased from Linco Research (St. Louis, MO) and the cortisol assay with materials purchased from Diasorin (Stillwater, MN). An antibody provided by the National Institutes of Health was used for the growth hormone assay. Plasma epinephrine and norepinephrine were measured with a single isotope derivative (radioenzymatic) method (30). Serum non-esterified fatty acids (15) and blood lactate (21) were measured with enzymatic techniques.

Glucose tracer methodology. Plasma proteins were precipitated with ice-cold acetone and lipids extracted with hexane. The aqueous phase was dried by Speed-Vac centrifugation (Savant Instruments, Farmingdale, NY). Samples were derivatized with 10% heptafluorobutyric (HFB) anhydride in ethyl acetate (30 min at 70°C). The tracer-to-tracee ratio (TTR) of HFB-glucose was measured by gas chromatography-mass spectrometry using electron impact ionization (ions of mass/charge ratio 519 and 521 for natural and [6,6-²H₂]glucose, respectively) on an Agilent 5973 system equipped with a 30 m × 0.32 mm HP-5MS column. Instrument response was calibrated using prepared glucose standards of known isotopic enrichment. Non-steady-state kinetic analysis to obtain the rates of appearance (Rₐ) and disappearance (Rₜ) of plasma glucose was performed (28,30) as follows:

\[ R_t(t) = \frac{F \cdot t \cdot \Delta V}{pV \cdot dE/dt} \]
\[ R_a(t) = R_t(t) - pV \cdot dC/dt \]

where \( R_a(t) \) and \( R_t(t) \) are the rates of appearance and disappearance of unlabeled glucose, respectively, as functions of time (µmol·kg⁻¹·min⁻¹); \( F \) is the infusion rate of [6,6-²H₂]glucose (µmol·kg⁻¹·min⁻¹); \( pV \) is the effective glucose volume of distribution (assumed to be 40 ml/kg); \( C \) is the concentration of unlabeled glucose (mmol/l); \( E \) is the isotopic enrichment (TTR); \( dE/dt \) is the rate of change of TTR; and \( dC/dt \) is the rate of change of unlabeled glucose concentration. Plasma glucose concentrations and TTR values were smoothed using a loess local polynomial smoothing function (Mathcad 11; Mathsoft Engineering & Education, Cambridge, MA) prior to calculations and to obtain the rates of change of TTR and concentration.

Statistical methods. Data are expressed as means ± SE, except where the standard deviation is specified. Condition and time-related data were analyzed by mixed-model repeated-measures analysis of variance. The Proc Mixed module of the SAS statistical package version 8.2 was used. P values <0.05 were considered to indicate significant differences.

RESULTS

Plasma concentrations of infused hormones. During saline infusion, plasma insulin concentrations tended to decline (Fig. 1), as did plasma glucose concentrations (Fig. 2). Insulin infusion in a dose of 0.20 mU·kg⁻¹·min⁻¹ raised plasma insulin concentrations approximately threefold \((P < 0.0001\) vs. saline; Fig. 1) from 3.8 ± 0.7 µU/ml at 0 min to 13.8 ± 2.1 µU/ml at 30 min and continued through 240 min. A dose of 0.10 mU·kg⁻¹·min⁻¹ raised plasma insulin concentrations approximately twofold \((3.5 ± 0.5 \mu U/ml at 0 min to 7.5 ± 1.0 \mu U/ml at 120 min, \( P = 0.0005\); Fig. 1\). A dose of 0.15 mU·kg⁻¹·min⁻¹ raised plasma insulin concentrations further \((10.2 ± 1.4 \mu U/ml at 240 min, \( P < 0.0001\); Fig. 1\). Glucagon infusion (1.0 ng·kg⁻¹·min⁻¹) appeared to raise plasma glucagon concentrations slightly, although not significantly \((P = 0.0158\); Fig. 1\). The levels were 113 ± 5 ng/ml at 0 min and 131 ± 6 ng/ml at 240 min. Growth hormone infusion (3.0 ng·kg⁻¹·min⁻¹) did not raise plasma growth hormone concentrations \((P = 0.0001\); Fig. 1\).

Plasma glucose concentrations and glucose kinetics. Insulin infusion in a dose of 0.20 mU·kg⁻¹·min⁻¹ lowered plasma glucose concentrations sharply \((P < 0.0001\) vs. saline; Fig. 2\) from 88 ± 2 mg/dl at 0 min to 72 ± 4 mg/dl at 60 min, 71 ± 3 mg/dl at 120 min, and 65 ± 3 mg/dl at 240 min. Insulin infusion in a dose of 0.10 mU·kg⁻¹·min⁻¹ lowered plasma glucose concentrations \((88 ± 2 ± 76 ± 1 mg/dl at 120 min; \( P = 0.0158\) and in a dose of 0.15 mU·kg⁻¹·min⁻¹ lowered plasma glucose concentrations further \((67 ± 2 mg/dl at 240 min, \( P < 0.0001\); Fig. 2\).

Insulin infusion in a dose of 0.20 mU·kg⁻¹·min⁻¹ decreased the relative (to baseline) \( R_a \) initially \((P = 0.0086\) vs. saline; Table 1\). Thus, glucose \( R_a \) was lower than glucose \( R_t \),
and the plasma glucose concentrations declined. Then, glucose $R_a$ increased and matched plasma $R_d$. A similar, but smaller, glucose kinetic pattern appeared to develop initially during insulin infusions in lower doses (Table 1), although that did not reach statistical significance ($P = 0.0562$). Glucose $R_a$ and $R_d$ drifted downward during infusion of saline (Table 1). They were unaltered compared with saline during infusions of glucagon and of growth hormone in the doses studied (data not shown). Thus, glucose $R_a$ and $R_d$ time course profiles were superimposable during infusions of saline, glucagon, and growth hormone.

**Neuroendocrine and other metabolic responses.** As plasma glucose concentrations decreased during infusion of insulin in a dose of 0.20 mU·kg$^{-1}$·min$^{-1}$, plasma C-peptide concentrations decreased sharply ($P < 0.0001$; Fig. 3) from 1.1 ± 0.1 ng/ml at 0 min to 0.7 ± 0.1 ng/ml at 60 min, 0.4 ± 0.1 ng/ml at 120 min, and 0.3 ± 0.0 ng/ml at 240 min. Indeed, as plasma glucose declined within the physiological range during infusion of insulin in a dose of 0.10 mU·kg$^{-1}$·min$^{-1}$, plasma C-peptide concentrations also declined (from 1.3 ± 0.1 ng/ml at 0 min to 0.7 ± 0.1 ng/ml at 120 min, $P = 0.0038$), and as plasma glucose concentrations fell further during infusion of insulin in a dose of 0.15 mU·kg$^{-1}$·min$^{-1}$, plasma C-peptide concentrations decreased further (to 0.4 ± 0.0 ng/ml at 240 min, $P < 0.0001$; Fig. 3).

Decrements in plasma glucose concentrations to subphysiological levels during infusion of insulin in a dose of 0.20 mU·kg$^{-1}$·min$^{-1}$ were associated with increments in the plasma concentrations of glucagon ($P = 0.0059$), epinephrine ($P = 0.0009$), and pancreatic polypeptide ($P < 0.0001$; Fig. 3). Plasma glucagon levels increased from 116 ± 7 pg/ml at 0 min to 144 ± 10 pg/ml at 240 min, epinephrine levels from 76 ± 9 to 292 ± 89 pg/ml, and pancreatic polypeptide levels from 73 ± 10 to 228 ± 41 pg/ml. Plasma norepinephrine concentrations (data not shown) increased ($P < 0.0001$) from 179 ± 7 to 226 ± 24 pg/ml. Plasma growth hormone ($P = 0.0318$) and cortisol ($P = 0.0002$) concentrations also increased (Table 2). Serum nonesterified fatty acid concentrations were suppressed ($P = 0.0163$) from 652 ± 54 μmol/l at 0 min to a nadir of 237 ± 59 μmol/l at 90 min and then rose to 532 ± 84 μmol/l at 240 min (Table 3). Blood lactate concentrations

![Image](http://ajpendo.physiology.org/10.220.31.on October 29, 2017)
mU H18528 to T H18528 concentrations increased during infusion of insulin in doses of 0.20 mU·kg\(^{-1}\)·min\(^{-1}\) through 240 min.

Table 1. *Mean relative* Ra and Rs before, during (0 through 240 min), and after infusion of saline and infusions of insulin

The values are means ± SE. Ra, rate of glucose appearance; Rs, rate of glucose disappearance. *0.10 mU·kg\(^{-1}\)·min\(^{-1}\) through 120 min, 0.15 mU·kg\(^{-1}\)·min\(^{-1}\) through 240 min.*

(443 ± 28 μmol/l at 0 min and 634 ± 112 μmol/l at 240 min) increased (P = 0.0117) (Table 3).

Decrement in plasma glucose concentrations within the physiological range during infusion of insulin in a dose of 0.10 mU·kg\(^{-1}\)·min\(^{-1}\) were not associated with increments in the plasma concentrations of glucagon, epinephrine, or pancreatic polypeptide (Fig. 3), of growth hormone or cortisol (Table 2), or of norepinephrine (data not shown). Serum nonesterified fatty acid concentrations remained suppressed (P = 0.0463), but blood lactate concentrations tended to increase (Table 3). On the other hand, decrements in plasma glucose concentrations to subphysiological levels during infusion of insulin in a dose of 0.15 mU·kg\(^{-1}\)·min\(^{-1}\) resulted in increases in plasma concentrations of glucagon, epinephrine, and pancreatic polypeptide (Fig. 3).
Table 3. Mean serum NEFA and blood lactate concentrations before, during (0–240 min), and after infusions of saline and insulin

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Saline Infusion</th>
<th>Insulin Infusions, mU·kg⁻¹·min⁻¹</th>
<th>Saline Infusion</th>
<th>Insulin Infusions, mU·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>-15</td>
<td>646±59</td>
<td>607±52</td>
<td>469±81</td>
<td>588±69</td>
</tr>
<tr>
<td>0</td>
<td>638±57</td>
<td>652±54</td>
<td>480±85</td>
<td>507±65</td>
</tr>
<tr>
<td>15</td>
<td>653±57</td>
<td>552±30</td>
<td>405±45</td>
<td>455±71</td>
</tr>
<tr>
<td>30</td>
<td>661±63</td>
<td>416±40</td>
<td>366±66</td>
<td>496±78</td>
</tr>
<tr>
<td>45</td>
<td>599±50</td>
<td>302±32</td>
<td>314±40</td>
<td>527±61</td>
</tr>
<tr>
<td>60</td>
<td>607±57</td>
<td>284±50</td>
<td>272±52</td>
<td>461±56</td>
</tr>
<tr>
<td>75</td>
<td>654±57</td>
<td>267±55</td>
<td>279±55</td>
<td>469±67</td>
</tr>
<tr>
<td>90</td>
<td>672±70</td>
<td>237±59</td>
<td>310±44</td>
<td>485±54</td>
</tr>
<tr>
<td>105</td>
<td>615±49</td>
<td>316±93</td>
<td>300±29</td>
<td>554±46</td>
</tr>
<tr>
<td>120</td>
<td>642±63</td>
<td>371±128</td>
<td>272±44</td>
<td>445±44</td>
</tr>
<tr>
<td>135</td>
<td>666±56</td>
<td>372±140</td>
<td>297±43</td>
<td>449±36</td>
</tr>
<tr>
<td>150</td>
<td>729±48</td>
<td>406±135</td>
<td>303±43</td>
<td>434±38</td>
</tr>
<tr>
<td>165</td>
<td>677±57</td>
<td>389±115</td>
<td>327±39</td>
<td>450±35</td>
</tr>
<tr>
<td>180</td>
<td>731±68</td>
<td>459±109</td>
<td>292±41</td>
<td>429±46</td>
</tr>
<tr>
<td>195</td>
<td>693±60</td>
<td>411±92</td>
<td>319±39</td>
<td>436±40</td>
</tr>
<tr>
<td>210</td>
<td>631±47</td>
<td>422±72</td>
<td>403±54</td>
<td>485±56</td>
</tr>
<tr>
<td>225</td>
<td>729±80</td>
<td>472±64</td>
<td>415±35</td>
<td>475±49</td>
</tr>
<tr>
<td>240</td>
<td>719±66</td>
<td>532±84</td>
<td>430±60</td>
<td>455±65</td>
</tr>
<tr>
<td>270</td>
<td>723±59</td>
<td>878±114</td>
<td>762±103</td>
<td>465±42</td>
</tr>
<tr>
<td>300</td>
<td>789±87</td>
<td>1,091±140</td>
<td>820±112</td>
<td>462±38</td>
</tr>
</tbody>
</table>

Values are means ± SE. NEFA, nonesterified fatty acids.
Table 4. Mean plasma insulin and epinephrine concentrations before, during (0–240 min), and after infusions of saline, glucagon, and GH

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Saline Infusion</th>
<th>Glucagon Infusion, 1.0 ng·kg⁻¹·min⁻¹</th>
<th>GH Infusion, 3.0 ng·kg⁻¹·min⁻¹</th>
<th>Glucagon Infusion, 1.0 ng·kg⁻¹·min⁻¹</th>
<th>GH Infusion, 3.0 ng·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>−15</td>
<td>3.9±0.4</td>
<td>3.6±0.6</td>
<td>4.0±0.7</td>
<td>55±10</td>
<td>69±12</td>
</tr>
<tr>
<td>0</td>
<td>3.6±0.5</td>
<td>3.8±0.5</td>
<td>4.4±0.9</td>
<td>61±9</td>
<td>67±11</td>
</tr>
<tr>
<td>15</td>
<td>3.5±0.4</td>
<td>4.2±0.6</td>
<td>4.1±0.7</td>
<td>59±10</td>
<td>70±12</td>
</tr>
<tr>
<td>30</td>
<td>3.8±0.5</td>
<td>3.6±0.5</td>
<td>3.4±0.5</td>
<td>54±9</td>
<td>70±12</td>
</tr>
<tr>
<td>45</td>
<td>3.3±0.5</td>
<td>3.7±0.6</td>
<td>3.6±0.5</td>
<td>58±9</td>
<td>62±10</td>
</tr>
<tr>
<td>60</td>
<td>3.2±0.5</td>
<td>3.4±0.4</td>
<td>3.3±0.5</td>
<td>58±10</td>
<td>58±10</td>
</tr>
<tr>
<td>75</td>
<td>3.5±0.5</td>
<td>3.3±0.5</td>
<td>3.5±0.5</td>
<td>58±9</td>
<td>70±10</td>
</tr>
<tr>
<td>90</td>
<td>3.5±0.4</td>
<td>3.2±0.4</td>
<td>3.8±0.8</td>
<td>60±9</td>
<td>65±11</td>
</tr>
<tr>
<td>105</td>
<td>2.9±0.4</td>
<td>2.9±0.3</td>
<td>3.5±0.5</td>
<td>58±8</td>
<td>64±10</td>
</tr>
<tr>
<td>120</td>
<td>3.0±0.4</td>
<td>2.9±0.3</td>
<td>3.4±0.6</td>
<td>64±10</td>
<td>66±10</td>
</tr>
<tr>
<td>135</td>
<td>2.8±0.2</td>
<td>3.0±0.4</td>
<td>3.5±0.6</td>
<td>65±10</td>
<td>62±9</td>
</tr>
<tr>
<td>150</td>
<td>3.0±0.4</td>
<td>3.0±0.3</td>
<td>3.1±0.5</td>
<td>73±15</td>
<td>66±10</td>
</tr>
<tr>
<td>165</td>
<td>2.7±0.3</td>
<td>3.0±0.4</td>
<td>2.8±0.4</td>
<td>61±8</td>
<td>68±11</td>
</tr>
<tr>
<td>180</td>
<td>2.8±0.5</td>
<td>3.4±0.6</td>
<td>2.6±0.4</td>
<td>61±10</td>
<td>69±12</td>
</tr>
<tr>
<td>195</td>
<td>2.9±0.4</td>
<td>3.0±0.4</td>
<td>3.1±0.6</td>
<td>66±10</td>
<td>72±12</td>
</tr>
<tr>
<td>210</td>
<td>3.0±0.4</td>
<td>2.8±0.4</td>
<td>3.0±0.4</td>
<td>62±9</td>
<td>83±16</td>
</tr>
<tr>
<td>225</td>
<td>2.6±0.4</td>
<td>2.5±0.3</td>
<td>3.0±0.5</td>
<td>70±11</td>
<td>81±13</td>
</tr>
<tr>
<td>240</td>
<td>2.5±0.3</td>
<td>2.8±0.4</td>
<td>4.1±1.0</td>
<td>68±11</td>
<td>76±10</td>
</tr>
<tr>
<td>270</td>
<td>2.9±0.4</td>
<td>2.7±0.5</td>
<td>2.3±0.5</td>
<td>67±9</td>
<td>80±13</td>
</tr>
<tr>
<td>300</td>
<td>2.6±0.4</td>
<td>2.6±0.3</td>
<td>3.4±0.6</td>
<td>79±10</td>
<td>87±12</td>
</tr>
</tbody>
</table>

Values are means ± SE. *To convert insulin to pmol/l, multiply by 6.0. **To convert epinephrine to pmol/l, multiply by 5.458.

DISCUSSION

These data indicate that the intravenous insulin infusion doses ranging from 0.14 to 0.24 mU·kg⁻¹·min⁻¹ often used to attempt to produce “basal” insulin replacement during suppression of endogenous insulin (and glucagon and growth hormone) secretion in the pancreatic (or islet) clamp technique in humans (e.g., see Refs. 1, 19, 24, 25, and 27) are excessive in healthy young adults. Infusion of insulin alone in a dose of 0.20 mU·kg⁻¹·min⁻¹ raised plasma insulin concentrations approximately threefold and drove plasma glucose concentrations down to below the postabsorptive physiological range of ~72–108 mg/dl (5). That activated physiological defenses against hypoglycemia (5). As plasma glucose concentrations declined within the physiological range, insulin secretion as assessed by plasma C-peptide concentrations decreased sharply. As plasma glucose concentrations fell further to levels below the glycemic

Table 5. Mean serum NEFA and blood lactate concentrations before, during (0–240 min), and after infusions of saline, glucagon, and GH

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Saline Infusion</th>
<th>Glucagon Infusion, 1.0 ng·kg⁻¹·min⁻¹</th>
<th>GH Infusion, 3.0 ng·kg⁻¹·min⁻¹</th>
<th>Glucagon Infusion, 1.0 ng·kg⁻¹·min⁻¹</th>
<th>GH Infusion, 3.0 ng·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>−15</td>
<td>64±49</td>
<td>536±56</td>
<td>544±35</td>
<td>588±69</td>
<td>675±93</td>
</tr>
<tr>
<td>0</td>
<td>638±57</td>
<td>498±49</td>
<td>588±46</td>
<td>507±65</td>
<td>715±84</td>
</tr>
<tr>
<td>15</td>
<td>653±57</td>
<td>564±84</td>
<td>595±31</td>
<td>455±71</td>
<td>620±68</td>
</tr>
<tr>
<td>30</td>
<td>661±63</td>
<td>503±56</td>
<td>534±28</td>
<td>496±78</td>
<td>663±62</td>
</tr>
<tr>
<td>45</td>
<td>599±50</td>
<td>489±62</td>
<td>598±36</td>
<td>527±61</td>
<td>639±64</td>
</tr>
<tr>
<td>60</td>
<td>607±57</td>
<td>531±59</td>
<td>600±59</td>
<td>461±56</td>
<td>674±65</td>
</tr>
<tr>
<td>75</td>
<td>654±77</td>
<td>491±38</td>
<td>611±46</td>
<td>406±67</td>
<td>530±73</td>
</tr>
<tr>
<td>90</td>
<td>672±70</td>
<td>515±44</td>
<td>713±76</td>
<td>485±54</td>
<td>590±86</td>
</tr>
<tr>
<td>105</td>
<td>615±49</td>
<td>589±56</td>
<td>624±34</td>
<td>554±46</td>
<td>581±80</td>
</tr>
<tr>
<td>120</td>
<td>642±63</td>
<td>610±53</td>
<td>698±41</td>
<td>445±44</td>
<td>546±70</td>
</tr>
<tr>
<td>135</td>
<td>666±56</td>
<td>607±60</td>
<td>760±42</td>
<td>449±36</td>
<td>502±70</td>
</tr>
<tr>
<td>150</td>
<td>729±48</td>
<td>614±53</td>
<td>821±62</td>
<td>434±38</td>
<td>497±61</td>
</tr>
<tr>
<td>165</td>
<td>677±57</td>
<td>674±83</td>
<td>714±40</td>
<td>450±35</td>
<td>483±74</td>
</tr>
<tr>
<td>180</td>
<td>731±68</td>
<td>641±67</td>
<td>774±47</td>
<td>429±46</td>
<td>499±61</td>
</tr>
<tr>
<td>195</td>
<td>693±60</td>
<td>652±58</td>
<td>750±49</td>
<td>436±40</td>
<td>538±62</td>
</tr>
<tr>
<td>210</td>
<td>631±37</td>
<td>639±50</td>
<td>770±48</td>
<td>485±56</td>
<td>531±69</td>
</tr>
<tr>
<td>225</td>
<td>729±80</td>
<td>671±50</td>
<td>795±42</td>
<td>475±49</td>
<td>516±61</td>
</tr>
<tr>
<td>240</td>
<td>719±66</td>
<td>720±68</td>
<td>840±45</td>
<td>455±65</td>
<td>524±70</td>
</tr>
<tr>
<td>270</td>
<td>723±59</td>
<td>755±50</td>
<td>832±44</td>
<td>465±42</td>
<td>551±65</td>
</tr>
<tr>
<td>300</td>
<td>789±87</td>
<td>729±51</td>
<td>810±42</td>
<td>462±38</td>
<td>545±63</td>
</tr>
</tbody>
</table>

Values are means ± SE.
thresholds for activation of glucose counterregulatory systems, the plasma concentrations of glucagon and epinephrine, as well as those of growth hormone and cortisol, increased. Were it not for these glucose counterregulatory defenses, plasma glucose concentrations would undoubtedly have fallen to even lower levels. Furthermore, infusion of insulin in a lower dose of 0.15 mU·kg⁻¹·min⁻¹ also drove plasma glucose concentrations down to subphysiological levels and activated glucose counterregulatory systems. Clearly, conclusions drawn from use of the pancreatic clamp technique with such doses that might be critically dependent on an excessive insulin replacement dose, including our own (27), need to be reassessed in light of these findings.

The lowest insulin dose tested, 0.10 mU·kg⁻¹·min⁻¹, raised plasma insulin concentrations approximately twofold and lowered plasma glucose concentrations within the physiological range. As a result, endogenous insulin secretion, as assessed by plasma C-peptide concentrations, decreased. It also suppressed lipolysis as assessed by serum nonesterified fatty acid concentrations. Clearly, that too is a biologically active dose. However, over the time frame studied, unlike both of the higher doses, it did not cause subphysiological glucose levels and did not suppress insulin secretion completely. It would likely have had less of a plasma glucose-lowering effect in the absence of endogenous insulin secretion. Thus, a peripheral intravenous insulin dose of 0.10 mU·kg⁻¹·min⁻¹ would appear to be a more appropriate dose for portal venous insulin replacement with the pancreatic clamp technique in humans.

The glucagon and growth hormone doses tested did not alter plasma glucose concentrations or any of the measured glucose regulatory factors. Infusion of glucagon in a dose of 1.0 ng·kg⁻¹·min⁻¹ did not raise plasma glucagon concentrations significantly. Mean values at 240 min were only 16% higher than those prior to glucagon infusion. Growth hormone infusion in a dose of 3.0 ng·kg⁻¹·min⁻¹ did not alter plasma growth hormone concentrations measurably. However, we have no measure of any effects of these infusions on endogenous glucagon or growth hormone secretion. In any event, these doses are not excessive. Indeed, they are somewhat low.

Insulin lowered plasma glucose concentrations by suppressing glucose production rather than by stimulating glucose utilization. The glucose Ra decreased initially and was lower than the glucose Rd and the plasma glucose concentrations, therefore, decreased. Then glucose Rd increased to match Rd, and the plasma glucose levels plateaued. The glucose kinetic method we used underestimates glucose Ra and Rd (9, 15) even during infusions of low doses of insulin (12). Therefore, our results are reported as those relative to baseline rather than as absolute rates. The observed decrement in glucose Rd was small compared with that during euglycemic clamps with similar doses of insulin (15), but the latter may well have also included a suppressive effect of glucose per se. Glucose turnover rates drifted downward during infusion of saline and were similar, with glucose Ra and Rd superimposable, during infusion of saline, of glucagon, and of growth hormone. Thus, in the doses tested, neither glucagon nor growth hormone raised plasma glucose concentrations.

These data confirm several principles of the physiology of glucose counterregulation, the mechanisms that normally prevent or rapidly correct clinical hypoglycemia, in humans (5, 13). First, the glycemic threshold for decrements in insulin secretion as plasma glucose concentrations decline lies within the postabsorptive plasma glucose concentration range. Second, the glycemic thresholds for increments in the secretion of the glucose counterregulatory hormones (glucagon, epinephrine, growth hormone, and cortisol) lie just below the physiological range. Third, in the setting of intact glucose counterregulatory systems, plasma glucose concentrations plateau at levels just below the physiological range and at levels higher than those required to produce symptoms of hypoglycemia despite ongoing hyperinsulinemia sufficient to decrease plasma glucose levels. In essence, a new steady state is established at plasma glucose concentrations low enough to maintain activation of glucose counterregulatory systems but higher than the glycemic threshold for symptoms of hypoglycemia.

In summary, these data indicate that putative basal intravenous insulin replacement doses of 0.20 mU·kg⁻¹·min⁻¹ and even of 0.15 mU·kg⁻¹·min⁻¹ are too high for use in the pancreatic (or islet) clamp technique in humans. A peripheral intravenous insulin dose of 0.10 mU·kg⁻¹·min⁻¹ would appear to be a more appropriate dose for portal venous insulin replacement. A glucagon replacement dose of 1.0 ng·kg⁻¹·min⁻¹ and a growth hormone replacement dose of 3.0 ng·kg⁻¹·min⁻¹ are not excessive; indeed, they are somewhat low. Thus, conclusions based on the use of this technique with excessive insulin replacement may need to be reassessed.

ACKNOWLEDGMENTS

We acknowledge the assistance of the staff of the Washington University General Clinical Research Center in the performance of this study; the technical assistance of Krishan Jethi, Cornell Blake, Gene Wade Sherrow, Michael Morris, Zina Lubovich, Sharon Travis, Sharon O’Neill, Freida Custodio, Jennifer Shew, and Dr. Adewole Okunade; and the assistance of Janet Dedede with the preparation of this manuscript.

GRANTS

This work was supported, in part, by United States Public Health Service/ National Institutes of Health Grants R37-DK-27085, MO1-RR-00036, P30-DK-56341, P60-DK-20579, and T32-DK-07120 and a fellowship award from the American Diabetes Association.

DISCLOSURES

P. E. Cryer has served on Advisory Boards for Novo Nordisk, Takeda Pharmaceuticals North America, MannKind, and Merck and as a consultant for Amgen, TolerRx, and Marcadia Biotech in recent years. S. M. Breckenridge, B. Raju, A. M. Arbelaez, B. W. Patterson, and B. A. Cooperberg have nothing to disclose.

REFERENCES

6. Edgerton DS, Lautz M, Scott M, Everett CA, Stettler KM, Neal DW, Chu CA, Cherrington AD. Insulin’s direct effects on the liver dominate.