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Research Article

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How Caffeine Affects Alpha-Amylase: An In Vivo Study

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Abstract

Background: Caffeine is a widely consumed psychostimulant with various effects, including weight and metabolic parameters. Caffeine consumption has been associated with increased blood glucose levels and reduced insulin sensitivity. Since xanthine derivatives like caffeine can activate alpha-amylase, an enzyme essential for carbohydrate-to-glucose conversion, this study investigates whether this translates to increased amylase activity in living organisms and explores potential gene expression changes. **Methods:** Male NMRI mice were divided to 5 groups with 6 mice per group (total of 30 mice): Control (C), sham (sh) and three treated groups T30, T50 and T60 (receiving 30, 50, or 60 mg caffeine, respectively, by gavage, once a day for two weeks. Body weight was measured at the end of the experiment, after which blood sugar, alpha-amylase levels and gene expression as well as cholesterol levels were measured. **Results:** Body weights of mice which had received the highest dose of caffeine (60 mg/mL) for three weeks had significantly decreased weight compared with the other groups. However, blood sugar, serum alpha-amylase and cholesterol levels showed no significant differences between various groups. Levels of hepatic alpha amylase gene expression were significantly higher in the T30 group. **Conclusions:** In conclusion, the findings suggest that caffeine's effect on hepatic alpha-amylase, an enzyme potentially important in metabolism, could hold significance for understanding and treating metabolic diseases.

Keywords:
caffeine; purine; alpha-amylase; Amy expression; NMRI mice; blood sugar

1. Introduction

Caffeine, a naturally occurring purine compound, is consumed globally in various beverages and is one of the most widely used substances in the world. It could be considered as the most available and used edible "drug", with various effects ranging from the well-known "alertness" and performance at work [1] to ergogenic effect in athletes [2], and potential neuroprotective ones in neurodegenerative diseases which may be related to its interaction with adenosine receptors [3–5].

Others reported helpful effects of caffeine include reduced risk of cardiovascular diseases leading to "improved survival" [6], reduced risk of liver cancer [7], possible improvement of alopecia when used in its topical form [8] and more recently, potentially reducing viral reproduction in SARS-CoV-2 [9]. As with any other substance, there are known adverse effects associated with

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caffeine, such as possibility of hypertension [10] or anxiety [11], and even withdrawal syndrome [12].

Numerous studies concern caffeine's effect on metabolic disorders, such as obesity and diabetes. Caffeine is reported to increase blood glucose levels and reduce insulin sensitivity in diabetic patients [13] and impair glucose tolerance in lean, obese and diabetic persons [14]. Actually, increase of blood glucose, when measured shortly after a single dose of caffeine consumption had been already observed since the late 60s [15]. Overall, glucose homeostasis is widely reported to be negatively affected by caffeine [16], and related to a decrease of glucose uptake by skeletal muscles [17].

In a previous (in vitro) study [18], it has been found that alpha-amylase could be activated by xanthine derivatives, including caffeine. Alpha-amylase is an enzyme that is involved in the production of glucose from carbohydrate; more specifically, it acts on substrates like starch which contain alpha-1,4-glycosidic bonds. It catalyzes the hydrolysis of those bonds, which results into the production of smaller sugar molecules such as maltose and maltotriose. It has been hypothesized that the observed increase of amylase activity could be related to the reported effect of caffeine on blood glucose-as one possible mechanism [18]. As a continuation of that study, the effect of caffeine ingestion on biochemical parameters was tested in NMRI mice (e.g., blood glucose levels and alpha-amylase) as well as liver alpha-amylase expression, since liver alpha-amylase was observed to increase in obese mice [19].

2. Material and Methods

2.1. Animals and Treatment

Mice (NMRI, male, a total of 30) were acquired from Pasteur institute (Karaj-Iran). One week was allowed for acclimatization of the mice to laboratory conditions. Throughout the three-week study period, the mice received a standard laboratory chow and had unrestricted access to water. Following a seven-day adjustment phase, their average weight was recorded at 25 ± 2 g. After their acclimatization period, the mice were allocated at random into five distinct groups, each consisting of six mice per group: the Control group (C), the Sham group (sh), and three experimental groups labeled T30, T50, and T60. These experimental groups were administered doses of 30, 50, and 60 mg of caffeine, respectively, via oral gavage on a daily basis over the course of two weeks.

The sham group received water instead, by gavage (as caffeine solvent), while the Control group was not exposed to any gavage.

The experimental protocol was approved by the ethics committee of Endocrinology and Metabolism Research Institute (EMRI) (code: EC00311).

2.2. Measured Factors

Body weight was measured weekly. Following the experimental period, the mice were humanely euthanized with an intraperitoneal injection of ketamine/xylazine at a dosage of 100/7.5 mg/kg [20]. The collected blood samples were then spun at 3000 rpm for a duration of 5 min. The concentrations of Blood Sugar (BS), Alpha-Amylase (Amy), and Cholesterol (Chol) in these samples were determined using Pars Azmoon Co., Iran commercial kits through a photometric analysis technique.

2.3. mRNA Extraction and cDNA Synthesis

1 mL TRizol reagent was added to micro tubes containing 1 mg homogenized mouse liver tissue. RNA extraction was then carried out by Trizol reagents (Invitrogen) according to manufacturer's instructions. RNA precipitates were reconstituted in 100 µL of DEPC-treated water and subsequently preserved at −80 °C. The assessment of RNA integrity and quantity was conducted by evaluating the OD260/280 ratios and OD260 values, utilizing a NanoDrop spectrophotometer (Thermo Scientific NanoDrop 2000). Samples exhibiting an OD260/280 ratio below 1.6 were excluded from the study.

For cDNA synthesis, First Strand cDNA Synthesis Kit (Thermo Science) was used as the manufacturer recommends.

Quantitative Real-Time PCR

To assess the expression level of Amy 1 mRNA, quantitative real-time PCR was performed using the SYBR Premix Ex Taq II kit (Takara, Japan) and an ABI StepOne™ Sequence Detection System (Applied Biosystems, CA, USA). The reactions were set up in 98-well plates with a final volume of 20 µL, comprising 10 µL of 2× SYBR Premix Ex Taq, DNAse, 1 µL of forward and reverse primers (1 µM each), 7.2 µL of deionized water, 0.2 µL of ROX dye, and 1.5 µL of 10-fold diluted cDNA. The housekeeping gene hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used for normalization. The procedure was based on kit manufacturer (ThermoFisher Scientific) guidelines. Each PCR reaction was performed in duplicate using specific oligonucleotide primers for amplification. Real-time PCR was performed with an initial denaturation step of 10 s at 95 °C, 40 cycles at 95 °C for 5 s and 61 °C for 30 s.

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2.4. Statistical Analysis

The data was analyzed through SPSS software version 15. *T*-test followed by sample independent *t*-test were used to compare difference between experimental groups. The criterion for statistical significance was $p \leq 0.05$. Also, results were presented as mean $\pm$ SD.

3. Results

3.1. Body Weight and Biochemical Factors

As observed in Table 1, body weight of mice which had received the highest dose of caffeine (60 mg/mL) for two weeks had decreased as compared with the other groups, while other parameters including blood glucose levels, cholesterol and alpha-amylase levels had no significant differences between various groups.

3.2. Alpha Amylase Gene Expression

As shown in Table 2, levels of hepatic alpha amylase gene expression are somewhat different (higher) in the treated groups compared with control group, although significance was found only in the T30 group.

4. Discussion

Although caffeine is generally reported to increase blood glucose levels via decreasing glucose uptake by skeletal muscles or affecting epinephrine, large scale studies seem to find a positive effect for caffeine in reducing the risk or delaying the appearance of diabetes. These findings could be related either to a difference between acute and chronic ingestion of caffeine, or the fact that these studies are usually about "coffee" that contains other substances [17]. A report on an animal model of diabetes suggests that chronic consumption of caffeine (60 days) can improve glucose tolerance and decrease blood glucose levels in diabetic animals (while no effect is seen in healthy ones). This effect could be related to either antioxidant, thermogenic, adenosine receptor antagonism or affecting glucose transport mechanism [21]. An eight-week study on cases with hypercholesterolemia has also demonstrated that green/roasted coffee beans could ameliorate metabolic syndrome-related parameters (blood glucose and lipid levels) [22]. As so, chronic *vs* acute use, as well as caffeine dose [23] may actually be of importance in amelioration or deterioration of the metabolic state. To name a recent study, acute consumption of coffee in humans was not affecting serum glucose levels; however, the study points to other carbohydrate-rich intake that existed throughout the study time [24]. In this study too, blood glucose levels were not found to be increased in the caffeine treated group compared with control group. On the other hand, a significant decrease in weight in the treated group that was receiving the highest dose of caffeine was found. A previous study on rats has shown that caffeine intake can improve muscle thermogenesis [25], while fat oxidation and decreased leptin has also been observed in humans upon regular caffeine intake [26], resulting into weight loss. Additionally, appetite suppression, which a recent study has shown to correlate with the rate of coffee metabolism, may be another contributing factor [27].

The effect of coffee on lipid profile is suggested to be dependent on the method of beverage preparation and the components that would be more present. Consequently, boiled coffee increases serum cholesterol, but due to the higher presence of diterpenes, while filtered coffee is devoid of this effect [28]. Previous studies suggesting a rise in cholesterol linked to "caffeine" might not have differentiated the effects of its individual components [29]. In this study as well, no effect was observed on the cholesterol levels of the animals upon caffeine consumption.

Finally, an effect of caffeine on alpha-amylase gene expression has been observed, but not on its serum levels. This is actually a potentially important effect, although only found in the T30 group, since a relation between liver alpha-amylase increase (both on serum and gene expression levels) and early stages of obesity have been previously suggested. Alpha-amylase inhibition is considered a potential aid in diabesity [30], while on the other hand, presence of low amylase levels in serum has been associated with insulin resistance [31]. This is an effect related to the disease itself, and has not been analyzed in details (especially with the definition of a threshold). What has been observed in the current study could be related to the duration of the treatment, dosage of the compound, and the fact that we had healthy mice. Based on these results, it could be interesting to find caffeine effect on obese mice alpha-amylase levels.

5. Conclusions

In summary, this study found no significant increase in blood glucose levels in caffeine-treated groups, but a notable decrease in body weight at the highest dose, aligning with previous findings on caffeine's role in weight loss. The impact on lipid profiles depends on coffee preparation methods, with no effect observed in the present study. The key finding was an increase in hepatic alpha-amylase expression in the T30 group. Unlike its more well-known counterparts in the pancreas and saliva, hepatic alpha-amylase is a relatively understudied enzyme, and it is

Table 1: Measured weight and biochemical parameters. $p < 0.05$ was considered significant (*).

Groups	Body Weight (mean ± SD)	BS. (mean ± SD)	Chol. (mean ± SD)	α-Amylase (mean ± SD)
Control	41 ± 2.7	202 ± 19.5	135 ± 28	2626 ± 354
Sham	40 ± 3	226 ± 23	139 ± 30	2596 ± 218
T30	40 ± 4	184 ± 61	151 ± 30	2513 ± 414
T50	38 ± 4	224 ± 18	180 ± 14	2455 ± 285
T60	37 ± 3 *	194 ± 27	149 ± 27	2277 ± 403

Table 2: Liver alpha-amylase gene expression in different groups. $p < 0.05$ was considered significant (*).

Groups	Control (mean ± SD)	Sham (mean ± SD)	T30 (mean ± SD)	T50 (mean ± SD)	T60 (mean ± SD)
Alpha-amylase gene expression	0.89 ± 0.40	1.29 ± 0.78	1.58 ± 0.79 *	1.53 ± 0.74	1.37 ± 0.65

now suggested to be important in metabolic processes, especially with regard to weight control. This warrants further investigation, especially in obese models.

List of Abbreviations

C	Control group
Sh	the Sham group
T30	Experimental group receiving 30 mg caffeine/day
T50	Experimental group receiving 50 mg caffeine/day
T60	Experimental group receiving 60 mg caffeine/day
BS	Blood Sugar
Amy	Alpha-Amylase
Chol	Cholesterol
HPRT	Housekeeping gene hypoxanthine-guanine phosphoribosyltransferase

Author Contributions

Conceptualization, A.E.-H. and E.M.; supervision, A.E.-H.; methodology, E.M. and F.B.; formal analysis, F.B., R.K. and E.M.; investigation, E.M., F.B. and R.K.; writing—original draft preparation, F.B. and E.M.; writing—review and editing, A.E.-H. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

All data has been reported in the article.

Consent for Publication

Not applicable.

Conflict of Interest

The authors have no conflict of interests.

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Ethics Committee Approval

This study was approved by the Ethics Committee of Endocrinology and Metabolism Research Institute (EMRI) in accordance with Helsinki declaration and guidelines of the Iranian Ministry of Health and Medical Education with the approval code EC-00311, and project code 1392-01-106-1483.

Animal Rights Statement

The study was conducted following the Basel Declaration and ARRIVE guidelines.

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