Improved tolerance to sequential glucose loading (Staub-Traugott effect): size and mechanisms

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Bonuccelli S, Muscelli E, Gastaldelli A, Barsotti E, Astiarraga BD, Holst JJ, Mari A, Ferrannini E. Improved tolerance to sequential glucose loading (Staub-Traugott effect): size and mechanisms. Am J Physiol Endocrinol Metab 297: E532–E537, 2009. First published June 16, 2009; doi:10.1152/ajpendo.00127.2009.—Improved glucose tolerance to sequential glucose loading (Staub-Traugott effect) is an important determinant of day-to-day glycemic exposure. Its mechanisms have not been clearly established. We recruited 17 healthy volunteers to receive two sequential oral glucose tolerance tests (OGTTs), at time 0 min and 180 min (Study I). The protocol was repeated on a separate day (Study II) except that plasma glucose was clamped at 8.3 mmol/l between 60 and 180 min. β-Cell function was analyzed by mathematical modeling of C-peptide concentrations. In a subgroup, glucose kinetics were measured by a triple-tracer technique (infusion of [6,6-2H2]glucose and labeling of the 2 glucose loads with [1-13C]glucose). In both Studies I and II, the plasma glucose response to the second OGTT equaled 84 ± 2% (P = 0.003) of the response to the first OGTT. Absolute insulin secretion was lower (37.8 ± 4.3 vs. 42.8 ± 5.1 mmol/m², P = 0.02), but glucose potentiation (i.e., higher secretion at the same glycemia) was stronger (1.08 ± 0.02- vs. 0.92 ± 0.02-fold, P = 0.006), the increment being higher in Study II (+36 ± 5% vs. Study I +19 ± 6%, P < 0.05). In pooled data, a higher glucose area during the first OGTT was associated with a higher potentiation during the second OGTT (rho = 0.60, P = 0.002). Neither insulin clearance nor glucose clearance differed between loads, and appearance of glucose over 3 h totalled 60 ± 6 g for the first load and 52 ± 5 g for the second load (P = not significant). Fasting endogenous glucose production [13.3 ± 0.6 μmol·min⁻¹·kg⁻¹ fat-free mass (FFM)⁻¹] averaged 6.0 ± 3.8 μmol·min⁻¹·kg⁻¹ FFM⁻¹ between 0 and 180 min and 1.7 ± 2.6 between 180 and 360 min (P < 0.03). Glucose potentiation and stronger suppression of endogenous glucose release are the main mechanisms underlying the Staub-Traugott effect.

Table 1. Anthropometric and metabolic characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (M/F)</td>
<td>17 (13/4)</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>37 ± 3</td>
<td>26–60</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.6 ± 0.8</td>
<td>17.4–27.2</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>48 ± 2</td>
<td>40–62</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>16 ± 2</td>
<td>5–27</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>5.1 ± 0.6</td>
<td>4.1–6.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.67 ± 0.24</td>
<td>3.58–7.25</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.54 ± 0.08</td>
<td>1.06–1.94</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>2.81 ± 0.28</td>
<td>1.68–5.86</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>0.76 ± 0.13</td>
<td>0.37–1.95</td>
</tr>
<tr>
<td>M-value, μmol·min⁻¹·kg FFM⁻¹*</td>
<td>36.8 ± 4.3</td>
<td>11.5–62.6</td>
</tr>
</tbody>
</table>

Mean ± SD: mean ± standard deviation of the study subjects; Range: range of values of the study subjects. *M-value measured in 11 subjects.

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IMPROVEMENT OF CARBOHYDRATE tolerance following repeated glucose administration was first reported by Hamman and Hirschman in 1919 (10) and subsequently confirmed by Staub in 1921 (24) and by Traugott in 1922 (27). This effect has since been known as the Staub-Traugott effect and has been demonstrated after oral (8, 24, 27) and intravenous administration of glucose (5–9).

Despite the fact that this facilitated glucose disposal is an important physiological determinant of overall glycemic exposure, its mechanisms have not been established conclusively. Increased plasma insulin response has been reported by some studies (2, 3, 25), but not others (1, 7, 14, 21, 23, 26, 30); decreased hepatic clearance of insulin (30) and enhanced insulin sensitivity (13, 14) have also been proposed.

On theoretical grounds, a lower glycemic response to a glucose load that follows another glucose challenge could be due to: 1) increased insulin secretion, 2) enhanced peripheral insulin sensitivity, 3) enhanced hepatic insulin sensitivity, 4) reduced absorption of oral glucose, or 5) a combination of the above. In the present study, we explored each of these mechanisms in healthy volunteers.

MATERIALS AND METHODS

Subjects. Seventeen healthy subjects (Table 1) volunteered for the study. They gave no history of preexisting metabolic disorder or familial diabetes and were not taking any medication. None of them had lost weight or changed dietary habits during the 3 mo preceding the study. All subjects had resting arterial blood pressure <140/90 mmHg and normal results for liver and renal function tests. The study protocol was approved by the local Ethics Committee, and all subjects gave their informed consent to participate.

Study protocol. Two studies were carried out in each subject after an overnight (12- to 14-h) fast, with a 2-wk interval. In the first study (Study I), subjects received two sequential oral glucose loads (75 g), the first at time 0 min and the second at time 180 min. Venous blood was sampled at timed intervals for plasma glucose, insulin, C-peptide, glucagon-like peptide (GLP)-1, and gastric inhibitory peptide (GIP) measurement. In the second study (Study II), plasma glucose concentrations were clamped at 8.3 mmol/l from 60 to 180 min by a 20% variable glucose infusion (hyperglycemic clamp technique). Study II was performed in 11 of the 17 subjects; their anthropometric and metabolic characteristics were superimposable on those of the whole group (data not shown).
In 11 participants in Study I, glucose fluxes were measured by a new triple-tracer technique. With this protocol, a primed (28 μmol/kg) constant (0.28 μmol·min⁻¹·kg⁻¹) infusion of [6,6-²H₂]glucose (CIL, Boston, MA) was administered for 2 h before glucose ingestion and continued throughout the study (0–360 min). At time 0, subjects drank a 75-g glucose solution containing 1.5 g of [1-²H]glucose, and at time 180 min the second oral glucose load was enriched with 1.5 g of [U-¹³C₆]glucose. Blood for determination of plasma tracer-to-tracee ratios (TTR) was sampled at timed intervals.

Fat-free mass (FFM) was measured by electrical bioimpedance using a Body Composition Analyzer Model TBF-300 (TANITA) (12).

Assays. Plasma glucose was measured by the glucose oxidase technique (Beckman Glucose Analyzers; Beckman, Fullerton, CA). Plasma insulin was measured in duplicate by RIA using a kit for human insulin with negligible cross-reactivity with proinsulin and its split products (Linco Research, St. Louis, MO). C-peptide was measured by RIA (Linco Research). Plasma triglyceride and serum high-density lipoprotein cholesterol were assayed in duplicate by standard spectrophotometric methods on a Synchron Clinical System CX4 (Beckman Instruments). Total C-terminal amidated GLP-1 was assayed by RIA using polyclonal antiserum no. 89390, raised in rabbits, which has an absolute requirement for the amidated COOH-terminus of GLP-1 and does not cross-react with COOH-terminally truncated metabolites nor with the glycine-extended forms. The assay cross-reacts <0.01% with GLP-1(7–35) and GLP-1(7–37) and 83% with GLP-1(9–36) amide and 100% with GLP-1(1–36) amide, GLP-1(7–36) amide, and GLP-1(8–36) amide. The assay has a detection limit of ~1 pmol/l and an ED₅₀ of 25 pmol/l. Intra- and interassay coefficients of variation are <6 and <15%, respectively (11, 20). The active (NH₂-terminal) GIP was assayed by RIA using a polyclonal antiserum 98171, raised in rabbits, that is NH₂-terminally directed and does not recognize NH₂-terminally truncated peptides. It has a cross-reactivity of 100% with human GIP(1–42) and <0.1% with human GIP(3–42), GLP-1(7–36) amide, GLP-1(9–36) amide, GLP-2(1–33), GLP-2(3–33), and glucagon. The detection limit is ~5 pmol/l and ED₅₀ 48 pmol/l. Intra- and interassay coefficients of variation are <6 and <15%, respectively (5).

[6,6-²H₂]glucose, [1-²H]glucose, and [U-¹³C₆]glucose enrichment were measured by gas chromatography-mass spectrometry (GC-MS). Briefly, after deproteinization, plasma samples were reacted with acetic anhydride and pyridine to form the penta-acetate derivative and measured by gas chromatography-mass spectrometry (GC-MS). The assay has a detection limit of ~0.2 pmol/l and an ED₅₀ of 25 pmol/l. Intra- and interassay coefficients of variation are <6 and <15%, respectively (11, 20).

Calculations. The model used to estimate β-cell function has been previously described (16). In brief, the model consists of the following three blocks: 1) a model for fitting the glucose concentration profile, the purpose of which is to smooth and interpolate plasma glucose concentrations; 2) a model describing the dependence of insulin (or C-peptide) secretion on glucose concentration; and 3) a model of C-peptide kinetics, i.e., the two-exponential model proposed by Van Cauter et al. (28), in which the model parameters are individually determined.

Table 2. **AUCᵢ, AUCᵢ₆, AUCᵢ₈, AUCᵢ₉, and AUCᵢ₀ in Studies I and II**

<table>
<thead>
<tr>
<th>Glucose Load:</th>
<th>Study I</th>
<th>Study II</th>
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<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td>AUCᵢ, mmol·l⁻¹·h⁻¹</td>
<td>1.222 (253)</td>
<td>1.121 (85)*</td>
</tr>
<tr>
<td>Incr. AUCᵢ, mmol·l⁻¹·h⁻¹</td>
<td>368 (134)</td>
<td>133 (208)*</td>
</tr>
<tr>
<td>AUCᵢ₆, mmol·l⁻¹·h⁻¹</td>
<td>267 (78)</td>
<td>259 (123)</td>
</tr>
<tr>
<td>Incr. AUCᵢ₆, mmol·l⁻¹·h⁻¹</td>
<td>190 (67)</td>
<td>60 (84)*</td>
</tr>
<tr>
<td>AUCᵢ₈, mmol·l⁻¹·h⁻¹</td>
<td>37 (12)</td>
<td>29 (9)*</td>
</tr>
<tr>
<td>Incr. AUCᵢ₈, mmol·l⁻¹·h⁻¹</td>
<td>29 (11)</td>
<td>13 (16)*</td>
</tr>
<tr>
<td>AUCᵢ₉, mmol·l⁻¹·h⁻¹</td>
<td>2.29 (1.44)</td>
<td>3.30 (1.87)*</td>
</tr>
<tr>
<td>Incr. AUCᵢ₉, mmol·l⁻¹·h⁻¹</td>
<td>0.20 (1.17)</td>
<td>0.29 (1.69)</td>
</tr>
<tr>
<td>AUCᵢ₀, mmol·l⁻¹·h⁻¹</td>
<td>8.27 (2.94)</td>
<td>8.37 (3.73)</td>
</tr>
<tr>
<td>Incr. AUCᵢ₀, mmol·l⁻¹·h⁻¹</td>
<td>7.16 (2.38)</td>
<td>1.93 (6.91)</td>
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</table>

Data are medians with interquartile ranges in parentheses. AUCᵢ, AUCᵢ₆, AUCᵢ₈, AUCᵢ₉, and AUCᵢ₀, area under the curve for glucose, C-peptide, insulin, glucagon-like peptide-1, and gastric inhibitory peptide, respectively; Incr. AUC, incremental AUC. P = 0.05 vs. first load by Wilcoxon signed rank (*) and vs. Study I (†).
adjusted to the subject’s anthropometric data. In particular, with regard to the insulin secretion block (block 2), the relationship between insulin release and plasma glucose concentrations is modeled as a dose-response function. The mean slope of the dose-response function is taken to represent β-cell glucose sensitivity. The dose response is modulated by a time-varying factor expressing a potentiation effect on insulin secretion, which accounts for several potentiating mechanisms (prolonged exposure to hyperglycemia, nonglucose substrates, gastrointestinal hormones, neurotransmitters). The potentiation factor is set to be a positive function of time and is constrained to average unity during the experiment; thus, it expresses the relative potentiation of the secretory response to glucose. In normal subjects, the potentiation factor typically increases from the baseline value to the end of a 2-h oral glucose tolerance test (OGTT; see Ref. 16). Total insulin secretion is calculated every 5 min for the whole 3-h period of each OGTT.

In Study I, insulin sensitivity was estimated from the plasma glucose and insulin responses to each oral glucose load by calculating the oral glucose insulin sensitivity (OGIS) index, which has previously been shown to be well correlated with the M value from the euglycemic hyperinsulinemic clamp (15). In Study II, insulin sensitivity was estimated as the M value between 60 and 180 min and expressed as micromoles per minute per kilogram FFM. Insulin clearance was calculated as the ratio of total insulin secretion (as reconstructed from C-peptide concentrations) to the insulin area under time-concentration curve (AUC) for corresponding time intervals.

During the last 20 min of the basal tracer equilibration period, plasma glucose concentrations and [6,6-2H2]glucose enrichment were stable in all subjects. Therefore, endogenous glucose production (EGP) was calculated as the ratio of [6,6-2H2]glucose infusion rate to the plasma TTR (mean of 3 determinations). The glucose concentration components resulting from the absorption of ingested glucose (oral glucose concentration) were determined from the product of total plasma glucose concentration and the ratio of plasma [1-2H]glucose and [U-13C6]glucose TTR to the TTR of the respective tracers in the ingested load. The plasma glucose concentration resulting from endogenous glucose release (endogenous glucose concentration) was obtained as the difference between total and oral glucose concentration. Glucose clearance was calculated from [6,6-2H2]glucose concentration during the entire experiment using the circulatory model described previously (17, 19).

Calculations were performed using the parameters of glucose kinetics derived in normal subjects (19). In brief, glucose clearance (ml·min⁻¹·kg FFM⁻¹) was represented as a piecewise-constant function over 2-min intervals and determined by regularized least squares by fitting the circulatory model to the [6,6-2H2]glucose concentration data throughout the experiment. From the model-determined glucose clearance, the rates of appearance of oral glucose and glucose production (μmol·min⁻¹·kg FFM⁻¹) were determined by fitting the model to the oral and endogenous glucose concentration components, using an analogous procedure.

AUCs were calculated by the trapezium rule. The Staub-Traugott effect was quantitated as the ratio of glucose AUC of the second glucose load to that of the first glucose load.

**Statistical analysis.** Data are given as means ± SE. Paired group values were compared by the Wilcoxon test; differences in time course between Studies I and II were analyzed by two-way ANOVA for repeated measures. Associations were tested by Spearman’s rho. A P value <0.05 was considered statistically significant.

**RESULTS**

**Study I.** Plasma glucose concentrations in response to the second glucose load were lower than those in response to the first, reaching a peak of 7.47 ± 0.28 mmol/l against one of 8.79 ± 0.38 mmol/l (Fig. 1). The corresponding AUC (Table 2) was significantly (P = 0.003) lower; the AUC ratio averaged 0.88 [0.12 (0.36 [0.86] when considering the ratio of the incremental areas). The C-peptide AUC was similar between the two loads (P = 0.76), whereas the insulin concentration AUC was slightly lower during the second compared with the first load (Fig. 1). The insulin secretion time course (Fig. 2) was flatter in response to the second than the first load, with the corresponding AUCs being 37.8 ± 4.3 and 42.8 ± 5.1 nmol/m², respectively (P = 0.02). In contrast, the potentiation factor, which increased on glucose stimulation, was significantly (P = 0.006) higher after the second load (1.08 ± 0.02-fold) than the first (0.92 ± 0.02-fold) (Fig. 2). β-Cell glucose sensitivity (calculated for the entire 360-min study period) averaged 88 ± 10 pmol·min⁻¹·m⁻²·mM⁻¹, whereas the OGIS index was similar between the two loads [433 ± 14 and 447 ± 25 pmol·min⁻¹·m⁻²·mM⁻¹, P = not significant (NS)]. Insulin clearance averaged 1.1 ± 0.1 l min⁻¹·m⁻² during both OGTTs.

The plasma GIP response to the two glucose loads was similar (Table 2), whereas the GLP-1 response was potentiated by the second load (Fig. 2).
Study II. Despite clamping plasma glucose at 8.3 mmol/l during the last 2 h following the first glucose load, the total glycemic response to the second load was similar to the corresponding response in Study I (but clearly reduced as the incremental area) (Table 2 and Fig. 3). The corresponding plasma insulin and C-peptide incremental AUCs were higher, presumably because of the preceding hyperglycemic plateau (Table 2). Likewise, insulin secretion in response to the second load tended to be higher in Study II than in Study I (AUC = 43.6 ± 6.6 vs. 37.8 ± 4.3 nmol/m², P = 0.07). The potentiation factor increased throughout the 6 h as was the case in Study I; the relative increment in potentiation between the first and the second load was significantly (P < 0.05) higher in Study II (from a mean value of 0.85 ± 0.02 to 1.15 ± 0.02, or +36 ± 5%) than in Study I (from 0.92 ± 0.02 to 1.08 ± 0.02, or +19 ± 6%). The M value measured during the hyperglycemic clamp period averaged 36.8 ± 4.3 μmol·min⁻¹·kg FFM⁻¹; the corresponding M/I ratio was 0.37 ± 0.06 μmol·min⁻¹·kg FFM⁻¹·pM⁻¹.

In the subjects receiving the triple tracer protocol, the ingested glucose labeled with [1-2H]glucose was still appearing in the systemic circulation at 180 min at sizeable rates (averaging 28.7 ± 3.4 μmol·min⁻¹·kg FFM⁻¹), slowly tending to zero thereafter. The second load labeled with [U-13C]glucose appeared in the circulation in amounts and time course almost superimposable to those of the first load (Fig. 4). Thus the total amounts of oral glucose appearing in the systemic circulation averaged 60 ± 6 g after the first load (0–180 min) and 52 ± 5 g after the second load (180–360 min) (P = NS); a further 8 ± 2 g of the first load had eventually appeared by the end of 6 h. Plasma glucose clearance rose from a fasting value of 2.9 ± 0.1 ml·min⁻¹·kg FFM⁻¹ to an average of 6.4 ± 0.4 in response to the first load, and remained at this stimulated level (averaging 5.5 ± 0.2 ml·min⁻¹·kg FFM⁻¹, P = NS) throughout the following 3 h. Basal EGP (13.3 ± 0.6 μmol·min⁻¹·kg FFM⁻¹) fell continuously with time, averaging 6.0 ± 3.8 μmol·min⁻¹·kg FFM⁻¹ during the first 180 min and 1.7 ± 2.6 μmol·min⁻¹·kg FFM⁻¹ during the final 180 min (P = 0.04) (Fig. 4).

In Study I, the second-to-first load ratio of glucose AUCs was inversely related to the corresponding ratio of mean potentiation factor (rho = 0.69, P = 0.006). In the pooled data
from Study I and Study II, a higher glucose area during the first OGTT was associated with a higher potentiation during the second OGTT (rho = 0.59, P = 0.002).

DISCUSSION

In this study, the Staub-Traugott phenomenon was clearly evident when using two sequential, equal oral glucose loads over a 6-h time period. The effect was somewhat accentuated when the priming provided by the first glucose load was reinforced by holding plasma glucose at high physiological levels with intravenous glucose (Study II). Thus, from previous studies as well as our results, it seems possible to conclude that, at the whole body level previous exposure to glucose, whether intravenous or oral (8, 24, 25, 27), with or without added nutrients (7) or whether comparing fasting with fed conditions (2, 3), generally improves the tolerance to subsequent glucose stimulation. How long after the priming the effect would last has not been established. Our protocol interposing 3 h between the glucose loads simulates the breakfast-lunch temporal sequence and therefore is relevant to physiological, free-living conditions.

With regard to the mechanisms, our results show that, not only was absolute insulin secretion not higher in response to glucose reloading, but it was actually somewhat (12%) lower, in keeping with the lower plasma glucose concentrations. However, we found that potentiation of insulin secretion was higher during the second than the first load (Study I) and was further heightened by antecedent steady hyperglycemia (Study II). In our model of β-cell function, the potentiation factor accounts for the fact that, during an acute stimulation, insulin secretion is higher on the descending phase of oral glucose-induced hyperglycemia than at the same glucose concentration on the ascending phase. The results of both Study I and Study II together indicate that one of the mechanisms of the Staub-Traugott effect is potentiation of insulin secretion despite lower absolute rates of insulin release: these would have been even lower had the β-cell not been primed by preceding hyperglycemia. In quantitative terms, a higher glycemic excursion in response to the first load was associated with a higher potentiation factor during the second load, suggesting that the priming effect of hyperglycemia was the basis for the subsequent potentiation of insulin secretion. Conversely, a greater increment in potentiation was associated with a larger Staub-Traugott effect, confirming the causative role of glucose potentiation in the secretory response to successive glucose challenges. In support of this interpretation is the finding that the GLP-1 response, but not the GIP response, also was potentiated by repeated glucose ingestion. The rates of appearance of the two oral loads were similar in amount and time course, implying that gastric contents were emptying in the gastrointestinal tract at similar rates. This also rules out the possibility that the Staub-Traugott effect itself might be due to the first load slowing down the transit of the second load. It is possible that GLP-1 release also is subject to glucose potentiation as is insulin release. We are not aware that this finding has been reported before, and it needs to be confirmed.

Neither tracer-derived glucose clearance nor OGIS were higher during the second than the first load. Thus sensitization of peripheral tissues by prior insulin exposure did not account for the Staub-Traugott effect, nor was there any change in whole body insulin clearance. In contrast, suppression of EGP by the first load was maintained and strengthened by the supervening load, thereby quantitatively accounting for the Staub-Traugott phenomenon. This finding is compatible with the notion that the dose-response curve of insulin-induced suppression of EGP is shifted to the left of the dose-response curve of insulin-mediated stimulation of glucose disposal (6). Thus the fact that peripheral plasma insulin levels at the end of the first absorptive period (180 min) were still 150% increased relative to baseline (136 ± 29 vs. 53 ± 6 pmol/l, P < 0.0001) ensured that EGP was tightly restrained and that the glycemic excursion due to the incoming second load was superseded on lower glucose levels (Fig. 4).

In conclusion, enhanced potentiation of insulin response and increased suppression of hepatic glucose production are the main mechanisms underlying the Staub-Traugott effect in healthy humans.

REFERENCES


