Effects of Capsaicin Coadministered with Eicosapentaenoic Acid on Obesity-Related Dysregulation in High-Fat-Fed Mice

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Obesity-induced inflammation contributes to the development of metabolic disorders such as insulin resistance, type 2 diabetes, fatty liver disease, and cardiovascular disease. In this study, we investigated whether the combination of eicosapentaenoic acid (EPA) and capsaicin could protect against high-fat diet (HFD)-induced obesity and related metabolic disorders. The experiments were performed using male C57BL/6J mice that were fed one of the following diets for 10 weeks: standard chow (5.3% fat content) (normal group), a HFD (32.0% fat content) (HFD group), or a HFD supplemented with either 4% (w/w) EPA (EPA group) or a combination of 4% (w/w) EPA and 0.01% (w/w) capsaicin (EPA+Cap group). Our results indicated that the body, fat and liver tissue weights and levels of serum glucose, insulin, total cholesterol, triglyceride, high-density lipoprotein-cholesterol, aspartate aminotransferase, and alanine aminotransferase were significantly higher in HFD group mice than in normal group mice (p<0.05 in all cases). However, the body and fat tissue weights and serum glucose levels and homeostasis model assessment of insulin resistance were significantly lower in EPA+Cap group mice group than in HFD and EPA group mice (p<0.05 in all cases). Thus, our study suggests that the combination of EPA and capsaicin might be beneficial for delaying the progression of obesity-related metabolic dysregulation and subsequent complications.

Key words eicosapentaenoic acid; capsaicin; high-fat diet; mouse; obesity

Methods

Animals Six-week-old male C57BL/6J mice (Japan SLC Inc., Shizuoka, Japan), weighing 18–20 g, were used for the study. The mice were fed a standard powder diet (MF® diet; Oriental Yeast, Tokyo, Japan) during a 1-week adjustment period and housed in a room maintained at 22±2°C on a 12:12 h light/dark cycle. The animals were randomly assigned to one of the following dietary interventions: standard chow (normal group), high-fat diet (HFD group), or HFD supplemented with either eicosapentaenoic acid (EPA group) or a combination of EPA and capsaicin (EPA+Cap group). The capsaicin diet was administered at 0.01% (w/w) in a combination with 4% (w/w) EPA per diet. The animals were sacrificed after 10 weeks of dietary intervention for metabolic syndrome, other dietary interventions, such as those targeting adipose tissue inflammation, are currently being explored. Previous reports have demonstrated the alleviation of hepatic steatosis by n-3 polyunsaturated fatty acids (n-3 PUFA) treatment. Further, dietary intake of n-3 PUFA has been observed to sensitize the liver and adipose tissue to insulin and improve insulin tolerance in obese mice, and long-chain n-3 PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been reported to possess anti-inflammatory properties. EPA, one of the major n-3 PUFAs has also been shown to exert anti-atherogenic and lipid-lowering effects. In numerous animal models, EPA supplementation of HFD was shown to prevent and to reverse obesity. Unlike DHA, EPA as a chronic dietary intervention may exert differential effects on outcomes such as plasma triacylglycerol concentrations, arterial stiffness, lipoprotein particle size, blood pressure, and heart rate.

Capsaicin, the pungent component of chili peppers, is known to have anti-obesity effects. Specifically, capsaicin has been reported to inhibit the expression and release of inflammatory adipocytokines, such as interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), from obese adipose tissues and isolated adipocytes, enhance the expression and release of adiponectin, and inhibit macrophage responses that are crucial for augmenting adipose tissue inflammation.

Therefore, the aim of this study was to determine whether the administration of EPA and capsaicin in combination could prevent and reverse HFD-induced obesity-related metabolic complications, such as insulin resistance. As regional body fat distribution may be an independent risk factor for metabolic and cardiovascular diseases, we also evaluated the effects of EPA and capsaicin on the indices of fat accumulation, namely, whole body, abdominal fat, and hepatic weights, and on commonly tested liver damage parameters, namely, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, in order to determine the potential clinical effects of the combination. Moreover, since previous studies demonstrated that mice or rats fed HFD supplemented with capsaicin were reduced obesity-induced metabolic dysregulation, we focused on combination effects of EPA and capsaicin to compared with the published effect of EPA alone.

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12-h light/dark cycle (diurnal time, 0800–2000h), with free access to chow and water. All experimental procedures were conducted in accordance with the Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals (approval No. 1007 (4)).

Treatment of Animals and Sample Preparation The mice were randomly divided into four groups (n=8 in each group), and those belonging to the normal group were fed a standard chow diet (360 kcal/100g fresh weight of food) composed of (by dry weight) 7.7% water, 23.6% protein, 5.3% fat, 2.9% fiber, and 54.4% nitrogen-free extracts. The HFD group mice, which were fed a HFD (508 kcal/100g fresh weight of food) composed of (by dry weight) 6.2% water, 25.5% protein, 32.0% fat, 2.9% fiber, and 29.4% nitrogen-free extracts (CLEA Japan, Inc., Tokyo, Japan) for 10 weeks, were randomly divided into three subgroups: a group fed HFD alone (HFD group), a group fed HFD supplemented with 4% (w/w) EPA (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) (EPA group), and a group fed HFD supplemented with 4% (w/w) EPA and 0.01% (w/w) capsaicin (Sigma-Aldrich, St. Louis, MO, U.S.A.) (EPA+Cap group). The mice had ad libitum access to chow and tap water. The body weights of the mice and food intake per cage were measured on a weekly basis. After 10 weeks, the mice were fasted for 12h and anesthetized under diethyl ether. Blood samples were collected from the inferior vena cava, and serum samples were separated by centrifugation (3000×g, for 15 min) and stored at −80°C. The mice were then killed using deep anesthesia with diethyl ether, and their livers and epididymal adipose tissues were excised, rinsed with cold saline, weighed, and stored at −80°C.

Analytical Methods AST, ALT, and glucose levels were determined using the Transaminase CII-Test Wako (for AST and ALT) and Glucose CII-Test Wako (for glucose) kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum insulin levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Morinaga, Yokohama, Japan). Serum triglyceride, high-density lipoprotein (HDL) cholesterol, and total cholesterol levels were determined using the Triglyceride E-Test Wako, HDL-Cholesterol E-Test Wako, and Cholesterol E-Test Wako kits (Wako Pure Chemical Industries, Ltd.), respectively. All assays were performed according to the manufacturer’s instructions.

Statistical Analysis Data are expressed as the mean±standard deviation (S.D.). Statistical analysis of the differences between mean values was performed via Tukey’s multiple comparison test using SPSS Statistics software (version 18.0, IBM Corporation, Armonk, NY, U.S.A.). Values of p<0.05 were considered statistically significant.

RESULTS The mean body weight of HFD and EPA group mice was significantly higher than that of normal group mice (p<0.01), in the 10th week (Fig. 1). However, the mean body weight of EPA+Cap group mice was significantly lower than that of HFD and EPA group mice, after 10 weeks of treatment (p<0.01) (Fig. 1). We also observed that the amount of food ingested was not altered by dietary capsaicin except during the 1st week. The mice were fed a standard chow or HFD containing 0.01% (w/w) capsaicin, which is similar to the daily diet in several Asian countries.7) The average food intake of mice in the HFD, EPA, and EPA+Cap groups was lower than that of normal group mice, with the average food intake of EPA+Cap group mice being lower than that of HFD and EPA group mice (data not shown). However, there were no relationship between the amount of food intake and the average body weight among four group mice during treatment. The mean liver wet weight/body weight ratio was significantly lower in HFD, EPA and EPA+Cap group mice than in normal group mice (p<0.05) (Table 1). The mean epididymal fat tissue weight/body weight ratio was significantly higher in HFD and EPA group mice than in normal group mice (p<0.01 in both cases). Further, this ratio was significantly lower in EPA+Cap group mice than in HFD and EPA group mice (p<0.01 in both cases) (Table 1).

The mean serum glucose and insulin levels and homeostasis model assessment of insulin resistance (HOMA-IR) index, which is an index of insulin resistance, of HFD and EPA group mice were significantly higher than those of normal group mice (p<0.01 in all cases) (Table 1). Interestingly, the mean serum glucose level and HOMA-IR index of EPA+Cap group mice were significantly lower, whereas the mean serum insulin level was higher, than those of mice in the HFD and EPA groups (Table 1).
The serum total cholesterol level of HFD group mice was significantly higher than that of normal group mice (p<0.01). However, the level was significantly lower in EPA and EPA+Cap group mice than in HFD group mice (p<0.01 in both cases) (Table 1). The mean serum triglyceride levels of HFD, EPA, and EPA+Cap group mice were significantly higher than that of normal group mice (p<0.01 in all cases) (Table 1). The mean serum HDL-cholesterol level of HFD group mice was significantly higher than that of normal group mice (p<0.01). However, the level was significantly lower in EPA and EPA+Cap group mice than in HFD group mice (p<0.01 in both cases) (Table 1). The mean serum AST and ALT levels were higher in EPA and EPA+Cap group mice than in HFD group mice (p<0.01 in both cases). However, these levels were significantly lower in EPA and EPA+Cap group mice than in HFD group mice (p<0.05 for both). Additionally, the mean serum AST and ALT levels of EPA+Cap group mice were lower than that of EPA group mice (Table 1).

**DISCUSSION**

Adiposity, which is the fraction of total body mass comprising neutral lipids stored in adipose tissue, is closely correlated with important physiological parameters such as systemic insulin sensitivity and serum triglyceride concentration. Obesity-induced inflammation is thus associated with the development of metabolic diseases such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease. HFD-induced obesity is also associated with fatty liver development in animal models. Further, medium hepatopathy is an important pathological stage in obesity-related complications such as insulin resistance, type 2 diabetes, hepatic steatosis, and cardiovascular disease.

Although our previous studies did not demonstrate EPA-mediated amelioration of insulin resistance in HFD-fed mice, Kalupahana et al. reported that high-dose, long-term EPA administration (36 g/kg for 11 weeks) prevented and improved HFD-induced insulin resistance. Therefore, the therapeutic effects of EPA may be dependent on the dosage and duration of administration. Although capsaicin may be useful for treating obesity-related complications such as insulin resistance that are triggered by obesity-induced inflammation, it remains unclear whether dietary capsaicin supplementation can effectively attenuate obesity-induced inflammation and subsequent complications. In recent reports, dietary capsaicin supplementation increased sensation of fullness in energy balance, and decreased desire to eat more after a meal in negative energy balance. Although the role of capsaicin in appetite suppression is not fully elucidated, neuronal circuits in the hypothalamus may be a pivotal target of capsaicin, as an agonist of transient receptor potential vanilloid 1 (TRPV1), which modulates food intake.

Therefore, in this study, we investigated the effects of administering a combination of EPA and capsaicin on HFD-induced obesity-related complications in mice. It has been shown that dietary capsaicin supplementation (0.014%) for 10 d lowers the adipose tissue weight and levels of serum triglyceride in obese rats. We set the dose of capsaicin in our study to 0.01% in consideration for long-term administration. EPA has been shown to significantly decrease weight gain associated with decreased adiposity in mice fed HFD enriched with EPA using a similar dose with the present study (4.0% EPA). Our results indicated that the combined administration of EPA and capsaicin decreased the body and fat tissue weights of obese mice, which suggests that the combination of

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<th>Table 1. Effects of EPA and Capsaicin on Tissue Weight and Biochemical Analysis of Serum in Mice Fed a HFD</th>
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*p<0.05, **p<0.01 compared normal group, †p<0.05, ‡p<0.01 compared with HFD group, §p<0.05, ¶p<0.01 compared with EPA group. AST, aspartate aminotransferase; ALT, alanine aminotransferase. Data are expressed as mean ± S.D. HOMA-IR = insulin (µU/mL) × glucose (mg/dL)/405 (insulin; 1 µg/L × 26= µU/mL).
EPA and capsaicin shows anti-obesity effects by reducing the food intake. Further, the combination of EPA and capsaicin also improved the hepatic function, as indicated by the serum AST and ALT levels, although these changes were not confirmed histologically or via the determination of hepatic total cholesterol and triglyceride levels after EPA and capsaicin administration. The serum total cholesterol but not triglyceride was significantly lower in EPA+Cap group mice than in HFD group mice. Moreover, the improvement in insulin resistance, as indicated by the HOMA-IR index, induced by both supplements was mainly owing to the reduced serum glucose and enhanced serum insulin levels, which may be attributed to the decrease in adipose tissue weight. Kang et al. indicated that dietary capsaicin reduce obesity-induced glucose intolerance by suppressing inflammatory responses and by enhancing fatty acid oxidation in adipose tissue and/or liver, both of which are important peripheral tissues affecting insulin resistance. Therefore, a combined capsaicin than EPA treatment may reduce body weight and visceral adipose fat in HFD-induced obese mice better than EPA alone. Hence, sustained supplementation of EPA and capsaicin in combination might delay the progression of HFD-induced obesity and subsequent inflammation and thus protect against metabolic dysregulation from the early stage of obesity.

In summary, our current study suggests that combined supplementation of EPA and capsaicin has the beneficial effects of reducing body and fat tissue weights, insulin resistance, and the levels of serum total cholesterol, AST, and ALT, in obese mice. However, further human and animal studies are needed to confirm these beneficial effects and elucidate the mechanism by which the combination of EPA and capsaicin protects against NAFLD progression.

**Conflict of Interest** The authors declare no conflict of interest.

**REFERENCES**


