The Protective Effect of BRAND'S Essence of Chicken (BEC) on Energy Metabolic Disorder in Mice Loaded with Restraint Stress

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We investigated the effects of BRAND'S Essence of Chicken (BEC) on the basic metabolism of plasma lipids in mice loaded with restraint stress. When a lipid emulsion was intravenously injected into mice, 20 hr restraint stress prolonged the elimination of plasma triglyceride (TG). The results indicated that lipid metabolism was definitely disrupted by stress, and that the use of TG as an energy source decreased. The plasma TG level was 320 ± 27 mg/dl 35 min after Intralipid® administration in restrained mice, while it was 253 ± 23 mg/dl in the restrained mice that were given 5 ml/kg of BEC. The improved plasma lipid metabolism was well explained by the finding that lipoprotein lipase in omental adipose tissue was remarkably improved by BEC. Our study shows that BEC improves metabolic dysfunction possibly by utilizing plasma lipids as energy sources and elevating lipoprotein lipase activity reduced by stress. The anti-stress effect of BEC may partly be related to improved plasma lipid metabolism for energy utilization.

Key words —— BRAND'S Essence of Chicken, energy metabolism, restraint-stress, Intralipid®, triglyceride

INTRODUCTION

Illness caused by stress has been recognized since ancient times. Stressors exist everywhere in our environment,[1)] and stress directly influences a number of peripheral physiological processes, including the secretion of hormones[2,3)] and suppression of the immune system.[4)] In addition, they can also cause acute organ dysfunction.[5)] For example, some kinds of stress reduce insulin secretion by impairing glucose transport in the pancreas of animals[6)] as well as lipoprotein lipase (LPL) activity.[7)] As a result, lack of glucose can cause not only fatigue but also various kind of physiological disorders. Therefore, it is important to alleviate the adverse effects of stress to maintain health. Many animal models have been used to investigate the effects of stress on the nervous, hormone and energy metabolic systems.[8)]

Generally, physiological processes require a source of energy to sustain tissue functions of the brain, central nervous system, heart, and muscles, *etc*., and this is mainly supplied by dietary glucose and lipids. Moreover, energy metabolism is important in various stress reactions. Previous studies found that stress causes remarkable changes in metabolic functions, and that the elimination of blood lipids is also affected by stress.[9)] The mechanism by which stress slows the elimination of blood lipids is unknown, but stress limits the supply of energy to the organs, which causes poor utilization of biological energy sources and leads to fatigue and various physiological disorders.[10)] Therefore, elevated levels of blood lipids in restrained mice may reflect degraded tissue function, and thus the elimination rate of blood lipids can be used as a marker of stress.[9)]

It is well-known that various foods affect physiological function.[11)] For example, BRAND'S Essence of Chicken (BEC), which is composed of water soluble substances extracted from gently cooked

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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.[12] It was reported that BEC increases 10% of resting energy expenditure in college students.[13] The relationship between metabolic stimulation and recovery from fatigue has also been examined.[14] BEC also increases the cerebrospinal fluid level of 5-hydroxyindolacetic acid in animals,[15] 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.

However, it is unknown whether BEC has any influence on the utilization of blood lipids. In this study, we examined the relationship between stress and blood lipid metabolism using a simple fat emulsion clearance test in mice exposed to restraint stress to clarify the anti-stress effects of BEC.[12] We thought that BEC might exert a protective effect on metabolic dysfunctions caused by stress.

Table 1. Composition of BEC

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

The data on BEC was provided by Cerebos Pacific Ltd.

MATERIALS AND METHODS

Animals —— Seven week-old female ICR mice were purchased from Charles River Japan Inc., Tokyo, Japan. In a previous study, we found that the metabolic response of female mice was more stable than that of male mice.[9] The animals were kept in a specific-pathogen-free animal room at 23 ± 1°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) 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 then bottled. The extract mainly consists of proteins, amino acids, and peptides as shown in Table 1.[16] For this study, it was generously provided by Cerebos Pacific Ltd., Singapore. Twenty % Intralipid®, a lipid emulsion that includes 20% soybean oil, 1.2% lecithin and 2.2% glycerol, was purchased from Pharmacia AB. Co., Ltd., Stockholm, Sweden. It was diluted with phosphate buffered saline (PBS) to produce a 10% soybean oil solution immediately before use. Gelatin was purchased from Nippi Ltd., Tokyo, Japan, and was dissolved in water immediately before use.

Isolation of Polypeptides from BEC —— The polypeptides were fractionated from a bottle (70 ml) of BEC. After preliminary fractionation by a 0.9 × 400 cm Sephadex G-25 gel filtration column, the extract was freeze-dried, and the high molecule peptide fraction [BEC (F1)], caramel containing fraction [BEC (F2)] and low molecule peptide fraction [BEC (F3)] totalling 2.4, 3.3 and 0.07 g were col-

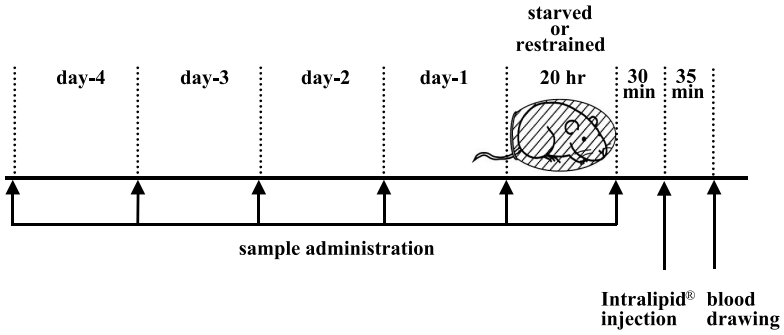

Fig. 1. The Plasma Lipid Tolerance Test

Seven week-old female ICR mice were placed in a restraint cage for 20 hr. BEC, gelatin or water was orally administered at 0.1 ml/10 g body weight daily for 5 days before and one time immediately after restraint stress loading. Intralipid® was dissolved with PBS to produce a 10% soybean oil solution and was injected into the tail vein in a volume of 0.1 ml/10 g body weight 30 min after 20 hr of restraint stress. The plasma TG concentration (mg/dl) was determined using an automatic serum analyzer.

lected, respectively. The isolated peptide fractions were then dissolved in distilled water before use.

Plasma Lipid Tolerance Test Procedure ——— The effects of restraint on triglyceride (TG) metabolism were investigated as follows. First, the mice were divided into three groups of five mice in each. The normal control group was fed, and was not exposed to the restraint stress before Intralipid® injection. In the starved group, the mice were deprived of food for 20 hr, 30 min before Intralipid® injection. In the restrained group, the mice were starved and confined to an oval metal restraint cage for 20 hr, 30 min before Intralipid® injection. Intralipid® was injected through the tail vein at 0.1 ml/10 g of body weight. Water, a gelatin solution composed of 7.2% gelatin in 0.3% caramel solution with the same caloric content as BEC, BEC and three fractions [BEC (F1), (F2) and (F3)] 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, 30 min before the administration of Intralipid® to mice that had been starved and restrained for 20 hr, respectively (Fig. 1). Thirty five min after Intralipid® injection, blood samples were taken from the heart under anesthesia with diethyl ether and collected into tubes containing 2% sodium heparin. The tubes were then centrifuged at 5000 rpm for 5 min and the supernatant was used for sampling. All samples were stored at –20°C until assay. Plasma TG was determined by an automatic serum analyzer (model 7070, Hitachi Ltd., Japan), by the glycerol kinase/glycerol-3-phosphate oxidase (GK-GPO) method,[17] and by an assay kit purchased from International Reagents Corp., Kobe, Japan.

Measurement of LPL Activity in Omental Adipose Tissue ——— At the end of restraint stress loading, the mice were killed by exsanguination under ether-induced anesthesia, and omental adipose tissues were quickly dissected out, perfused with PBS, and then weighed. A hundred milligrams of each adipose tissue was minced with scissors and homogenized in a homogeneizer (Physcotron, NS-51K, Microtec Co. Ltd., Japan) at maximum setting for 30 sec in 1 ml of ice-cold solution containing 0.25 M sucrose, 1 mM EDTA, 10 mM Tris–HCl and 12 mM deoxycholate at pH 7.4. The homogenate was collected by centrifugation at 12000 rpm and 4°C for 20 min, and the fraction between the upper fat layer and the bottom sediment was removed after tube slicing. It was then diluted with 4 volumes of the homogenization solution and decanted. Afterward, it was quickly stored at –20°C before LPL activity measurement. The LPL activity measurement was carried out by following the BALB-DTNB method[18] using a commercial kit (Lipase Kit; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and an automatic serum analyzer (model 7070, Hitachi Ltd.). Tissue LPL activity was expressed as units per gram tissue protein, in which one unit is equivalent to 1 μM of fatty acid released per min at 37°C. The protein contents of the tissue extracts were determined by the Lowry's method.[19]

Statistical Analysis ——— Data were expressed as means ± 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.

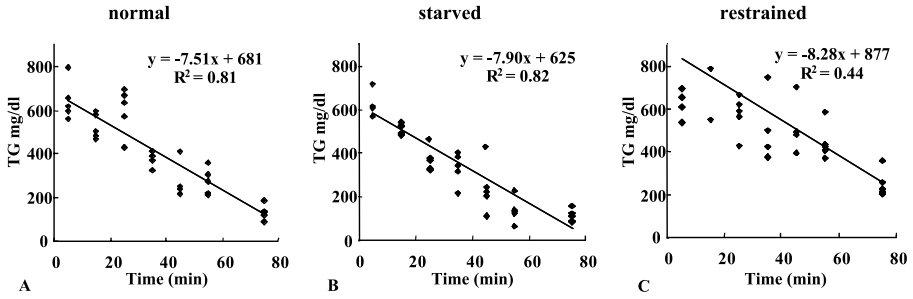

Fig. 2. Chronological Changes in the TG Plasma Concentration after the Injection of Intralipid®

The respective points represent the mean value of the plasma TG concentration (mg/dl) which was obtained from five mice for the respective times (min) after Intralipid® injection. Intralipid was dissolved with PBS to produce a 10% soybean oil solution and was injected into the tail vein in a volume of 0.1 ml/10 g body weight. Mice in the normal control group were fed and not exposed to the restraint stress. In the starved group, the mice were deprived of food for 20 hr, 30 min before Intralipid® injection. In the restrained group, the mice were starved and confined to an oval metal restraint cage for 20 hr, 30 min before Intralipid® injection.

RESULTS

Effect of Stress on Plasma TG Elimination

Figure 2 shows chronological changes of the plasma TG concentration after the injection of Intralipid®. The correlation coefficient of the chronological changes for the normal control group was $R^2 = 0.81$ (Fig. 2, A), and the average level was 375 ± 37 mg/dl 35 min after injection. In the starved group, the correlation coefficient slightly increased ($R^2 = 0.82$), and the average level was slightly decreased to 331 ± 74 mg/dl after 35 min as shown in Fig. 2 (B). The plasma TG levels were higher, and the clearance rate was lower in the restrained group that was exposed to stress for 20 hr than for the starved group. The concentration of TG was 509 ± 166 mg/dl after 35 min, and the correlation coefficient was $R^2 = 0.44$ (Fig. 2, C).

Effect of BEC and its Isolated Fractions on Plasma TG Elimination

Figure 3 shows the results of plasma TG levels 35 min after the injection of Intralipid®, where the starved group was 227 ± 40 mg/dl. In the water group fixed in the restraint cage for 20 hr, the plasma TG level was 320 ± 27 mg/dl, which was remarkably decreased. The concentration of plasma TG was 253 ± 23 mg/dl for 5 ml/kg BEC, 261 ± 27 mg/dl for 10 ml/kg BEC and 290 ± 27 mg/dl for 20 ml/kg BEC. The plasma TG metabolism in these mice under restraint stress was improved by BEC, but the effect was not dose–dependent. Finally, 10 ml/kg gelatin, which has the same caloric content as BEC, had no effect on the levels of plasma TG.

The administration of 200 mg/kg BEC (F1) and BEC (F3) resulted in 305 ± 35 and 325 ± 21 mg/dl, respectively. TG metabolism was elevated by 13 and 9%, respectively, 35 min after the injection of Intralipid®. In contrast, 200 mg/kg BEC (F2) resulted in 483 ± 59 mg/dl. In this case, the water group was 371 ± 26 mg/dl as shown in Fig. 4.

As shown in Fig. 5, BEC (F1) decreased the plasma TG levels 35 min after the injection of Intralipid® in a dose–dependent manner. Here, 100 and 200 mg/kg of BEC (F1) were improved by 18% (281 ± 35 mg/dl) and 21% (273 ± 31 mg/dl) compared with the water group, respectively.

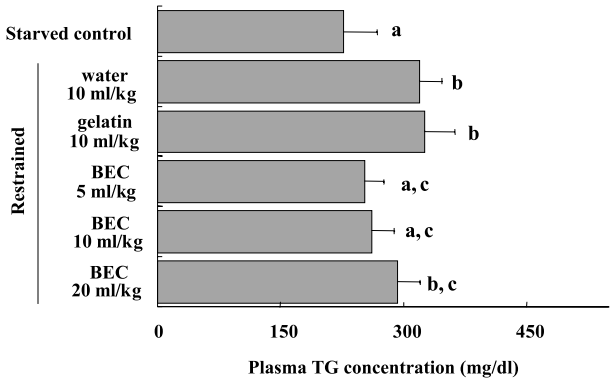

Fig. 3. The Effects of BEC on TG Elimination in the Plasma Obtained from ICR Mice Loaded with Restraint Stress

Seven week-old female ICR mice were placed in restraint cages and starved for 20 hr. The results represent the mean ± S.D. of seven mice in each group. Intralipid® was diluted with PBS to produce a 10% soybean oil solution and was injected into the tail vein in a volume of 0.1 ml/10 g body weight. The TG concentration in the plasma was measured 35 min after Intralipid® injection. BEC, gelatin solution or water was given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and the solutions were given at the end of restraint stress loading, respectively. The different letters indicate significant differences among the groups at $p < 0.05$.

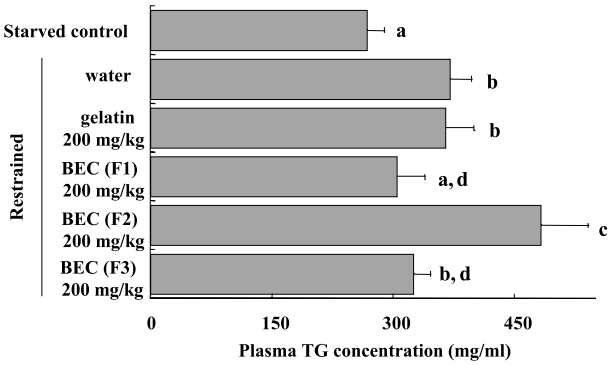

Fig. 4. The Effects of BEC Extract Fractions on TG Elimination in the Plasma Obtained from ICR Mice Loaded with Restraint Stress

Seven week-old female ICR mice were placed in restraint cages and starved for 20 hr. The results represent the mean ± S.D. of seven mice in each group. Intralipid® was diluted with PBS to produce a 10% soybean oil solution and was injected into the tail vein in a volume of 0.1 ml/10 g body weight. The TG concentration in the plasma was measured 35 min after Intralipid® injection. BEC (F1), (F2), (F3), gelatin solution or water was given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and the solutions were given at the end of restraint stress loading, respectively. The different letters indicate significant differences among groups at $p < 0.05$.

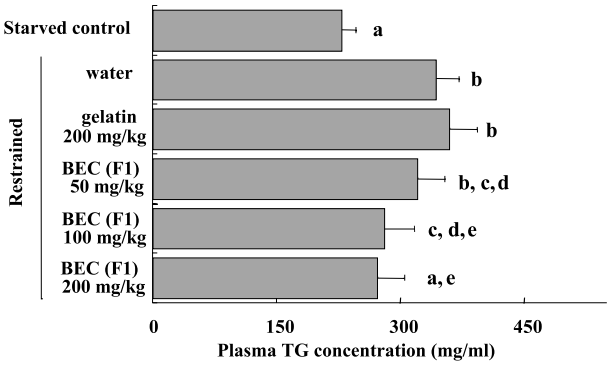

Fig. 5. The Effects of BEC (F1) on TG Elimination in the Plasma Obtained from ICR Mice Loaded with Restraint Stress

Seven week-old female ICR mice were placed in restraint cages and starved for 20 hr. The results represent the mean ± S.D. of seven mice in each group. Intralipid® was diluted with PBS to produce a 10% soybean oil solution and was injected into the tail vein in a volume of 0.1 ml/10 g body weight. The TG concentration in the plasma was measured 35 min after Intralipid® injection. BEC (F1), gelatin solution or water were given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, and the solutions was given at the end of restraint stress loading, respectively. The different letters indicate significant differences among the groups at $p < 0.05$.

Effects of BEC and its Isolated Fractions on LPL Activity in Omental Adipose Tissue

Figure 6 shows the LPL activity in omental adipose tissue, where the basal value was 680 ± 111 U/g

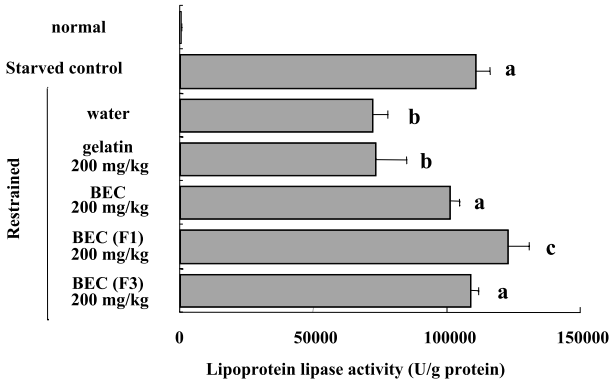

Fig. 6. The Effects of BEC and its Fractions (F1 and F3) on LPL Activity in Omental Adipose Tissue

Seven week-old female ICR mice were placed in restraint cages and starved for 20 hr. BEC, BEC (F1), BEC (F3), gelatin solution or water was given orally at 0.1 ml/10 g body weight daily for 5 days before the restraint began, respectively. At the end of restraint stress loading, the mice were killed, and omental adipose tissues were dissected out and homogenized to measure LPL activity. The LPL assay was carried out by the BALB-DTNB method using a commercial kit and an automatic serum analyzer. Tissue LPL activity was expressed as units per gram tissue protein, in which one unit is equivalent to 1 μM of fatty acid released per min at 37°C. The different letters indicate significant differences among the groups at $p < 0.05$.

in the normal control and 111054 ± 5094 U/g in the starved group. Starving significantly increased the LPL activity. When mice were fixed in the restraint cage for 20 hr, the LPL activity was 72380 ± 5675 U/ml (the water group). The administration of 200 mg/kg BEC increased the activity (101407 ± 3498 U/g). BEC (F1) and (F3) showed the same potency (122997 ± 7852 and 109015 ± 2948 U/g, respectively), but the administration of 200 mg/kg gelatin was not effective (73743 ± 11087 U/g).

DISCUSSION

Fatigue and illness caused by stress has been recognized since ancient times,[20] and these effects indicate that stress limits the supply of energy, resulting poor utilization of biological energy sources and leading to neurological or other tissue dysfunction.[21] Blood lipid utilization as an energy source plays an important role in the recovery of fatigue and various physiological disorders induced by stress.[22] Therefore, elevated blood lipid levels may also reflect disrupted energy metabolism, and so the elimination rate of blood lipids is a good marker for stress. In the present study, we investigated the basic metabolism of lipids in mice treated with restraint

stress. As shown in Fig. 2, the TG elimination rate after loading stress was definitely prolonged for the starved *vs*. restrained group. This result indicates that plasma TG metabolism was disrupted by the stress and thus decreased the utilization of TG as an energy source.[9] When glucose utilization is insufficient, stored lipids are consumed as an energy source. Energy metabolism preferentially involves fatty acids that sustain physiological processes of the brain, central nervous system, heart, and muscles, *etc*.

BEC significantly decreased the plasma TG levels at 5 and 10 ml/kg compared against the water control, but administration of 20 ml/kg was not significant for the water group, the 5 and 10 ml/kg groups (Fig. 3). As shown in Fig. 4, BEC (F1) significantly decreased the plasma TG compared against the water group, while a higher plasma TG level was significantly observed in BEC (F2) than for the water group. BEC (F2) could not improve the utilization of plasma TG as an energy source. Therefore, a quantitative balance between BEC (F1) and (F2) may be the reason why BEC did not show any dose-response. Although the administration of 20 ml/kg BEC was not significantly improved to the plasma TG concentration of the starved group, it was likely to decrease the plasma TG as compared with the water group. Thus, these results suggest that the anti-stress effect of BEC is partially due to its improvement of lipid utilization as an energy source. This effect may be caused by the acceleration of lipid metabolism and not the simple addition of nutrients or calories because gelatin used as a nutritional supplement showed no remarkable effect.

The LPL activity was significantly improved to 40% for BEC administration compared with the water group (Fig. 6), showing that the promoting activity of BEC on lipid metabolism is partly due to improved adipose LPL activity. Generally, LPL is the rate-limiting enzyme involved in the hydrolysis of lipoprotein TG, and thus controls the uptake of fatty acids into tissues.[23] Physiological LPL expression in adipose tissue may optimize lipid metabolism, and thus a defect in LPL synthesis would therefore not only interfere with the release of fatty acids but also with intracellular lipolysis. BEC improves lipid metabolic dysfunction possibly by elevating LPL activity and increasing the utilization of TG as an energy source. Furthermore, omental adipose tissue consumes a large quantity of oxygen to supply energy used in various biochemical reactions, and the tissue is susceptible to oxidative stress.[24] Physical or mental stress increases the oxygen concentration in the organs, followed by the generation of active oxygen molecules and free radicals, and this process causes a chain reaction of lipid peroxide generation in membranes.[25] As a result, free radicals degrade tissue function and directly affect the secretion of hormones. Previous studies suggested that LPL expression can be induced by cytokines and hormones such as tumor necrosis factor and leptin.[26,27] It is speculated that LPL levels are reduced in adipose tissue damaged by stress.

The high molecule peptide fraction [BEC (F1)] elevated the plasma TG concentration in a dose–dependant manner (Fig. 5). The plasma lipid elimination can be explained by the improved LPL activity (Fig. 6). On the other hand, the low molecular fraction [BEC (F3)] also showed the same activity (Fig. 6). It was reported that BEC contains a variety of proteins, amino acids and peptides including anserine and carnosine, which are anti-oxidants.[28,29] These might contribute to the protective effect of LPL activity in adipose tissue against oxidative damage induced by stress. Also, BEC (F3) contains these low molecular compounds, and may enhance the plasma lipid elimination.

BEC, a hot water extract of chicken muscle, is used in the East as a remedy and nutritional supplement for physical and mental stress.[12] BEC may activate a 5-HT-dependent physiological process that promotes recovery from physical fatigue.[15] It improves LPL activity and alleviates lipid utilization in stress-loaded mice. 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.[13] Thus, BEC may be a useful and healthy food for the prevention of some diseases related to stress.

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