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# Lemon detox diet reduced body fat, insulin resistance, and serum hs-CRP level without hematological changes in overweight Korean women

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ABSTRACT

The lemon detox program is a very low-calorie diet which consists of a mixture of organic maple and palm syrups, and lemon juice for abstinence period of 7 days. We hypothesized that the lemon detox program would reduce body weight, body fat mass, thus lowering insulin resistance and known risk factors of cardiovascular disease. We investigated anthropometric indices, insulin sensitivity, levels of serum adipokines, and inflammatory markers in overweight Korean women before and after clinical intervention trial. Eighty-four premenopausal women were randomly divided into 3 groups: a control group without diet restriction (Normal-C), a pair-fed placebo diet group (Positive-C), and a lemon detox diet group (Lemon-D). The intervention period was 11 days total: 7 days with the lemon detox juice or the placebo juice, and then 4 days with transitioning food. Changes in body weight, body mass index, % body fat, and waist-hip ratio were significantly greater in the Lemon-D and Positive-C groups compared to the Normal-C group. Serum insulin level, homeostasis model assessment insulin resistance scores, leptin, and adiponectin levels decreased in the Lemon-D and Positive-C groups. Serum high-sensitive C-reactive protein (hs-CRP) levels were also reduced only in the Lemon-D group. Hemoglobin and hematocrit levels remained stable in the Lemon-D group while they decreased in the Positive-C and Normal-C groups. Therefore, we suppose that the lemon detox program reduces body fat and insulin resistance through caloric restriction and might have a potential beneficial effect on risk factors for cardiovascular disease related to circulating hs-CRP reduction without hematological changes.



*Abbreviations:* BMI, body mass index; BUN, blood urea nitrogen; DEXA, dual-energy X-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; Hb, hemoglobin; Hct, hematocrit; HOMA-IR, homeostasis model assessment insulin resistance; hs-CRP, high-sensitive C-reactive protein; IF, intermittent fasting; IGF-1, insulin-like growth factor-1; HDL, high-density lipoprotein; LCD, low-calorie diet; LDL, low-density lipoprotein; RBC, red blood cell count; RIA, radioimmunoassay; VLCD, very low-calorie diet; WHR, waist-hip ratio.

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## 1. Introduction

The recent economic growth in Korea resulted in significant changes in the lifestyle of Koreans, especially related to their diet. Due to a Westernized diet, the intake of food containing complex carbohydrates and vegetables have decreased, while the intake of animal products and processed foods have increased [1]. Therefore, fat intakes and the prevalence of obesity have been rising rapidly. According to the Korean National Health and Nutrition Examination Survey, the prevalence of obesity in Korean adults (aged ≥19 years) was 30.8% in 2010. This constituted an increase of approximately 4.8% compared to the 1998 prevalence of 26.0% [2]. Obesity is an established risk factor for various chronic diseases such as hypertension, cardiovascular disease, diabetes, and cancer. Therefore, the increase in obesity rates is considered a major problem for individuals and society as a whole that should be dealt with thoroughly [3,4]. The World Health Organization announced that obesity should not be merely regarded as a risk factor for chronic diseases but as a disease itself [5].

Recently, there has been an increasing interest in diets and weight loss programs to prevent obesity. Various well-known methods to treat obesity, including diet, exercise, drugs, and surgical therapy, exist. Synthetic drugs such as dexfenfluramine and phentermine, which are serotonergic and catecholaminergic agents respectively, have been commonly used to treat obesity. However, drug therapies are known to have side effects including anxiety, insomnia, headache, dizziness, elevated blood pressure, and digestive disorders [6,7]. In addition to the enormous economic burden, surgical therapy has been reported to cause pain and also have side effects [8]. Various diet programs for weight loss using restricted caloric intake such as the Atkins, Denmark, low-calorie diet (LCD), and lemon detox diet exist. However, the efficacy and safety of these diet programs need to be carefully examined.

The Atkins and Denmark diets are known as low-carbohydrate and high-protein diets. Since insufficient carbohydrate intake can induce gluconeogenesis and body protein loss, these high-protein diets have been reported to prevent lean body mass loss [9]. However, restricting carbohydrate intake (≤100 g/day) can cause severe ketosis, electrolyte loss, dizziness, orthostatic hypotension, fatigue, bad breath, and a rise in serum uric acid levels caused by water diuresis [10]. In Koreans, 65% of the daily caloric intake stems from carbohydrates; hence, these diet programs are hardly acceptable for Koreans.

Since the consumption of a low-carbohydrate and high-protein diet can lead to increased caloric intake from fat and saturated fatty acids, it potentially affects coronary heart disease risk [11]. Research on LCD has asserted that it can reduce body weight and fat levels of obese individuals [12]. LCDs for weight loss impose restrictions on caloric intake: 1200–1500 kcal/d for men and 1000–1200 kcal/d for women [13]. Thus, these diets can be used as long-term interventions. Recently, intermittent fasting (IF) was introduced as a calorie restriction method that can increase insulin sensitivity and prevent against oxidative damage of proteins, lipids, and DNA by reducing oxidative stress [14]. Furthermore, IF was reported to increase oxidation resistance, metabolic stress, and immune function. Caloric restriction diets, including IF, change serum insulin-like growth factor-1 (IGF-1), insulin, and cortisol levels in the blood, and protect from tumor growth [15]. However, calorie-restricted diets, especially very low-calorie diets (VLCDs) that only allow consumption of <500 kcal/d are hard to maintain. Moreover, they can lead to vitamin and mineral loss during the early intervention period [16]. VLCD treatment leads to rapid weight loss, but frequent complaints include headache, dizziness, fatigue, muscular weakness, gastrointestinal problems, and nausea [17,18].

The lemon detox program, which provides 800 to 1000 kcal per a day, is a VLCD for reducing body weight and fat. The lemon detox program, known as the Master Cleanse or Lemonade Diet, was first proposed by Stanley Burroughs in the 1940s. It became popular with the publication of Beyer [19]. The program was composed of Neera syrup and lemon juice. The Neera syrup is a blend of maple and palm tree syrups (maple syrup 63%-67%, Coconut Palm 8%-12%, Nipah Palm 8%-12%, Palmyra Palm 3%-7%, Arenga Palm 3%-7%, Kitul Palm 3%-7%) and is designed to have high amounts of minerals and trace elements. Therefore, the lemon detox program can provide higher minerals and vitamins than other VLCDs. In addition to having detoxification effects, it is proposed to eliminate cravings for junk food, sodas, coffee, and other unhealthy beverages [19]. However, the mechanism of this detoxification in the body is not clear. Sun et al reported elevated levels of an endotoxemia marker in obese individuals [20], and Cani et al proposed that metabolic endotoxemia is decreased during the fasting status [21]. Weight gain has been shown to play a leading role in elevating the circulation of endotoxins due to a dysfunction of the liver [22]. Adipokines are secreted from adipose tissues and can influence insulin sensitivity, reactive oxygen species generation, and inflammatory responses [23]. Thus, body fat reduction is hypothesized to have positive effects on detoxification in the body by inhibiting oxidative stress and endotoxin production, and altering inflammatory and pro-inflammatory responses.

The lemon detox program has been introduced in Korea several years ago. However, despite a high social interest, little is known about its efficacy and safety. Since it can provide more minerals and vitamin C than other VLCDs during abstinence period, we hypothesized that the lemon detox program would reduce body weight and body fat mass, and it could improve insulin resistance and reduce several risk factors of cardiovascular disease such as serum lipid profile and circulating adipokines. To test the hypothesis, the current study aimed to investigate changes of body weight, body composition, homeostasis model assessment of insulin resistance (HOMA-IR) scores, circulating adipokines related to insulin resistance and cardiovascular disease risk factors by the lemon detox intervention trial in overweight premenopausal Korean women.

## 2. Methods and materials

### 2.1. *Participants and study design*

Participants were recruited in October 2013 through online advertisements on the homepages of the Seoul Women's University and Cencorp Korea Co, Ltd, located in Seoul, Korea.

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**Table 1 – Nutrient composition of the supplements**

| Nutrients | Neera syrup | Maple Syrup | Lemon juice | Nutrients formula | Nutrients liquid |
|---|---|---|---|---|---|
| Calories (kcal) | 279 | 366.7 | 13.38 | 353 | 249 |
| Protein (g) | 1 | 0 | 2.92 | 22.3 | 0.2 |
| Fat (g) | 0.3 | 0 | 0.03 | 6.9 | 2.3 |
| Carbohydrates (g) | 67 | 90 | 2.92 | 60 | 57 |
| Fiber (g) | 0.5 | - | 0.53 | 19.38 | 0.22 |
| Cholesterol (mg) | 1 | - | 0 | 0.2 | 0.0 |

All nutrient contents are per 100 g and data were received from manufacturers. Analysis of nutrients for the formulas and liquids were performed at the N-tech Research Institute (Jinju, Gyeongsangnam, Korea).

We selected 100 subjects who were aged 20–50 years old, being premenopausal, healthy without diabetes or any other major medical conditions, consuming the average Korean diet, and a body mass index (BMI) ≥23 kg/m$^2$. All clinical trial protocols were approved by the Institutional Review Board of the Seoul Women's University (IRB-2013A-4), and informed consent for participation was obtained from all participants. This study used double-blind, randomized, and, controlled experiments. The subjects were randomly distributed into the following 3 groups based on similar mean BMIs: (1) a normal control group consisting of women who consumed a diet without restrictions (Normal-C); (2) a pair-fed diet group who was provided with a placebo juice that had a calorie restriction similar to the lemon detox group but did not contain lemon juice (Positive-C); and (3) a lemon detox diet group (Lemon-D) who was provided the lemon detox juice. The number of person, which was considered a statistical validity and drop rate, in each group was similar as 33 people for Lemon-D or Positive-C groups and 34 people for Normal-C group.

One day before the clinical intervention, we measured anthropometric indices and collected 12-hours fasting blood samples, 24-hour dietary recall, and general information. The clinical trial period was 11 days (7 days with the lemon detox juice or the placebo juice, and then 4 days with transitioning food). The diets consisted of 2 L of lemon detox juice containing 140 g Neera syrup, 140 g lemon juice, and water per day for the Lemon-D group, and same amount of placebo juice containing 110 g maple syrup, lemon-flavored juice, and water for the Positive-C group. The daily caloric intake was 409.3 kcal in the Lemon-D and 403.3 kcal in the Positive-C group. The diets for the transitioning period consisted of packs of nutrient formula, nutrient liquid, and soybean milk. These were provided to both the Lemon-D and Positive-C groups 3 times per day. All diets and ingredients were supplied by Cencorp Korea Co. Ltd. (Seoul, Korea). Table 1 shows the nutrient contents for each ingredient. The dietary intakes of the Lemon-D and Positive-C groups are shown in Table 2. After the end of clinical intervention, we measured anthropometric indices and collected 12-hours fasting blood again for biochemical analysis. Dietary records of subjects for intervention periods were also collected to analyze nutrients intakes. Selection process of subjects and the procedure of our experimental intervention trial were presented in Fig. 1.

### 2.2. *Anthropometric and blood pressure measurements*

Height was measured using an automatic meter (DS-103, JENIX; Seoul, Korea) before and after the experiment. Waist circumference was measured 1 cm below the navel by specifically trained researcher twice before and after the experiment. Body composition (weight, body fat, lean body mass, body fat percentage, BMI, and waist-hip ratio [WHR]) was measured by a multi-frequency bioimpedance analysis (In Body 720, Biospace; Seoul, Korea).

All subjects were examined for anthropometric indices including body fat mass and bone density using dual energy X-ray absorptiometry (DEXA) in the Sky Hospital (Seoul, Korea) before and after the clinical intervention. Blood pressure was measured on the left arm with an automated blood pressure monitor (TM-2655P, A&D Co.; Tokyo, Japan) after subjects were relaxed for 5 minutes. Blood pressure was measured twice for each participant to eliminate errors.

### 2.3. *Blood sample collection and preparation*

We collected blood samples for biochemical analysis before and after the experiment. Overnight fasting blood samples from brachial veins were collected in vacuum tubes (Becton Dickinson; Meylan, France) without anticoagulant for biochemical analysis and with ethylenediaminetetraacetic acid for hematological analysis. The hematological analysis was immediately performed after sample collection using a hematology analyzer (BC-3600, Mindray Co.; Shenzhen, China) and included white blood cell count, red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Hct) levels.

**Table 2 – Daily nutrient intakes for the Lemon-D and positive-C groups**

| Stage | Nutrient composition | Lemon-D | Positive-C |
|---|---|---|---|
| Abstinence period (7 days) | Calories (kcal) | 409.3 | 403.3 |
| | Protein (g) | 5.5 (9.9) | 0.0 |
| | Fat (g) | 0.5 (8.4) | 0.0 |
| | Carbohydrates (g) | 97.9 (29.4) | 99.0 (29.8) |
| | Fiber (g) | 1.4 (0.6) | - |
| | Cholesterol (mg) | 1.4 (0.7) | - |
| Transition period (4 days) | Calories (kcal) | 990.0 | 990.0 |
| | Protein (g) | 45.1 (81.2) | 45.1 (81.2) |
| | Fat (g) | 27.5 (53.7) | 27.5 (53.7) |
| | Carbohydrates (g) | 153.0 (45.9) | 153.0 (45.9) |
| | Fiber (g) | 24.1 (96.6) | 24.1 (96.6) |
| | Cholesterol (mg) | 0.0 ( 0.0) | 0.0 (0.0) |

Figures in parenthesis denote a percentage of dietary reference intake for Korean.

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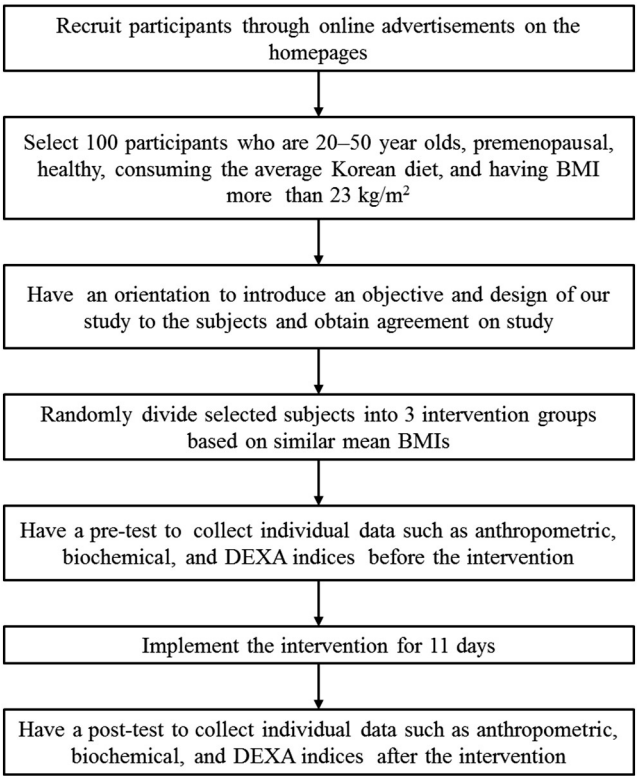


**Fig. 1 – Flows of the study.**

Blood samples without the anticoagulant were immediately centrifuged at 3000 rpm (4°C) for 20 minutes to separate the serum and stored at –80°C before the analysis.

### 2.4. *Biochemical analysis*

Serum lipid profiles including triglyceride, cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, free fatty acid, albumin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), bilirubin, blood urea nitrogen (BUN), creatinine, and uric acid levels were examined using an automatic blood biochemistry analyzer (Selectra E, Vital scientific; Dieren, Netherlands).

Fasting serum glucose concentrations were measured using glucose-hexokinase method (Glu Reagent kit, Roche Diagnostics Ltd.; Mannheim, Germany) by chemistry analyzer (Modular PE, Modular Analytics, Roche Diagnostics Ltd.; Mannheim, Germany). Serum insulin levels were determined using electrochemiluminescence immunoassay (Elecsys Insulin kit; Roche Diagnostics Ltd, Mannheim, Germany) by Roche Modular Analyzers (Modular Analytics E170; Roche Diagnostics Ltd). $HbA_{1c}$ levels were detected by high-performance liquid chromatography (Variant II Turbo, Bio-Rad Laboratories Inc.; Berkeley, CA, USA). Insulin resistance was expressed as a HOMA-IR score, calculated as fasting serum glucose levels (mmol/L) multiplied by serum insulin levels (mU/L) divided by 22.5 [24]. We also measured the serum leptin concentration with a radioimmunoassay (RIA) method using a γ-counter (Cobra; Packard Bioscience Co, Meriden, CT, USA). Serum IGF-1 levels were detected by a chemiluminescence immunoassay method. Analyses of serum adiponectin, homocysteine, and high sensitive-C reactive protein (hs-CRP) levels were performed by enzyme-linked immunosorbent assay (ELISA), chemiluminescent microparticle immunoassay, and immunoturbidimetric assay methods, respectively. We used the following commercial kits: leptin: Human Leptin RIA kit, Linco Research Inc., Saint Charles, Montana, USA; IGF-1: Liaison IGF-1 kit, Diasorin, Italy; adiponectin: Human Adiponectin ELISA kit, Biovendor, Czech Republic; and homocysteine: Architect Homocysteine assay kit, Abbott Lab, Green Oaks, IL, USA. Serum vitamin C levels were analyzed using a spectrophotometer (Uvikon 930, Kontron; Montigny, Le Bretonneux, France) using the 2,4-dinitrophenyl hydrazine method [25].

### 2.5. *Participant demographics and dietary intakes*

We conducted a demographic analysis of the participants. The women were required to fill out a questionnaire asking items about obesity-related factors prior to the experiment. Socio-demographic variables including age, sex, education level, and marital status were also examined.

Typical dietary intakes of subjects were collected by a self-recording method using a 24-hour recall before the clinical trial started in October 2013. All participants directly recorded their dietary intakes using a meal diary during the intervention period. Energy and nutrient intakes were calculated by a nutrient analysis software (Computer Aided Nutritional Analysis for professionals version 4.0 [CAN pro 4.0], Nutritional Assessment Program, 2011; The Korean Nutrition Society, Seoul, Korea).

### 2.6. *Statistical analyses*

Statistical programs available in the SPSS software (version 10, SPSS Inc.; Chicago, Illinois, USA) were utilized for data analysis. Anthropometric and biochemical indices are presented as means ± SD. Comparisons of data before and after the clinical trial were assessed by paired t-test. To confirm significant differences in the degrees of changes between the 3 clinical intervention groups, one-way ANOVA analyses and Duncan's multiple range tests were performed. General characteristics are presented as numbers and percentages and were verified by $\chi^2$ tests between experimental intervention groups. The statistical significance was set at $P < .05$ unless otherwise stated.

## 3. Results

### 3.1. *Participant demographics*

Initially, 100 premenopausal women aged 20–50 years and with a BMI ≥ 23 kg/m$^2$ participated in the clinical trial. However, only data from 84 women were used for analysis, because 16 women dropped out of the program due to failure of controlling their appetite, personal schedules, and stress. The average age of the participants was 24.2 years (Lemon-D; 24.1 ± 7.4, Positive-C; 23.6 ± 6.3, and Normal-C; 24.9 ± 8.8 years) and most of the women were students (72.6%) and not married (86.9%). The proportion of participants whose household income per month was greater than 5 000 000 won was 39.0%. The proportions of women whose education level was

**Table 3 – General characteristics of the subjects in the intervention groups**

| Group | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) | $X^2$-value |
|---|---|---|---|---|---|
| Occupation | | | | | |
| Student | 21(80.8) | 16(57.1) | 24(80.0) | 61(72.6) | 11.65 |
| Housewife | 2(7.7) | 0(0.0) | 1(3.3) | 3(3.6) | |
| Office job | 2(7.7) | 9(32.1) | 3(10.0) | 14(16.7) | |
| Merchant | 1(3.8) | 1(3.6) | 1(3.3) | 3(3.6) | |
| Others | 0(0.0) | 2(7.1) | 1(3.3) | 3(3.6) | |
| Income of your family per month (ten thousands won) | | | | | |
| Less than 100-300 | 4(16.0) | 8(28.6) | 10(34.5) | 22(26.8) | 2.81 |
| 300-500 | 9(36.0) | 9(32.1) | 10(34.5) | 28(34.1) | |
| More than 500 | 12(48.0) | 11(39.3) | 9(31.0) | 32(39.0) | |
| No answer | 1(3.8) | 0(0.0) | 1(3.3) | 2(2.4) | |
| Education level | | | | | |
| Elementary school | 0(0.0) | 0(0.0) | 1(3.3) | 1(1.2) | 5.09 |
| High school | 1(3.8) | 5(17.9) | 3(10.0) | 9(10.7) | |
| University (include college) | 23(88.5) | 20(71.4) | 24(80.0) | 67(79.8) | |
| Above graduate school | 2(7.7) | 3(10.7) | 2(6.7) | 7(8.3) | |
| Marital status | | | | | |
| Unmarried | 24(92.3) | 24(85.7) | 25(83.3) | 73(86.9) | 1.04 |
| Married | 2(7.7) | 4(14.3) | 5(16.7) | 11(13.1) | |

Values are number and percentage of subjects per group.
Tests of significance among 3 intervention groups are based on $\chi^2$ test ($P < .05$)

university graduation and below high school graduation were 79.8% and 11.9%, respectively. No significant differences between the 3 clinical trial groups were found regarding the majority of the participants' general characteristics (Table 3).

### 3.2. *Anthropometric indices and blood pressure*

The average height and body weight of the subjects were 162.2 cm and 68.3 kg, respectively. All subjects in this study had a BMI ≥23 kg/m$^2$, and their mean BMI was 25.9 ± 2.7 kg/m$^2$. More significant changes in body weight were observed in the Lemon-D and Positive-C groups than the Normal-C group. In our study, body weight, BMI, body fat percentage, WHR, and waist circumference significantly decreased in all 3 groups. However, the Lemon-D and Positive-C groups had a greater reduction in body weight, BMI, and total body fat mass than the Normal-C group and there were no significant differences on all anthropometric measurements between the Lemon-D and Positive-C groups. Systolic and diastolic blood pressure decreased by all 3 intervention trials, and no statistically significant difference between the 3 groups was found (Table 4).

In terms of data measured by DEXA, no significant changes in total and trunk fat masses were found in the Normal-C group, while a significant reduction was found in the Lemon-D and Positive-C groups. We observed no significant differences between Lemon-D and Positive-C groups. However, the ratio of trunk fat to total fat only exhibited a significant decrease only in the Lemon-D group after intervention trials. In addition, a significant difference in the changes of the ratio of trunk fat to total fat was found in the Lemon –D group compared to the Normal-C group (Table 5).

### 3.3. *Serum nutrient levels, liver, and renal function*

Serum albumin level significantly decreased as a result of the lemon detox intervention. Serum total protein concentration was reduced in the Lemon-D and Positive-C groups, and vitamin C levels were significantly decreased in the Lemon-D and Normal-C groups. However, there were no statistically significant differences in the changes of serum total protein, albumin, and vitamin C levels between the 3 groups (Table 6).

Significant decreases on serum GOT and GPT levels were not observed in Lemon-D group. The total bilirubin concentration significantly increased in the Normal-C group and showed a tendency for a decrease in the Lemon-D and Positive-C groups. BUN levels significantly decreased in all 3 groups, and serum creatinine levels increased in the Lemon-D group only. Lastly, serum uric acid levels significantly increased in the Positive-C group (Table 6).

### 3.4. *Serum lipid profiles*

Serum total cholesterol, HDL cholesterol, and LDL cholesterol levels were significantly reduced in the Lemon-D and Positive-C groups, whereas serum triglyceride levels significantly decreased only in the Lemon-D group. Changes in serum total cholesterol and HDL cholesterol levels were significantly greater in the Lemon-D and Positive-C groups compared with the Normal-C group, and the decrease in LDL cholesterol levels was significantly greater in the Positive-C group compared with the Normal-C group. Serum free fatty acid levels significantly increased in all 3 groups (Table 7).

### 3.5. *Serum glucose, insulin, and insulin resistance levels*

Serum glucose levels significantly decreased in the Positive-C group. However, no significant changes were observed in the Normal-C or Lemon-D groups. We found a more significant change in glucose levels in the Positive-C compared to the Normal-C group. However, $HbA_{1c}$ and serum insulin levels were significantly reduced in the Lemon-D and Positive-C

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**Table 4 – Changes in anthropometric measurements of subjects by groups and the total for all subjects**

| Group | | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) |
|---|---|---|---|---|---|
| Height (cm) | | 161.6 ± 4.7 | 161.7 ± 5.3 | 163.2 ± 5.9 | 162.2 ± 5.3 |
| Weight (kg) | Pre | 69.5 ± 9.3*** | 67.7 ± 8.9*** | 67.9 ± 7.2* | 68.3 ± 8.4*** |
| | Post | 66.9 ± 9.0 | 65.1 ± 8.7 | 67.3 ± 7.5 | 66.4 ± 8.4 |
| | Change | −2.6 ± 1.2[b] | −2.6 ± 1.6[b] | −0.6 ± 1.3[a] | −1.9 ± 1.7 |
| BMI (kg/m$^2$) | Pre | 26.6 ± 3.0*** | 25.9 ± 2.8*** | 25.5 ± 2.3* | 25.9 ± 2.7*** |
| | Post | 25.6 ± 2.9 | 24.9 ± 2.8 | 25.2 ± 2.4 | 25.2 ± 2.7 |
| | Change | −1.0 ± 0.5[b] | −1.0 ± 0.6[b] | −0.2 ± 0.5[a] | −0.7 ± 0.6 |
| Skeletal muscle (kg) | Pre | 23.27 ± 2.65* | 22.87 ± 2.47 | 23.35 ± 2.65** | 23.16 ± 2.57 |
| | Post | 22.99 ± 2.52 | 22.64 ± 2.10 | 23.67 ± 2.69 | 23.12 ± 2.47 |
| | Change | −0.28 ± 0.55[b] | −0.23 ± 0.79[b] | 0.32 ± 0.62[a] | −0.05 ± 0.71 |
| Total body fat (kg) | Pre | 26.78 ± 6.08*** | 25.64 ± 5.83*** | 25.01 ± 5.20*** | 25.77 ± 5.68*** |
| | Post | 24.65 ± 6.05 | 23.43 ± 6.32 | 23.96 ± 5.31 | 24.00 ± 5.84 |
| | Change | −2.13 ± 0.86[b] | −2.21 ± 1.20[b] | −1.05 ± 1.03[a] | −1.77 ± 1.16 |
| Body fat percentage (%) | Pre | 38.17 ± 4.42*** | 37.56 ± 3.74*** | 36.50 ± 5.39** | 37.37 ± 4.59*** |
| | Post | 36.45 ± 4.74 | 35.56 ± 4.49 | 35.37 ± 5.11 | 35.77 ± 4.76 |
| | Change | −1.73 ± 1.11[ab] | −2.00 ± 1.58[b] | −1.13 ± 1.62[a] | −1.60 ± 1.50 |
| Waist-hip ratio | Pre | 0.844 ± 0.036*** | 0.835 ± 0.034*** | 0.833 ± 0.039 | 0.837 ± 0.036*** |
| | Post | 0.837 ± 0.037 | 0.826 ± 0.036 | 0.830 ± 0.043 | 0.831 ± 0.038 |
| | Change | −0.007 ± 0.007[ab] | −0.009 ± 0.010[b] | −0.003 ± 0.010[a] | −0.006 ± 0.009 |
| Waist circumference (cm) | Pre | 84.4 ± 7.5* | 82.4 ± 8.2*** | 83.6 ± 6.9*** | 83.4 ± 7.5*** |
| | Post | 82.8 ± 9.1 | 79.0 ± 9.0 | 81.5 ± 6.6 | 81.1 ± 8.3 |
| | Change | −1.6 ± 3.2 | −3.3 ± 3.7 | −2.0 ± 2.9 | −2.3 ± 3.3 |
| Systolic blood pressure (mmHg) | Pre | 117.4 ± 11.2*** | 116.1 ± 9.5*** | 113.5 ± 10.7** | 115.5 ± 10.5*** |
| | Post | 110.6 ± 11.1 | 109.4 ± 7.4 | 109.4 ± 8.3 | 109.8 ± 8.9 |
| | Change | −6.7 ± 7.3 | −6.7 ± 7.6 | −4.1 ± 7.1 | −5.8 ± 7.4 |
| Diastolic blood pressure (mmHg) | Pre | 72.7 ± 10.5** | 72.9 ± 8.7*** | 71.1 ± 8.8* | 72.2 ± 9.2*** |
| | Post | 68.1 ± 9.3 | 67.9 ± 7.4 | 68.2 ± 8.0 | 68.1 ± 8.1 |
| | Change | −4.6 ± 7.0 | −5.0 ± 6.0 | −2.9 ± 6.7 | −4.1 ± 6.6 |

Values are means ± SD.
*, **, ***: Significantly different at $P < .05$, $P < .01$, and $P < .001$, respectively, between pre and post by paired $t$ test.
[a,b,c]: Means with different superscript letter are significantly different at $P < .05$ among 3 intervention groups by ANOVA and Duncan's multiple range test.

groups. No significant differences were observed for changes in $HbA_{1c}$ and serum insulin levels between the 3 groups. The HOMA-IR score showed a significant decrease in the Lemon-D and Positive-C groups after intervention trials (Fig. 2).

### 3.6. *Adipokines and inflammatory markers*

Serum leptin level significantly decreased in the Lemon-D and Positive-C groups, while no significant change was seen in the Normal-C group. No significant difference between the Lemon-D and Positive-C groups was found in the changes of serum leptin (Fig. 3).

Serum IGF-1 and adiponectin levels were significantly reduced in the Lemon-D and Positive-C groups. Serum homocysteine levels increased significantly in the Lemon-D and Positive-C groups, while they significantly decreased in the Normal-C group. Serum hs-CRP levels only exhibited a statistically significant decrease in the Lemon-D group after intervention trials (Fig. 3).

**Table 5 – DEXA determinations of body fat contents of subjects pre and post intervention**

| Group | | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) |
|---|---|---|---|---|---|
| Trunk fat (kg) | Pre | 15.58 ± 3.69*** | 14.60 ± 3.39*** | 14.43 ± 3.23 | 14.84 ± 3.43*** |
| | Post | 14.54 ± 3.80 | 13.48 ± 3.03 | 14.52 ± 3.19 | 14.18 ± 3.34 |
| | Change | −1.05 ± 0.89[b] | −1.11 ± 0.81[b] | 0.10 ± 0.69[a] | −0.66 ± 0.97 |
| Total fat (kg) | Pre | 27.28 ± 6.66*** | 27.27 ± 5.24*** | 26.71 ± 5.65 | 27.07 ± 5.79*** |
| | Post | 25.75 ± 6.86 | 25.54 ± 5.45 | 26.75 ± 5.39 | 26.04 ± 5.86 |
| | Change | −1.53 ± 1.19[b] | −1.73 ± 0.85[b] | 0.04 ± 0.86[a] | −1.03 ± 1.26 |
| Trunk fat/total fat ratio | Pre | 0.549 ± 0.038** | 0.534 ± 0.45 | 0.540 ± 0.032 | 0.541 ± 0.039* |
| | Post | 0.541 ± 0.038 | 0.529 ± 0.041 | 0.542 ± 0.035 | 0.537 ± 0.038 |
| | Change | −0.008 ± 0.014[b] | −0.005 ± 0.019[ab] | 0.002 ± 0.016[a] | −0.004 ± 0.017 |

Trunk/Total ratio, ratio of trunk fat to total fat.
Values are means ± SD.
*, **, ***: Significantly different at $p<0.05$, $p<0.01$, and $p < 0.001$, respectively, between pre and post by paired $t$ test.
[a,b,c]: Means with different superscript letter are significantly different at $p < 0.05$ among 3 intervention groups by ANOVA and Duncan's multiple range test.

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**Table 6 – Serum measurements from subjects at pre and post intervention**

| Group | | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) |
|---|---|---|---|---|---|
| Total protein (g/dL) | Pre | 8.26 ± 0.45* | 8.28 ± 0.44* | 8.21 ± 0.62 | 8.25 ± 0.51** |
| | Post | 8.02 ± 0.38 | 8.05 ± 0.38 | 8.08 ± 0.33 | 8.01 ± 0.36 |
| | Change | −0.24 ± 0.57 | −0.23 ± 0.48 | −0.12 ± 0.64 | −0.20 ± 0.57 |
| Albumin (g/dL) | Pre | 4.66 ± 0.29* | 4.53 ± 0.24 | 4.56 ± 0.28 | 4.58 ± 0.27** |
| | Post | 4.53 ± 0.26 | 4.44 ± 0.20 | 4.48 ± 0.17 | 4.48 ± 0.21 |
| | Change | −0.013 ± 0.30 | −0.09 ± 0.27 | −0.07 ± 0.31 | −0.09 ± 0.29 |
| Vitamin C (μmol/L) | Pre | 90.86 ± 20.55*** | 87.53 ± 27.18 | 93.29 ± 37.79** | 90.62 ± 29.53*** |
| | Post | 68.40 ± 23.91 | 75.62 ± 20.23 | 70.44 ± 26.21 | 71.54 ± 23.55 |
| | Change | −22.46 ± 20.96 | −11.91 ± 36.03 | −22.85 ± 33.6 | −19.08 ± 31.23 |
| GOT (U/L) | Pre | 20.16 ± 8.15 | 23.92 ± 26.71 | 22.35 ± 6.34* | 22.20 ± 16.38* |
| | Post | 18.26 ± 5.49 | 17.54 ± 3.16 | 19.77 ± 4.64 | 18.56 ± 4.56 |
| | Change | −1.90 ± 5.03 | −6.38 ± 26.06 | −2.58 ± 5.72 | −3.64 ± 15.61 |
| GPT (U/L) | Pre | 17.66 ± 12.81 | 14.67 ± 8.06* | 19.44 ± 16.01* | 17.30 ± 12.81*** |
| | Post | 14.55 ± 8.01 | 11.08 ± 5.60 | 14.64 ± 11.43 | 13.43 ± 8.83 |
| | Change | −3.16 ± 7.93 | −3.59 ± 6.84 | −4.76 ± 11.42 | −3.87 ± 8.96 |
| Total bilirubin (mg/dL) | Pre | 0.54 ± 0.21 | 0.56 ± 0.20 | 0.51 ± 0.23* | 0.53 ± 0.21 |
| | Post | 0.50 ± 0.17 | 0.48 ± 0.18 | 0.57 ± 0.21 | 0.52 ± 0.19 |
| | Change | −0.04 ± 0.14[b] | −0.08 ± 0.22[b] | 0.07 ± 0.17[a] | −0.02 ± 0.19 |
| BUN (mg/dL) | Pre | 9.71 ± 2.68*** | 8.53 ± 3.78** | 9.07 ± 3.94*** | 9.09 ± 3.53*** |
| | Post | 3.68 ± 0.59 | 4.76 ± 5.42 | 4.16 ± 0.75 | 4.22 ± 3.17 |
| | Change | −6.02 ± 2.60 | −3.77 ± 6.36 | −4.90 ± 3.75 | −4.87 ± 4.58 |
| Creatinine (mg/dL) | Pre | 0.729 ± 0.068* | 0.743 ± 0.081 | 0.758 ± 0.076 | 0.744 ± 0.075 |
| | Post | 0.769 ± 0.068 | 0.757 ± 0.084 | 0.757 ± 0.097 | 0.761 ± 0.084 |
| | Change | 0.040 ± 0.074 | 0.014 ± 0.089 | −0.002 ± 0.178 | 0.017 ± 0.93 |
| Uric acid (mg/dL) | Pre | 4.68 ± 0.77 | 4.43 ± 0.82*** | 4.78 ± 1.00 | 4.63 ± 0.88*** |
| | Post | 4.97 ± 0.96 | 5.10 ± 1.20 | 4.83 ± 1.04 | 4.96 ± 1.07 |
| | Change | 0.29 ± 0.77[ab] | 0.67 ± 0.89[a] | 0.06 ± 0.71[b] | 0.33 ± 0.81 |

Values are means ± SD.
*, **, ***: Significantly different at $P < .05$, $P < .01$, and $P < .001$, respectively, between pre and post by paired $t$ test.
[a,b,c]: Means with different superscript letter are significantly different at $P < .05$ among 3 intervention groups by ANOVA and Duncan's multiple range test.

### 3.7. *Hematological changes*

RBC, Hb, and Hct levels did not significantly change in the Lemon-D group, but a statistically significant decrease was seen in the Positive-C group. Significant decreases in Hb and Hct levels were also found in the Normal-C. WBC level significantly decreased only in Lemon-D group. No significant differences in the changes of the hematological indices were observed in the 3 groups (Table 8).

**Table 7 – Serum lipid measurements of subjects at pre and post intervention**

| Group | | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) |
|---|---|---|---|---|---|
| Triglyceride (mg/dL) | Pre | 96.04 ± 35.05** | 77.84 ± 33.84 | 106.67 ± 82.27 | 93.77 ± 57.05** |
| | Post | 78.23 ± 20.02 | 68.32 ± 32.65 | 93.83 ± 61.39 | 80.50 ± 43.59 |
| | Change | −17.81 ± 28.01 | −9.52 ± 37.50 | −12.83 ± 44.41 | −13.27 ± 37.34 |
| Cholesterol (mg/dL) | Pre | 184.29 ± 21.96*** | 184.43 ± 28.28*** | 184.88 ± 36.30 | 184.55 ± 29.43*** |
| | Post | 167.04 ± 26.32 | 159.46 ± 25.51 | 179.63 ± 35.18 | 169.01 ± 30.42 |
| | Change | −17.25 ± 22.00[b] | −24.96 ± 19.29[b] | −5.25 ± 20.92[a] | −15.54 ± 22.11 |
| HDL-cholesterol (mg/dL) | Pre | 57.47 ± 11.45*** | 62.35 ± 11.47*** | 59.90 ± 11.76 | 59.96 ± 11.60*** |
| | Post | 49.77 ± 10.43 | 56.07 ± 11.37 | 60.29 ± 11.45 | 55.62 ± 11.80 |
| | Change | −7.70 ± 9.17[b] | −6.28 ± 5.81[b] | 0.39 ± 7.91[a] | −4.34 ± 8.43 |
| LDL-cholesterol (mg/dL) | Pre | 92.42 ± 13.50* | 88.82 ± 16.89*** | 88.08 ± 21.99 | 89.67 ± 17.89*** |
| | Post | 86.42 ± 16.80 | 76.89 ± 16.78 | 87.67 ± 21.42 | 83.69 ± 18.99 |
| | Change | −6.00 ± 11.92[ab] | −11.93 ± 12.28[b] | −0.42 ± 14.10[a] | −5.98 ± 13.58 |
| Free fatty acid (mg/dL) | Pre | 644.81 ± 227.13** | 633.21 ± 216.15*** | 565.07 ± 182.68** | 612.46 ± 208.99*** |
| | Post | 910.38 ± 441.85 | 920.14 ± 400.28 | 836.63 ± 337.85 | 887.30 ± 390.21 |
| | Change | 265.58 ± 435.07 | 286.93 ± 381.72 | 271.57 ± 394.49 | 274.83 ± 398.58 |

AI, atherogenic index.
Values are means ± SD.
*, **, ***: Significantly different at $P < .05$, $P < .01$, and $P < .001$, respectively, between pre and post by paired $t$ test.
[a,b,c]: Means with different superscript letter are significantly different at $P < .05$ among 3 intervention groups by ANOVA and Duncan's multiple range test.

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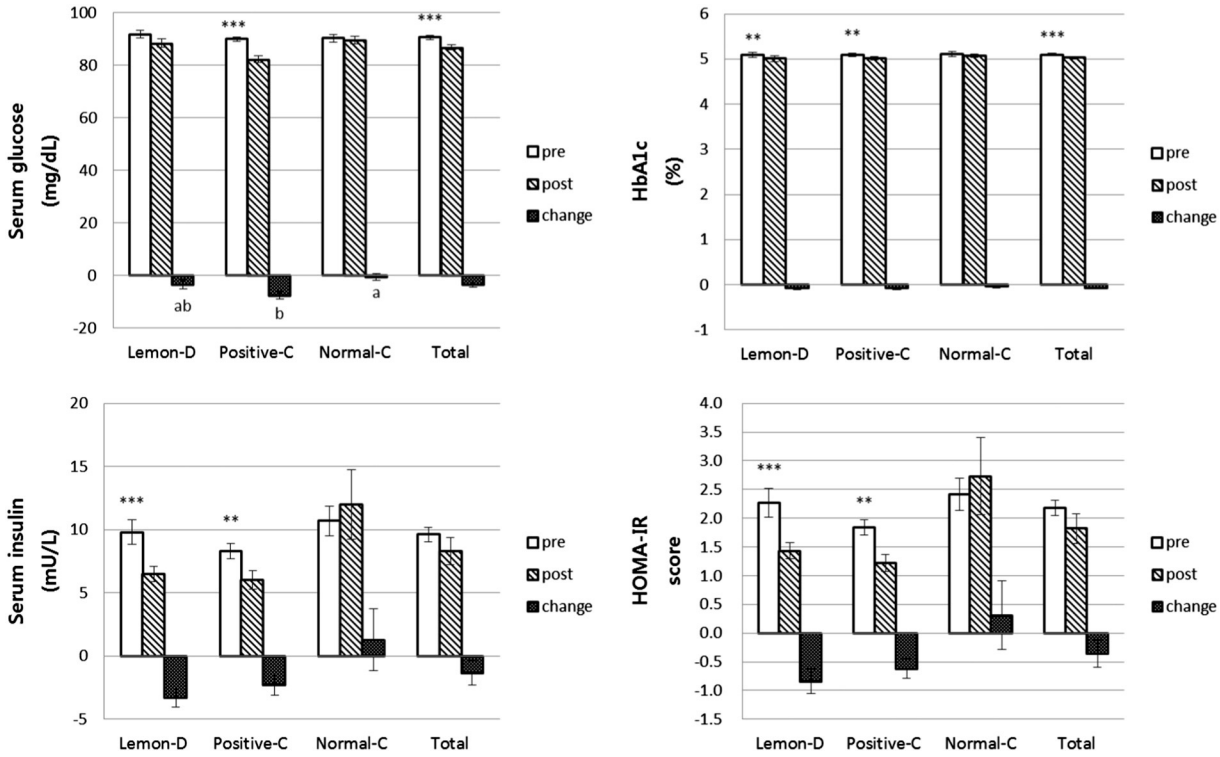


**Fig. 2 – Serum glucose, $HbA_{1c}$, insulin and HOMA-IR scores of subjects at pre and post intervention.**

## 4. Discussion

This study was conducted to prove that the lemon detox program would reduce body weight, body fat mass, insulin resistance, and cardiovascular disease risk factors. Furthermore, we tried to confirm the safety of lemon detox program for liver and renal functions and hematological indices.

In this study, serum total protein, albumin (indicators for protein nutritional status), and BUN levels significantly decreased, and serum creatinine levels significantly increased in the Lemon-D group. However, we observed similar changes in the Positive-C group, and no significant differences in the changes of these indicators between the Lemon-D and Positive-C groups. These results suggest that serum levels of nutrients and biomarkers for renal function might be altered by caloric restriction. In addition, the mean values for systolic and diastolic blood pressure, blood glucose levels, serum lipid composition, and blood biochemical indices related to liver and renal function were within the normal range both before and after the lemon detox intervention. There was no significant difference between the clinical intervention groups. Based on these observations, we suggest that the lemon detox program does not cause damage in body functions.

Most clinical studies of calorie-restricted diets with 9- to-20 week intervention periods have reported that energy consumption of less than 1,000 kcal/d causes a reduction in body weight, BMI, abdominal visceral fat, and intermuscular fat [26,27]. Although our intervention period was shorter than these studies, significant decreases in body weight, BMI, body fat percentage, WHR, and waist circumference were observed in all experimental groups. In our current study, there were no significant changes in total and trunk fat masses and the ratio of trunk fat to total fat as a result of the intervention in the Normal-C group. However, total and trunk fat masses significantly decreased through caloric restriction in the Lemon-D and Positive-C groups. Since we found no significant differences on them between the Lemon-D and Positive-C groups, we assumed that the effects of the lemon detox program on body weight and body fat reduction might be the result of caloric restriction.

Furthermore, we found that the ratio of trunk fat to total fat only in the Lemon-D group decreased significantly, while the other groups did not show any changes. This result indicates that trunk fat was reduced more significantly than total fat in the Lemon-D group. Several human studies have reported that visceral fat accumulation is a common and major risk factor for coronary artery disease. A reduction in intra-abdominal visceral fat has been shown to be effective for improving coronary risk factors [28]. Moreover, abdominal obesity is considered as the most prevalent risk factor for atherogenic dyslipidemia [29]. Therefore, a considerable decrease in trunk fat might cause a protective effect against chronic diseases such as atherogenesis and coronary heart disease.

Caloric restriction has been shown to alter adipokine (eg, adiponectin and leptin) production from adipocytes [30] and improve insulin sensitivity [27]. Kmiec et al showed that serum leptin, insulin, and glucose levels significantly decreased after 48 hours of fasting [30]. Lee et al reported that an LCD of 1,200 kcal/d for 12 weeks decreased serum levels of glucose, insulin, and leptin, and HOMA-IR scores [31]. In our study, we also observed 3.7%, 33.7%, 37.3%, and 37.0% decreases in serum glucose, insulin, leptin, and HOMA-IR levels, respectively, caused by the lemon detox program. We

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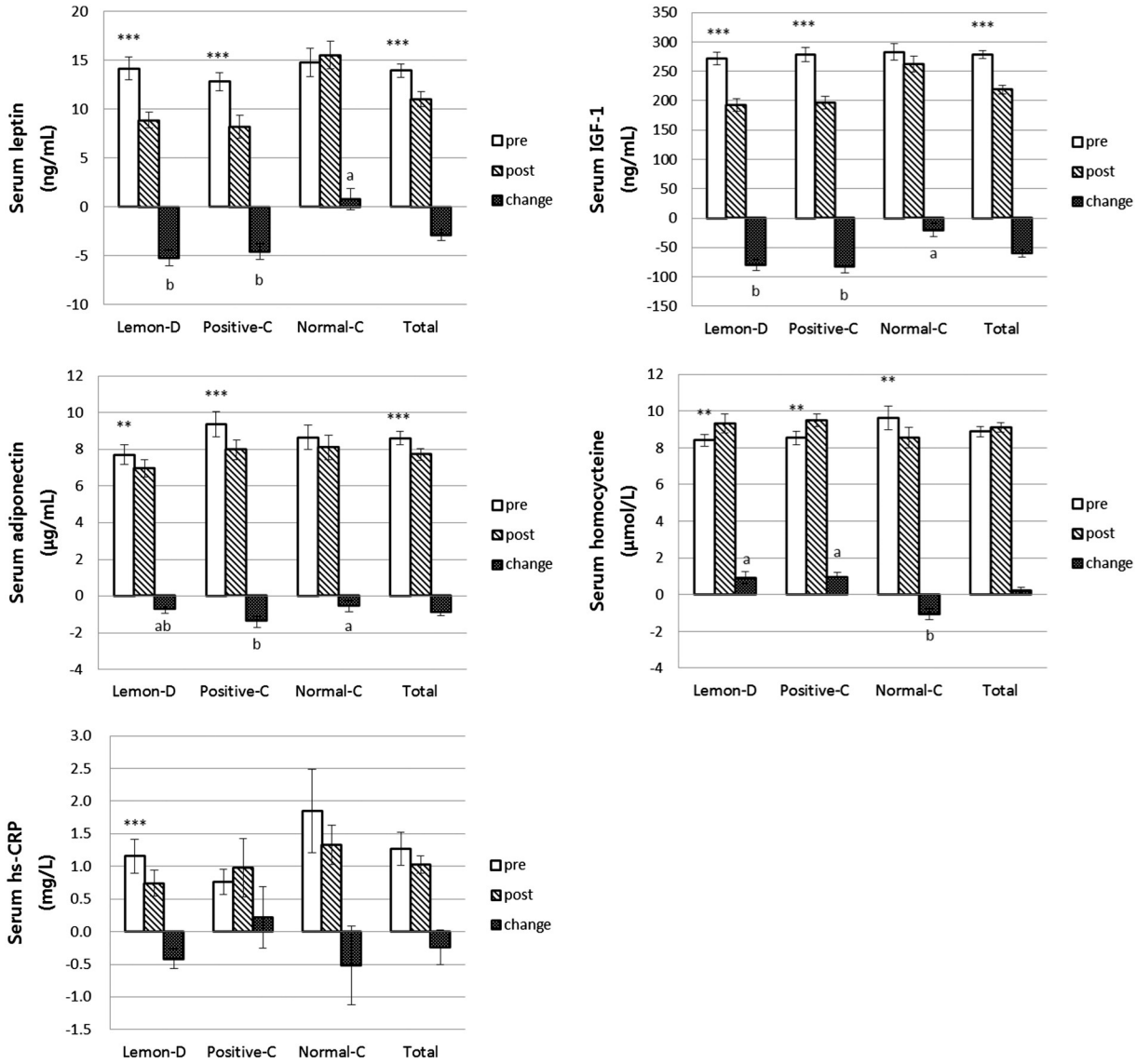


**Fig. 3 – Serum adipokines and inflammatory markers related to cardiovascular disorder of subjects at pre and post intervention.**

**Table 8 – Hematological indices of subjects at pre and post intervention**

| Group | | Lemon-D (n = 26) | Positive-C (n = 28) | Normal-C (n = 30) | Total (n = 84) |
|---|---|---|---|---|---|
| WBC ($10^3$ cells/μL) | Pre | 6.43 ± 2.18* | 6.23 ± 1.59 | 7.14 ± 1.87 | 6.61 ± 1.91* |
| | Post | 5.55 ± 1.86 | 5.65 ± 1.43 | 6.85 ± 1.86 | 6.05 ± 1.81 |
| | Change | −0.78 ± 1.68 | −0.58 ± 1.85 | −0.29 ± 2.41 | −0.57 ± 2.01 |
| RBC ($10^6$ cells/μL) | Pre | 4.46 ± 0.40 | 4.40 ± 0.30** | 4.45 ± 0.27 | 4.44 ± 0.32** |
| | Post | 4.36 ± 0.34 | 4.28 ± 0.27 | 4.41 ± 0.28 | 4.35 ± 0.30 |
| | Change | −0.10 ± 0.36 | −0.12 ± 0.21 | −0.05 ± 0.27 | −0.09 ± 0.28 |
| Hb (g/dL) | Pre | 13.11 ± 1.19 | 12.84 ± 1.04* | 13.08 ± 0.69** | 13.01 ± 0.98*** |
| | Post | 12.80 ± 0.87 | 12.54 ± 0.96 | 12.77 ± 0.76 | 12.70 ± 0.86 |
| | Change | −0.31 ± 0.89 | −0.31 ± 0.65 | −0.31 ± 0.58 | −0.31 ± 0.70 |
| Hct (%) | Pre | 38.85 ± 3.06 | 38.32 ± 2.66** | 38.89 ± 1.88* | 38.69 ± 2.54*** |
| | Post | 37.97 ± 2.39 | 37.30 ± 2.55 | 38.13 ± 2.00 | 37.80 ± 2.32 |
| | Change | −0.88 ± 2.69 | −1.02 ± 1.58 | −0.76 ± 1.80 | −0.89 ± 2.03 |

Abbreviation: WBC, white blood cell.
Values are means ± SD.
*, **, ***: Significantly different at $P < .05$, $P < .01$, and $P < .001$, respectively, between pre and post by paired $t$ test.
[a,b,c]: Means with different superscript letter are significantly different at $P < .05$ among 3 intervention groups by ANOVA and Duncan's multiple range test.

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found a similar reduction in those indicators in the Positive-C group. Leptin is an adipokine known to induce insulin resistance in humans [23]. Thus, our findings support the hypothesis that the lemon detox program might have a beneficial effect on insulin sensitivity by reducing circulating leptin levels and insulin resistance (detected as HOMA-IR) despite the short-term intervention period. In addition, serum IGF-1 levels, an important regulator of β-cell function, which shares signaling pathways with insulin [32], significantly decreased in all calorie-restricted groups in our study. According to a study of Kaaks et al, IGF-1 is activated by insulin, it has been suggested that prolonged fasting decreases circulating IGF-1 levels by increasing growth hormone resistance [33]. Anson et al suggested that caloric restriction can influence serum IGF-1 levels through altering the growth hormone-IGF-1 axis and the insulin signaling pathway [15]. As the IGF-1 pathway is known to be down-regulated by nutritional interventions including diet restriction [34], our results indicate that the caloric restriction of the lemon detox and placebo juice diets can reduce serum IGF-1 levels through reducing circulating insulin levels. This is in line with the findings of Kaaks et al [33].

Obesity can cause cardiovascular diseases such as atherosclerosis by causing an increased inflammatory response including enhanced tumor-necrosis factor-α expression, increased leptin, and decreased adiponectin production from adipocytes [35,36]. It is well known that high blood pressure, elevated homocysteine, C-reactive protein, and LDL cholesterol levels are risk factors for cardiovascular disease [37]. Fasting for 48 hours can depress leptin and adiponectin mRNA levels by 30% and 40%, respectively [38]. Moreover, caloric restriction can lower blood pressure by decreasing plasma norepinephrine and catecholamine levels [39], and long-term caloric restrictions (>12 weeks) have been shown to reduce serum hs-CRP levels [40]. We also observed decreased blood pressure, serum leptin, adiponectin, and hs-CRP levels in women receiving the lemon detox intervention, although our intervention was a short-term one. Serum hs-CRP levels in the Positive-C group were not significantly altered but showed a slight trend for an increase. Hs-CRP, an inflammation marker, is a strong predictor for cardiovascular diseases such as myocardial infarction [41]. Increased hs-CRP levels have been shown to be associated with a higher BMI and might enhance cardiovascular risk [42]. However, we could not observe any decrease of hs-CRP in Positive-C group that was also calorie-restricted group. Therefore, decreased hs-CRP level by the lemon detox program might have a beneficial effect against cardiovascular diseases regardless of calorie restriction.

Higher homocysteine levels are also a risk factor for myocardial infarction [43]. Gallistl et al reported that serum homocysteine levels significantly increased after short-term caloric restriction and were associated with decreased plasma folate levels [44]. However, Henning et al suggested that a rise in serum homocysteine levels during weight loss was inhibited by adequate oral intake of vitamins [45]. Our result also showed increased serum homocysteine levels in all calorie-restricted groups, but we did not find a protective effect of vitamins in the lemon detox group (whose participants were expected to consume more vitamins by drinking lemon juice). Unexpectedly, serum vitamin C levels decreased as a result of the lemon detox program in our current study. Vitamin C contents in citrus juices are affected by temperature, maturity, processing factors, packaging, and storage time [46,47]. Our findings regarding the decreased vitamin C levels in the Lemon-D group might be due to using a processed product of lemon juice.

We failed to find an improving effect of the lemon detox intervention on the serum lipid profile. Since strict caloric restriction affects not only caloric intake but also various types of food and nutrient intakes, HDL cholesterol levels might decrease after the intervention. However, serum triglyceride, total cholesterol, and LDL cholesterol levels decreased in women receiving a calorie-restricted diet.

The manufacturer of the Neera syrup reported that the syrup used in the Lemon-D diet had higher mineral contents (20.3 mg magnesium, 390 mg potassium, 1.2 mg manganese, 2.69 mg zinc, and 4.05 mg ferritin per 100 g) than the maple syrup used in the Positive-C group. We also observed significant decreases in Hb and Hct levels in the Positive-C and Normal-C groups, but no such changes were found in the Lemon-D group. A 25% reduction in food intake was reported to decrease Hct, Hb, and mean corpuscular volume levels in dogs [48]. Even though we did not present in this result, the average energy intakes per day (during the 11-day intervention) were 771.5, 771.3, and 1287.2 kcal in the Lemon-D, Positive-C, and Normal-C groups, respectively. Notably, the calorie intake of the Normal-C group during the experimental period was 39% lower than that before the clinical trial. Hence, decreased Hb and Hct levels in the Positive-C and Normal-G groups were likely caused by the 61.3% and 39.1% restrictions in caloric intakes. However, the Lemon-D group showed no changes in the hematological indices, although the women's level of dietary restriction was the same as in the Positive-C group. These results support our assumption that the lemon detox diet offers a higher mineral intake and thus does not disrupt blood mineral maintenance during the short-term intervention despite the calorie restriction. Moreover, WBC level can routinely indicate total circulating lymphocyte count which is an inflammatory marker. Its level is positively correlated with BMI, insulin resistance, and inflammatory adipokines related to cardiovascular diseases [49]. Since weight loss and reduced BMI caused a decrease in WBC level [49], the lemon detox intervention might reduce WBC level by weight loss. Noticeably, we observed a significant decrease of WBC only in the Lemon-D group whereas no change was detected in the Positive-C group. Therefore, we assumed that the lemon detox intervention might be more effective for reducing the risk factors of cardiovascular disease by decreasing circulating lymphocytes than other VCLDs.

Overall, our results suggest that the lemon detox program might have several beneficial effects such as body fat reduction and improving insulin resistance through caloric restriction. In addition, it might have a potential beneficial effect on risk factors for cardiovascular disease related to the reduction of circulating hs-CRP reduction and changes in adipokine production. Since the intervention period of our study was short (only 11 days) and did not cause hematological damage, the lemon detox program might be safe and easily acceptable. However, the effect of the lemon detox diet

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on cardiovascular disease is still unclear because of the observed unexpected changes in serum lipid profiles and homocysteine levels.

The limitation of our study was that we could not completely control the participants' diet and physical activity. In addition, the Normal-C group which was supposed to maintain normal and regular diet showed a caloric intake reduction of 39%. We could not serve fresh lemon juice in the Lemon-D group. Therefore, more elaborate studies should be warranted. In addition, further research is needed to elucidate the exact mechanism of reduction in circulating hs-CRP by the lemon detox program.

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