Influence of acute hyperglycemia in human sepsis on inflammatory cytokine and counterregulatory hormone concentrations

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Supported by the Key Project of the Tenth-Five-year plan Foundation of PLA, No. 01Z011

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Received: 2002-12-28 Accepted: 2003-02-16

Abstract

AIM: In human sepsis, a prominent component of the hypermetabolite is impaired glucose tolerance (IGT) and hyperglycemia. Elevations in plasma glucose concentration impair immune function by altering cytokine production from macrophages. We assessed the role of glucose in the regulation of circulating levels of insulin, glucagon, cortisol, IL-6 and TNF-α in human sepsis with normal or impaired glucose tolerance.

METHODS: According to the results of intravenous glucose tolerance test, forty patients were classified into two groups: control group (n=20) and IGT group (n=20). Plasma glucose levels were acutely raised in two groups and maintained at 15 mmol/L for 3 hours. Plasma insulin, glucagon and cortisol levels were measured by radioimmunoassay, the levels of TNF-α and IL-6 were detected by ELISA.

RESULTS: In IGT group, the fasting concentrations of plasma glucose, insulin, glucagon, cortisol, IL-6 and TNF-α levels were significantly higher than those in control group (P<0.05). During clamp, the control group had a higher average amount of dextrose infusion than the IGT group (P<0.01). In control group, plasma insulin levels rose from a basal value to a peak at an hour (P<0.05) and maintained at high levels. Plasma glucagon levels descended from a basal value to the lowest level within an hour (P<0.01) and low levels were maintained throughout the clamp. In IGT group, plasma insulin was more significantly elevated (P<0.01) and plasma glucagon levels were not significantly declined. Plasma cortisol levels were not significantly changed in two groups. In control group, plasma IL-6 and TNF-α levels rose (P<0.01) within 2 hours of the clamp and returned to basal values at 3 hours. In IGT group, increased levels of plasma cytokine lasted longer than in control group (3 hours vs. 2 hours, P<0.05), and the cytokine peaks of IGT group were higher (P<0.05) than those of control group.

CONCLUSION: Acute hyperglycemia pricks up hyperinsulinemia and increases circulating cytokine concentrations and these effects are more pronounced in sepsis with IGT. This suggests a potential modulation of immunoinflammatory responses in human sepsis by hyperglycemia.
Severity of sepsis and underlying diagnosis
Sepsis was defined by the American College of Chest Physicians-Society of Critical Care Medicine consensus statement by an identifiable site of infection and evidence of a systemic inflammatory response manifested by at least three of the following criteria: (1) temperature, >38 °C or <36 °C; (2) heart rate, >90 beats per minute; (3) respiratory rate, >20 breaths per minute; (4) white blood cell count, >12 000/mm3 or <4 000/mm3[19]. Patients meeting enrollment criteria were entered within 24 h. The cause of sepsis was severe acute pancreatitis (n=11), colorectal anastomotic dehiscence (n=9), perforated diverticular disease (n=9), gastroduodenal perforation (n=7), gallbladder perforation (n=3) and spontaneous splenic abscess (n=1). Sepsis severity was scored using the method of Elebute and Stoner[20]. This scoring procedure takes into account the site of infection, bacteriology, body temperature, secondary effects (e.g. jaundice) and various haematological and biochemical variables, such as white cell count and plasma albumin concentration.

Study protocol
After 12-hour fast overnight, the patients were placed in a supine comfortable position with the sickroom temperature between 20 °C and 24 °C. Intravenous lines were inserted into a large antecubital vein of one arm for infusions and into a dorsal vein of the contralateral arm for blood sampling. Patency was preserved in a slow saline infusion (0.9 % NaCl). After withdrawal of baseline blood samples, plasma glucose concentrations were acutely raised with a bolus injection of 0.25 g/kg glucose followed by a varying 30 % glucose infusion to achieve steady-state plasma glucose concentrations of about 15 mmol/L for 180 minutes.

Analysis
Samples for analysis of plasma glucose were collected in tubes containing a trace of sodium fluoride. Plasma glucose was determined according to the glucose oxidase method with an autoanalyzer (Beckman Instruments). Serum samples for measuring hormone and cytokine level were stored at -80 °C until assay. Commercially available kits were used for radioimmunoassay of plasma insulin, glucagon and cortisol concentrations. Serum concentrations of TNF-α, IL-6 were determined in duplicate with commercially available kits (R&D Systems). Dilution curves of serum samples were parallel to those of standard. Intra-assay and interassay coefficients of variation were 3.8 % and 5.8 % for TNF-α, 2.8 % and 3.3 % for IL-6.

Statistical analysis
Results were given as mean ±SD. One-way ANOVA was used to compare baseline data, followed by Scheffé’s test for pairwise comparisons. Multiple comparison tests were made with ANOVA, followed by post hoc analysis (Student-Newman-Keuls test) to locate the significant difference indicated by ANOVA. A value of P<0.05 was considered statistically significant. Data were analysed using Statistical Package for the Social Sciences computer software (SPSS 11.0).

RESULTS
During the 7-month study period 46 patients were collected, however analysis was limited to 40 patients with complete and available data. IGT group had higher admission severity scores than control group (APACHE II, 10 (7-15) vs. 7 (4-10), sepsis score, 13 (8-19) vs. 8 (6-13); P<0.05). In IGT group, the levels of fasting plasma glucose, insulin, glucagon, cortisol, IL-6 and TNF-α levels were significantly higher than those in control group (Table 1). During the clamp, plasma glucose became stabilized at 15 mmol/L with oscillations not exceeding 5 % of the prefixed value. The control group had a higher average amount of dextrose infusion than IGT group (0.68±0.31 g/kg vs. 0.42±0.16 g/kg; P<0.01).

Table 1 Details of control and IGT groups

| Variable          | Control group (n=20) | IGT subject (n=20) |
|-------------------|----------------------|--------------------|
| Age, y            | 41±4                 | 42±6               |
| Sex, M/ F, n      | 13/ 7                | 12/ 8              |
| Body mass index, kg/ m2 | 21.11±1.22          | 20.12±1.43        |
| Plasma glucose, mmol/ L | 5.22±0.89          | 7.23±0.73         |
| Plasma insulin, pmol/ L  | 72.00±14.83         | 82.95±10.29       |
| Plasma glucagon, pmol/ L   | 80.75±12.98         | 90.90±15.54       |
| Plasma cortisol, pmol/ L    | 0.68±0.11          | 0.79±0.12         |
| IL-6, pg/ ml       | 3.18±0.64           | 3.63±0.43         |
| TNF-α, pg/ ml      | 4.66±0.70           | 5.99±0.76         |
| APACHE II score    | 7(4-10)             | 10(7-15)          |
| Sepsis score       | 10(8-16)            | 15(10-25)         |

All data were the mean (s.d.), except APACHE II score and sepsis score which were the median (range). APACHE, acute physiology and chronic health evaluation. *P<0.05 vs. control group. †P<0.01 vs control group.

Counterregulatory hormone
During the clamp, plasma insulin levels increased from a basal level of 72.00±14.83 pmol/L to a peak of 83.40±14.29pmol/L within one hour (P<0.05) and maintained at high levels in control group. Whereas, plasma insulin levels increased more significantly in IGT group (P<0.01) (Figure 1). In control group, plasma glucagon levels decreased from a basal value of 80.75±12.98 pmol/L to the lowest level of 74.70±11.40 pmol/L within an hour (P<0.01) and low levels maintained, and was not significantly declined in IGT group during the entire observation period (Figure 2). Plasma cortisol was not significantly changed in two groups (Figure 3).

Figure 1 Circulation insulin levels during hyperglycemia clamps in 20 patients of control group (○-○) and in 20 patients of IGT group (△-△). Mean ±S.E.M. in human sepsis. Plasma insulin levels rose from a basal value to a peak within an hour (P<0.01) and high levels maintained in two groups.
In IGT patients, a short term of hyperglycemia after glucose infusion resulted in increased levels of plasma cytokines, with a peak of 3.67±0.57 pg/mL within 1 hour compared to a peak of 3.18±0.64 pg/mL in control group. In IGT patients, increased plasma cortisol levels and IL-6 levels were observed during hyperglycemia, with a peak within 1 hour (5.4±0.64 pg/mL vs. 4.66±0.70 pg/mL, p<0.01). In IGT group, increased level of plasma TNF-α-1, IL-6 during the clamping lasted longer than in control group (3 hours vs. 2 hours, p<0.05) (Figure 4, 5).

**Inflammatory cytokine**

In control group, plasma IL-6 levels increased from a basal value of 3.18±0.64 pg/mL to a peak of 3.67±0.57 pg/mL within 1 hour (p<0.01) and returned to baseline at 3 hours. Fasting plasma TNF-α levels were 4.66±0.70 pg/mL, they peaked at 1 hour (5.4±0.64 pg/mL, p<0.01), and returned to baseline at 3 hours. In IGT group, increased levels of plasma cytokine during the clamp lasted longer than in control group (3 hours vs. 2 hours, p<0.05) (Figure 4, 5).

**DISCUSSION**

In this study, we found that IGT group had higher admission severity scores than control group, and higher plasma concentrations of counterregulatory hormones and inflammatory cytokines. During the clamp, IGT group had a lower average amount of dextrose infusion than control group. In IGT patients, a short term of hyperglycemia after glucose infusion failed to adjust the plasma concentration of counterregulatory hormones to maintain glucose homeostasis. Acute hyperglycemia in control and in IGT patients induced an increase in plasma IL-6, TNF-α concentrations and insulin levels, and the effect was amplified by IGT group. These results indicate that hyperglycemia in human sepsis with IGT is more easy to be revoked, and plays a potential modulation role in immunoinflammatory responses.

The mechanisms for “stress IGT” are well known. Human sepsis is accompanied by a marked increase in plasma concentration of counterregulatory hormones, i.e. glucagons, epinephrine, cortisol and growth hormone that affect glucose homeostasis. These hormones can lead to significant reductions in insulin sensitivity through poorly understood mechanisms likely related to alterations in insulin signal pathway. Counterregulatory hormones also enhance lipolysis and level of free fatty acids (FFA) which may contribute additional defects to the defective insulin action. Cytokine, TNF-α and IL-6 may exert their influence indirectly by stimulating counterregulatory hormone secretion and by direct action themselves. TNF-α and IL-6 individually and synergistically increase net glucose flux through resistance to insulin actions in muscle and liver via poorly understood post-receptor mechanisms. Hepatic insulin resistance leads to ongoing glucose production even in hyperglycemia. Peripheral insulin resistance decreases skeletal muscle glucose uptake and reduces glucose clearance, which leads to the development of IGT and even hyperglycemia.

After glucose infusion, in normal group, glucagon and glucose concentrations reduced by host insulin were sufficient and inhibited hepatic gluconeogenesis and glycolysis and prevented glucose production. In contrast, in IGT group, glucose infusion failed to suppress endogenous glucose production despite accompanying hyperinsulinemia. Using stable isotopes, it was demonstrated that hepatic glucose production was -150 % of the normal resting post-absorptive values of healthy subjects in spite of provision of total parenteral nutrition with dextrose at rates exceeding the basal energy expenditure.

Glucose-based nutrition in septic patients may cause marked hyperinsulinemia due to peripheral insulin resistance. Hyperinsulinemia associated with glucose-based nutrition in sepsis might augment proinflammatory cytokine production and stress response in septic patients. The present findings
also demonstrated that acute hyperglycemia affected concentration of plasma cytokines in human sepsis, and this effect was more pronounced in IGT patients. *In vitro* studies using supraphysiological glucose concentration (>22 mmol/l) showed an increase in TNF-α and IL-6 secretion from healthy human mononuclear cells. Furthermore, increased synthesis of TNF-α has been reported both in rat uterine cells cultured *in vitro* with increasing concentrations of glucose and in placental tissue explants from women with gestational diabetes incubated with high glucose (25 mmol/l). Human monocytes produced by IL-6 in healthy volunteers increased during 24-hour incubation in high-glucose medium. These findings are in accordance with our observations in sepsis, suggesting a potential modulation of immunoinflammatory response by carbohydrates.

In summary, IGT in sepsis is associated with marked changes in plasma concentrations of counterregulatory hormones and inflammatory cytokines, and these changes partially account for the fact that IGT easily develops acute hyperglycemia during glucose infusion. Acute hyperglycemia pricks up hyperinsulinemia and increases circulating cytokine concentrations. This suggests a potential role of hyperglycemia in immunoinflammatory responses in human sepsis.

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Edited by Ren SY and Wang XL