

Effect of Caffeine Co-Ingested with Carnitine on Weight, Body-Fat Percent, Serum Leptin and Lipid Profile Changes in Male Teen Soccer Players: a Randomized Clinical Trial

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Abstract

Background: Weight loss and decreasing the Body fat percentage (BF%) is motivated to optimize performance. In order to achieve these, many supplements are used by athletes, however the possible negative or synergic effects have not been fully described in the literature, specifically in humans. The present study was conducted to investigate the co-administration effects of two common used supplements in body weight and BF% management to recommend athletes for safe weight and BF% reduction.

Materials and Methods: In the present double-blind, randomized, parallel, placebo-controlled study, the effect of six-week co-administration of caffeine and carnitine was determined on changes in body weight (BW), BF%, serum leptin concentration and lipid profile (triglyceride, HDL Cholesterol, LDL Cholesterol and Total Cholesterol), fasting blood glucose (FBG), and free fatty acid (FFA) changes. Twenty eight male teen soccer players from Ahvaz-Iran, were divided in three groups (group CafPlc, caffeine (6 mg/kg/day) + dextrose; group CafCar, caffeine (6 mg/kg/day) + carnitine (2g); and group Plc, dextrose).

Results: Caffeine-carnitine had a lowering effect on BW ($P=0.02$) and BF% ($P=0.03$), compared to caffeine alone and placebo in male teen soccer players (mean age of 16.92 ± 0.76 years). TG was significantly decreased in CafCar ($P=0.04$). FFA levels were increased in CafCar ($P=0.04$) and there was significant differences between CafCar and Plc groups ($P=0.01$). FBG was increased in both CafPlc and CafCar ($P=0.01$ and $P=0.02$, respectively), with no significant differences between groups.

Conclusion: The synergistic effect of caffeine-carnitine might be suggested to decrease the BF% and BW, besides it may prevent the increment of FFA levels; however it should be prescribed cautiously since it increased FBG levels.

Key Words: Adolescents, Aggression, Children, Life Satisfaction, Self-rated Health.

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1- INTRODUCTION

Weight loss and decreasing the body fat percent (BFP) in athletes is generally motivated by a desire to optimize performance by improving power-to-weight ratio, making or losing weight to compete in a certain weight category (1). Unsafe weight management practices can compromise athletic performance and negatively affect health. Athletes and clients often attempt to lose weight by not eating, limiting caloric or specific nutrients from the diet and specifically by using nutritional supplements. It is estimated that 89% of college athletes have used or currently use nutritional supplements to enhance their performance (2-4). Agents for weight management, including caffeine, ephedrine, and green tea have been proposed as strategies for weight loss and weight maintenance, since they may increase energy expenditure (EE) counteract the decrease in metabolic rate that is present during weight loss (5-8).

Since caffeine has been removed from the list of prohibited substances by the World Anti-Doping agency in 2004 (9), it is used widely as an ergogenic aid and a general stimulant and a fatty acid mobilizer from adipose tissues (9, 10). It has been hypothesized that fat-mobilizing effects of caffeine and other methylxanthines elevate blood free fatty acid (FFA) concentration and spare carbohydrate stores (10, 11). Caffeine also possesses thermogenic effects and can stimulate fat oxidation in vitro and in humans, in part via sympathetic activation of the central nervous system (12-14). The thermogenic response may be limited by activation of cyclic adenosine monophosphate (cAMP) or intracellular feedback inhibition by phosphodiesterase enzymes (5, 15). However caffeine has documented potential side effects at high doses such as gastrointestinal distress, dizziness, anxiety, irritability and an inability to focus (16, 17). Carnitine is a facilitator of fatty acid oxidation by virtue

of its role in interorganelle translocation of fatty acids (10, 18). It is previously mentioned that non-essential fatty acids (NEFAs) acutely cause systemic and muscle insulin resistance by enhancing muscle oxidative stress through mitochondrial reactive oxygen species (ROS) generation and nuclear factor kappa-B (NFκB) activation. It might be possible that caffeine supplementation alone may cause some negative effects via increment of FFA levels in blood (19) and increase the risk of atherosclerotic coronary artery disease (CAD) (20); therefore, it would be worth paying attention to use of the heart health related supplements with caution.

Leptin, a protein released from adipose tissue, is thought to play a role in the regulation of body weight, EE and food intake (21). Many factors affect the leptin levels in blood such as fasting and energy restriction (22). The increased circulating leptin has been implicated as a marker of leptin resistance (23). Yamashita presented that as increased leptin levels may have effects on tissues and organs that remain sensitive to high leptin concentrations, the decrease of leptin due to coffee consumption may attenuate such effects. However, The exact mechanism is not entirely clear (24).

According to National Athletic Trainers' Association (NATA) recommendation, to manage body composition and maintain good health a regular exercise program should be combined with a dietary plan and safe supplements (25). This study is in line with our previous research on the effects of supplementation and exercise on changes in serum markers and performance in athletes (26-29).

Langin (30) suggested utilizing molecules that stimulate lipolysis and entrance of fatty acid into mitochondria and oxidation of the released fatty acids might be a good approach to decreasing fat stores. On this basis, the combined effects of caffeine,

stimulants for lipolysis, and carnitine, stimulants for fatty acid entrance into mitochondria, were investigated in present study. We rationalized that supplementation of caffeine with carnitine, beside its positive effects on performance would prevent the possible complications of caffeine alone.

To our knowledge there is no study investigating the combined effect of caffeine and carnitine to make weight-loss interventions as effective as possible, with no side effect on cardiovascular status and performance. Therefore, we combined caffeine supplementation with carnitine to investigate the alleviation of possible negative consequences on lipid profile.

2- MATERIALS AND METHODS

2-1. Study design and intervention

Using a double-blind, randomized, parallel, placebo-controlled protocol, male teen subjects (mean age of 16.92 ± 0.76 years old, height: 1.73 ± 0.06 cm) were assigned to 1 of 3 groups:

- Caffeine (6 mg/kg/day) + dextrose (CafPlc; n=10) group;
- Caffeine (6 mg/kg/day)+carnitine (2g) (CafCar; n=10) group; and
- Dextrose (Plc; n=8) group.

Since six weeks is approximately the period required to achieve a steady state in response to exercise, the participants undergo 6-week supplementation and exercise programme (31). Each participant take four capsules, identical in appearance, on training days. A package containing capsules and instructions to use was given to subjects weekly. Capsules given for each group were as follow:

- CafPlc: 1 capsule containing caffeine + 3 capsules containing dextrose,
- CafCar: 1 capsule containing caffeine + 3 capsules containing 666.66 mg carnitine,

- Plc: 4 capsules containing dextrose.

Subjects were included if, they to train 3 times per week since two years ago. Participants signed a consent form explaining the procedures, which had been approved by the ethical committee of Ahvaz Jundishapur University of Medical Sciences. The study was registered in Iranian registry of clinical trials (IRCT registration number: IRCT2014100619411N1).

Participants were randomly assigned to 3 groups in the random blocks (the size of block was 6), on the blocked randomization method. The sequence of permuted blocks was generated with a computer random number generator. Stratification has not been done.

An investigator with no clinical involvement in the trial weighed the caffeine, carnitine and dextrose precisely, filled the capsules and packed the supplements in numbered pockets based on the random list. The other person, who was not involved in the trial and not aware of random sequences, assigned the subjects to the numbered pockets. At the end of the study, participants were asked about the possible adverse effects of supplementation, but none of the volunteers report any adverse effect.

2-2. Participants

Thirty-one male teen soccer players (aged 16-18 years old) without any cardiovascular or muscular disease were eligible to participate in the study. The flow diagram of the progress is shown in **Figure.1**. Exclusion criteria included history of thyroid or heart disease, hypertension, smoking, drinking and current use of a restricted-calorie or vegetarian diet or any supplements such as creatine, amino acid or protein supplements or weight loss medications 8 weeks prior to the study. Participants consuming more than 300 mg of caffeine daily (described as caffeine

users) (32) were excluded from the study. Participants were told to maintain their current caffeine intake thorough the duration of the study. In addition participants were required to record all physical activity. Three-day food logs, analyzed prior to the start and the end of the investigation. The subjects were instructed to refrain from strenuous exercise 48 hours before the first session. Maximal oxygen consumption (VO_{2max}) (using cooper test), anthropometric measurements and fasting blood samples (to evaluate serum leptin, LDL, TG, TC, HDL, FBG and FFA) were collected before the initiation of the intervention and after 6-week intervention. Body composition analyzer (InBody720, Biospace, Korea) was used to assess body composition. The subjects should step on

the foot electrode in barefoot and hold the handles and the procedure was held as mentioned in the manual (3).

The training program performed 3 times per week, 2 hours each session at the sport club. Each session consisted of 20 minutes warm-up, 85 Minutes professional workouts and 15 minutes cool down. After 6 weeks intervention VO_{2max} (using cooper test), weight and height (with no shoes and heavy clothing, subjects standing with both feet in the center of the scale), fasting blood samples were collected in a room located next to the sport club. Body-weight was measured to the nearest 0.1 kg on a Seca digital weighing scale (made in USA), and a stadiometer was used for measuring height to the nearest 0.1 cm with bare feet.

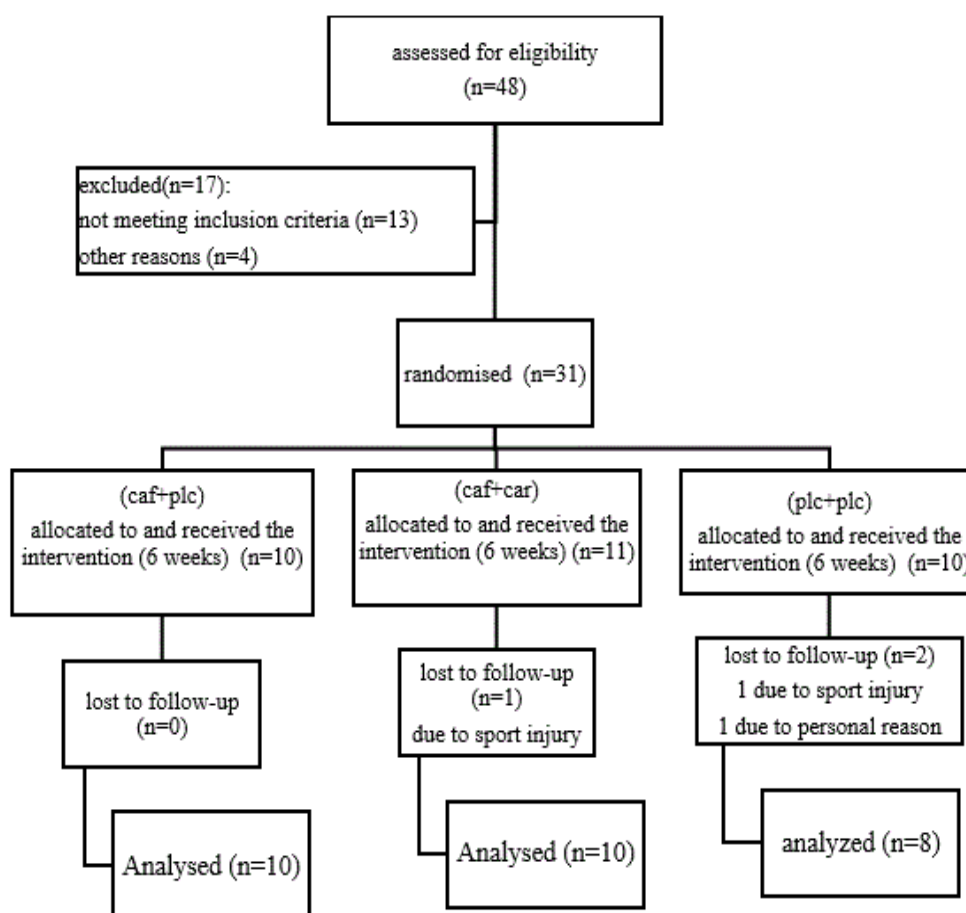


Fig1: Flow diagram of the progress

2-3. Blood sampling and variables

Sera and plasma variables were measured from 10 mL of blood drawn with stasis via venipuncture of an antecubital vein. All blood samples were taken in the morning at approximately the same time of day (i.e., between 8 am and 9 am for all subjects). Serum was separated by centrifugation at 3,000 rpm at 25°C for 15 min and stored at -70 °C until analysis. Assays were performed a week after sampling (33).

Leptin level and lipid profile determined by ELISA method (Diagnostic Biochem Canada Inc. kit, Cat. No. CAN-L-4260) and Pars Azmon kits respectively.

2-4. Statistical analysis

Statistical analysis was carried out by using a SPSS (11.5th version). Results were expressed as means \pm standard deviation (SD), and $P < 0.05$ was considered statistically significant. The normality test was assessed using Kolmogorov-smirnov statistic. Paired t-test was used to test the significance of changes in each group after intervention.

If the data showed significant differences by the ANOVA one-way test between 3 groups, Post hoc comparisons (the Tukey's test) was used. Beta=0.05, alpha=0.05 was used to estimate the sample size (34) and 10 subject was allocated in each group (**Figure.1**).

3-RESULTS

Thirty-one healthy male teen subjects entered the study; 28 (90.32 %) completed the entire 6 weeks. Three individuals (9.68 %) dropped out for reasons unrelated to the intervention such as sport injuries or personal reasons.

No significant differences in V_{O2max} ($P=0.449$), weight ($P=0.853$) and BF% ($P=0.273$) were noted at baseline among the 3 study groups. The baseline characteristics of participants are presented in **Table.1** (please see the end of paper). The

macronutrient intake for within groups, at baseline (pre-intervention) and at the end of study based on 3-day food recalls is summarized in **Table.2** (please see the end of paper). Over the 6-week period, subjects in group CafCar lost a significant amount of body weight ($P=0.02$) compared with subjects in the CafPlc and Plc groups. In addition to losing body weight, group CafCar subjects also achieved a significant reduction in body fat ($P=0.03$) (**Table.3**) (please see the end of paper).

However there was no significant differences between groups for weight (df: 2, $F=1.26$, $P=0.30$) and BF% (df: 2, $F=2.97$, $P=0.07$). There was no significant differences between groups for leptin (df:2, $F=0.84$, $P=0.44$) (**Table.3**), LDL (df:2, $F=1.64$, $P=0.21$), HDL (df:2, $F=0.40$, $P=0.67$), TC (df:2, $F=0.24$, $P=0.35$) and TG (df:2, $F=0.24$, $P=0.78$) (**Table.4**) (please see the end of paper). However TG was decreased in CafCar group after supplementation ($P=0.04$).

FFA values (**Table.4**) were increased in CafPlc group, with no within changes in two other groups. However, ANOVA analysis showed significant differences between groups (df: 2, $F=4.82$, $P=0.01$) and subsequently, post-hoc analysis released that there were significant differences between CafPlc and PLC groups ($P=0.01$), however there was no significant differences between CafPlc and CafCar nor CafCar and Plc groups ($P=0.08$ and 0.67 , respectively). No relevant side effects was observed in any group. FBG was increased significantly in CafCar and CafPlc groups ($P=0.02$ and 0.01 , respectively), however there was no significant differences between groups (df: 2, $F=0.85$, $P=0.43$).

4- DISCUSSION

The results from the current investigation demonstrate that caffeine co-ingested with carnitine during the days of training had a lowering effect on bodyweight and body fat percentage, compared to the ingestion of

caffeine alone and placebo. The leptin levels and lipid profile was unchanged in all groups except for TG which was significantly lower in CafCar group. FFA levels were increased in CafPlc group. Reduced sympathetic nervous system (SNS) functioning translates into reduced adrenaline-induced (norepinephrine and epinephrine) thermogenesis. Norepinephrine and epinephrine physiologically affect many systems such as on metabolism, specifically of carbohydrates and lipids. The beta-adrenergic receptors are involved in the pathways of lipolysis, glycogenolysis, and thermogenesis; many studies use a methylxanthines, such as caffeine or theophylline, to potentiate thermogenesis (15). Caffeine, which is also present in tea, possesses thermogenic effects and can stimulate fat oxidation (FO) in vitro and in humans, in part via sympathetic activation of the central nervous (12). The hormones of adrenalin and noradrenalin induce lipolysis by stimulating hormone-sensitive lipase activity in the adipose tissue. This leads to the release of free fatty acids into the circulation (35). On the other hand carnitine is a facilitator of fatty acid oxidation by virtue of its role in interorganelle translocation of fatty acids(10, 36).

However chronically elevated plasma FFA appears to have pathophysiological consequences, such as insulin resistance (37). Several factors may contribute to increased lipid deposition in muscle. An increase in fatty acid uptake without any change in oxidation could lead to cytosolic lipid accumulation (38). Conversely, an impaired ability to utilize fat as a fuel source because of reduced activity of enzymes of oxidative metabolism and fatty acid utilization could also result in increased cytosolic lipids (39). Recently, the concept of defective fatty acid oxidation causing insulin resistance has been challenged. Insulin-resistant skeletal

muscle can also manifest reduced efficiency of fat oxidation during fasting metabolism despite elevated levels of plasma FFA, a condition which commonly occurs in obesity and type 2 diabetes mellitus (DM) (19, 40). Rarely, adverse reactions to caffeine are severe; for example, hypertension, cholesterol abnormalities, arrhythmias, coma and death (41). Although caffeine has been demonstrated to exert major effects on lipolysis (42, 43), our data show that, caffeine per se did not have any significant effect on body weight and BF%. The changes of body weight and body fat percent was significantly lower in CafCar group compared with two other groups, whereas there were no significant differences in leptin levels between groups, the significant decrease in BW and BFP of the CafCar group might be due to increased FFA release and oxidation (due to caffeine and carnitine co-administration) with no effect of caffeine on leptin to a loss of appetite neither in the CafPlc nor CafCar groups. Although in the study conducted by Eun-Young Choi et al., coffee solution decrease final body weights in rats, food intakes was also decreased by coffee solution (36). Moreover in contrast to our study a positive relation between satiety and daily caffeine intake has been shown in men and women (44). Since in a study conducted by Williams et al., no association between decaffeinated coffee consumption and adiponectin was reported, it is speculated that caffeine contained in the coffee may have acted to increase adiponectin levels (45).

Cowwan et al., also showed that coffee consumption in high-fat-fed rats was associated with decreased body weight, adiposity and energy intake. Despite a more favorable body composition, rats displayed profound systemic insulin resistance, likely due to caffeine (46). Leptin production in white adipose tissue is regulated by the sympathetic nervous system through β 3

adrenoceptors (37). The stimulating effect of caffeine on the sympathetic nervous system may lead to down-regulation of leptin release (38). A negative association of CafCar consumption with weight and BF% is consistent with previous report from Hongu N study. However in contrast to our study, Hongu et al., released that the reduction in body fat was accompanied with reduction in the endogenous marker of adiposity, leptin, which was significantly lower in the caffeine, carnitine and choline supplemented group (10). Yamashika also showed a statistically significant inverse association between coffee consumption and leptin concentration (24). However in consistence with our study there was no notable relationship, in elderly men, between leptin and coffee drinking in a study conducted by Lagiou (47).

Although previous studies reported an inverse association of coffee consumption with triglycerides and a positive association with high-density lipoprotein cholesterol levels (24), we did not find any effect of supplementation on lipid profile, except for TG in CafCar group. Consistent with this finding, Karabudak et al. reported no significant changes in the serum levels of lipid profile following coffee consumption (48). In contrast to our study, the study conducted by Choi et al. showed that there were no significant differences in TG levels between coffee and placebo groups in rats, however TC and HDL-C levels were significantly increased and decreased, respectively in coffee ingested group (36). In accordance to our results, FFA was increased after caffeine ingestion in Bloomer et al study (49). In the present study, there was significance differences between CafPlc and PLC groups with neither differences between CafPlc and CafCar nor between CafCar and Plc. These results might be due to increased lipolysis following caffeine ingestion, however since carnitine facilitate FA entrance into mitochondria, there was no differences

between CafCar and Plc groups. Increased concentrations of Cyclic adenosine monophosphate (cAMP) have been shown to increase glycogenolysis, which may be partially responsible for the significantly impaired glucose tolerance seen after consumption of the caffeinated coffee compared with both the control and the decaffeinated coffee beverages in Johnston study (50).

Caffeine is also an adenosine receptor antagonist and therefore can inhibit muscle glucose uptake, even in the presence of insulin. Plasma glucose concentrations was significantly higher after consumption of caffeinated coffee than after consumption of the control beverage or decaffeinated coffee. It would be better if the insulin levels, insulin and leptin resistance were also measured in our study. To monitor body composition, it would be better if other devices such as calipers were also used. Besides to increase the generalizability of the findings, the study should be conducted in different types of sports, with different exercise protocols and in both genders.

4-1. Limitations of the study

It would be better if the study was also conducted in female athletes and the results compared with male and the hormonal changes were also investigated. Besides the time period of present study was relatively short, it is recommended to investigate the supplementation effect in long-term studies.

5. CONCLUSION

Caffeine co-administered with carnitine decrease body weight and BF% compared with caffeine alone. Since FFA levels were increased in CafPlc group with no changes in CafCar group, the BF% and weight lowering effect of this combination may be due to increased lipolysis and consequently facilitated entrance of fatty acids into mitochondria and fat oxidation. CafCar also

has lowering effect on TG levels with no any other negative effect on lipid profile. However caffeine supplementation with or without carnitine increased FBG levels. Altogether, the caffeine and carnitine might have synergistic effect and it would be recommended with the aim of decreasing body fat and weight besides facilitating the FFA entrance to mitochondria and preventing the increased levels of FFA, however it should be prescribed cautiously since it increases FBG levels.

6- CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest in the authorship or publication of this contribution.

7-ACKNOWLEDGMENTS

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Table 1: The comparison of baseline characteristics of participants (Mean \pm SD)

Variables	CafPlc (n=10)	CafCar (n=10)	Plc (n=8)	P-value
Age (year)	17 \pm 0.81	16.70 \pm 0.82	17.12 \pm 0.64	0.48
Height (cm)	1.73 \pm 0.08	1.74 \pm 0.04	1.73 \pm 0.05	0.982
Weight (kg)	65.98 \pm 7.54	64.28 \pm 9.16	66.65 \pm 10.33	0.853
Total body fat (%)	11.52 \pm 2.16	13.13 \pm 3.93	14.63 \pm 5.1	0.273
Vo _{2max} (ml.kg ⁻¹ .min ⁻¹)	78.38 \pm 5.82	75.71 \pm 6.95	74.69 \pm 6.29	0.449
LDL (mg/dl)	68.00 \pm 15.22	64.80 \pm 12.99	66.00 \pm 9.31	0.863
HDL (mg/dl)	48.86 \pm 17.61	48.38 \pm 11.25	45.78 \pm 6.60	0.889
TC (mg/dl)	147.40 \pm 26.01	146.90 \pm 26.61	149.50 \pm 17.29	0.978
TG (mg/dl)	80.90 \pm 21.50	88.00 \pm 22.24	107.50 \pm 28.70	0.109
FFA (nmol/ml)	1.73 \pm 1.24	3.35 \pm 2.00	3.70 \pm 3.32	0.152
Glc (mg/dl)	80.50 \pm 6.24	78.50 \pm 5.14	77.500 \pm 7.25	0.602
Leptin (ng/ml)	2.32 \pm 1.40	2.76 \pm 1.92	3.60 \pm 3.57	0.536

LDL: Low density lipoprotein; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; FFA: Free fatty acid.

Table- 2: The within and between comparison of Macronutrient intake (based on 2 × 3-day food recalls

Group Macronutrient	CafPlc			CafCar			Plc		
	Baseline	End of the study	P-value*	Baseline	End of the study	P-value*	Baseline	End of the study	P-value*
Energy (Kcal/day)	2502.01±62.22	2399.03±284.25	0.74	2519.03±146.87	2528.50±162.82	0.85	2497.62±169.47	2481.70±199.03	0.73
CHO (%)	57.92±3.50	57.82±4.50	0.91	56.24±4.20	56.01±4.29	0.56	58.18±5.09	57.88±4.51	0.48
Fat (%)	25.29±4.00	25.26±5.45	0.98	31.67±2.68	31.07±2.14	0.15	28.42±4.28	28.10±4.45	0.46
Pr (%)	16.78±0.50	16.91±0.95	0.75	12.08±4.56	12.90±4.60	0.08	13.39±4.89	14.00±5.18	0.21

CHO: Carbohydrate; Pr: Protein; *: Results are presented for paired t-test.

Table-3: The weight, body fat percent (BF%) and Leptin changes within and between intervention groups

Group Variable	CafPlc			CafCar			PLC			ANOVA (P-value)**	EF
	Pre (Mean±SD)	Post (Mean±SD)	P-value*	Pre (Mean±SD)	Post (Mean±SD)	P-value*	Pre (Mean±SD)	Post (Mean±SD)	P-value*		
Weight (kg)	65.98±7.54	65.84±7.29	0.59	66.17±9.65	65.78±9.67	0.02	66.72±11.16	66.82±11.18	0.28	0.30	0.107
BF (%)	11.52±2.16	10.86±2.15	0.13	13.61±3.98	12.47±4.47	0.03	14.63±5.18	15.48±3.94	0.39	0.07	0.229
Leptin (ng/ml)	2.45±1.42	2.30±2.77	0.90	2.83±2.11	3.04±2.65	0.79	3.88±3.83	1.98±0.95	0.24	0.44	0.077

CafPlc: Caffeine+placebo; CafCar: Caffeine+carnitine; PLC: Placebo, EF: Effect size; *: Results are presented for paired t-test (differences between pre and post.); **: Results are presented for one-way ANOVA test (Differences between groups).

Table-4: Paremeters changes within and between intervention groups

Group Variable	CafPlc			CafCar			PLC			ANOVA (P-value)**	EF
	Pre (Mean±SD)	Post (Mean±SD)	P-value*	Pre (Mean±SD)	Post (Mean±SD)	P-value*	Pre (Mean±SD)	Post (Mean±SD)	P-value*		
LDL (mg/dl)	68.00±15.22	71.40±15.54	0.32	63.5±13.90	60.12±15.00	0.19	64.40±9.44	63.00±11.57	0.43	0.21	0.141
TG (mg/dl)	80.90±21.50	79.60±24.50	0.88	85.25±24.35	76.75±25.24	0.04	100.40±25.53	92.40±42.59	0.60	0.78	0.024
TC (mg/dl)	147.40±26.01	151.00±25.78	0.37	145.38±28.36	143.00±27.40	0.64	148.80±19.24	142.40±15.43	0.34	0.35	0.097
HDL (mg/dl)	48.86±17.61	47.37±7.89	0.75	49.71±12.21	49.70±11.25	0.99	47.38±5.95	50.92±4.75	0.18	0.67	0.039
FBG (mg/dl)	80.50±6.24	90.40±5.92	0.01	78.22±5.38	91.77±13.32	0.02	79.87±8.59	86.12±11.44	0.07	0.43	0.066
FFA(nmol/ml)	1.73±1.24	3.47±2.37	0.04	3.35±2.00	2.84±2.41	0.11	3.70±3.32	2.27±1.15	0.23	0.01	0.278

CafPlc: Caffeine+placebo. CafCar: Caffeine+carnitine. PLC: Placebo, EF: Effect size, LDL: Low density lipoprotein, TG: Triglyceride. TC: Total cholesterol, HDL: High density lipoprotein.*: Results are presented for paired t-test (Differences between pre and post). **: Results are presented for one-way ANOVA test (Differences between groups.).