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ORIGINAL ARTICLE

The Effect of Simultaneous Consumption of Coffee Caffeine and Sleep Deprivation on Plasma Ghrelin and Leptin Levels

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ABSTRACT

Background: Unlike sleep deprivation, caffeine in coffee has been shown to yield positive effects on appetite, body weight, and risk of developing symptoms of metabolic syndrome. However, the combined effects of caffeinated coffee and sleep deprivation are unknown. The aim of this study was to determine the simultaneous effects of coffee caffeine intake and sleep deprivation on leptin and ghrelin.

Methods: This was a randomized clinical trial comprised three-day treatments with two-week washout interval. Forty-two healthy men, habitual caffeinated coffee consumers (1-3 cups/day) and good sleepers (based on the Pittsburgh Sleep Quality Index) were recruited. Subjects were randomly assigned to three groups: three nights of deprived sleep (4 hrs. in bed) plus 3×150 mL/cup of boiled water (BW treatment), decaffeinated coffee (DC treatment, without sugar, 99.9% caffeine-free), and caffeinated coffee (CC treatment, without sugar, 65 mg caffeine/cup). DC and CC treatments were blinded. At the end, fasting serum leptin and plasma ghrelin were measured and compared.

Results: No significant differences were found between the treatments in the leptin level but a significant difference was shown between the treatments in plasma ghrelin (P=0.048). Pairwise comparisons test showed that the CC treatment led to lower plasma ghrelin as compared to the DC treatment (P=0.006).

Conclusion: Caffeinated coffee with sleep deprivation simultaneously manipulated the level of ghrelin towards an anorexigenic effect (reduced plasma ghrelin). However, further investigations are required to support caffeinated coffee as an appetite/weight loss recommendation.

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Introduction

A remarkable reduction in sleep duration has frequently been reported over the past few decades (1). The changes in sleep pattern have been recognized as one of the underlying causes of adverse metabolic health. Both the epidemiological

and experimental studies have shown that on an average the less people sleep, the more they eat (2). Likewise, there are huge amount of nutritional and physiological evidences indicating that sleep loss may contribute to increase the plasma concentrations of ghrelin (the hunger hormone) and decrease those of leptin (the satiety hormone). These hormones are known as appetite-regulating hormones with a major impact on energy balance and such changes contribute to an increase in appetite, food intake, and weight gain (1).

In contrast, caffeinated coffee, the most common beverage that people usually drink when tends to restrict their sleep duration (3), has been raised to inversely affect adiposity and appetite regulation. This may indicate a positive impact to modulate peripheral metabolism and obesity (4). Coffee has also been shown to have a major contribution to the changing of energy balance over the decades, particularly among normal weight people (5). Regular coffee caffeine consumption (containing up to 450 mg caffeine) and acute caffeine ingestion (approximately up to 10 mg caffeine/body weight) have been shown to cause doubled lipids turnover, up to 13% increase in resting energy expenditure (REE) and a moderately increase in free fatty acids (FFA) oxidation (6).

To our knowledge, despite evidence indicating the converse effects of sleep deprivation and coffee caffeine consumption on appetite and body weight, the simultaneous effects of these two variables have remained unknown yet. This would be more important in a country like Iran, where coffee is increasingly being consumed (7), particularly amongst people who has an intention to reduce the number of hours of sleep they get at night (8). Therefore, the objective of this study was to determine the simultaneous effects of sleep deprivation and coffee caffeine consumption on the levels of two appetite-regulating hormones of leptin and ghrelin among healthy adult Iranian men. This randomized crossover study tested the hypothesis that the simultaneous effects of coffee caffeine consumption and sleep deprivation would not change the levels of appetite-regulating hormones of leptin and ghrelin.

Materials and Methods

In this randomized clinical trial, a total of 57 men aged 20 to 40 years with a Pittsburgh Sleep Quality Index (9) (PSQI)≤5 at a sleep screening visit conducted in Tehran, the capital city of Iran, were initially recruited. The Ethics Committee of Tehran University of Medical Sciences approved the study protocol. Subjects signed a written informed consent and were invited to the research unit for further screening performed by an interview and anthropometric measurements including age, weight, height, body mass index (BMI), waist, and hip circumferences (HC), waist circumference (WC), waist to hip circumference (WHR), and body fat percentage (BF%).

The ratio of waist to hip was obtained and body fat percentage (BF%) was calculated using the age and sex prediction formula; BF%=(1.20×BMI)+(0.23×Age)–(10.80×Gender)–5.40, where gender was considered equal to 1 for adult men (10, 11). Subjects with 24.9 $kg/m^2 \leq BMI \leq 18.5\ kg/m^2$, physical demand activities and a shift-working job were excluded from the study. A complete nutrition and medical history was obtained from subjects for screening purposes. Inclusion criteria were non-smoking, habitual coffee consumption of not more than 3 cups/day, without having travelled across the time zone during 4 weeks before the study, with no family history of serious medical problems such as any type of diabetes, lung or heart disease, arthritis, other rheumatic disease, cancer or other chronic condition, and medications, supplements or diet prescribed. A total of 51 healthy adult men were qualified to continue the study.

Subjects who met the study criteria were asked to stay one night (adaptation night) in the research unit to familiarize themselves with the study protocols. They were instructed to fix their bedtimes (between 23.00 pm to 7.00 am) and eating times (8.00 am, 13.00 pm and 20.00 pm), during the next two weeks. A deviation of not more than 30 minutes from the specified times was permitted. Subjects were asked to avoid having travelled across the time zone, heavy physical activities, and caffeine-containing foods based on the list they were given, during the next two weeks. However, they did not need to observe any special changes in their usual food habits or to follow a particular diet. Fasting serum leptin and plasma ghrelin were measured after a one-night stay in the research unit (baseline values), to ensure that subjects with normal levels of appetite-regulating hormones participated in the study.

Subjects were then permitted to return to their usual life for a two-week run-in period, observing all study principles instructed. The run-in period was considered for washout of caffeine and to ensure that subjects would be able to endure caffeine depletion (12, 13). Meanwhile, subject could also find enough opportunity to adapt with study's protocols and instructions that they had received during the adaptation night, such as fixed bedtimes and mealtimes. Besides, they were requested to complete three 24-hour dietary recalls/week (so a total of 6 dietary recalls), during the two-week run-in period.

which contributed to evaluate the dietary intake of the subjects. These recalls involved call interviews.

The average Reported Energy Intake (rEI) obtained through the six 24-hour dietary recalls during the run-in period for each subjects was compared with his own average Predicted Energy Intake (pER) calculated using Harris-Benedict equation (14) as Energy requirement (for male)=[66.5+(13.75×W)+(5×H)–(6.77×Y)]×PAL, where W was actual weight in kilograms, H was height in centimeters, Y was age in years and PAL was the subject's Physical Activity Level. Subjects with rEI<70%pER (under-reported) and rEI>130%pER (over-reported) were excluded from the study (15). Finally, 44 healthy adult men were recruited to participate in this randomized crossover clinical trial.

Forty-four healthy adult men that met all inclusion criteria were randomized to one of the three groups to participate in a randomized crossover clinical trial including three treatments separated by two-week washout intervals. The two-week intervals were intended to washout caffeine from coffee intake and to minimize carry-over effects that might have arisen due to treatment sequences (12, 13). Each treatment comprised three consecutive nights of sleep deprivation with only 4 hours in bed and three 150 mL/cup of boiled water (BW treatment), decaffeinated coffee (DC treatment), or caffeinated coffee (CC treatment), which were given in one-cup doses at 20.00, 20.40 and 21.20 pm.

The selected caffeinated/decaffeinated coffee brand had no added sugar and was Nescafe - original instant black coffee. While decaffeinated coffee was 99.9% caffeine-free (16), each cup of 150 mL of the selected caffeinated coffee (2 g coffee) contained 65 mg caffeine (16). Therefore, subjects received an amount of 195 mg caffeine/night regarded as safe as 3 cups of caffeinated coffee in the CC treatment group (17, 18). Subjects were blinded to decaffeinated and caffeinated coffee in the DC and CC treatment groups, but naturally not to boiled water in the BW treatment group.

To provide a one-night partial sleep deprivation of 4 hours in bed, subjects were scheduled to go to bed at 2:45 am each night, and the lights were kept switched off until next day 07:00 am. For identical conditions, all subjects were asked to stay in the research unit for the entire three days in each treatment. Subjects were only allowed to participate in sitting activities such as playing games or watching movies but not exciting ones, studying, interacting with each other or the study staffs during the day, and were asked not to engage in any other task. They were not permitted to do any moderate or heavy physical activities or to nap during the day.

The energy requirement for each participant was calculated to obtain a reasonable estimate for the food amount that subject required to receive during the treatments. The study was designed to keep subjects in a condition mirroring their typical lifestyle with an energy intake and physical activity as close as possible. The prolonged waking hours due to sleep deprivation protocol was covered with only sitting activities. Therefore, a fixed PAL of 1.55 (14) was used to calculate energy requirements during the treatments. The energy requirements were divided into five meals along the day, including breakfast (at 8.00 am), first snack (at 10.00 am), lunch (at 13.00 pm), second snack 43 (at 16.00 pm), and diner at (19.00 pm). However, subjects were not given breakfast in the morning of third day, to measure fasting levels of leptin and ghrelin.

Fasting levels of leptin and ghrelin were measured at baseline (in the morning after a one-night stay in the research unit) and end of each treatment (in the morning of third day). Enzyme-linked immunosorbent assay (ELISA) and Recombinant ELISA kit (Phoenix, Burlingame, USA) with the sensitivity of 0.312 ng/ml was used for the quantitative determination of serum leptin. To measure the quantitative amount of plasma ghrelin, enzyme immunoassays (EIA) and ghrelin EIA Kit (Phoenix, Burlingame, USA) with a minimum detectable concentration of 0.13 ng/ml was employed.

An initial sample size of 15 subjects per group with an attrition rate of approximately 20% was able to produce a sample size of approximately 12 subjects with a statistical actual power of 0.86 to detect a significant difference ($P<0.05$). The sample size was calculated using the software G power 3.1.0, considering three treatments for three groups in a crossover design (19). Consecutive sampling method, a reasonable alternative of non-probability sampling method (20), was used to facilitate the sampling procedure. This means that by study, everybody who was available as a good representative of overall population was recruited in a reasonable period of time (20).

Statistical analysis was performed using analysis of variances with SPSS software (version 21, Chicago, IL, USA). Descriptive statistics were calculated for all variables as means, standard deviation, minimum, and maximum. Kolmogorov-Smirnov test was used to show that the levels of appetite-regulating hormones were normally distributed. Thus, general linear model one-way repeated measures (ANOVA) was employed to evaluate the null hypothesis that there was no significant beverage effect on the leptin and ghrelin levels, when sleep deprivation was

simultaneously coupled with boiled water or one of the study beverages of caffeinated and decaffeinated coffee. The statistical differences between the treatments (within-subjects effects) were reported at a significance level of 0.05. When $P<0.05$, the statistical difference between three treatments was considered significant.

However, as ANOVA does not identify where differences occur, when ANOVA indicated a statistically significant difference, the Fisher's Least Significant Difference (LSD) was used in order to determine which specific means (pairwise treatments) differed in a statistically significant manner. In other words, the follow-up comparisons of the LSD test determined the differences between groups.

Results

Regarding baseline characteristics and anthropometric measurements, a flowchart of study enrollment and study design was presented in Figure 1. Fifty-seven subjects with a PSQI≤5 agreed to participate in the study, of which a total of 44 subjects who met the study criteria were randomly assigned to two groups of 15 and one group of 14. However, one was withdrawn because of personal reasons and one left the study due to common cold. Therefore, the overall attrition rate in the present study was approximately 5% (14% for group A only). The 42 subjects who completed the study were compliant with the treatments. Mean basic characteristics of 42 subjects who competed the study were presented in Table 1. Table 1 also shows the mean baseline characteristics data for subjects after randomization in three groups. There were no statistically significant dereferences between three groups of BW, DC and CC for baseline

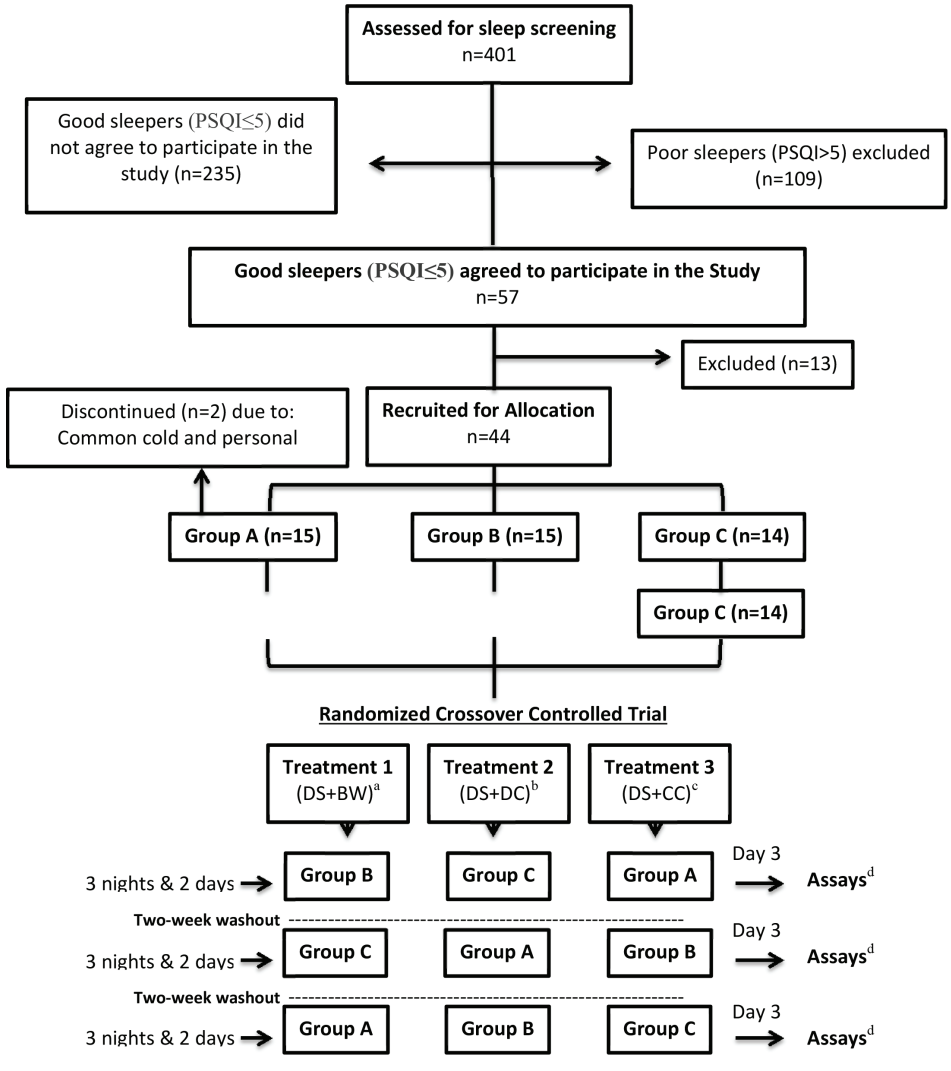

Figure 1: Flowchart of subjects' recruitment, the attrition and study design of the randomized crossover trial determining the simultaneous effects of coffee caffeine consumption and sleep deprivation on the levels of leptin and ghrelin among healthy adult Iranian men. [a]BW Treatment (DS+BW): Deprived Sleep (3 nights as 4 hrs. in bed)+Boiling Water (3 cups of 150 mL). [b]DC Treatment (DS+DC): Deprived Sleep (3 nights as 4 hrs. in bed+Decaffeinated Coffee (3 cups of 150 mL- 99.9% caffeine-free). [c]CC Treatment (DS+CC): Deprived Sleep (3 nights as 4 hrs. in bed) and Caffeinated Coffee (3 cups of 150 mL- 65 mg caffeine/cup). [d]Fasting serum leptin and plasma ghrelin

Table 1: Mean baseline physical and appetite-regulating levels of 42 subjects who completed the study (overall and by group)

Parameters	Mean±SD				[1]P value
	All subjects n=42	Group A n=13	Group B n=15	Group C n=14	
Age *(y)*	27.92±4.45	26.75±4.76	27.95±3.97	29.59±4.79	0.216
Weight *(kg)*	72.96±7.02	71.32±6.60	73.44±7.54	73.64±7.63	0.698
Height *(cm)*	178.78±6.61	178.75±6.05	179.27±6.81	178.34±7.48	0.940
BMI *(kg/m²)*	22.77±1.41	22.46±1.68	22.82±1.45	23.10±1.12	0.583
WC *(cm)*	88.29±3.24	87.99±3.58	88.64±3.39	88.60±2.94	0.864
HC *(cm)*	95.95±5.23	95.65±5.98	97.40±4.91	95.12±5.21	0.531
WHR *(%)*	0.92±0.03	0.92±0.03	0.91±0.03	0.94±0.04	0.076
[2]BF *(%)*	17.55±1.96	16.91±2.09	17.61±1.96	18.33±1.81	0.226
[3]PSQI	2.88±1.84	3.38±1.85	2.31±1.70	3.38±1.71	0.281
Leptin *(ng/mL)*	7.67±1.56	7.94±1.37	7.30±1.70	7.82±1.61	0.486
Ghrelin *(ng/mL)*	0.5417±0.0698	0.58±0.0675	0.53±0.0642	0.51±0361	0.096

[1]Baseline values were not significantly different between three groups of A, B and C. [2]Body Fat Percent for adult men: (1.20×BMI)+(0.23×Age)–(10.80×1)–5.40. [3] Pittsburgh Sleep Quality Index, using PSQI questionnaires. BMI: body mass index; WC: waist circumference; HC: head circumference; WHR: waist to hip circumference; BF: body fat; PSQI: Pittsburgh Sleep Quality Index.

characteristics including age, weight, height, BMI, waist and hip circumferences, WHR, body fat percentage, PSQI, leptin and ghrelin levels.

Figure 2a shows the levels of fasting serum leptin during different study treatments. The figure shows that the highest fasting serum leptin was in the BW treatment group, when subjects were sleep deprived (as 4 hrs. in bed during 3 consecutive nights) and received 3×150 mL/cup of boiled water. The lowest mean glucose was found in the DC treatment group, when the same subjects were given 3×150 mL/cup of decaffeinated coffee, no added sugar, and 99.9%

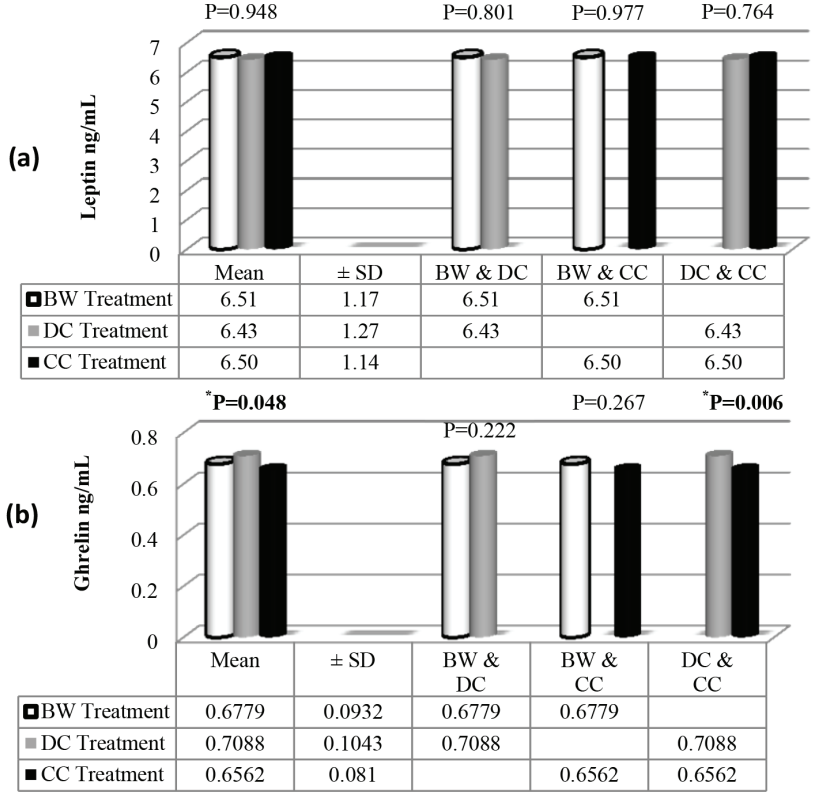

Figure 2: Analysis of variances for the levels of fasting serum leptin (a) and plasma ghrelin (b) in a randomized crossover clinical trial determining the simultaneous effects of coffee caffeine consumption and sleep deprivation on the levels of leptin and ghrelin among healthy adult Iranian men (n=42). BW Treatment: Deprived Sleep (3 nights as 4 hrs. in bed)+Boiled Water (3 cups of 150 mL). DC Treatment: Deprived Sleep (3 nights as 4 hrs. in bed)+Decaffeinated Coffee (3 cups of 150 cc- 99.9% caffeine-free). CC Treatment: Deprived Sleep (3 nights as 4 hrs. in bed) and Caffeinated Coffee (3 cups of 150 cc- 65 mg caffeine/cup). *Significant.

caffeine free.

A repeated measures ANOVA indicated no statistically significant beverage effect on mean leptin levels [F(2,82)=0.053, P=0.948, Figure 2a]. Thus, no significant evidence was found to reject the null hypothesis, suggesting that boiled water, decaffeinated coffee and caffeinated coffee did not significantly change fasting serum leptin, when subjects were simultaneously requested to be sleep deprived. Although not required, a post hoc test of LSD did not denote to any statistical differences (Figure 2, P>0.05) in the fasting serum leptin between the BW and DC treatment groups (P=0.801), BW and CC treatment groups (P=0.977), or DC and CC treatment groups (P=0.764).

As shown in Figure 2b, plasma ghrelin of deprived sleep subjects fluctuated opposite to serum leptin with a peak in the DC treatment group and a bottom in the CC treatment group, when subject who were sleep deprived received 3×150 mL/cup of no added sugar caffeinated coffee containing 65 mg caffeine/cup. One-way repeated measures analysis of variances determined that mean fasting plasma ghrelin differed significantly between the treatments [F(2,82)=3.154, P=0.048, Figure 2b]. Follow-up comparisons were made to discover where the differences occurred. LSD tests determined that the DC treatment resulted in a slightly increase in fasting plasma ghrelin as compared to the BW treatment (0.7088±0.1043 vs. 0.6779±0.0932 ng/mL, respectively), which was not statistically significant (P=0.222). However, when subjects received the CC treatment, fasting plasma ghrelin decreased to 0.6562±0.081 ng/mL, which was statistically significantly different to the DC treatment (P=0.006) but not to BW treatment (P=0.267).

Discussion

We found no significant changes in fasting serum leptin following consumption of caffeinated, or decaffeinated coffee, or boiled water among healthy adult Iranian men who were simultaneously requested to be sleep deprived for 3 consecutive nights with 4 hours in bed. In terms of the effects of boiled water or study beverages of caffeinated and decaffeinated coffee on plasma ghrelin, the results of the present study showed a lower fasting plasma ghrelin only following caffeinated coffee as compared to decaffeinated.

To our knowledge, this is the first study to determine the simultaneous effects of sleep deprivation and coffee caffeine consumption. Both experimental and epidemiological sleep studies with no coffee consumption intervention have previously reported a decrease in leptin level caused by sleep restriction (2, 21). A shortened sleep period as 4 hours in bed even for only two consecutive nights has been reported to contribute to almost 18% decrease in the levels of leptin. These hormonal change has been shown to be followed by a rise in appetite, thus a higher level of hunger (2). Considering our study, nothing but sleep duration was manipulated in BW treatment, this could conceivably be assumed to be in agreement with previously reported studies indicating a decreased leptin level caused by sleep deprivation.

However, some different results were found when administrating different treatments. This study yielded no significant beverage effects for the leptin levels during the treatments. This finding appeared to be inconsistent with former studies that revealed an inverse association between leptin and caffeine (22) or coffee caffeine levels (23). Studies have shown that dose- and gender- dependent effects of caffeine may contribute to create a part of differences in the impacts of coffee caffeine on leptin levels (24). In addition, the techniques used to prepare coffee (25) and even the time of drinking coffee (26) have shown as debated issues affecting the caffeine-independent effects of coffee. However, in the present study, a certain group of subjects were recruited through a controlled screening procedure, while they were matched for major part of variables related to coffee consumption such as type, ingredients, preparation technique and amount of coffee consumed. In fact, findings in this study that found no effects for study beverages on the leptin levels may suggest that some metabolic factors control circulating leptin level that may be affected by sleep deprivation when it is coupled with coffee caffeine consumption, simultaneously. However, it remains to be elucidated that how sleep deprivation or the interaction between sleep deprivation and coffee consumption might do so.

Despite limited knowledge of leptin's pathophysiologic effects, its blood level has been reported to be directly associated with obesity and inflammatory markers (27). Elevated leptin levels has been reported to stimulate some tissues and organs such as liver, pancreas, platelets, and myocardium which are responsive to high concentrations of leptin (28). A higher circulating leptin has been recognized as an indicator of leptin resistance in both aging and obesity (29).

If previously reported, impact of coffee caffeine on decreased leptin level is translated to the role of coffee caffeine against leptin resistance, findings in the present study that failed to find any significant changes in serum leptin level may indicate the probable impact of sleep deprivation to attenuate some benefits of coffee caffeine consumption such as those related to leptin resistance and eventually

insulin resistance, which their association is required for keeping a balance in human health (30). To note, any acute drop in leptin signals may lead to increased food intake, thus higher fat storage and leptin resistance. This condition may end up on the abdominal organ, liver (31).

The present study showed that the mean value of ghrelin in BW treatment group was approximately 25% higher than its baseline mean value. This was in agreement with previous findings (2, 32) indicating up to 28% increase in the morning plasma ghrelin concentrations in healthy men who spent 4 hours in bed as compared to who spent 10 hours in bed. Notably, even one night of 8-hour wakefulness may cause elevated plasma ghrelin as compared to a sleep condition for the similar period of time in healthy male participants (33).

Limited findings aimed at determining the relationship between ghrelin and coffee caffeine, while to the best of our knowledge, no study has been carried out yet to show the effects of coffee, either caffeinated or decaffeinated, on ghrelin when it is coupled with sleep deprivation, simultaneously. The findings in this study indicating lower level of plasma ghrelin amongst deprived sleep subjects who simultaneously received caffeinated coffee may suggest a probable appetite-inhibitory role for caffeinated coffee mediated by decreased plasma ghrelin. This however, was in contrast to previously reported evidence demonstrating a positive association between coffee caffeine (on its own) and the ghrelin levels and appetite (17).

Besides the effects of sleep deprivation and probable mechanism(s) following short sleep duration, a part of different results in the present study may be related to ghrelin function and interaction with other mechanisms. The gut-derived hormone of ghrelin has been reported to be involved in a wide range of physiological mechanisms in human. Ghrelin has been shown to be associated with growth hormone, appetite regulation, food intake (the orexigenic role) and body weight managing as well as lipolysis (33). Therefore, it is not surprising to find some incompatible results, particularly that the impacts of sleep a mechanism affecting ghrelin, is not still well elucidated in details.

Furthermore, one area of interest in ghrelin studies has recently been driving at the critical role of ghrelin in glucose homeostasis. Not all but most of these studies have proposed a bilateral inverse relationship between ghrelin concentration and insulin secretion. In one hand, ghrelin has been demonstrated to have an inhibitory effect on insulin secretion. This function may result in an increased circulating glucose, indicating a diabetogenic effect for ghrelin (34). On the other hand, insulin has also been known to suppress ghrelin concentration, independent from glucose (35), indicating an anorexigenic function for insulin.

In both insulin secretion inhibitory role and anorexigenic function, ghrelin needs to be octanoylated by ghrelin O-acyltransferase (GOAT) into its active form, the acyl ghrelin (32). When obesity is due to type 2 diabetes the manipulation of this pathway has been considered as a novel concept in obesity treatment; a therapeutic function affecting both energy and glucose homeostasis via an antagonist-signaling pathway (36). However, due to an inverse association between insulin resistance and ghrelin levels, caffeinated coffee which in present study showed to cause lower plasma ghrelin, does not appear to be enough justified for an appetite/weight loss recommendation among healthy individuals who have normally shortened sleep duration (4).

As a limitation, our study excluded the potential sex- and age- specific impacts, findings could not be generalized to women or other age groups. Second, this study did not show the differences between good sleepers and poor sleepers or moderate habitual coffee consumers and individuals who rarely consume coffee or excessive coffee consumers. It would also be beneficial to compare different diurnal pattern of coffee drinking to identify any possible time-response relationship. Third, the sleep time and sleep duration were recorded by experimenters, whereas polysomnography could provide more appropriate and more accurate sleep records. Nevertheless, a continuous monitoring and strict list of criteria that was employed during subject recruitment was of strength of the present study. Moreover, as unique features, while most coffee studies are designed to examine only the effects of caffeinated and decaffeinated coffee, the boiled water reference in the present study provided a control group affected neither by caffeinated nor decaffeinated coffee.

Conclusion

When simultaneously coupled with sleep deprivation, coffee caffeine consumption lowered plasma ghrelin but did not change the level of leptin in healthy adult Iranian men. This suggests that some possible metabolic factor(s) in the control of circulating leptin may be affected in simultaneous effects of coffee consumption and sleep deprivation. We defined an anorexigenic role, mediated by lower plasma ghrelin, when caffeinated coffee was consumed by healthy adult men who were simultaneously sleep deprived. Nevertheless, due to an inverse association between insulin resistance and ghrelin levels, further investigations are needed

to support caffeinated coffee as an appetite/weight loss recommendation, particularly in individuals with shortened sleep duration.

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Conflict of Interest

None declared.

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