Improved Glucose Disappearance Following Repeated Glucose Administration

Serum Insulin, Growth Hormone and Free Fatty Acid Levels During the Staub-Traugott Effect

Andrew John Szabo, M.D., C.M., F.R.C.P.(C), Jacqueline J. Maier, M.D., Olga Szabo, B.S., Rafael A. Camerini-Davalos, M.D., New York

SUMMARY

The Staub-Traugott effect, the phenomenon of improved tolerance to repeated glucose administration, was investigated in fifteen healthy volunteers. Two glucose loads were administered intravenously forty-six minutes apart. Blood samples were taken periodically after each glucose infusion. The second glucose load was given immediately after the first sampling period. The glucose disappearance rate (K) improved after the second infusion from 2.04 ± 0.23 to 2.87 ± 0.16 ($p < 0.001$).

Serum immunoreactive insulin levels rose promptly after the glucose infusions, but while a decrease was noted following the first peak, the levels remained persistently elevated after the second glucose load. Growth hormone levels decreased and were slightly lower yet six to nine minutes after completion of the infusions.

A striking reduction of free fatty acid levels followed the first glucose load; values fell to 50 per cent of the fasting levels prior to, and decreased even further following, the second infusion. Thus, the improved K of the second glucose tolerance test was associated with already diminished serum free fatty acid levels. DIABETES *18*:232-37, April, 1969.

Improvement of carbohydrate tolerance following repeated glucose administration was reported in 1919 by Hamman and Hirschman[1] and confirmed independently by Staub[2] in 1921 and Traugott[3] in 1922. This finding was utilized in the development of the double load glucose tolerance test of Exton and Rose,[4] as a sensitive diagnostic tool for diabetes prior to the cortisone era.

In spite of its recognition over fifty years ago, the phenomenon now known as the Staub-Traugott effect has never been satisfactorily explained. The present investigation was undertaken to study whether the Staub-Traugott effect is caused by augmented insulin secretion, or by a decrease of anti-insulin factors, such as growth hormone and free fatty acids.

From the Department of Medicine, and Diabetes Center, New York Medical College, New York, N. Y.

SUBJECTS AND METHODS

All subjects were healthy volunteers between twenty and thirty years of age. They gave no history of pre-existing metabolic disorder or of familial diabetes, and they were not taking any medication. Their weight was within ± 20 per cent of their estimated normal.[5] There were thirteen male and two female subjects (table 1).

After an overnight fast the subjects rested for approximately one hour and a fasting venous blood sample was obtained. Then glucose 0.5 gm./kg. body weight was infused intravenously as a 25 per cent solution over a period of 3 min. Venous blood samples were obtained 6, 9, 12, 16, 26, 36 and 46 min. after starting the glucose infusion. Immediately following collection of the 46-min. sample, a second glucose load was infused. Concentration, volume and duration of the second infusion was identical to the first. Venous blood samples were collected for another 46 min. after starting the second infusion, with timing of the samples corresponding to those obtained after the first glucose load. Repeated sampling of blood was achieved through a "Y" tube attached to an 18 gauge needle which was kept open in the vein by a slow 0.9 per cent NaCl drip. Prior to taking the sample, the saline drip was clamped and approximately 5 ml. of blood was withdrawn and discarded to avoid sample dilution by the saline. The 15 ml. of blood samples thereafter collected were allowed to clot at 4° C. and were centri-

TABLE 1

Anthropometric data and glucose disappearance rates (K_1 and K_2) after repeated glucose infusion in fifteen healthy volunteers

Case No.	Age (years)	Sex	Weight (lbs.)	Height (feet)	K_1	K_2
I	23	F	126	5'6"	3.01	4.92
II	23	M	155	5'9"	1.30	1.92
III	21	M	137	5'8"	2.51	3.12
IV	26	F	150	5'10"	4.63	4.78
V	27	M	160	5'8"	2.43	3.98
VI	22	M	116	5'8"	1.29	2.04
VII	24	M	165	6'0"	1.76	2.79
VIII	24	M	190	6'1"	1.19	2.56
IX	23	M	158	5'10"	1.09	1.71
X	21	M	165	6'0"	1.98	3.62
XI	26	M	175	5'11"	2.31	3.12
XII	24	M	170	5'7"	1.91	2.51
XIII	25	M	185	6'0"	1.39	2.14
XIV	23	M	155	5'6"	2.04	2.06
XV	30	M	183	6'2"	1.28	1.81
Mean ± S.E.M.					2.04 ± 0.23 p value: < 0.001	2.87 ± 0.16

fuged at 25,000 rpm for 15 min., within two hours after their collection. Sera were stored frozen in small aliquots at —20° C.

Serum glucose concentrations were determined in duplicates, immediately following centrifugal separation, on a Technicon AutoAnalyzer using a modification of the method of Hoffman.[6] Insulin and growth hormone concentrations were determined immunologically employing the double antibody assay of Morgan and Lazarow,[7] and Morgan.[8] Free fatty acid levels were determined on a Technicon AutoAnalyzer adapted to the procedure of Antonis.[9] With this method, mean fasting serum free fatty acid concentrations of forty-seven healthy controls (age four to fifty-one years) was 519 ± 35 μEqu./L., values ranging from 95 to 900 μEqu./L.

Glucose values were plotted on semilogarithmic paper and disappearance rate (k) was calculated according to the method of Conard.[10] If the decreasing glucose values during either observation period did not fit a straight line on the semilogarithmic paper, the slope of the last 30 min. of the testing time was used. This was necessitated by the wide range of the immediate postinfusion values which reflected equilibration in plasma and extracellular volume rather than actual tissue glucose uptake.[11] Statistical significance was calculated with the Student *t* test.[12]

RESULTS

The initial glucose removal rate (K_1) was low in Subject IX, within normal limits in all others. The K values improved in fourteen out of fifteen volunteers after the second glucose load except for Subject XIV who had similar rates for the two infusions. Subject IV had a very high initial K_1 value (4.6), which improved only minimally after the second infusion. It is not improbable that her K_1 approximated the maximal rate of utilization she was able to reach at these ranges of glucose concentrations. The mean K_1 was 2.04 ± 0.23, and the mean K_2 was 2.87 ± 0.16 and the difference was statistically significant $p < 0.001$ (table 1).

The mean serum glucose level (figure 1) immediately prior to the second infusion was 161 mg./100 ml., almost twice as high as the fasting value (84 mg./100 ml). Therefore, even though the first glucose peak was somewhat lower then the second (340 ± 21 mg./100

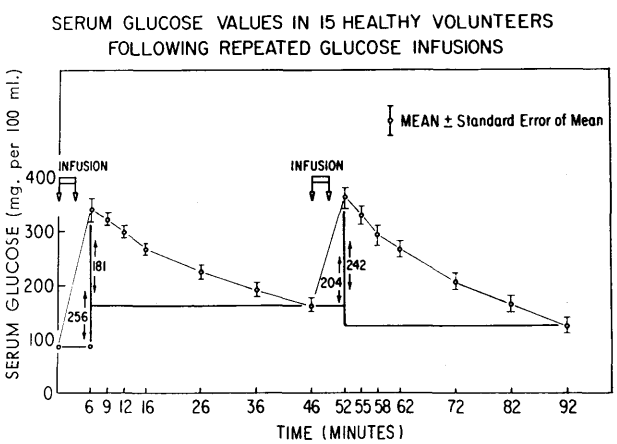

FIGURE 1

ml. and 365 ± 17 mg./100 ml. respectively), the increment over preinfusion level was appreciably greater following the first glucose load, than after the second (256 mg./100 ml. and 204 mg./100 ml., $p < 0.025$). The mean serum glucose fell 181 mg./100 ml. in 43 min. after completion of the first, and 242 mg./100 ml. after the second glucose load ($p < 0.005$, figure 1).

The serum insulin levels underwent wide individual variations. While in some subjects the postinfusion values were only a few microunits higher than the fasting levels, in others rises as much as 90 μU. were noted after the glucose loads.

The mean serum insulin levels reached a peak in 6 min. after the start of the glucose infusion (figure 2). The highest values were of similar magnitude, 42 ± 7 and 39 ± 4 μU./ml. respectively for the first and second infusions. However, while rapid decrease followed the first peak, the levels remained persistently elevated after the second peak. Mean insulin values 16, 26, and 36 min. after the start of the second glucose infusion were significantly higher ($p < 0.025$) than in the corresponding samples obtained after the first glucose load.

The fasting serum growth hormone levels (figure 3) were in the low normal range. Slight decreases were noted shortly after both glucose infusions. The levels returned to near preinfusion values in 15 to 20 min.

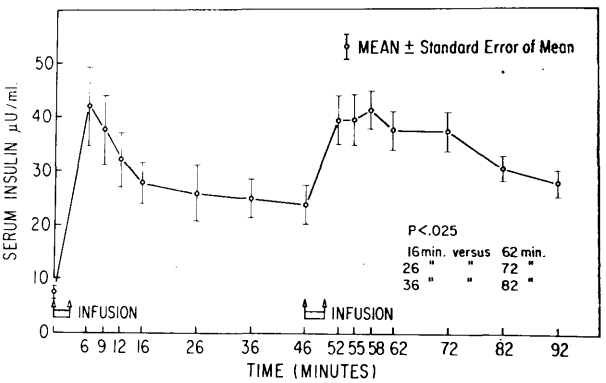

FIG. 2. Serum insulin levels following repeated intravenous glucose infusions in 15 healthy volunteers.

following the glucose loads. One subject (X), had a marked growth hormone rise 26, 36 and 46 min. following the second infusion, reaching values of 14, 28 and 24 mμg./ml. of serum, respectively. Figure 3 shows mean growth hormone values with Subject X excluded (straight line) and included (interrupted line).

Initial free fatty acid levels (352 ± 30 μEq./L.) after an insignificant rise, fell to 174 ± 13 μEq./L. after the first glucose infusion, and remained below that level throughout the rest of the study. Thus, when the

SERUM GROWTH HORMONE LEVELS FOLLOWING REPEATED INTRAVENOUS GLUCOSE INFUSIONS IN 15 HEALTHY VOLUNTEERS

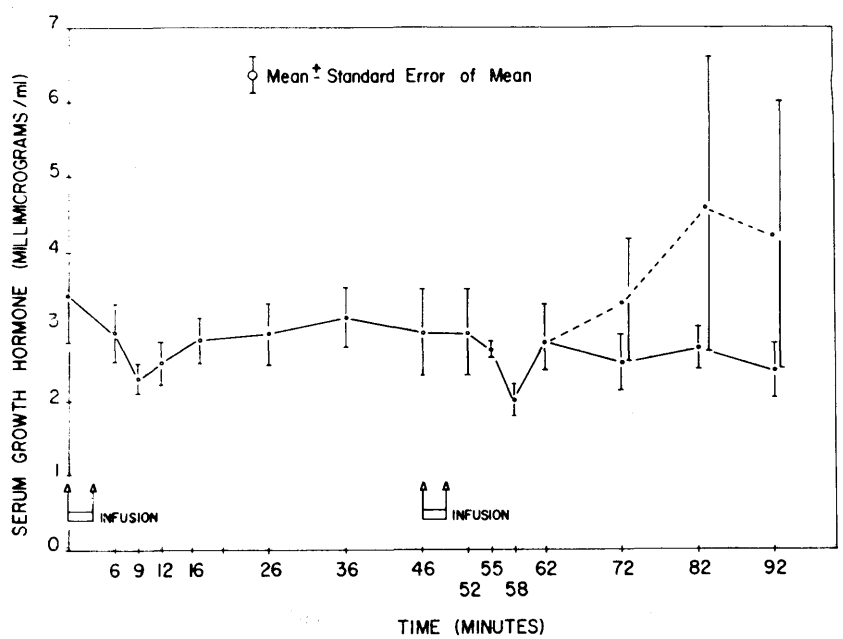

FIG. 3. Serum growth hormone levels following repeated intravenous glucose infusions in fifteen healthy volunteers. Solid line: Mean and S.E.M. with Subject X excluded after the 62 min. sample. Interrupted line: Mean and S.E.M. Subject X included.

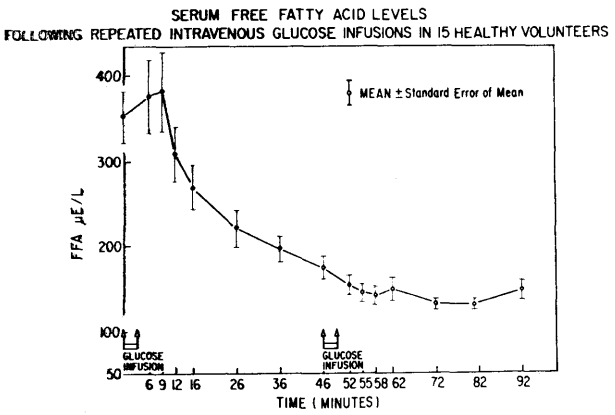

FIGURE 4

second glucose infusion was started, free fatty acid levels were already markedly decreased and following the second glucose load, the values fell to even lower levels (figure 4).

DISCUSSION

When glucose was administered orally in successive doses to human subjects, Hamman and Hirschman,[1] and Staub[2] and Traugott[3] observed paradoxical improvement of tolerance. In the present series of experiments, repeated glucose loads were administered intravenously. This was done to exclude differences in absorption, to shorten the time of the experiments, and to render data which can be expressed by a single number (K) that is characteristic for the whole curve. The relatively short time interval between successive infusions (43 min. from the completion of the first to the beginning of the second glucose load) was chosen following the suggestion of Somersalo[13] that the second tolerance test should start before a rebound after the first could take place. It may be because of this timing, and the choice of our subjects, that a positive Staub-Traugott effect was noted in fourteen out of our fifteen cases, contrary to the experience marked in the recent literature.[14,15]

The improvement following successive glucose administration was marked. This is emphasized by the fact that not only the rate of disappearance (K) improved, but that the mean of the actual values reached 43 min. after completion of the second infusion was 38 mg./100 ml. lower than the glucose level at the corresponding time following the first infusion. The importance of the latter is stressed by the observation of Forbath and Hetenyi[16] that K values do not necessarily correlate with actual glucose utilization.

The rise of serum glucose over preinfusion level was much smaller after the second than after the first sugar load (figure 1). This was surprising, since dosage, time of infusion, and concentration of the solutions were identical for successive tests in any individual subject. Increase of the fluid volume equilibrating with the infused glucose, incomplete mixing, substantial urinary glucose loss or increased rate of glucose utilization during the second infusion could possibly explain the finding. None of these possibilities was explored in the present study.

There were wide variations in serum immunoreactive insulin from one subject to another, and there were fluctuations within individual tolerance tests as well. This is reflected in the large standard errors of the means shown in figure 2. High and low responses in serum insulin levels of healthy subjects have been commented upon by Cerasi and Luft[17] and by Welborn et al.[18] and thought to be a normal biological variant.

In the present study serum immunoreactive insulin levels did not return to the baseline prior to the second infusion. Thus, the second glucose load was received by tissues already exposed to high insulin levels. Furthermore, the area under the second insulin curve is larger than the area under the first. It is tempting to associate the higher immunoreactive insulin levels with the improved glucose utilization, but there are several reasons for opposing this conclusion.

Although the effect of insulin on glucose metabolism has been firmly established, a simple correlation between insulin secretory responses and glucose disappearance has not been found.[19,20] Recently a mathematical approach has been developed applicable to specific experimental conditions and utilizing a digital computer.[21]

Soskin in 1934 reported that injection of glucose into pancreatectomized dogs receiving constant infusion of insulin, resulted in a normal glucose disappearance pattern.[22] Repeated injections of glucose to the same animal resulted in typical Staub-Traugott effect. Thus it is evident that while the presence of insulin is necessary for normal glucose tolerance, additional amounts of insulin may not necessarily be required to dispose of glucose loads. Indeed, insulinization of the tissue may be more important than circulating insulin levels. In acute experiments immediately following total pancreatectomy, normal tolerance to glucose was reported in animals not given any insulin,[23] while tolerance was abnormal in those who were maintained subtotally pancreatectomized for a longer period of time prior to total acute pancreatectomy.[24] Finally, serum insulin levels should not be causally equated with secretion, since changes in re-

moval rate may be equally important.[25]

In view of the above studies, and because our immunoassay may not differentiate the biologically inactive proinsulin[26] from active insulin, we elected to consider factors other than elevated insulin levels as causing the improvement of K afrer the second glucose load. Indeed, it has been reported that immunoreactive insulin levels at the tail-end curves following glucose stimulation may contain up to 40 per cent "big insulin."[27] In the present study elevated insulin levels were seen in the latter part of the experiment.

Since Soskin et al. had reported that the hypophysis was indispensable in Staub-Traugott effect,[28] serum growth hormone levels were determined in our experiments. The initial levels were low, as only overnight fast preceded the tests and the majority of our subjects were male.[29] A slight fall was noted immediately after the infusions, but growth hormone concentrations returned to near preinfusion levels in the subsequent 15 to 20 min. A single subject (X) had a marked rebound of growth hormone in the last 30 min. of the second postinfusion period, which reached levels several times higher than that of the fasting value. A metabolic effect of this GH rise was not however reflected by the other variables. Growth hormone rebound three hours following intravenous glucose tolerance test (IVGTT) in prediabetes has recently been described.[30] The relative insensitivity of the growth hormone assay at the low levels which were seen in fourteen out of fifteen subjects in these studies is disturbing enough to caution against overly zealous interpretation of present results. We are, therefore, not prepared to suggest that changes in growth hormone levels are responsible for the improvement of tolerance to a second glucose load. This of course does not exclude the importance of other pituitary or hypothalamic systems.

The most significant differences seen in the variables studied after successive glucose loads was the reduction of free fatty acid levels after the first infusion (figure 4). Levels fell to 50 per cent of the fasting values by the end of the first sampling period (prior to the second glucose infusion) and decreased even further following the second glucose load. Thus, the improved second K was associated with already markedly diminished serum free fatty acid levels.

Free fatty acids have been reported to impair glucose utilization by muscle in vitro.[31]

This observation led to the hypothesis of the "glucose fatty acid cycle."[32] Investigators, who attempted to validate the hypothesis in vivo, induced elevation of free fatty acid levels by heparin infusion with or without a fat meal.[33,34] While the results generally supported the hypothesis, such maneuvers may themselves have an effect on metabolism, not necessarily mediated by free fatty acid levels. Recently, it was reported that infusion of free fatty acid to dogs promoted insulin secretion and resulted in a marked drop of blood glucose.[35] Though this introduces doubt in the validity of the Randle hypothesis in vivo, it should be mentioned that in those experiments the amounts of free fatty acid infused were clearly of the pharmacological magnitude, and that one animal, with its serum free fatty acid only doubled, did not have insulin hypersecretion, nor hypoglycemia.

The present findings are consistent with the observation of Soskin et al.[28]: that an intact hypophysis is necessary to elicit a successful Staub-Traugott effect. In hypopituitary patients fasting free fatty acid levels being low, a glucose infusion may not result in as marked a decrease of free fatty acid as we have observed in normals. If lowering of free fatty acid levels by the first infusion were instrumental in eliciting improvement of K in the second glucose tolerance test, the lesser reduction of the already low fasting free fatty acid in the hypopituitary patient may be the cause of the absence of the Staub-Traugott phenomenon which was noted by the above authors.

While in the present studies cause and effect relationship cannot be claimed for the decrease of free fatty acid and improvement of glucose utilization, the correlation is striking and necessitates a reappraisal of the glucose fatty acid metabolic interactions within the range of physiological concentrations of these substances.

In this discussion, alterations in glucose utilization, insulin, growth hormone and fatty acid levels have been looked upon as direct metabolic effects of the infused glucose. Alternatively, it may be considered that all these effects are the result of suppression of epinephrine levels by the glucose infusion. Interpretation and pathophysiology of the Staub-Traugott effect may be of clinical importance, since the experiment has a close resemblance to "nibbling",[36] which was advocated as a dietary habit preferrable to "gorging."

It is concluded that the Staub-Traugott effect can be reproduced following intravenous glucose tolerance test in young adults. Improvement of the glucose disappearance rate following the second infusion was associated with persistent elevation of serum immunoreactive insulin and a small decrease of growth hormone and markedly lowered serum free fatty acid levels.

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