

Anti-Stress Effect of BRAND'S Essence of Chicken (BEC) on Plasma Glucose Levels in Mice Loaded with Restraint Stress

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We investigated the effect of BRAND'S Essence of Chicken (BEC) on the basic metabolism of blood glucose in mice loaded with restraint stress. In the glucose intravenously treated mice, the stress prolonged the half-life period ($T_{1/2}$) of elimination for blood glucose from 51.7 to 62.2 min. The elimination rate decreased to 82.5% per min in stressed mice compared with a starved control. The $T_{1/2}$ of blood glucose was 55.2 min in the BEC treated group. BEC alleviated a stress-induced decrease in blood glucose. The improved glucose metabolism was well explained by the findings that insulin levels were elevated and glycogen synthesis in liver was remarkably activated by BEC. Our study demonstrates that BEC improves metabolic dysfunction of blood glucose by elevating insulin levels reduced by restraint stress.

Key words — BRAND'S Essence of Chicken, glucose metabolism, restraint-stress

INTRODUCTION

It has been widely reported that stress is involved in various diseases and there are many stressors in our environment.¹⁾ There have been a number of recent studies about the mechanisms of the development of various symptoms caused by stress. A response induced by stress is transmitted to the organs through the autonomic nervous system and hormones.²⁾ Stress is well known to directly affect the secretion of hormones,³⁾ to suppress the immune system⁴⁾ and to cause acute organ dysfunction.⁵⁾ For example, some kinds of stress reduce insulin generated by the pancreas in animals and induce an impairment of glucose metabolism.⁶⁾ As a result, less utilization of glucose as energy can induce not only fatigue but also various physiological disorders. Therefore, it is important to alleviate the adverse

effects of stress to maintain health, and many animal models have been used to investigate the effects of stress on the nervous, hormone and energy metabolic systems.⁷⁾

It is well-known that various foods affect physiological function.⁸⁾ For example, BRAND'S Essence of Chicken (BEC), which is composed of water soluble substances extracted from gently cooked chicken, is a popular health supplement, and is consumed particularly by Chinese communities and in Southeast Asia as a traditional health food. Recent studies suggest that it enhances mental efficiency and recovery from postpartum sickness and mental fatigue.⁹⁾ It was reported that BEC increases 10% of resting energy expenditure in college students.¹⁰⁾ The relationship between metabolic stimulation and recovery from fatigue has also been examined.¹¹⁾ BEC also increases the cerebrospinal fluid level of 5-hydroxyindolacetic acid in animals,¹²⁾ and consumption of BEC may lead to the activation of serotonin-dependent physiological processes like sleep improvement, mood elevation, analgesia, facilitation of motor output and regulation of the circadian rhythm.

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However, it is unknown whether BEC has any influence on glucose metabolism. In this study, we demonstrated the relationship between stress and blood glucose metabolism using a simple glucose solution clearance test in mice exposed to restraint stress to clarify the anti-stress effects of BEC. We thought BEC might exert a protective effect on metabolic dysfunctions caused by stress.

MATERIALS AND METHODS

Animals — Seven week-old female ICR mice were purchased from Charles River Japan Inc., Tokyo, Japan. In a preliminary study, we found that the metabolic response of female mice was more stable than that of male mice.¹³⁾ The animals were kept in a specific-pathogen-free animal room at $23 \pm 1^\circ\text{C}$ with a 12-hr light-dark cycle of lights on from 6:00 to 18:00 and were fed standard laboratory chow (CE-2; Clea Japan Inc., Tokyo, Japan) and tap water. They were kept for a week before the experiment. In the restraint stress experiment, each mouse was confined to an oval metal restraint cage for 20 hr before the assay. The animals were taken care of and treated according to the guidelines established by the Japanese Society of Nutrition and Food Science, Law No. 105 and Notification No. 6 of the Japanese government. This study was planned and performed at Institute for Health Care Science, Suntory Ltd., Osaka, Japan.

Chemicals — BEC (70 ml/bottle, 21.6 kcal) is produced via a water extraction process from chicken meat for several hours under high-temperature conditions. After removing the fat, it is concentrated and bottled. The extract mainly consists of proteins, amino acids, and peptides as shown in Table 1, and was generously provided by Cerebos Pacific Ltd., Singapore. D-(+)-glucose was purchased from Nacalai Tesque Inc., Kyoto, Japan. Gelatin was purchased from Nippi Ltd., Tokyo, Japan. These samples were dissolved in a water solution immediately before use.

Glucose Tolerance Test Procedure — The effects of restraint on glucose metabolism were investigated as follows. First, mice were divided into four groups of seven mice in each. In the starved group, the mice were deprived of food for 20 hr, 35 min before glucose administration. In the restrained group, the mice were starved and confined to an oval metal restraint cage for 20 hr, 35 min before glucose

Table 1. Composition of BEC

| Ingredient | Amount |
|----------------------|------------------------|
| protein (peptide) | 83.0 mg/ml |
| free amino acid | 3.1 mg/ml |
| L-anserine | 2.3 mg/ml |
| L-carnosine | 0.8 mg/ml |
| taurine | 0.7 mg/ml |
| hexose | 0.8 mg/ml |
| phosphatidyl choline | 0.4 mg/ml |
| minerals | |
| calcium | 26 $\mu\text{g/ml}$ |
| iron | 1 $\mu\text{g/ml}$ |
| zinc | 2 $\mu\text{g/ml}$ |
| magnesium | 32 $\mu\text{g/ml}$ |
| sodium | 1740 $\mu\text{g/ml}$ |
| natrium | 550 $\mu\text{g/ml}$ |
| chlorine | 1340 $\mu\text{g/ml}$ |
| phosphorus | 480 $\mu\text{g/ml}$ |
| sulfur | 500 $\mu\text{g/ml}$ |
| copper | 2 $\mu\text{g/ml}$ |
| manganese | 5 $\mu\text{g/ml}$ |
| selenium | 0.05 $\mu\text{g/ml}$ |
| vitamins | |
| vitamin B2 | 1.0 $\mu\text{g/ml}$ |
| vitamin B6 | 0.37 $\mu\text{g/ml}$ |
| vitamin B12 | 0.002 $\mu\text{g/ml}$ |
| niacin | 6.4 $\mu\text{g/ml}$ |
| falacin | 0.15 $\mu\text{g/ml}$ |
| vitamin C | 15 $\mu\text{g/ml}$ |

The data on BEC was provided by Cerebos Pacific Ltd.

administration. Water, a gelatin solution composed of 7.2% gelatin in 0.3% caramel solution with the same caloric content as BEC⁹⁾ and BEC were given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and those solutions were also given at the end of restraint stress loading, 35 min before glucose injection to mice that had been starved and restrained for 20 hr, respectively (Fig. 1). For the glucose tolerance test, a 20% glucose solution was administered to animals intravenously at 0.1 ml/10 g body weight 35 min after 20 hr restraint stress. Twenty micro liters of blood samples were taken from the tail vein, and blood glucose was measured with an ANTISENSE II Blood Glucose Analyzer (Sankyo Co. Ltd., Tokyo, Japan). The elimination half-life ($T_{1/2}$) for blood glucose was calculated by linear regression analysis of the time course of glucose concentration after glucose loading,^{14,15)} and seven animals were used at each time point. For analyzing metabolic functions, water, the gelatin

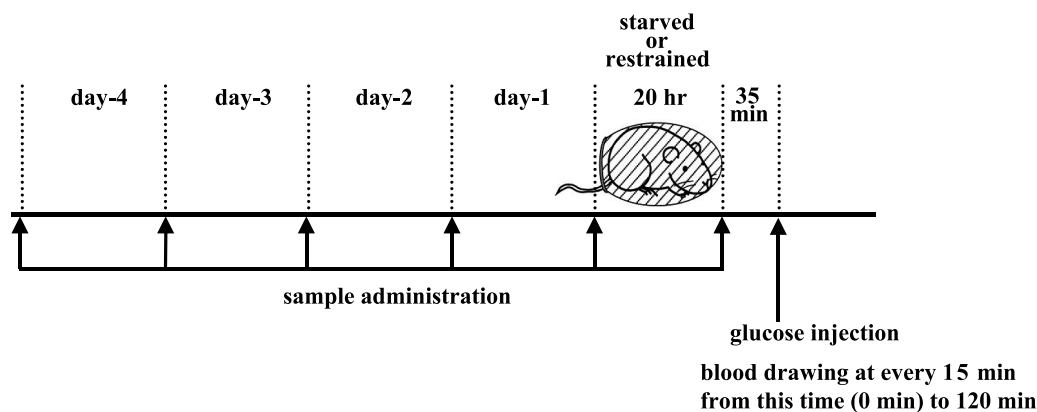


Fig. 1. Procedure for the Glucose Tolerance Test

Seven week-old female ICR mice were placed in a restraint cage for 20 hr. BEC, gelatin or a water solution (the restrained control) was administered orally at a dose of 0.1 ml/10 g body weight daily for 5 days before and one time immediately after restraint stress loading. A 20% glucose solution was administered to animals intravenously at 0.1 ml/10 g body weight 35 min after 20 hr restraint stress. The glucose elimination rate was calculated based on the chronological change in blood glucose levels.

solution and BEC were given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and those solutions were also given at the end of restraint stress loading. After 35 min, these animals were sacrificed to analyze the following metabolic parameters.

Ketone Bodies and Insulin Measurements —

Plasma acetoacetic acid (ACAC) analysis was carried out by the autokit ketone bodies (Wako Pure Chemicals Industries Ltd., Osaka, Japan) method¹⁶⁾ using an automatic serum analyzer (model 7070, Hitachi Co. Ltd., Tokyo, Japan). Insulin was measured by radioimmunoassay. The RAT Insulin [¹²⁵I] Assay System (Amersham Bioscience, Uppsala, Sweden) was used for the analysis of mouse insulin.

Liver Glycogen Measurements — A 20% glucose solution was orally administered after starving for 20 hr at a dose of 0.1 ml/10 g body weight. After 35 min, the mice were sacrificed for the measurement of glycogen synthesis activity in the liver. The liver was excised out and homogenized. Then 500 μ l of 30% aq. KOH were added to 200 mg of the homogenized liver and heated at 100°C for 20 min. Six hundred micro liters of cold 95% ethanol were added after cooling on ice bath and the mixture was kept at -20°C for 30 min. After cooling, it was centrifuged at 8000 rpm at 4°C for 15 min. The supernatant was removed and the precipitate was suspended in 400 μ l of distilled water. Five hundred micro liters of cold 95% ethanol were added and the mixture was kept at -20°C for 30 min. Again it was centrifuged at 8000 rpm at 4°C for 15 min. The precipi-

tate was dried at 70°C, 200 μ l of 1 N H₂SO₄ were added, and the mixture was heated at 100°C for 2 hr. Then, 200 μ l of 1 N NaOH were added to the mixture for neutralization. The concentration of glucose liberated from glycogen in the reaction mixture was measured.

Statistical Analysis — Data were expressed as means \pm S.D. and evaluated by analysis of variance (ANOVA) using SPSS software (SPSS Inc. Japan, Tokyo, Japan). Differences between the group means were considered to be significant at $p < 0.05$ using the Tukey procedure generated by this program. Statistical significance between two groups was evaluated by the Student's *t*-test.

RESULTS

Effect of Stress on Glucose Tolerance

Figure 2 shows the chronological change in the blood glucose levels of the restrained and starved mice. The clearance rate was lower in mice exposed to stress than the starved mice.

Effect of BEC on Glucose Tolerance

Table 2 shows the results of blood glucose elimination as $T_{1/2}$ using linear regression analysis. The $T_{1/2}$ for glucose in blood was 51.7 min and the elimination rate was 0.97% per min in the starved mice. In the restrained group, in which water was administered (the water group), the $T_{1/2}$ of plasma glucose was prolonged to 62.2 min (120.3% of the starved control) and the elimination rate remarkably de-

creased to 0.80% per min (inhibition of 17.5% compared with the starved control). BEC shortened the $T_{1/2}$ from 62.2 (the water group) to 55.2 min. BEC increased the elimination rate to 0.91% per min, which was 113.8% compared with the water group, and improved the elimination rate of plasma glucose. While, gelatin was weaker than BEC. The $T_{1/2}$ of gelatin was 59.1 min and the elimination rate was 0.85% per min.

Effect of BEC on Ketone Bodies (ACAC)

Figure 3 shows the change in plasma ACAC levels after loading stress. The mean level was 322.1 ± 39.9 nmol/ml in the water group. It was 250% compared with the starved control (128.1 ± 13.1 nmol/ml). Plasma ACAC levels were improved to 26.3% (236.7 ± 28.7 nmol/ml) when BEC was given. This is statistically significant against the water group. Gelatin was rather weak (275.3 ± 40 nmol/ml) as

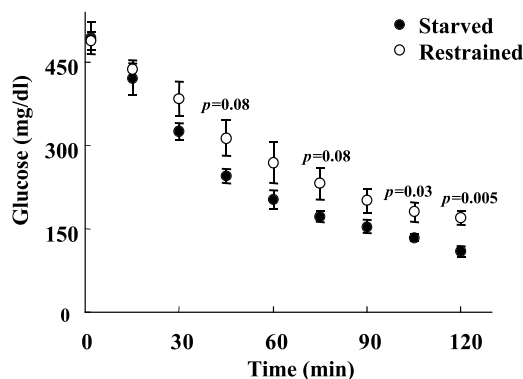


Fig. 2. Changes with Time in Blood Glucose Levels in Restrained and Starved Mice

Seven week-old female ICR mice were placed in a restraint cage and starved for 20 hr before the assay. The respective points represent the mean value of the plasma glucose concentration (mg/dl), which was obtained from seven mice for the respective times (min) after glucose injection. Two g/kg of glucose was administered to the restrained and starved mice in a volume of 0.1 ml/10 g of body weight. p = number shows statistical significance between the starved and restrained mice by the Student's t -test at each point.

compared with BEC.

Effect of BEC on Insulin Levels

The basal value of the serum insulin concentration obtained in the starved control was 35.0 ± 6.6 ng/ml as shown in Fig. 4. When mice were fixed in the restraint cage for 20 hr, the concentration was 7.7 ± 0.8 ng/ml (the water group). This is significantly lower than the starved control. BEC significantly improved the insulin level (14.7 ± 3.2) compared with the water group. Gelatin group was weaker than BEC and it was not significant against the water group.

Effect of BEC on Glycogen Synthesis in Liver

We examined liver glycogen content in the four groups (Fig. 5). The amount of liver glycogen in the starved control was 10.3 ± 1.8 mg/g. The amount in the BEC group was remarkably increased (11.4 ± 1.1 mg/g), compared with the water group (4.4 ± 1.2 mg/g). Gelatin also showed the same potency (9.8 ± 3.0 mg/g).

DISCUSSION

Fatigue and illness caused by stress has been recognized since ancient times,¹⁷⁾ and these effects indicate that stress limits the supply of energy, resulting poor utilization of biological energy sources. As an energy source, glucose utilization plays an important role in recovery from fatigue and various physiological disorders induced by stress.¹⁸⁾ Therefore, the elevated blood glucose levels may also reflect disrupted energy metabolism, and so the elimination rate of blood glucose is a good marker for stress. We found that the blood glucose elimination rate was remarkably lower in the stressed mice (the water group) than the starved control by comparing the respective $T_{1/2}$ calculated by linear regression analysis (Table 2). The results indicate that glucose

Table 2. Effects of BEC on Glucose Metabolism Blood Obtained from ICR Mice

| Treatment | $T_{1/2}$ (min) | Elimination rate (min) | % of control |
|----------------------|-----------------|------------------------|--------------|
| Starved control | 51.7 | 0.97% | 100.0% |
| Restrained + water | 62.2 | 0.80% | 82.5% |
| Restrained + BEC | 55.2 | 0.91% | 93.8% |
| Restrained + gelatin | 59.1 | 0.85% | 87.6% |

Seven week-old female ICR mice were placed in the restraint cage for 20 hr. Water, a gelatin solution and BEC were given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and those solutions were also given at the end of restraint stress loading. After 35 min, glucose was injected.

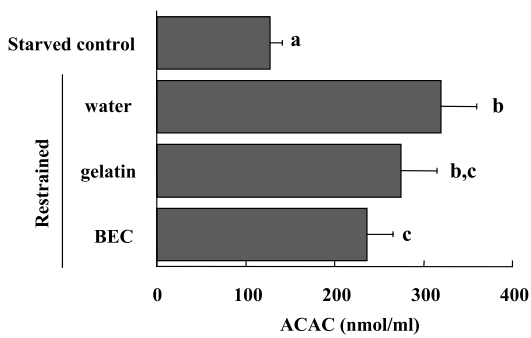


Fig. 3. Effect of BEC on Plasma Ketone Body (ACAC) Levels

Seven week-old female ICR mice were placed in a restraint cage for 20 hr. BEC, gelatin or a water solution (the restrained control) was administered orally at a dose of 0.1 ml/10 g body weight daily for 5 days before and one time immediately after restraint stress. After 35 min, the mice were sacrificed and plasma ACAC analysis was carried out by the autokit ketone bodies. The results represent the mean \pm S.D. obtained for 7 animals. The different letters indicate significant differences among the groups at $p < 0.05$.

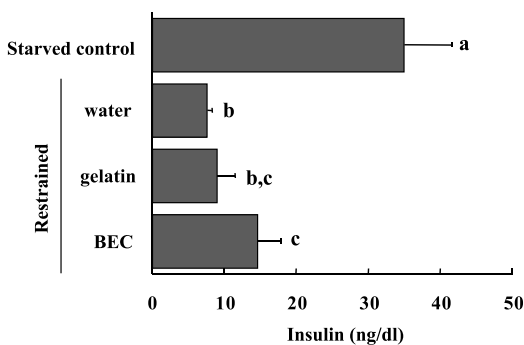


Fig. 4. Effect of BEC on Insulin Levels

Seven week-old female ICR mice were placed in a restraint cage for 20 hr. BEC, gelatin or a water solution (the restrained control) was administered orally at a dose of 0.1 ml/10 g body weight daily for 5 days before and one time immediately after restraint stress. After 35 min, the mice were sacrificed and the plasma insulin level was measured by the RAT Insulin [125 I] Assay System. The results represent the mean \pm S.D. obtained for 7 animals. The different letters indicate significant differences among the groups at $p < 0.05$.

metabolism was definitely disrupted by stress and decreased the utilization of glucose as an energy source. In the present study, we also found that BEC shortened the $T_{1/2}$ of elimination for blood glucose from 62.2 to 55.2 min, and the elimination rate increased to 0.91% per min. The results suggest that the anti-stress effect of BEC seems to be due to its improvement of glucose utilization as an energy source. On the other hand, gelatin used as a nutritional supplement exhibited no remarkable effect.

Generally, when glucose utilization is insufficient, the stored lipid is consumed as an energy source. The ketone bodies, which are imperfect me-

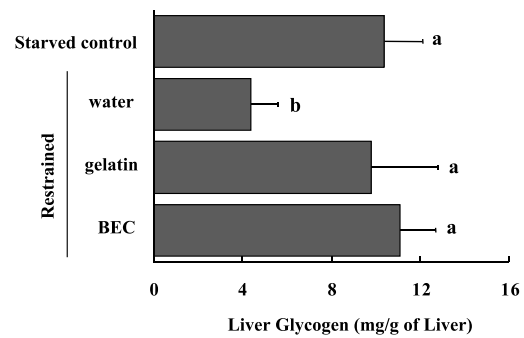


Fig. 5. Effect of BEC on Glycogen Synthesis in Liver

Seven week-old female ICR mice were placed in a restraint cage for 20 hr. BEC, gelatin or a water solution (the restrained control) was administered orally at a dose of 0.1 ml/10 g body weight daily for 5 days before and one time immediately after restraint stress. A 20% glucose solution was also orally administered at the same time. After 35 min, the mice were sacrificed for the measurement of glycogen synthesis activity in the liver. The liver was excised out and homogenized. Then, the concentration of glucose liberated from glycogen was measured. The results represent the mean \pm S.D. obtained for 7 animals. The different letters indicate significant differences among the groups at $p < 0.05$.

tabolites of fatty acids accumulated in the blood. This fact indicates the energy metabolism is preferred to fatty acids.¹⁹⁾ As shown in Fig. 3, the plasma ACAC level improved to 26.3% by BEC compared with the water group. The result suggests that BEC promotes energy metabolism by alleviating adverse effects on glucose utilization.

It is controversial whether stress reduces insulin levels and inhibits glucose utilization. It is demonstrated that stress (the water group in Fig. 4) reduced insulin levels in blood, possibly due to the functional damage associated with impairment of the oxidation process induced by stress. Previous studies reported that stress caused free radical reactions to produce deleterious modifications in membranes and damages cells.²⁰⁾ As a result, free radicals degrade tissue function. The protective effect of BEC on the blood insulin level might be explained by the properties such as antioxidative action rather than by the addition of nutrients or calories. It was reported that BEC contains protein, amino acids and peptides including anserine and carnosine, which are antioxidative substances.²¹⁻²³⁾ The anti-stress effect of BEC may contribute to both of them. We demonstrated the protective effects of BEC in insulin level and glucose metabolism. It suggested that both anserine and carnosine may promote the pancreas function lowered by oxidative stress.

It is well known that liver tissue consumes a large quantity of oxygen to supply energy in various bio-

chemical reactions, and the tissue is susceptible to oxidative stress. Stress loading requires a large quantity of glucose as an energy source,²⁴⁾ which is mainly supplied by liver glycogen synthesized from carbohydrates. Our results indicate that BEC remarkably improved lowered glycogen synthesis in stress-loaded mice (the water group) as shown in Fig. 5. Although gelatin had the same effect, it was likely lower than BEC. BEC evidently improves liver function and activates glycogen synthesis.

BEC, a hot water extract of chicken muscle, is familiar in the East as a remedy and nutritional supplement for physical and mental stress. It was reported that BEC is able to increase central 5-hydroxytryptamine (5-HT) activity in tested animals.¹²⁾ BEC may activate a 5-HT-dependent physiological process to recover physical fatigue. Moreover, BEC recovered serum cortisol decreased by a mental workload test in human.⁹⁾ It alleviates glucose utilization in stress-loading. This supports the conclusion that BEC improves stress-suppressed energy metabolism by increasing the metabolic rate by nearly 10% of baseline in terms of resting energy expenditure.¹⁰⁾ Thus, BEC may be a useful and healthy food for the prevention of some diseases related to stress.

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