Mini Review

Placebo Controlled Study to Investigate The Relationship Between Lowering Serum Zonulin Levels And Improved Body Weight Composition Using a Daily Oral Dose of Phenylcapsaicin

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ABSTRACT

Leaky gut syndrome is associated with intestinal dysbiosis. Phenylcapsaicin, a novel analogue of capsaicin, at a low dose of 0.560 mg/day lowered serum zonulin levels from 80.78(ng/mL) to 75.50(ng/mL) after 54 days of daily oral supplementation. Lowered serum zonulin levels were correlated to improved body weight composition in the low dose treatment group but not in the high dose treatment group versus placebo in normal healthy subjects. Low dose treatment with phenylcapsaicin may be considered as a potential tool to alleviate the symptoms associated with leaky gut syndrome and commensurate healthy weight loss.

Keywords: Zonulin; Phenylcapsaicin; Body weight; Disorders

Introduction

Gastrointestinal disorders are on the rise in the United States, with some studies estimating that around twenty million Americans suffer from chronic digestive disorders [1]. Our gut is made up of over 4,000 square feet of intestinal lining or epithelium. This lining acts as a barrier to limit interaction between luminal contents, such as intestinal microbiota, the immune system, and the rest of the body, while also supporting the transportation of nutrients, water and waste through this lining [2]. When the gut microbiome is unbalanced, the cells in the intestinal wall can become compromised and permeable, allowing the passage of toxins, antigens and bacteria to the enter the bloodstream, causing a 'leaky gut' [3]. Leaky gut syndrome can dramatically impact an individual's well being and their quality of life, with disruptions in the gut microbiome affecting an individual's immunity and increasing the risk factor for infectious disease [4]. Chronic inflammation throughout the body caused by leaky gut syndrome can cause gastrointestinal symptoms such as bloating cramps and constipation, as well as skin problems, like acne rashes and eczema. While certain treatments have shown some promise, such as the administering of pre and probiotics, restrictive diets and dietary supplements, there is no official recommendation regarding treatment or management regimes for the symptoms of leaky gut syndrome [5-7].

It might seem incongruous to talk about hot and spicy chili peppers and gut health - after all, natural capsaicin is the major bioactive compound in chili peppers. Recent research has shown a relationship between dietary capsaicin and gut microbiota population. Kang et al. showed that capsaicin may prevent microbial dysbiosis (a reduction in microbial diversity), gut barrier dysfunction and chronic low-grade inflammation, which also resulted in an anti-obesity effect [8]. Though chili peppers have been a part of the human diet for years, and are popular worldwide, the difficult extraction process and variability of capsaicinoids in naturally occurring peppers, has led researchers

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to focus on finding alternatives that are readily available from chemical synthesis. We herein report the results on obesity and the leaky gut biomarker, zonulin after daily oral treatment with phenylcapsaicin. Phenylcapsaicin is a synthetic analogue of capsaicin, developed by aXichem.

The increased attention in gut health has led to a host of rapidly expanding in vivo, in vitro and ex vivo techniques and methods for investigation of the intestinal barrier. One such method focuses on zonulin, an endogenous human analogue of the bacterial enterotoxin zonula occludens toxin [9]. By disassembling the tight junctional protein complexes in the intestinal lining, zonulin is believed to modulate intestinal permeability [10]. Research has shown high serum levels of zonulin may point to the presence of increased intestinal permeability, disrupted gut barrier function and an altered immune response [11, 12]. Elevated circulatory levels of zonulin are also directly associated with a number of conditions, such as celiac disease, diabetes, and psychological distress [13, 14]. Unfortunately, few drugs have shown a direct reduction of circulatory zonulin levels, and a potential mitigation of leaky gut symptoms. This paper measures the effect of phenylcapsaicin on serum levels of zonulin at two doses (high and low), and the resultant outcome of reduced body weight and improved body composition in treated subjects.

We aim to evaluate the effect of a novel analogue of capsaicin, phenylcapsaicin, on serum levels of zonulin, weight loss and body composition changes along with diet and lifestyle modifications in healthy adults in a randomized, double-blind, parallel-group clinical trial of male and female subjects. We also aimed to evaluate the anti-obesity effect of phenylcapsaicin on the sample population.

In 2021, an eight-week study titled, "Effects of Phenylcapsaicin on Weight Loss and Body Composition," was conducted at the Center for Applied Health Sciences in Canfield, Ohio. The study was designed as a randomized, double-blind, parallel-group clinical trial of male and female subjects recruited at a single investigational center in Northeast Ohio. The primary objective of this placebo-controlled, double-blind study was to determine the effects of daily supplementation with phenylcapsaicin (phenylcapsaicin) on body weight and body composition during an 8-week diet and exercise program. The primary variables therefore were average body weight and body fat composition.

In addition, as a synthetic version of capsaicin, phenylcapsaicin was expected to function similarly to the naturally occurring compound. Natural capsaicin has been shown to have potent agonist activity in TRPV1, known as the 'capsaicin receptor,' which is broadly distributed in tissues throughout the body, including in the gastrointestinal tract. , for example demonstrating potent agonist activity in TRPV1 (also known as the "capsaicin receptor"), which is broadly distributed in tissues throughout the body, including in the gastrointestinal tract [14, 15]. Yang et al. describe the function of the mechanism accordingly: capsaicin stabilizes TRPV1's open state by 'pull-and-contact' interactions between the vanillyl group and the S4-S5 linker [14-16]. Studies in mice showed that the interaction between dietary capsaicin and gut microbiota had an anti-obesity effect, through the prevention of microbial dysbiosis, gut barrier dysfunction and chronic low-grade inflammation Therefore, a secondary objective of the study was to collect information on insulin resistance, appetite, cravings for sweet foods, mental clarity, mood, fatigue, as well as information via clinical chemistry panels of serum and plasma, with a specific focus on the the change in serum levels of zonulin present in subjects at the beginning and the end of the 8-week study, using zonulin as a proxy marker of intestinal permeability.

Methods

After giving informed consent and being cleared for participation by passing a screening physical, 39 overweight recreationally active men and women were randomly assigned to receive either phenylcapsaicin or a placebo. Following baseline testing, all groups underwent eight weeks of daily supplementation, a calorie restricted diet (500 calories less than their estimated daily requirement), and an increase in habitual physical activity (30 minutes of walking exercise 3 days per week). All subjects were then tested for changes in body weight, body girths, body composition and insulin resistance. They were also tested for changes in general markers of health, including heart rate, blood pressure, and comprehensive clinical chemistry panels of serum and plasma before and after the eight weeks of supplementation. This study specifically measured the change in the serum levels of zonulin to ascertain the impact

of phenylcapsaicin on intestinal permeability, and correlation with weight loss when combined with a prescribed diet and an increase in exercise and physical activity.

The study was conducted as a randomized, double-blind, parallel-group clinical trial of male and female subjects recruited at a single investigational center: the Center for Applied Health Sciences in Canfield, Ohio. The study included male and female subjects between 18 years and 45 years (inclusive), with a body mass index between 27 and 35, a normal resting heart rate (<90 bpm), and normotensive. The study excluded subjects that exercised more than three times a week, as well as patients with health conditions that affected their metabolism, weight loss or gain, reproductive health, and general health conditions such as heart disease and blood pressure. The study was conducted following ICH-GCP guidelines to ensure subject safety and scientific integrity of the data.

Settings

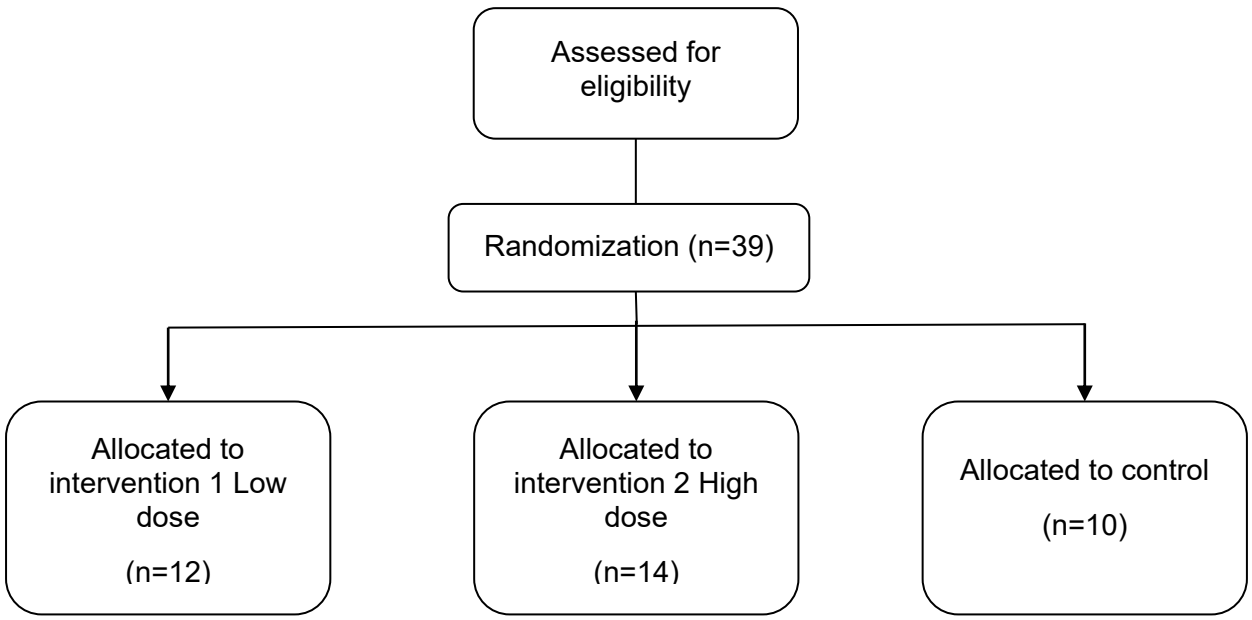
Diet: All subjects were placed on a "Zone" type diet that provides approximately 500 kcals per day less than their estimated energy requirements (via the Mifflin St. Jeor equation). The research dietitian met with each subject to explain the proper procedures for recording dietary intake and provide examples of the types of foods they can consume. Each subject's baseline diet (3 days: two weekdays & one weekend day) was analyzed via Nutri Base IX (Clinical Edition) to determine its energy and macronutrient content. Additional 3-day diet records were analyzed prior to the last day of testing (i.e. during week 8) to verify that eating habits remained consistent throughout the study.

Exercise and Physical Activity: All subjects were asked to increase their habitual physical activity to 30 minutes of walking exercise at least 3 days per week. To monitor compliance to the exercise regimen, each subject was required to document their walking exercise in a training log that was brought to each visit. Each subject's physical activity was assessed via a standardized questionnaire at baseline and again on the last day of testing (during the week 8 visit).

Supplementation: After qualifying for the study, subjects were matched according to gender (sex) and BMI prior to being randomly assigned to receive, in a double-blinded manner, either phenylcapsaicin or the placebo. Subjects randomized to the active product consumed either a low dose (0.560 mg) or a high dose (1.12mg) of phenylcapsaicin every day in the morning for eight weeks. Subjects randomized into the placebo group consumed microcrystalline cellulose. Supplements will be prepared in capsule form, matched for flavor and consistency, and packaged in coded generic containers for double-blind administration.

Results

The study enrolled 54 participants, of which 72% completed the 8-week study. 26 of the participants were female, and 13 of the



participants were male, with an average age of 46. All subjects were screened and evaluated at a single center in Ohio, and the following pieces of data were collected from each subject after screening and after the 8-week study.

- Height, weight, HNS Score
- Medical evaluation data: systolic BP, diastolic BP, heart rate, waist circumference, hip circumference
- Body Composition Values using an InBody 570 Body Composition Analyzer: Body Water percentage, Lean Body Mass percentage, Body fat percentage (PBF), Fat mass (kg), Body Mass Index (BMI)
- Laboratory Analyses: Hb (g/l), Ferritin (ug/l), Ht (%), WBC (x ul), RBC (mcL), MCH (pg), MCHC (g/L), RCDW (%), Platelet (x10e3/ml), HbA1c (%)FBG (mg/dL), IL-6 (pg/mL), IL-8 (pg/mL), IL-12B (pg/mL), IL-17 (pg/mL), TNFα (pg/mL), Zonulin (ng/mL), Gherlin (pmol/L).

The results of the study showed that participants treated with both the high and low dose of phenylcapsaicin experienced improved body composition as measured through BMI, Body fat percentage, Lean Body Mass percentage and Fat Mass (**Table 1**). Subjects treated with a low dose of phenylcapsaicin saw a decrease in BMI of 1.1 over the course of the eight weeks, as compared to placebo subjects that lost 0.6 (**Figure 1**). All body composition metrics also showed improvement,

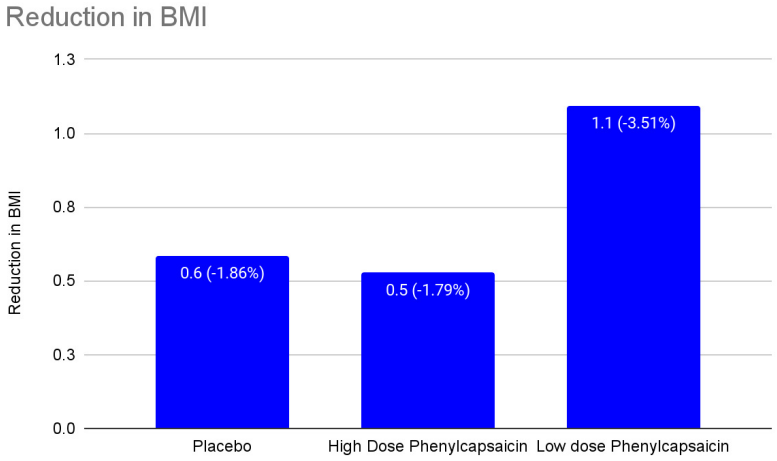
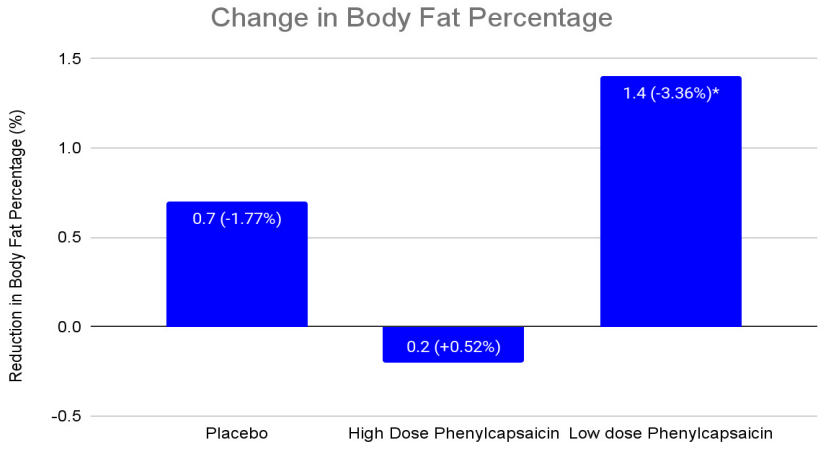


Figure 1: Change in BMI in subjects receiving low dose of phenylcapsaicin compared to placebo group.



*Statistically significant with a single tailed Student T-test ($p<0.01$)

Figure 2: Change in body fat percentage in subjects receiving low dose of phenylcapsaicin compared to placebo group.

and this was statistically significant for body fat percentage, lean body mass percentage and fat mass. Supplementary phenylcapsaicin was found to be most effective at the lower dose with subjects experiencing an 3.36% decrease in body fat percentage (**Figure 2**).

The study also showed a change in serum levels of zonulin for subjects who received phenylcapsaicin. (Figure 3) Over the 8 week study period, zonulin levels decreased by a statistically significant average of 9.2ng/ml (p=0.088) for the low dose phenylcapsaicin treatment group. Subjects treated with a low dose of phenylcapsaicin showed a decrease of 8.3ng/ml (12.4%) over the course of the eight weeks, as compared to placebo subjects that showed a decreased of 0.6ng/ml (1.1%).This suggests that phenylcapsaicin can impact the microbiome and potentially improve gut health for individuals fighting inflammation or obesity. In the high dose population, subjects also showed a 5.5% decrease in serum levels of zonulin.

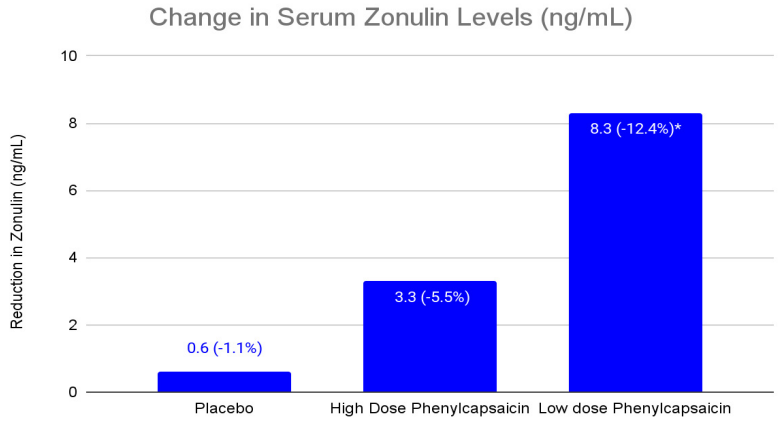
Discussion

The study effectively isolated the effect of phenylcapsaicin in a controlled environment, with a focus on understanding both the impact of the compound on metabolism, body composition and body weight, and the mechanism by which phenylcapsaicin might influence the gut microbiome, likely contributing to weight loss by reducing inflammation and preventing dysbiosis. By measuring the anti-obesity of phenylcapsaicin at both a high and a low dose, the study gained richer data into the trending effects of supplementation, and into the possible optimizations that were possible in dosing. In further studies on phenylcapsaicin, researchers could recruit a larger and more diverse sample size to see how the compound might affect individuals of various age, ethnic or geographic cohorts. More real-world data could be gathered to determine phenylcapsaicin's effectiveness at varying levels of adherence to supplementation, changes in diet or exercise to determine the

Table 1: Grouped Body Composition Changes

Group	Placebo		High Dose Phenylcapsaicin		Low Dose Phenylcapsaicin	
Average data	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
Body Weight (kg)	89	87	85.7	83.9	90.5	88.21
Heart Rate	68.8	70.6	68.9	70.9	71.3	71.3
Waist Circumference	99.6	97.2	95.9	95.4	97.9	95
Hip Circumference	110.6	110.1	108.6	110.4	110.8	108.7
Body Fat Percentage (%)	39.5	38.8	38.4	38.6	41.7	40.3*
Lean Mass (kg)	33.7	32.6	54.9	54.4	51.1	51.2
Lean Mass: Fat Mass Ratio (kg)	1.63	1.68	1.66	1.63	1.46	1.56*
Fat Mass (kg)	33.7	32.6	33.9	34	36	34.1*
BMI (kg/m2)	31.4	30.8	29.4	28.9	31.1	20
Zonulin (ng/mL)	56.4	55.8	59.9	56.6	67.1	58.8*

* Statistically significant with a single tailed Student T-test (p<0.01)



* Statistically significant with a single tailed Student T-test (p<0.01)

Figure 3: Change in Serum Zonulin in percentage of subjects receiving a low dose of phenylcapsaicin compared to placebo group.

optimal dosage of the compound. Some recent research has been skeptical of zonulin as a postulated biomarker of intestinal barrier integrity; further studies might incorporate the measurement of other serum level biomarkers of intestinal permeability, such as dual-sugar assays, and use immunohistochemistry and expression profiles of zonula occludens proteins [17].

The study was designed to examine the effect of phenylcapsaicin supplementation on weight loss. The study showed that, similar to naturally occurring capsaicin, at a low dose, phenylcapsaicin led to weight loss, improved body composition. The study showed that phenylcapsaicin reduced body weight and fought obesity, increasing metabolism and lowering energy intake. The compound was also shown to reduce gut inflammation and balance intestinal flora. One crucial outcome of the study was the reduced serum levels of zonulin, the critical gut barrier biomarker. As a proxy biomarker for intestinal permeability and gastrointestinal barrier health, serum zonulin levels have been shown to be significantly higher in patients with gastrointestinal disorders such as Crohn's and IBD, making phenylcapsaicin a strong viable treatment to promote improved gut health. The study also demonstrated an excellent safety and tolerability profile at both high and low doses, though phenylcapsaicin proved to be most effective at the lower dose. Regulatory organizations, such as the European Food Safety Authority, have also deemed phenylcapsaicin to be safe for use among adolescents and adults [18].

Kang et al. showed that capsaicin was shown to prevent microbial dysbiosis (a reduction in microbial diversity), gut barrier dysfunction and chronic low-grade inflammation, which resulted in an anti-obesity effect [19]. Though chili peppers have been a part of the human diet for years, and are popular worldwide, the difficult extraction process and variability of capsaicinoids in naturally occurring peppers, has led researchers to focus on finding alternatives that are readily available from chemical synthesis. Phenylcapsaicin is the active ingredient in aXivite®, a supplement formulation being developed by aXichem. It is readily available in its pure form through a chemical synthesis process, and is a promising new dietary ingredient, which in further formulations into finished products has the potential to significantly improve gastrointestinal health.

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