

# Supplement for controlling blood lipids and maintaining anthropometric indicators in obese women: Zinc or *Lactobacillus casei*

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## ABSTRACT

Dietary plans, pharmaceutical therapies, physical activity, and psychological approaches are some of the methods that have been suggested to address weight excess and its negative effects. Food supplements that are now on the market have several claimed modes of action, including enhanced energy expenditure or lipolysis, improved glucose metabolism, and decreased appetite. Based on previous studies, it seems reasonable to assume that Probiotic and Zn supplementation may have favorable effects on weight loss or reversing obesity-related comorbidities. Therefore, this study was designed to compare the effects of daily intake of 30 mg Zinc (Zn) supplement and 6,5x10<sup>9</sup> CFU/day *Lactobacillus casei* (LCS) as Probiotic along with RCD on anthropometric measurements and lipid profiles in obese individuals. For 30 days, 84 participants were randomly assigned to one of four groups: zinc (30 mg/day), LCS (6,5x10<sup>9</sup> CFU/day), "zinc and LCS," or placebo. Anthropometric markers, food intake, stress levels, physical activity, and lipid profiles were assessed before and after the intervention. After controlling by confounding variable, in this study was dietary intake, the greatest reduction in total cholesterol levels was in the Zn group. The largest increase in HDL-C levels was in the Zn+LCS group. The largest decrease in LDL-C levels was in the combined Zn+LCS group. The greatest reduction in triglyceride levels was in the Zn group. The largest decrease in waist circumference was found in the Zn group. Likewise, the largest decrease in percent body fat after was in the Zn group.

**Keywords:** Supplement, Blood lipid profiles, Anthropometric indicators, Obese, Zinc, *Lactobacillus casei*

## Introduction

Globally, the prevalence of obesity has been steadily increasing over the past few decades [1], which has led to an increase in the prevalence of numerous weight-excess complications.

Dietary plans [2-4], pharmaceutical therapies [5, 6], physical activity [7], and psychological approaches [8] are some of the

methods that have been suggested to address weight excess and its negative effects. While some have raised concerns [9], the majority are safe [10, 11]. Nevertheless, the main problem is the occurrence of unfavorable events and decreased compliance, even though it has improved many situations. Because of the widespread belief that natural chemicals may be synonymous with balance and health, so-called super foods and food supplements have been very popular in recent years. Even if this is partially untrue, indeed, many of the dietary supplements that are currently on the market have almost no serious side effects, considering the numerous negative outcomes that might result from consuming natural ingredients [12].

Food supplements that are now on the market have a number of claimed modes of action, including enhanced energy expenditure or lipolysis, improved glucose metabolism, and decreased appetite. There is a lot of literature available, but it is sometimes

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of poor quality, making it difficult to navigate and difficult to provide appropriate recommendations to people who seek help. Additionally, it is important to keep the dose in mind because it is widely recognized that dosage can determine whether a supplement is poisonous or beneficial, and too frequently, commercially accessible supplements offer wildly varying dosages without even adequately disclosing this information on their labels [12].

Food for special medical purposes (FSMPs) are designed to provide full or partial nourishment to people with unique medical needs or those whose capacity to metabolize food is impaired [13]. Functional foods that use probiotics to enhance immune function, cognitive responsiveness, and general well-being are another facet of nutrition as a health-promoting idea [14]. Probiotic preparations are classified as either food supplements or functional food ingredients under European law [15]. Probiotics are employed as live biotherapeutic products (LBPs) as well as health-promoting food supplements (nutraceuticals), according to the US Food and Drug Administration [16]. The European Pharmacopoeia Commission has established the quality assurance of probiotics as LBPs on the European market [17]. Probiotics are utilized to strengthen the immune system and enhance intestinal health. The FAO/WHO definition of a probiotic as live microorganisms that, when given in sufficient quantities, promote the host's health has been upheld and approved by the International Scientific Association for Probiotics and Prebiotics (ISAPP) [18]. Previous studies have illustrated the positive effects of probiotic supplementation: decreased BMI, total body fat, markers of metabolic disorders, increased numbers of beneficial gut microorganisms, and higher levels of short-chain fatty acids [19].

Zinc (Zn) deficiency is one of these micronutrients that is frequently problematic in obese people [20]. Zn has also been identified as a limiting nutrient in Restricted calorie diets (RCD) [21]. Additionally, prior research has shown that obese people have inadequate nutritional consumption and plasma zinc levels. Therefore, if the zinc deficiency is not addressed, it appears that additional weight gain or the emergence of obesity-related illnesses may occur. According to a previous study, taking 30 mg of zinc gluconate every day for a month resulted in a considerable drop in body weight body mass index (BMI), and profile lipids [22]. Two important hypotheses about the potential mechanisms by which zinc supplementation may contribute to weight loss include regulating appetite and reducing insulin resistance (IR) [23]. The positive effects of dietary zinc intake and plasma zinc levels on inflammatory status are another significant factor that merits attention [24].

So, based on previous studies, it seems reasonable to assume that Probiotic and Zn supplementation may have favorable effects on weight loss or reversing obesity-related comorbidities. Therefore, this study was designed to compare the effects of daily intake of 30 mg Zn supplement and  $6,5 \times 10^9$  CFU/day *Lactobacillus casei* (LCS) as Probiotic along with RCD on anthropometric measurements and lipid profiles in obese individuals.

## Materials and Methods

### *Study design and participants*

This double-blind randomized clinical trial was conducted from August to October 2023. The sample size was calculated as 21 subjects for each group. In this two-arm parallel study with two-tailed testing, a power  $(1-\beta)$  of 80% and  $\alpha = 0.05$  was used. Eight four healthy women with obesity and  $25 < \text{BMI} < 35 \text{ kg/m}^2$  in the age range of 30-50. In our study, exclusion criteria were the presence of pregnancy or lactation, not currently undergoing weight loss therapy, and consuming other supplements outside the intervention during the study. The primary outcomes were anthropometric measurements, serum lipid (Total cholesterol, HDL-C, LDL-C, and Tryglyceride), stress levels, and physical activity scores. This research has received approval from the Health Research Ethics Commission of the Makassar Health Polytechnic with Ethical Exemption number 0681/M / KEPK-PTKMS / VII / 2023.

### *Intervention*

During this study, there were four groups. First was the control group, and second was subjects in the Zn group who received 30 mg zinc monohydrate. Third was the LCS group which received  $6,5 \times 10^9$  CFU/day, and the fourth was the Zn+LCS group which received 30 mg zinc monohydrate +  $6,5 \times 10^9$  CFU/day *Lactobacillus casei* supplement. In addition, subjects in Zn, LCS, and Zn+LCS groups received a restricted calorie diet (RCD) with  $\sim 500$  kcal lower than the estimated energy requirement based on the Mifflin-St Jeor equation to reduce their weight by about 1 kg per month [25]. A qualified dietitian evaluated diet adherence once a week. Participants were urged to continue their typical level of physical activity and were monitored by phone calls to make sure they were complying.

### *Statistical analysis*

The anthropometric and food consumption variables were subjected to the intention-to-treat principle. Body weight and percentages of body fat were determined using a bioelectrical impedance analysis (BIA) device (TANITA BC-542). A stadiometer (Seca) was used to measure height without shoes. Weight (in kilos) divided by height (in meters squared) yielded the BMI. A stadiometer (Onemed) was used to measure waist circumference (WC). The biochemical data was analyzed using the Thermo Scientific TM Indiko™ Plus Clinical Chemistry Analyzer. Kolmogorov-Smirnov test for normal distribution of data ( $n > 50$ ). SPSS 26 was used to statistically analyze the data. Since there were fewer than 50 samples, the Shapiro-Wilk test was used to check for normality in the data. Use the Wilcoxon (abnormal) and paired t-test (normally distributed data) to compare the data before and after the intervention. One-way ANOVA can be used to test across groups if the data is normally distributed; if not, the Kruskal-Wallis test can be used. Use GLM to check for confounding variables.

## Results and Discussion

Throughout the 30-day experiment, 84 participants in total—21 in each group—completed it. The follow-up was not lost by

anyone. The characteristics of subjects at the beginning of the study showed on **Table 1**.

**Table 1. General Characteristics of Subjects at the Beginning of the Study**

Characteristics	Control (n = 21)	Zn (n = 21)	LCS (n = 21)	Zn + LCS (n = 21)
Age (years)	35,90±2,76	35,86±3,12	36,15±3,39	36,14±3,39
Weight (kg)	74,44±11,64	74,80±8,35	77,72±13,74	73,98±7,4
BMI (kg/m <sup>2</sup> )	32,65±5,15	31,96±3,86	33,87±5,89	31,49±3,47
Physical Activity (PAL)	1,57 ± 0,139	1,57±0,139	1,53 ± 0,11	1,57± 0,14
Stress Level (Score)	9,71 ± 2,87	9,67 ± 3,47	9,43 ± 2,98	9,71 ± 2,87

Values are mean ± SD.

The mean BMI of the control group was 32.65 ± 5.15 kg/m<sup>2</sup>, the zinc supplementation group was 31.96 ± 3.86 kg/m<sup>2</sup>, the LCS supplementation group was 33.87 ± 5.89 kg/m<sup>2</sup>, the combined group was 31.49 ± 3.47 kg/m<sup>2</sup> (p=0.592). This mean BMI is included in the research inclusion criteria, namely obese nutritional status (BMI >25.0 kg/m<sup>2</sup>).

### *Dietary intake, physical activity, and stress level*

Dietary intakes of energy, carbohydrate, fat, fiber, saturated fatty acids (SAFA), polyunsaturated fatty acids (PUFA), cholesterol, and Zn were significantly different between the intervention groups at baseline and the end of the month [22]. This occurred

due to calorie restriction in the intervention group (Zn, LCS, and Zn+LCS)

As is shown in **Table 2**, No significant changes were observed in physical activity and stress levels between the four groups during the study.

Subjects' physical activity measured by physical activity level (PAL) was categorized into light (sedentary lifestyle) = 1.40-1.69, moderate (active or moderately active lifestyle) = 1.70-1.99, and heavy (vigorous or vigorously active lifestyle) = 2.00-2.40 [26]. Subjects' stress levels were measured with the Depression Anxiety Stress Scale 42 (DASS 42) questionnaire by Lovibond (1995) which was categorized into normal (0-14), mild (15-18), moderate (19-25), severe (26-33), and very severe (≥34) [27].

**Table 2. Comparison of Physical Activity and Stress Levels Between and within Intervention Groups**

Characteristics	Control (n = 21)	Zn (n = 21)	LCS (n = 21)	Zn + LCS (n = 21)	P-Value
Physical Activity (PAL)					
Pre-intervention	1,57 ± 0,139	1,57 ± 0,139	1,53 ± 0,11	1,57 ± 0,139	0,436 <sup>b</sup>
Post-intervention	1,57 ± 0,139	1,57 ± 0,139	1,54 ± 0,12	1,55 ± 0,129	0,436 <sup>b</sup>
P-value	1,000 <sup>c</sup>	1,000 <sup>c</sup>	0,518 <sup>d</sup>	0,741 <sup>d</sup>	
Stress Level (Score)					
Pre-intervention	9,71 ± 2,87	9,67 ± 3,47	9,43 ± 2,98	9,71 ± 2,87	0,988 <sup>a</sup>
Post-intervention	9,48 ± 2,23	9,10 ± 2,51	9,00 ± 2,30	9,48 ± 2,23	0,865 <sup>a</sup>
P-value	0,497 <sup>c</sup>	0,588 <sup>c</sup>	0,234 <sup>c</sup>	0,497 <sup>c</sup>	

<sup>a</sup>one-way ANOVA <sup>b</sup>Kruskal Wallis Test <sup>c</sup>paired t-test <sup>d</sup>Wilcoxon Signed Ranks Test

**Table 3** shows that most of the subjects' physical activities measured by PAL were in the light category. **Table 3** also shows that there was no difference in the physical activity of the subjects grouped into light, medium, and heavy between the treatment and control groups with a p>0.05 value. Most subjects' physical activity was in the light category (90.48% in the control group, 100% in the zinc and *Lactobacillus casei* supplementation group, and 95.24% in the combined group). This light activity could be due to the type of work of the subjects, all of whom work as housewives and mostly do light activities such as sitting, chatting, and lying down.

Apart from the lack of physical activity, another factor that affects obesity is stress, according to research states that too much stress can harm everyone [28, 29]. Hormonal changes in someone who is depressed or stressed trigger increased secretion of cortisol which can lead to increased accumulation of body fat, especially in the abdominal area [30]. Someone who has an overweight and obese body mass index has a cortisol concentration that tends to be higher and will activate fat storage enzymes and signal hunger to the brain. The main hormonal response to stress is the activation of the corticotrophin-releasing hormone-adreno corticotropic hormone cortisol system. The process that occurs includes stimulation of the hypothalamus causing the secretion of

corticotrophin-releasing hormone (CRH), further stimulating the anterior pituitary to secrete ACTH. Increased secretion of CRH and ACTH causes the adrenal cortex to release excessive cortisol. Cortisol is the main hormone during adaptation to stress. When the body experiences stress, the body will indirectly release the cortisol hormone. High levels of the hormone will stimulate the body to secrete insulin, leptin, and the neuropeptide Y (NPY) system that causes hunger so that

there is a desire to eat. This results in the accumulation of visceral fat and can increase appetite stimulation by decreasing the work of appetite suppressants by melanocortin / POMC so that it will bind to receptors in the lateral hypothalamus causing active melanin-concentrating hormone (MCH) and will have an effect on increasing appetite through the medial and insular prefrontal cortex [31, 32].

**Table 3. Differences of Study Subjects According to Physical Activity and Stress Level During the Study between Intervention groups**

Characteristics	Control (n = 21)		Zn (n = 21)		LCS (n = 21)		Zn + LCS (n = 21)		P-Value
	n	%	n	%	n	%	n	%	
Physical Activity (PAL)									
Light	19	90,48	21	100	21	100	20	95,24	0,909 <sup>a</sup>
Moderate	2	9,52	0	0	0	0	1	4,76	
Stress Level (Score)									
Normal	20	95,24	21	100	21	100	20	95,24	0,923 <sup>a</sup>
Mild	1	4,76	0	0	0	0	1	4,76	

<sup>a</sup>one-way ANOVA

### *Lipid profiles, waist circumference, and body fat percentage*

In the groups that received zinc, LCS, and combined zinc and LCS intake, all lipid profiles, and anthropometric indicators showed significant differences before and after the intervention ( $p < 0.05$ ). Lipid profiles and anthropometric indicators of the study subjects obtained at the beginning of the study and the end of the study were further analyzed to compare changes and differences within and between groups. Comparison of lipid profiles in the control group showed no significant differences in total cholesterol, HDL-C, LDL-C, and triglyceride levels before and after the intervention ( $p > 0.05$ ), but waist circumference and percent body fat showed significant differences ( $p < 0.05$ ). In the groups that received zinc, LCS, and combined zinc and LCS intake, all lipid profiles, and anthropometric indicators showed significant differences before and after the intervention ( $p < 0.05$ ). Between intervention groups, there were significant differences in cholesterol levels, HDL-C, LDL-C, Triglycerides, waist circumference, and percent body fat in the control, zinc, LCS, and combined zinc and LCS supplementation groups before and after the intervention ( $p < 0.05$ ) [22, 33].

Micronutrient supplementation can be effective for weight regulation. Zinc supplementation significantly reduces total cholesterol, LDL cholesterol-C, and triglycerides, therefore, has the potential to minimize obesity-related consequences. For instance, zinc supplementation significantly increased serum zinc and decreased fasting glucose, insulin, and HOMA-IR. Conflicting results in different studies could be due to variations in dosage, supplementation period, differences in the chemical form of zinc, as well as supplementation having been conducted in different population groups. In addition, zinc supplementation can restore zinc expression and reduce not only inflammatory cytokine production but also oxidative stress markers, in obese populations [34].

There are several human studies that have assessed the effects of probiotics on obesity. One month of intervention with synbiotics significantly reduced body weight and BMI in obese children and adolescents [35]. Eight weeks of intervention with synbiotics without any lifestyle manipulation reduced z-scores, waist circumference, and waist-hip ratio [36]. Eight weeks of intervention with *Lactobacillus rhamnosus* in obese children with liver disease showed that *L. rhamnosus* strain GG altered bacterial composition without remarkable effects on z-scores or visceral fat [37].

Another 12-week intervention with probiotics (*Lactobacillus gasseri* BNR17) showed a slight decrease in body weight and significant waist and hip circumference among obese adults [38] and a 12-week intervention with *L. gasseri* SBT2055 showed a favorable impact on abdominal adiposity and body weight among adults [39, 40]. Similarly, supplementation with *Lactobacillus casei*. The differences in results with other studies may be due to the diversity of probiotic strains, supplement composition and dosage, subject characteristics, geographical differences, and duration of intervention.

With mixed results, several other studies also showed positive results regarding the activity of *Lactobacillus* probiotics in lowering cholesterol when tested in vitro. The suspected mechanism is the ability of probiotics to degrade cholesterol into coprostanol. In addition, probiotics also produce an enzyme called Bile Salt Hydrolase (BSH), which is an enzyme that can deconjugate bile salts. The bile salts will then be excreted through the feces so that the amount of bile acids returning to the liver is reduced. The enzyme Bile Salt Hydrolase (BSH) is responsible for the deconjugation of bile acids, where glycine or taurine is separated from steroids, resulting in free or conjugated bile salts. To balance the concentration of bile salts, the body will take blood cholesterol as its precursor material. This process will eventually lower the overall blood cholesterol level. The ability to deconjugate bile salts is strongly related to the activity of the

enzyme bile salt hydrolase (BSH) produced by probiotics. Previously, in order to perform its function properly, probiotics must first be resistant to bile salts secreted into the gut. Another

advantage of probiotics successfully deconjugating bile salts is that cholesterol is more easily attached to the bacterial cell wall, reducing the body's ability to absorb cholesterol [41, 42].

**Table 4. Effect of Confounding Variables on Changes in Lipid Profile and Anthropometric Indicators of Subjects in the Treatment Group**

Characteristics	Zn + CDR (n = 21)	LCS + CDR (n = 21)	Zn + LCS +CDR (n = 21)	Confounding Variables
Δ Total cholesterol (mg/dl)	<b>-47,853</b>	-28,921	-43,040	SAFA
P-Value	0,010	0,049	0,005	0,211
Δ HDL-C (mg/dl)	15,372	25,226	<b>28,692</b>	Fat (% of energy)
P-Value	0,000	0,000	0,000	0,002
Δ LDL-C (mg/dl)	-44,683	-38,842	<b>-58,963</b>	Fat (% of energy)
P-Value	0,003	0,001	0,000	0,996
Δ Triglyceride (mg/dl)	-57,610	-46,124	-33,658	SAFA
P-Value	0,003	0,012	0,076	0,370
Δ Waist circumference (cm)	-5,210	-3,469	-4,187	Carbohydrate(% of energy)
P-Value	0,000	0,000	0,000	0,406
Δ Body fat percentage (%)	-0,929	-0,672	-0,768	Fat (% of energy)
P-Value	0,000	0,000	0,000	0,259

The confounding variables in this study included intake, physical activity, and stress level. Since physical activity and stress level showed no difference before and after the intervention both within and between groups while dietary intake showed a significant difference, intake was a confounding variable in this study. Effect of confounding variables on changes in lipid profile and anthropometric indicators of subjects in the treatment group showed on **Table 4**. Based on the GLM test with covariates including % TKE, %KH, % Fat, Zinc intake, Fibre intake, Cholesterol intake, Trans Fat intake, and Unsaturated fat intake, it was found that % fat influenced the decrease of HDL-C level ( $p=0.002$ ).

The greatest reduction in total cholesterol levels after controlling for saturated fat intake was in the zinc group. Each saturated fatty acid has diverse biological and cholesterol-raising effects with the chain length of the saturated fatty acid playing an important role in determining the effects on lipid and lipoprotein levels. The most commonly consumed saturated fatty acids are palmitic acid (main sources: vegetable oils, dairy products, and meat), stearic acid (meat, dairy products, and chocolate), myristic acid (dairy products and tropical oils, especially coconut oil) and lauric acid (dairy products and tropical oils) [43].

The largest increase in HDL-C levels after controlling for fat intake (%) was in the combined zinc and *Lactobacillus casei* group. The largest decrease in LDL-C levels after controlling for fat intake (%) was in the combined zinc and *Lactobacillus casei* group. For a given individual, many factors may modify the slower and/or less efficient absorption response and desaturation of stearic acid to oleic acid, including lifestyle factors such as overall dietary composition and physical activity, clinical conditions such as obesity, insulin resistance, and hypertriglyceridemia, as well as genetic factors [44]. Saturated

fatty acids from the diet have been shown to decrease hepatic LDL-C receptor activity, protein, and mRNA levels and this leads to increased LDL-C levels. In addition, decreased LDL-C receptors may lead to increased conversion of intermediate-density lipoproteins to LDL-C. Saturated fatty acids have been shown to decrease cholesterol ester formation, a reaction catalyzed by the enzyme acyl CoA: cholesterol acyltransferase (ACAT). Free cholesterol in the endoplasmic reticulum is a key regulator of sterol receptor binding protein (SREBP) activation, which translocates to the nucleus and increases LDL-C receptor transcription. Increased cholesterol levels in the endoplasmic reticulum prevent SREBP activation. When free cholesterol is esterified to cholesterol esters, it no longer prevents SREBP activation and increases LDL-C receptor expression. Thus, saturated fatty acids by reducing cholesterol ester formation and increasing free cholesterol may lead to downregulation of LDL-C receptor expression.

The greatest reduction in triglyceride levels after controlling for saturated fat intake was in the zinc group. A possible mechanism associated with the effect of zinc supplementation on lipid metabolism is that zinc inhibits hormone-sensitive lipases through 3-kinase-Akt/PKB signaling by phosphoinositide-dependent complexes. Regarding this finding, it is hypothesized that zinc supplementation results in improved glucose utilization and lipid metabolism [45]. Also, it has been investigated that zinc also plays a role in lipoprotein lipase and lecithin cholesterol ester transferase pathways; thus, ZNF202 may be a gene susceptible to the development of dyslipidemia in the human body and participates in the construction of an adipokine called zinc- $\alpha$ 2-glycoprotein, zinc increases adiponectin secretion and inhibits leptin secretion in the human body. If zinc metabolism is

impaired, the regulatory role of zinc on these adipokines is impaired [46].

The largest decrease in waist circumference after controlling for carbohydrate intake (%) was found in the zinc group. Likewise, the largest decrease in percent body fat after controlling fat intake

(%) was in the zinc group. According to Tejoyuwono *et al.*, excess waist circumference is a cause of health problems that arise when people live a poor lifestyle, such as eating foods with excessive fatty consumption without being balanced with exercise [45].

**Table 5. Mean Blood Lipid Levels and Anthropometric Indicators at the End of Treatment**

Characteristics	Control (n = 21)	Zn (n = 21)	LCS (n = 21)	Zn + LCS (n = 21)	Normal Value
Total cholesterol (mg/dl)	205,87	200,11	196,10	192,38	<200
HDL-C (mg/dl)	46,21	57,10	68,77	72,33	>40
LDL-C (mg/dl)	122,01	119,18	97,71	93,52	<100
Triglyceride (mg/dl)	188,26	116,76	143,10	130,23	<150
BMI (kg/m <sup>2</sup> )	32,65	31,64	33,87	31,50	18,5 - 25,0
Waist circumference (cm)	100,76	96,23	100,90	96,29	<80,00
Body fat percentage (%)	43,37	42,33	44,92	41,20	20 - 32

After 30 days of intervention, the blood lipid levels in the treatment groups decreased to normal blood lipid levels. Except the mean levels of total cholesterol and LDL-C in the zinc group combined with calorie restriction were still slightly above normal. However, the decrease in total cholesterol and LDL-C levels in the group was the largest compared to before the intervention (**Table 5**).

In the anthropometric indicators of the treatment group, the reduction in BMI, abdominal circumference and percent body fat had not reached normal levels, although there was a significant decrease when compared to before the intervention.

## Conclusion

After controlling by confounding variable, in this study was dietary intake, the greatest reduction in total cholesterol levels was in the Zn group. The largest increase in HDL-C levels was in the Zn+LCS group. The largest decrease in LDL-C levels was in the combined Zn+LCS group. The greatest reduction in triglyceride levels was in the Zn group. The largest decrease in waist circumference was found in the Zn group. Likewise, the largest decrease in percent body fat after was in the Zn group.

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