# Metabolic Profiles of Thermal Trauma

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**The study was designed to establish where significant correlations exist in a variety of metabolic substrates and hormone mediators in patients sustaining thermal injury. The factors studied were insulin, human growth hormone, cortisol, glucagon, free fatty acid, triglyceride and glucose. Incorporated into this design was an evaluation of the impact of quantitated severity of injury upon these correlations. In patients sustaining a low severity of injury (Probability of death (p = 2.2 to 33.9) there appeared a loss of glucose regulation in conjunction with insulin resistance without significant interplay of other factors studied. In contrast, patients sustaining high severity injury (p = 46.9 to 100) evidenced correlations between glucagon and glucose (negative), cortisol and free fatty acid indicating a significant role of hyperglucagonemia in these patients. A discriminant function analysis was employed to incorporate all significant variables into a probability model. Only insulin, glucose and glucagon appeared in the optimal classification equation.**

THERMAL TRAUMA is accompanied by an increase in the metabolic rate and elevated blood glucose levels.[1] Investigations have suggested that these alterations are mediated by endocrine changes in which there is impaired insulin secretion and subsequent insulin resistance,[1,24–26,32] increased concentrations of glucocorticoids,[3,4,10] increased catecholamine elaboration,[4,5,12,31] and increased pancreatic secretion of glucagon.[19,23–25,30] Elevated catecholamine levels have been shown to increase lipolysis with a resultant elevation of blood free fatty acid concentration.[6,7,14,22] It has been suggested that fat breakdown supplies energy needs following injury, and postinjury protein breakdown occurs to supply elements for hepatic gluconeogenesis.[17] Thus, the metabolic consequences of injury involve many factors. However, their respective activities are not isolated or independent but act in concert to effect the ultimate level of blood glucose. Further, both increase in metabolic level and protein catabolism have been shown to be proportional to the severity of the injury.[11,20]

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This study was designed to evaluate a variety of metabolic substrates and hormone mediators and to establish where significant correlations exist. It was also designed to evaluate the contribution of severity of injury upon these correlations.

## Materials and Methods

Thirteen patients with thermal injury were studied (Table 1). Patients were adults ranging in age from 18 to 60 years, nine male and four female, and total body surface area burn ranged from 16 to 91%. Peripheral blood samples were collected each morning before breakfast at two to three day intervals starting usually on Day 2 to 3 postburn and continuing as long as possible up to 30 days postburn. The number of determinations varied from three to 12 among the patients.

Blood samples were drawn as follows: clean tubes for serum to be used in the insulin, human growth hormone, and cortisol assays, tubes containing 15% EDTA and 200 units Trasylol (Aprotinin 10,000 Kallikrein inactivator units/ml FBA Pharmaceuticals New York, New York) per 0.5 ml plasma for the glucagon assay, tubes containing potassium oxalate for blood glucose assay and tubes containing 100 units of heparin/ml blood for free fatty acid and triglyceride assays. Specimens were immediately placed on ice, centrifuged at 5° when necessary, dispensed and frozen until tested. All samples from an individual patient were tested together. Insulin was tested in the coated charcoal immunoassay[15] and glucagon was assayed by radioimmunoassay using 30K antibody from Roger Unger, M.D., Department of Internal Medicine, University of Texas. Human growth hormone was quantitated in the charcoal dextran radioimmunoassay[18] and cortisol was tested in the competitive protein binding radioimmunoassay.[21] Free fatty acids were measured in a two-phase extraction and colorimetric determination[2] and plasma triglycerides were determined as previously described.[8] Blood glucose was assayed by the hexokinase procedure.

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TABLE 1. *Patient Data*

| Patient No. | Sample Data: No. Samples | Sample Data: Period (Post Burn Day No.) | Age (yrs)/ Sex | Burn: TBS % | Burn: FT % | p |
|---|---|---|---|---|---|---|
| 1 | 7 | 6–24 | 29 F | 16 | 0 | 4.2 |
| 2 | 8 | 2–19 | 23 F | 47 | 15 | 19.4 |
| 3 | 6 | 5–17 | 26 F | 28.5 | 5 | 2.2 |
| 4 | 9 | 7–26 | 36 M | 50 | 0 | 8.3 |
| 5 | 12 | 8–34 | 18 M | 48 | 16 | 6.7 |
| 6 | 10 | 4–27 | 29 M | 38.5 | 7.5 | 28.8 |
| 7 | 10 | 3–24 | 60 M | 40 | 6 | 13.8 |
| 8 | 7 | 4–18 | 56 M | 26.5 | 9.5 | 33.9 |
| 9 | 4 | 10–17 | 36 M | 54 | 50 | 98.2 |
| 10 | 5 | 2–12 | 57 M | 88 | 80 | 100 |
| 11 | 4 | 4–11 | 51 M | 82.5 | 18 | 84.0 |
| 12 | 5 | 5–14 | 56 F | 19 | 18 | 70.0 |
| 13 | 6 | 13–25 | 21 M | 91 | 10 | 46.9 |

Median values were computed from all test samples of an individual patient for each of the laboratory measurements. Those values were used to represent a patient in all subsequent data analysis. These data were analyzed using the All Possible Regressions routine of the Biomedical Computer Programs (BMDP). The dependent variable was glucose and the remaining quantities were potential independent variables. A second regression analysis included information about the severity of the burn as additional independent quantities. The percentage of full and partial thickness burn, a subjective burn index,[27] and the probability of death (p) computed from a discriminant analysis[9] were utilized. A third analysis attempted to discriminate between two groups of patients on the basis of laboratory variables. The low severity group was composed of patients Numbers 1–8 with a range of p = 2.2 to 33.9 and a high severity group composed of patients Numbers 9–13 with a range of p = 46.9 to 100. None of the low severity group were fatalities while all the high severity group were fatalities. The All Possible Regressions routine was used for analysis of variables in these two groups of patients. The stepwise Discriminant Analysis Program of BMDP was used to identify the best set of discriminatory variables.

TABLE 2. *Data Summary Statistics*

| Variable | Mean | S.D. | Range |
|---|---|---|---|
| Insulin | 44.4 | 52.7 | 7–199 |
| HGH | 1.3 | 1.8 | 0.0–6.3 |
| Cortisol | 25.1 | 6.6 | 13.9–39.2 |
| Glucagon | 260.1 | 264.5 | 74.0–1101.5 |
| FFA | 317.9 | 113.1 | 183.0–509.0 |
| TG | 124.8 | 40.3 | 48.5–196.0 |
| p | 37.6 | 37.9 | 2.2–100.0 |
| Glucose | 147.2 | 30.8 | 95.0–200.0 |

## Results

Means for each variable were calculated from the median values for each patient studied (Table 2). The Pearson correlation coefficients were then determined among these laboratory measurements (Table 3). Strongly positive correlations ($p < 0.05$) were noted between cortisol and probability of death (0.743), cortisol and glucagon (0.715) and glucagon and free fatty acid (0.595). Free fatty acid and triglyceride (0.526), cortisol and free fatty acid (0.486), insulin and glucose (0.482) and free fatty acid and probability of death (0.472) were correlated where $p < 0.1$. The most strongly negative correlations existed between insulin and other variables tested, particularly probability of death (−0.487) and free fatty acid (−0.482) but were not highly significant statistically ($p < 0.1$).

The computer programs examined the potential predictor variables to find the best subset to predict blood glucose concentration. All possible regressions were calculated: R-squared, adjusted R-squared and Mallow's Cp were used to select the best subset.

The optimal subset of laboratory variables for predicting glucose included insulin, cortisol, and glucagon. The regression equation was:

$$\text{Glucose} = 35.07 + 0.42(\text{Insulin}) + 4.55(\text{Cortisol}) - 0.08(\text{Glucagon}).$$

TABLE 3. *Correlation Matrix of Median Patient Observation (N = 13)*

| | Insulin | Human Growth Hormone | Cortisol | Glucagon | Free Fatty Acid | Triglyceride | Probability of Death | Glucose |
|---|---|---|---|---|---|---|---|---|
| Insulin | 1.000 | | | | | | | |
| Human growth hormone | −.343 | 1.000 | | | | | | |
| Cortisol | −.374 | .208 | 1.000 | | | | | |
| Glucagon | −.176 | .022 | .715 | 1.000 | | | | |
| Free fatty acid | −.482 | −.100 | .486 | .595 | 1.000 | | | |
| Triglyceride | −.304 | .150 | .318 | .349 | .526 | 1.000 | | |
| Probability of death | −.487 | .042 | .743 | .422 | .472 | .160 | 1.000 | |
| Glucose | .482 | −.310 | .211 | −.122 | −.086 | −.103 | .334 | 1.000 |

The multiple $R^2$ for this relationship was 0.64.

When the burn severity data was entered, probability of death (p) replaced cortisol in the relationship:

$$\text{Glucose} = 108.08 + 0.73(p) + 0.50(\text{Insulin}) - 0.04(\text{Glucagon}).$$

The multiple $R^2$ for these variables was 0.76. The replacement of cortisol by p should not be surprising in view of the fact that the correlation between these two quantities was 0.743 (Table 3).

The previous data analyses considered all patients studied as one group. Further analysis considered contrasts in the metabolic responses between the low and high severity groups of patients. Means of the low and high severity groups for each variable were calculated from the median values for each patient (Table 4). T statistics are given to indicate differences between each variable studied of the two groups. The Pearson correlation coefficients were then determined among these laboratory measurements for low severity patients as a group (Table 5) and high severity patients as a group (Table 6). Because of the limited number of patients in these subgroups, large values were required for significance. In the low severity group analysis only two relationships were significant at $p < 0.05$. These were insulin-glucose (+0.858) and p-cortisol (0.679). In contrast, in the high severity group, four relationships were significant at $p < 0.05$. These were: cortisol-glucagon (+0.844), FFA-glucagon (+0.806), glucose-glucagon (−0.798), and FFA-glucose (−0.918). Interestingly, the two relationships shown to be significant in the low severity group, *i.e.*, insulin-glucose and p-cortisol, had no correlation in the high severity group, being −0.010 and +0.107 respectively.

Discriminant analysis, which distinguishes between two groups using a collection of discriminatory variables from each group, was then used in the analysis of these two groups. This analysis produced a discriminant function which is a linear combination of this set of variables. Means of the low and high severity groups (Table 4) was used in this analysis. A regression analysis on all laboratory variables identified insulin, glucagon and glucose as predictor variables in the optimal classification function. $R^2$ for this relationship was 0.78.

TABLE 4. *Means of Laboratory Measurements for Low and High Severity Patient Groups*

| | Low Severity | High Severity | |
|---|---|---|---|
| N | 8 | 5 | t |
| Insulin | 63.8 | 13.4 | 1.84 |
| Human growth hormone | 1.2 | 1.5 | 2.80 |
| Cortisol | 21.5 | 30.8 | 3.32 |
| Glucagon | 167.7 | 408.9 | 1.33 |
| Free fatty acid | 268.1 | 397.7 | 2.36 |
| Triglyceride | 114.4 | 141.6 | 1.21 |
| Glucose | 140.0 | 158.8 | 1.08 |

The resulting discriminant function was given by:

$$Y = 0.198 \times \text{Glucose} - 0.121 \times \text{Insulin} + 0.013 \times \text{Glucagon} - 26.688.$$

Patients whose values of glucose, glucagon and insulin result in $y > 0$ are classified as "expected fatalities" while those with $y < 0$ are "expected survivors." The above equation correctly classified all 13 patients.

## Discussion

One of the major obstacles to understanding the hypermetabolic response to trauma is the cause and effect relationships that exist between carbohydrate, protein and lipid substrates and the metabolic hormones. Numerous studies have elucidated relationships in segments of the metabolic consequences of injury. The results remain, however, disconnected.

Multiple regressions analysis of a variety of the variables involved in the metabolic response of a patient or group of patients is a way of determining where significant relationships exist of all the possibilities present. In addition, by including an evaluation of the severity of the trauma, the contributions of this variable to the total response can be determined.

TABLE 5. *Correlation Matrix of Low Severity Group Observation (N = 8)*

| | Insulin | Human Growth Hormone | Cortisol | Glucagon | Free Fatty Acid | Triglyceride | Probability of Death | Glucose |
|---|---|---|---|---|---|---|---|---|
| Insulin | 1.000 | | | | | | | |
| Human growth hormone | −.356 | 1.000 | | | | | | |
| Cortisol | −.085 | .440 | 1.000 | | | | | |
| Glucagon | .359 | .509 | .298 | 1.000 | | | | |
| Free fatty acid | −.333 | −.243 | −.083 | −.075 | 1.000 | | | |
| Triglyceride | −.207 | .074 | −.133 | −.312 | .330 | 1.000 | | |
| Probability of death | −.183 | .034 | .679 | −.249 | −.305 | −.436 | 1.000 | |
| Glucose | .858 | −.402 | .192 | .377 | −.133 | −.123 | −.037 | 1.000 |

TABLE 6. *Correlation Matrix of High Severity Group Observation (N = 5)*

| | Insulin | Human Growth Hormone | Cortisol | Glucagon | Free Fatty Acid | Triglyceride | Probability of Death | Glucose |
|---|---|---|---|---|---|---|---|---|
| Insulin | 1.000 | | | | | | | |
| Human growth hormone | −.425 | 1.000 | | | | | | |
| Cortisol | .279 | −.519 | 1.000 | | | | | |
| Glucagon | .075 | −.405 | .844 | 1.000 | | | | |
| Free fatty acid | −.345 | .123 | .433 | .806 | 1.000 | | | |
| Triglyceride | −.146 | .544 | .431 | .441 | .606 | 1.000 | | |
| Probability of death | −.552 | −.448 | .107 | .031 | −.087 | −.423 | 1.000 | |
| Glucose | −.010 | −.039 | −.410 | −.798 | −.918 | −.520 | .381 | 1.000 |

The results obtained in the present study confirm the changes in metabolic substrates and hormone mediators seen in other studies of the hypermetabolic response to thermal injury. Increased secretion of human growth hormone, cortisol and glucagon was quantitated. There was evidence of both impaired and elevated secretion of insulin. Results of these alterations were seen in abnormal caloric substrate regulation.

Regression analysis of mean values for all parameters studied for all patients established several pairs as significant relationships in control of glucose concentration. Most highly correlated was probability of death as a measure of severity of injury and cortisol level. It has been suggested that cortisol may be responsible for early post injury metabolic responses.[3] The strong correlation between cortisol and glucagon similarly supports previous work which suggests glucagon as a mediator of later post metabolic responses.[3,25,28]

Of importance to this study was the analysis of correlations when considering severity of injury. It became evident that correlations of marginal significance when considering all patients as a group had no correlation in one severity group but were highly correlated in the other group. For example, the correlation of insulin and glucose was only significant at $p = 0.1$ for all patients had no correlation in the high severity group but were significantly correlated ($p < 0.05$) in the low severity group.

In the low severity group, not surprisingly, cortisol and probability of death were related. However, the only other significant correlation was insulin and glucose. This group characteristically also had elevated levels of insulin (mean 63.8) similar to insulin resistance reported in other severe stress.[3,13] It, thus, appears that a loss of glucose regulation occurs in conjunction with insulin resistance without significant interplay from other factors in the group of patients with low severity injury.

In marked contrast was the correlations between variables in the high severity group. Insulin did not correlate with glucose concentration but was replaced by a strongly negative correlation between glucose and glucagon. These inappropriately low insulin levels are consistent with the decreased insulin secretion seen in other severe stress states.[1,16] Additionally, relationships existed between glucagon and cortisol or free fatty acid. This data supports the suggestion that hyperglucagonemia plays a significant role in hyperglycemia[25] but only in severely injured patients. Interestingly, growth hormone and cortisol were not seen to act significantly with glucagon to affect glucose level. The negative correlation between free fatty acid and glucose may be the result of fat utilization as an energy source[17] again only seen in severely injured patients.

The physical limitation of patient samples precluded evaluation of blood catecholamine levels or investigation of protein breakdown for gluconeogenesis. The relationship of catecholamine to factors studied here, as well as that of protein catabolism[1,29,31] have shown the significant roles played by these factors. Inclusion of these factors would certainly have brought forth additional data. The data analysis previously discussed evaluated each variable in a correlation matrix where glucose was the dependent variable. Thus, any one factor could be assessed in its correlation to any one other factor in affecting glucose concentration. In an effort to expand on this, a discriminate function analysis was employed. This allowed any of the variables studied to enter the equation when it made a contribution, even though it may not be significantly different between each of the two severity groups individually. In this analysis, glucose, insulin and glucagon only were selected as predictor variables. It was not intended that this function be used, prospectively but was used in a retrospective analysis of the data to identify the interaction of all significant variables. However, it may facilitate management decisions in the experimental treatment of hypermetabolic responses.

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