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Long-Term Consumption Of Energy Drinks Induces Hormonal And Non-Hormonal Biomarkers Alteration In Young People

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ABSTRACT

Energy drinks containing significant quantities of caffeine, taurine, and sugar are increasingly consumed, particularly by adolescents and young adults. Excessive consumption of energy drinks and accumulation of the above ingredients, as well as their mutual interactions, can be hazardous to the health of young adults. This study aimed to assess the effect of acute consumption of energy drinks on body weight, blood glucose, insulin, leptin, and ghrelin hormones.

The study involved 50 volunteers, healthy young adults (ages 19-22 years), who were divided into two groups: the first consumed energy drinks, and the second did not consume energy drinks. All participants had their serum glucose and insulin, leptin, and ghrelin hormones measured. In addition to calculating body mass index (BMI), the homeostasis model assessment estimated IR (HOMA-IR) and leptin/ghrelin ratio.

In the above experiment, the consumers of energy drinks presented a significant increase in BMI, serum glucose, and insulin resistance (HOMA-IR) compared to those who did not consume energy drinks. No significant changes were noted in the insulin hormone and leptin/ghrelin ratio. Consumption of energy drinks caused a significant decrease ($p < 0.001$) in leptin and ghrelin levels.

In conclusion, energy drink consumption significantly affects insulin resistance and leptin-ghrelin levels. More studies are needed to evaluate the effects of energy drink consumption in healthy, young, and normal-weight individuals.

Introduction

Energy drinks consumption has increased dramatically in the past few decades by young adolescents and adults. Energy drinks are marketed for their energy-boosting effect to enhance physical and mental performance (Ismail et al., 2018). Energy drinks first appeared in Europe and Asia in the 1960s. An exponential sales growth was observed after Red Bull's introduction to the European market in 1987, which was also observed 10 years later in the United States of America. Energy drinks have become the fastest-growing product in the beverage category since the introduction of bottled water (Erdmann et al., 2021).

Energy drinks are widely available beverages containing caffeine, taurine, glucuronolactone, ginseng, and other ingredients and are said to improve physical or cognitive performance and should not be confused with isotonic drinks or sports drinks (Higgins et al., 2018). These substances, most of which act as stimulants, are not included in the list of materials under regulation by the Food and Drug Administration (FDA) of the USA. The levels of these stimulants vary amongst different brands of energy drinks and, in most cases, are higher than the values allowable (Mansy et al., 2017). Usually, one can of an energy drink contains as much caffeine as three to five cans of Coke (34 mg) (Elbendary

et al., 2023).

A comprehensive literature review summarizes that a single dose of 200 mg of caffeine, or less, by healthy people without comorbidities and pharmacokinetic disturbances is usually not associated with toxic effects.

However, a dose above 300 mg at once can cause caffeine intoxication, the symptoms of which are mainly related to its stimulating effect. The most common ones are restlessness, nervousness, excitement, insomnia, facial flushing, increased urination, gastrointestinal disorders, muscle tremors, chaotic flow of thoughts and speech, irritability, arrhythmia, tachycardia, and psychomotor agitation. The severity of the undesirable effects of caffeine consumption is dose-dependent. The threshold of caffeine toxicity appears to be about 400 mg/day in healthy adults of 19 years or older, 100 mg/day in healthy adolescents of 12–18 years old, and 2.5 mg/kg/day in healthy children of less than 12 years old (Rodak et al., 2021).

In the last few years, caffeine use increased, especially as energy drinks, and unfortunately, there are limited studies about the long-term effects of energy drink ingredients on humans; therefore, this study aimed to explore caffeine intake habits and the perception of its impact on health among college students by analyzing the effects of caffeine on some physiological parameters related to human health.

Materials and Methods

Participants

The study was conducted in the College of Education at Salahaddin University-Erbil. Fifty male students aged 19-22 years with no chronic metabolic diseases and not taking any medications were recruited for this observational survey. Thirty subjects were consuming energy drinks (consumer group), mean weight was 73.733±8.374 kg, and twenty subjects were not consumers of energy drinks (non-consumer group), mean weight was 67.719±5.669 kg. The mean weekly intake of energy drinks in the consumer group was five bottles.

Blood sample collection and preparation

Five milliliters of fasting blood was collected into a plain gel tube from each subject. The blood was allowed to clot at room temperature and then centrifuged at 3,000 rpm for 15 min within 30 min of sample collection. The serum obtained was stored at -20∘C until used for biochemical assays.

Biochemical assay

Serum lipid profile

Serum lipid concentrations were determined in both consumer and non-consumer groups by colorimetric method using the commercial kit Biolabo-France. Total cholesterol, triglyceride, and high-density lipoprotein cholesterol (HDL-C) are measured on a COBAS INTEGRA 400 plus system analyzer serviced by Roche Diagnostics (Deutschland). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation (Friedewald et al., 1972), as shown in the following: LDL-C = TC − (HDL-C + total TG/5) mg/100 ml.

Serum fasting glucose

Serum glucose concentration was estimated by the glucose-oxidase colorimetric method by using a kit supplied by Biolabo (France).

Triglyceride-glucose (TyG) index

The triglyceride-glucose (TyG) index has been identified as a reliable alternative biomarker of insulin resistance (Tao et al., 2022). The TyG index, calculated as TyG index = Ln [Fasting triglyceride (mg/dl) × fasting glucose (mg/dl)]/2, is a composite indicator composed of fasting triglyceride (TG) and fasting glucose (FG) levels (Simental-Mendía et al., 2008).

Kidney function tests

Serum uric acid, urea, and creatinine activity were assayed using a commercial kit (BIOLABO, France).

Serum alkaline phosphatase (ALP)

The activity of alkaline phosphatase (ALP) in serum was assayed by the commercial kits (NS, BIOTECH Co., Egypt).

Serum lactate dehydrogenase (LDH)

Serum lactate dehydrogenase (LDH) levels were estimated using a Cobas-e411 analyzer (Roche Diagnostics, Mannheim, Germany).

Hormonal assay

Using the commercially available ELISA kits method (enzyme-linked immunosorbent assay, Sunlong Biotech CO., LTD, China), serum fasting insulin (mU/L), total serum ghrelin (pg/mL), and serum leptin (pg/mL) levels were determined for each subject according to the manufacturer's protocol.

BMI and assessments of insulin resistance

Body mass index (BMI) was calculated as weight in kg divided by height in m^2. The homeostasis model assessment-estimated IR (HOMA-IR) was used to estimate insulin sensitivity using the fasting plasma glucose and insulin concentrations. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula; HOMA-IR = serum glucose (mg/dL) × plasma insulin (μU/mL)/405 (Matthews et al., 1985).

Statistical analysis

Data were analyzed using the GraphPad Prism (GraphPad Software version 9, San Diego, CA, USA) and expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was applied to check for normal distribution. All the analyzed variables were normally distributed ($p > 0.05$). Statistical analysis was conducted using a student *t*-test to compare consumer and non-consumer groups. A value of $p<0.05$ was considered statistically significant.

Results and Discussion

The 50 participants (30 consumers and 20 non-consumers) in this study ranged in age from 19-22 years. Among consumers, energy drink intake was five bottles per week. Energy drinks consumption led to a gain in body weight (calculated as BMI, Figure 1) compared to non-consumers (consumer: 23.961 ± 2.311; non-consumer: 22.218 ± 2.101, ***p=0.0083***).

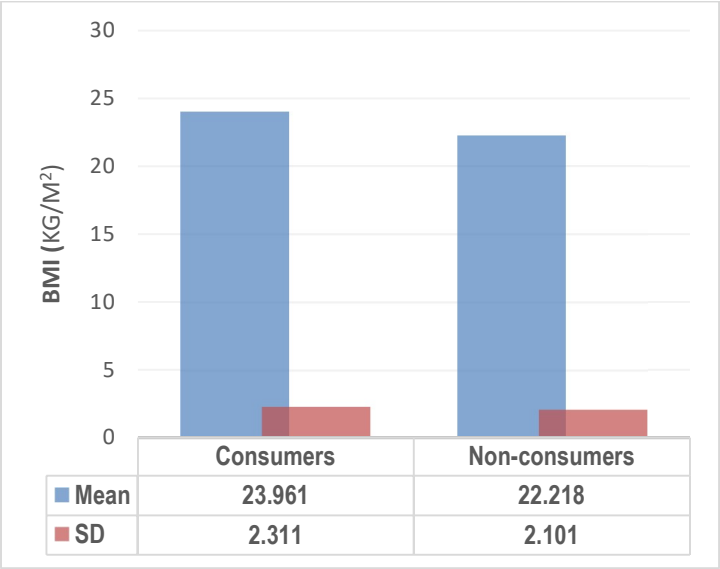

Figure 1: BMI change in response to energy drinks consumption (p=0.0083)

Serum lipid profile

Table (1) represents serum levels of total cholesterol, triglyceride, HDL-C, and LDL-C for both groups. Analysis of serum lipids and lipoproteins showed a significant increase in total cholesterol (154.882 ± 26.134 mg/dl), triglyceride (106.925 ± 37.788 mg/dl), and LDL-C (98.428 ± 32.113 mg/dl) upon energy drinks consumption as compared to the non-consumers group (total cholesterol: 118.475 ± 19.769 mg/dl, triglyceride: 71.265 ± 16.503 mg/dl, LDL-C: 61.750 ± 17.453 mg/dl). However, caffeinated energy drinks significantly (p=0.0003) decreased HDL-C (31.307 ± 10.213 mg/dl) in comparison to non-consumer individuals (HDL-C: 46.475 ± 11.515 mg/dl).

Table 1: Lipid profile changes in response to energy drinks consumption.

Parameters	Non-Consumers	Consumers	P-value
Total cholesterol (mg/dl)	118.475 ± 19.769	154.882 ± 26.134	<0.0001
Triglyceride (mg/dl)	71.265 ± 16.503	106.925 ± 37.788	0.0003
HDL-C (mg/dl)	46.475 ± 11.515	31.307 ± 10.213	<0.0001
LDL-C (mg/dl)	61.750 ± 17.453	98.428 ± 32.113	<0.0001

The values are represented as mean ± standard deviation (SD).

Lipid profile is important for dyslipidemia and associated with atherosclerosis, diabetes, obesity, and other degenerative disorders. HDL-C level has been inversely linked to the risk of cardiovascular diseases by several studies. However, although the effects on total cholesterol and LDL-C may not signal the adverse health impact of caffeinated energy drink consumption, it is noteworthy that we observed a significant decrease in HDL-C induced by caffeinated energy drinks. In addition, triglyceride, an atherogenic plasma lipid rich in apo C-III, increased (Famurewa et al., 2015).

The effect of a decrease in HDL-C and an increase in triglyceride may be significantly amplified in a chronic consumption of these drinks. Currently, limited literature is available on the effect of energy drinks on lipid profiles to compare results obtained in this study. The available literature by Ugwuja (2014), although inconsistent with our finding for HDL-C, reported that energy drinks might not have an important effect on lipid profile after administering a different energy drink. However, over the past research, there is a line of evidence on the effect of caffeine, the main content of an ideal energy drink, on lipid profile and predisposition to atherogenesis. Several experimental and human studies have shown significant alterations in lipid parameters induced by caffeine consumption. Conclusions of other studies pointed out that it is very likely that most of the observed effects after consuming energy drinks are mainly produced by caffeine content (Famurewa et al., 2015). Caffeine is known to stimulate adrenal glands increasing gluconeogenesis in the liver and lipolysis in adipose tissue, causing a rise in free fatty acids in the serum and futile recycling of triglyceride, which could be responsible for the increase of glucose and triglyceride in the serum (Raj et al., 2009).

Triglyceride-glucose index (TyG)

The subjects consuming energy drinks had a significant increase (p= 0.0002) in blood glucose (94.842 ± 11.235 mg/dl), concomitantly with a significantly elevated serum triglyceride (106.925 ± 37.788 mg/dl) in comparison to the subjects did not consume energy drinks (glucose: 83.495 ± 6.921 mg/dl; triglyceride: 71.265 ± 16.503 mg/dl) (Figure 2 and Table 1).

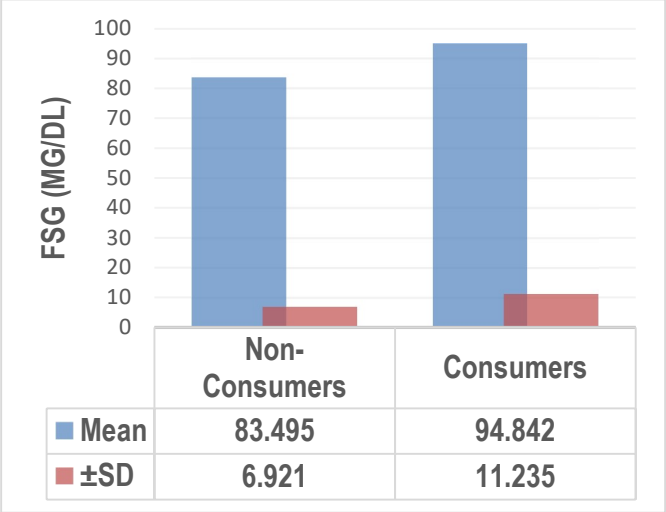

Figure 2: FSG change in response to energy drinks consumption (p= 0.0002)

Triglyceride glucose index (TyG) is increasingly utilized as a marker of insulin resistance and diabetes. TyG was significantly elevated ($p<0.0001$) in the subjects that consumed energy drinks (4.582 ± 0.161) compared to the subjects not consuming (4.331 ± 0.138) (Figure 3).

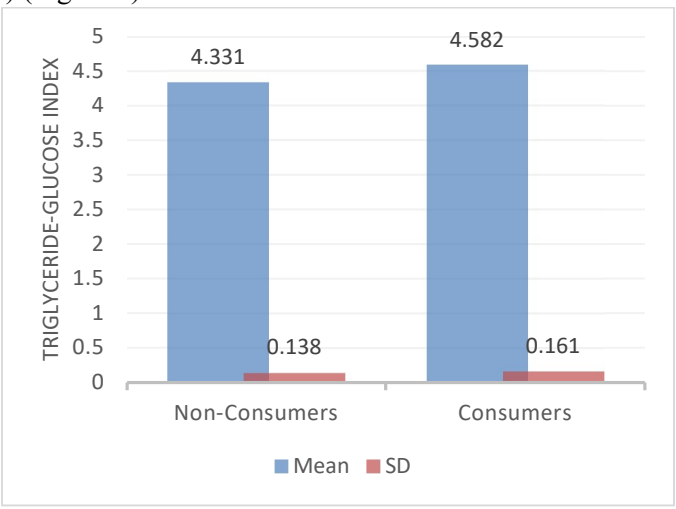

Figure 3: Effects of energy drinks consumption on TyG index (p<0.0001)

Our study observed a significant increase in fasting blood glucose in the consumer group compared to the non-consumers group. These findings agree with previously reported results that revealed a significant alteration in blood glucose after energy drink administration (Nowak et al., 2018). Moreover, energy drinks provide the consumer with a blood glucose increase that may stimulate an insulin response and enhance the glucose pool in body cells (MacDonald, 2016). Consumption of high sugar levels causes various detrimental effects on health, especially inducing insulin resistance, which is closely associated with developing metabolic disorders such as obesity or type 2 diabetes. In addition, high blood glucose levels may cause oxidative stress by overproduction of reactive oxygen species (Nagai et al., 2014).

Furthermore, some studies have proved that caffeine may be important in regulating insulin release and related metabolic disorders. González-Domínguez et al. (2017) showed that healthy young adults who consumed sugar-sweetened drinks with caffeine had a significant increase in blood glucose and insulin levels after 20–30 min. The authors concluded that adverse effects might result from the synergic effect of caffeine and sugar. Furthermore, a recent meta-analysis found that caffeine consumption increases blood glucose and prolongs the period of high blood glucose. Our study also found that a

marker of insulin resistance, TyG, was significantly elevated in subjects who consumed caffeinated energy drinks. These data collectively suggest that despite the lower carbohydrate intake, chronic consumption of sugar-free energy drinks may similarly promote insulin resistance to sugared energy drinks or soft drinks (Dewar and Heuberger, 2017).

Kidney function tests

Plasma urea, creatinine, and uric acid were significantly ($p<0.05$) affected by energy drinks (Table 2). However, plasma urea (24.546 ± 6.041 mg/dl), creatinine (0.796 ± 0.160 mg/dl), and uric acid (4.821 ± 1.049 mg/dl) were generally higher in the subjects who consumed energy drinks than in subjects who did not (plasma urea: 17.295 ± 5.150 mg/dl; creatinine: 0.692 ± 0.114 mg/dl; uric acid 3.955 ± 1.190 mg/dl).

A study conducted by Ugwuja (2014) on rats revealed that energy drink consumption had been associated with significantly higher urea, uric acid, and creatinine, consistent with the present findings. Furthermore, elevated plasma urea, uric acid, and creatinine in subjects who consumed energy drinks compared to non-consumer subjects suggest renal involvement. Both urea and creatinine are products of protein metabolism, which accumulate in the blood when the kidneys are affected (Ugwuja, 2014).

Table 2: Changes induced by the consumption of energy drinks on kidney function tests.

Parameters	Non-Consumers	Consumers	P-value
Blood urea (mg/dl)	17.295 ± 5.150	24.546 ± 6.041	<0.0001
Serum creatinine (mg/dl)	0.692 ± 0.114	0.796 ± 0.160	0.0168
Serum uric acid (mg/dl)	3.955 ± 1.190	4.821 ± 1.049	0.0105

The values are represented as mean ± standard deviation (SD).

Previous case reports revealed acute kidney injury induced by excessive ED consumption thought to be due to taurine (Greene et al., 2014). The doses that caused the injuries varied, probably due to interactions with other ingredients. According to Mansy et al. (2017), chronic consumption of energy drinks for 12 weeks increases creatinine and uric acid levels. Besides alterations in liver function tests, the observed effects were due to free radical production and oxidative stress. The results of this study are consistent with the results of another study conducted on a Red Bull that showed a significant increase in urea and creatinine levels after the administration of a drink (Al-badry, 2018).

Liver function enzyme (Alkaline phosphatase)

Figure 4 shows the effects of consuming caffeinated energy drinks on the alkaline phosphatase (ALP), commonly used as a marker to diagnose liver damage. The results show that caffeinated energy drink consumption significantly (p-value = 0.0015) increased the ALP level in the consumer group (68.107 ± 24.217) when compared with the non-consumers group (49.75 ± 10.977).

These results agree with Akande and Banjoko (2011), who reported increased ALP concentration in rats' serum after treatment with caffeinated energy drinks.

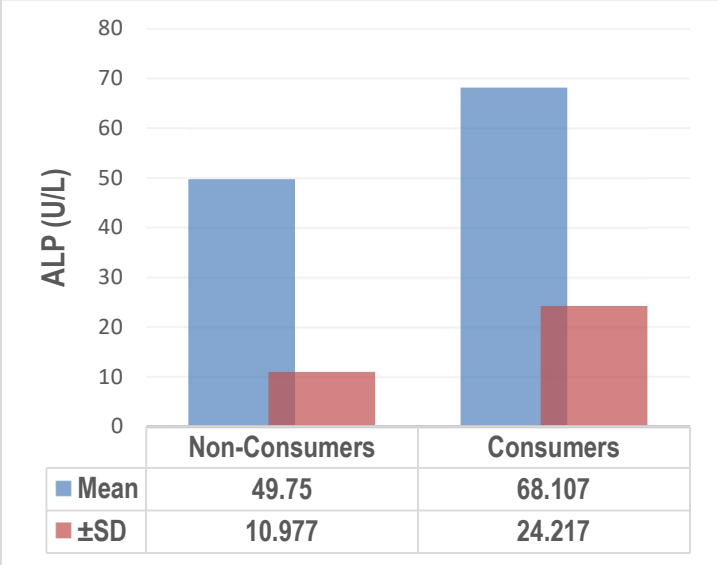

Figure 4: Alkaline phosphatase change in response to energy drinks consumption (P-value: 0.0015)

Many studies showed increased serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) during prolonged use of energy drinks (Kutia et al., 2020). However, the study performed by Costa-Valle et al. (2018), in which the energy drink "Red Bull" was singly administered to rats in a dose of 10 mL/kg, showed that the levels of these enzymes are within the reference range.

Lactate dehydrogenase

The result of the effects of caffeinated energy drinks on lactate dehydrogenase (LDH) level on students investigated showed that there was an increase in the level of LDH in consumers (154.607 ± 36.588 U/L) when compared with non-consumer subjects (119.450 ± 21.979 U/L) and the increase is statistically significant (p-value = 0.0004, Figure 5).

Lactate dehydrogenase (LDH) is a ubiquitous cytoplasmic cellular enzyme present in essentially all major organ systems and the extracellular space. Although there is no other metabolic function in this space, they are still beneficial because they serve as indicators suggestive of disturbances of cellular integrity induced by pathological conditions. It is known to be retained by viable cells with intact plasma membranes and released from cells with damaged membranes. LDH catalyzes the reversible conversion of pyruvate to lactate within cells during anaerobic glycolysis (Mubarak et al., 2018, Klein et al., 2020).

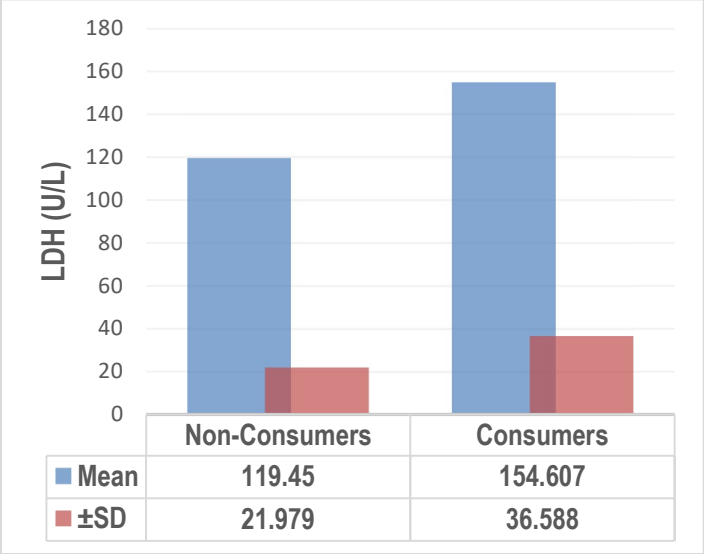

Figure 5: Lactate dehydrogenase (LDH) change in response to energy drinks consumption (P-value: 0.0004)

Insulin resistance

Energy drinks consumption did not significantly (***p=0.078***) change insulin concentration compared to non-consumer individuals (8.179 ± 4.484 mU/L; 6.348 ± 0.858 mU/L, respectively, ***p=0.0788***). Furthermore, significant differences in fasting plasma glucose (consumer: 94.842 ± 11.235 mg/dL; non-consumer: 83.495 ± 6.921 mg/dL, ***p=0.0002***) and insulin resistance (consumer: 1.939 ± 1.218; non-consumer: 1.314 ± 0.242, p=0.0289), were observed between consumers and non-consumers of energy drinks (Table 3).

Table 3: Effects of energy drinks consumption on insulin resistance

Parameters	Non-Consumers	Consumers	*p*-value
Fasting insulin (mU/L)	6.348 ± 0.858	8.179 ± 4.484	0.0788 ^{ns}
Fasting plasma glucose (mg/dL)	83.495 ± 6.921	94.842 ± 11.235	0.0002 **
HOMA-IR	1.314 ± 0.242	1.939 ± 1.218	0.0289 *

The values are represented as mean ± standard deviation (SD).

According to a review article on the content and safety of energy drinks, the amount of sugar in one can (or 500 mL) is typically about 13 teaspoons (slightly more than 1⁄4 cup of sugar). Sugar and caffeine have been noted to have synergistic effects causing a significantly high increase in blood glucose and insulin after their consumption (Nowak et al., 2018). Long-term consumption of these energy drinks containing excessive amounts of simple sugars can lead to obesity and insulin resistance. Reduction in insulin sensitivity causes pancreas beta cells to increase insulin secretion. Over time, beta cells cannot secrete sufficient insulin to maintain normal blood glucose levels, causing diabetes, and diabetes is a major risk factor and one of the most common causes of cardiovascular disease (Kaur et al., 2022). Concerningly, long-term exposure of the body to excessive amounts of simple sugar is associated with the development of obesity, insulin resistance, possibly fatty liver disease, and ultimately type 2 diabetes (Moran et al., 2022).

Effect of energy drink intake on Leptin/Ghrelin hormone

Intake of energy drinks was not only exhibited in increased body weight but also led to significantly (p-value<0.0001) decreased levels of leptin in plasma (716.276 ± 155.379 pg/ml) when compared to non-consumers of energy drinks (850.056 ± 187.293 pg/ml). However, plasma levels of ghrelin were significantly decreased (969.739 ± 185.612 pg/ml) in response to energy drinks consumption compared to subjects who did not consume energy drinks (1279.658

± 206.821 pg/ml).

After calculating the leptin/ghrelin ratio (data presented in Table 4), we should mention that no statistical significance was achieved. The mean leptin/ghrelin ratio was highest in subjects who consumed energy drinks compared to subjects who did not (0.745 ± 0.127 in consumers vs. 0.673 ± 0.135 in the non-consumer group).

Table 4: Effects of energy drinks consumption on leptin/ghrelin hormones

Parameters	Non-Consumers	Consumers	*p*-value
Leptin hormone (pg/ml)	850.056 ± 187.293	716.276 ± 155.379	0.0097 **
Ghrelin hormone (pg/ml)	1279.658 ± 206.821	969.739 ± 185.612	0.0001 ***
Leptin/ghrelin ratio	0.673 ± 0.135	0.745 ± 0.127	0.0647 ns

The values are represented as mean ± standard deviation (SD).

Ghrelin is secreted mainly in the stomach. It stimulates appetite and increases food intake. Leptin is a hormone, synthesized primarily in fat cells, that has been shown to decrease food intake and increase energy expenditure (Adamska-Patruno et al., 2018). Leptin and ghrelin are the main hormones working together but in opposite manners, regulating reciprocally and influencing appetite and hunger sensations (Arabi et al., 2019).

Leptin is secreted by adipose tissue but also by the stomach and mammary glands. It influences dietary intake, regulates food intake, and energy consumption, induces the satiety sensation—"a satiety hormone", and, consequently, determines the number of adipose deposits. At the same time, it is considered a pro-inflammatory adipokine, being involved in low inflammation associated with an increased amount of fat tissue. Most forms of obesity are associated even with leptin resistance (Sitar-Tăut et al., 2021).

Ghrelin, a stomach-derived hormone secreted by P/D1 cells, also has an important role in short-term appetite regulation and stimulation but is also involved in lipogenesis, insulin sensitivity, having anti-inflammatory properties, blocking the renin-angiotensin system, decreasing sympathetic activity, influencing blood pressure and heart rate, and finally being involved in cardiovascular disease development (low values being associated with increase global cardiovascular risk) (Sitar-Tăut et al., 2021). Previously reported data suggested that a low ghrelin level could be one of the pathogenetic pathways of type 2 diabetes development (Poykko et al., 2003).

The results presented in the current study found low leptin and ghrelin levels in the consumer group compared to the non-consumer group. We do not have a clear explanation for these discrepancies.

The leptin/ghrelin ratio appears to be a hunger regulator, with a higher ratio associated with hunger and decreased appetite (Adamska-Patruno et al., 2018). The previous hypothesis suggested that the leptin/ghrelin ratio can be used to identify subjects with an unfavorable evolution after obesity weight-loss therapeutic treatment, with weight regain after successful weight loss (Crujeiras et al., 2014). Arabi et al. (2019) demonstrated that patients with a high leptin/ghrelin ratio compared to those with a low leptin/ghrelin ratio had higher body mass index and were more likely to be diabetic.

Limited findings aimed at determining the relationship between ghrelin and caffeine, while to the best of our knowledge, no study has been carried out yet to show the effects of energy drinks on leptin and ghrelin. The findings in this study indicating a lower level of plasma ghrelin amongst subjects who simultaneously received energy drinks may suggest a probable appetite-inhibitory role for caffeinated drinks mediated by decreased plasma ghrelin (Rasaei et al., 2019).

Conclusions

The major finding of our study is that energy drinks, when consumed long-term and in high concentrations, interfere negatively with insulin resistance, lipid profile, creatinine, urea, uric acid, ALP, and LDH. However, energy drinks increase plasma insulin and glucose and decrease ghrelin and leptin hormones. The decrease in insulin sensitivity we documented due to caffeine ingestion is close to the magnitude of the increase in insulin sensitivity that can be

achieved with glucose-lowering agents and is clinically relevant. It has been ruled out that energy drinks, in their respective doses, may result in hepatic, renal, and cardiac damage. Further studies on a larger cohort are needed to specifically locate the mode of action of energy drinks, either directly on endocrine hormones or other metabolic pathways.

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