Arch. Bas. App. Med. 10 (2021):17-23
www.archivesbamui.com

Research Article

Effect of short- and long-term administration of caffeine on salivary flow rate, amylase, IgA and C-reactive protein in male Wistar rats

Lasisi T. J.[1, 2], *Shittu S. T.[1], Kolade B. A.[1], Akobi A. M.[1], Usman A. A.[1]

[1.] *Department of Physiology, College of Medicine, University of Ibadan, Nigeria*
[2.] *Department of Oral Pathology, College of Medicine, University of Ibadan, Nigeria*

Accepted: January 29, 2022

Abstract
Background: Despite several reports on the effects of caffeine on various body systems, those on short- and long-term effect on salivary secretion are lacking. Aim: To evaluate the short- and long-term effect of caffeine on salivary secretion in rats.
Methods: Seventy-two male Wistar rats (110 - 150 g), randomly divided into two main groups (subdivided into 6 groups of 6 animals) were included. The short- and long-term groups were given different doses (3 - 40 mg/kg bw) of caffeine for 7 days and 28 days respectively. After treatments, stimulated saliva samples were collected and assessed for lag-time, flow rate, alpha amylase activity, secretory immunoglobulin A (IgA) and C reactive protein (CRP) concentrations. Results: Salivary lag-time was significantly lower in the 20 mg/kg and 40 mg/kg groups only compared to the control group ($p < 0.001$ for both) after short-term caffeine administration. Also, salivary amylase activity was significantly higher in the 40 mg/kg groups compared to the control group ($p = 0.01$) after short-term caffeine administration. Salivary flow rate was significantly lower in the 40 mg/kg group compared to the control group ($p < 0.01$) following long-term caffeine administration. No changes were observed in the levels of salivary IgA and CRP for both short- and long-term administration of the different doses of caffeine. Conclusion: Whereas short-term caffeine administration was associated with lower salivary lag-time and higher salivary amylase activity also at high doses, long-term caffeine administration was associated with reduced salivary flow rate at high dose only.

Key Words: Caffeine, Salivation, Immunoglobulin A, C-Reactive Protein, Rat

INTRODUCTION

Caffeine is a known stimulant of the central nervous system by blocking the action of adenosine on its receptors (Nehlig, 1992). It also stimulates the sympathetic division of the autonomic nervous system by increasing the plasma concentration of catecholamines (Shah, 2016). Some of the physiologic functions of caffeine include stimulation of the central nervous system, stimulation of the heart together with increased blood supply, relaxation of smooth muscle, bronchial dilatation, increased renal blood flow, renin secretion and diuresis (Leonard *et al*., 1987; Liu *et al*., 2018; Velickovic *et al*., 2019; Nehlig, 2022;). Similarly, it has been documented that caffeine has anti-inflammatory, antioxidant, antiproliferative and pro-motility effects on the gastrointestinal system. In addition, it stimulates gastric acid, biliary and pancreatic secretions, reduces risk of gallstone and causes changes in the composition of gut microbiota (Iriondo-DeHond *et al*., 2020; Nehlig, 2022). Despite the numerous data on the effect of caffeine on the gastrointestinal system, reports on salivary secretion, composition and function are scarce.

Saliva is a complex fluid produced by major and minor salivary glands to maintain the oral homeostasis in human and animals. Saliva serves many physiologic functions that are made possible by the numerous qualitative and quantitative properties (Mandel, 1989). The constituents of saliva have been implicated in its specific functions such as mechanical cleansing of the oral cavity, dilution of harmful substances, buffering, antimicrobial effect, mineralization of teeth, taste sensation, facilitation of swallowing and speech, initiation of digestion and promotion of healing following tissue injury (de Almeida *et al*., 2008; Proctor, 2016). Salivary secretion has its unique properties and numerous constituents of which salivary flow rate, secretory immunoglobulin A and amylase play significant roles. Hence, factors or conditions affecting these salivary parameters could impair oral homeostasis. Many studies have shown that salivary secretion is affected by various conditions, of which diet and food ingredients are important (Watson and Antal., 1980; Johannsson and Ryberg., 1991; Johnson *et al*., 2018; Lasisi *et al*., 2018). Caffeine and its effects on biological system have been widely studied and in spite of these ubiquitous reports, questions on the potential health benefits/risks associated with caffeine consumption still persist. For example, most reports on the effect of caffeine on salivary secretion were inconclusive. While some studies documented reduced salivary flow rate following caffeine consumption (Barasch and Gordon, 2016; Hala Zakaria and

**Author for Correspondence:* Tel: +2348052644680
E-mail: *toyinsts@yahoo.com*

Akram, 2019), others reported no change (Imamura *et al*., 2015; Masoumi *et al*., 2016; Hildebrandt *et al*., 2019). Similarly, Klein *et al*, (2010) reported a dose-dependent increase in salivary amylase activity following caffeine ingestion and acute stress in healthy young adult males. Other studies reported lack of changes in the salivary amylase levels following caffeine intake (Nater and Rohleder, 2009; Klein *et al*., 2014). Furthermore, most studies on the effect of caffeine on salivary secretion (Imamura *et al*., 2015; Barasch and Gordon, 2016; Masoumi *et al*., 2016; Hala Zakaria and Akram, 2019; Hildebrandt *et al*., 2019) were carried out following short-term (30 to 60 minutes) caffeine administration despite the several reports on caffeine habituation which tends to mask the appreciable functional differences occasioned by short-term caffeine exposure (Bangsbo *et al*., 1992). To the best of our knowledge, reports on the long-term effect of caffeine on salivary secretion are scarce hence, this study.

MATERIALS AND METHODS

Experimental animals and grouping: All experimental procedures and animal handling were carried out in strict adherence with the institutional ethical guidelines which were in accordance with the NIH publication
No. 85–23 guidelines. Seventy-two male Wistar rats weighing between 110 and 150 g were included in this study. The animals were housed in plastic cage with perforated covering lid at the central animal house of the College of Medicine, University of Ibadan. They were fed with standard pellet feed (Ladokun feeds®) which contained 67.9 % carbohydrate, 21 % protein, 3.5 % fat, 6 % fibre, 0.8 % minerals and 0.8 % vitamins. Drinking water was given to them ad libitum. The protocols of the experiment are shown in figure 1. The rats were acclimatized for two weeks, maintained under standard conditions. After acclimatization, the rats were randomly divided into two main groups of 36 rats each which were subsequently subdivided into 6 groups of 6 rats each as indicated in figure 1. The caffeine preparation and administration were done according to the method previously described (Angelucci *et al*., 2002) with slight modifications. The short-term groups were given caffeine for 7 days while the long-term groups were administered caffeine for 28 days. The control groups were given equal volume of normal saline. Water and feed were given to all the groups ad libitum throughout the period of experiment. Body weights were checked using a weighing scale (Citizen, China).

Saliva collection: After the completion of caffeine administration, stimulated whole saliva samples were collected between 8 and 10 am from all groups. Following overnight fasting with access to water only, animals were anaesthetized with ketamine (100 mg/kg i.p.) and Zylazine (5 mg/kg body weight i.m.) (Lasisi *et al*., 2018). After anesthesia, stimulated saliva sample was initiated using pilocarpine hydrochloride (10 mg/kg body weight i.p.; (Zigma, USA) (Lasisi *et al*., 2014). Saliva samples were collected into graduated sterile plain bottles (placed on ice cubes) over a period of 10 minutes. Samples were stored at -20^{o}C.

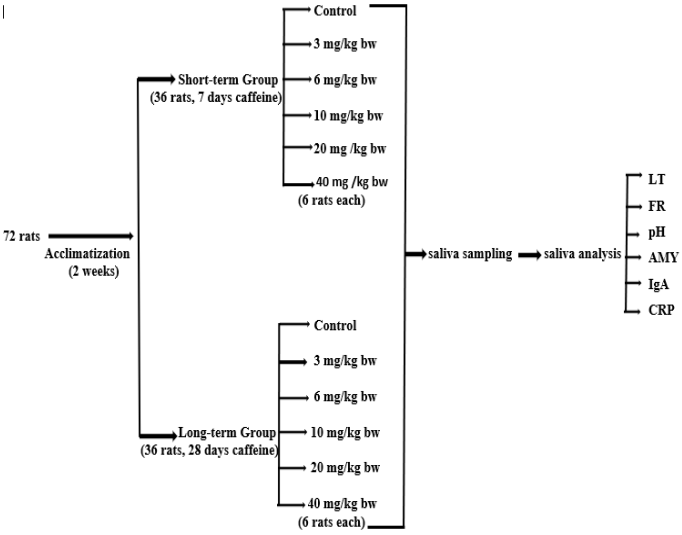

Figure 1:
Experimental design
LT = Lag-time, FR = Flow rate, AMY = Amylase, IgA = Immunoglobulin A, CRP = C reactive protein

Lag-time and flow rate determination: The salivary lag-time was evaluated as the duration of time between the administration of pilocarpine and the onset of salivation as indicated by wetness of the floor of the mouth (Lasisi *et al*., 2018). The flow rate of saliva samples was determined by dividing the volume of saliva secreted by ten minutes (the period for saliva collection).

Determination of salivary amylase, IgA and CRP concentrations using ELISA: Concentrations of amylase was determined using kinetic enzyme reaction whilst concentrations of IgA and CRP in saliva samples were determined using ELISA with strict adherence to the manufacturer's instructions provided in the kits (Salimetrics, UK)
Reagent preparation: All reagents were brought to room temperature and mixed before use. The plates were brought to room temperature. Appropriate volume (as indicated in the manual) of salivary amylase, immunoglobulin A (SIgA), and c-reactive protein (CRP) diluents were prepared for conjugate dilution. Wash buffer, diluents and stop solutions were prepared. Standards were also diluted serially.

Procedure for salivary alpha-amylase activity: The saliva samples and reagents were brought to room temperature and other procedures were carried out as instructed by the manual given in the Salimetrics® salivary alpha-amylase kinetic enzyme kit, (Suffolk, UK). The assay procedure was based on the method of Wallenfels *et al*. (1978) which utilizes 2-chloro-p-nitrophenol linked with maltotriose as a chromogenic substrate to determine the activities of alpha amylase. The alpha-amylase activity of the sample is directly proportional to the increase in absorbance at 405 nm. For ease of use, the reaction was read in a 96-well microtiter plate. The assay was standardized by running the low and high salivary alpha-amylase controls provided by the kit manufacturer with each assay. The micro plate reader was set up to read in center measurement kinetic mode initially at 1 minute, then again 2 minutes later. A 1:10 dilution of saliva was prepared by combining 10 µL saliva with 90 µL of alpha-amylase diluent.

The mixture was diluted further by pipetting 10 µL of the 1:10 dilution with 190 µL of additional alpha-amylase diluents. This gave a final concentration of 1:200. The alpha-amylase solution in the microtiter plate was incubated at 37°C. 8 µL of prediluted controls and samples were added to their respective wells. 320 µL of preheated alpha-amylase was added to each well using a multichannel pipette. The microtiter plate was then placed in the plate reader and read at exactly 1 minute and 3 minutes at 405 nm. The absorbance value obtained at 1 minute was subtracted from the absorbance value obtained at 3 minutes. The resulting absorbance value was multiplied by the standard conversion factor. Results were expressed in U/mL

Calculations
(ΔAbs/min x TV x DF)/MMA x SV x LP = U/mL of α-amylase activity in sample
Where:
ΔAbs./min = Absorbance difference per minute
TV = Total assay volume (0.328 mL)
DF = Dilution factor
MMA = Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
SV = Sample volume (0.008 mL)
LP = Light path = 0.97 (specific to plate received with kit)

Sample preparation for IgA determination: Saliva samples were diluted into 1:5 (25 µL saliva into 100 µL SIgA diluents). 10 µL of standards, controls, and 1:5 diluted samples were added to separate tubes containing 4 mL of SIgA diluents. 10 µL SIgA diluents were added to zero tube.
Initial incubation with conjugate in tubes: 1:120 dilution of conjugate (25 µL into 3 mL SIgA diluent) was prepared. 50 µL was immediately added into a tube for each standard, control, or unknown. Then invert to mix. Tubes was incubated for 90 minutes at room temperature.

Transfer of Incubated samples to assay plate: Tubes were mixed by inversion again. 50 µL was transferred from each tube to the assay plate according to the layout. 50 µL SIgA diluents were added to the Non-specific binding wells. Then the plate was covered. The plate was incubated for 90 minutes at room temperature with continuous mixing at 400 rpm. Plate was washed 6 times with wash buffer and blotted. 50 µL tetramethyl benzidine solution was added to each well and mixed 5 minutes at 500 rpm. Plate was incubated in dark at room temperature for an additional 40 minutes. 50 µL stop solution was added to each well and mixed for 3 minutes at 500 rpm. Finally, the bottom of the plate was wiped clean and the optical density was read on a standard plate reader at 450 nm within 10 minutes of adding stop solution.

Procedure for C-reactive protein analysis: analysis was carried out according to the manufacturer's instruction (Salimetrics, UK). Briefly, saliva samples were diluted in the ratio 1:10. 50 µL of CRP standard, CRP control and diluted saliva samples were pipetted into appropriate wells. 50 µL of sample diluent was pipetted into two wells to serve as the zero standard. 1:250 dilution of the CRP Antibody Enzyme Conjugate was prepared from which 150 µL was immediately added to into each well of CRP standard, CRP control or diluted saliva samples. Plate was covered with the provided adhesive cover, mixed and incubated for 2 hours at room temperature. Plate was washed 4 times with wash buffer and blotted after the last wash. 200 µL of TMB substrate solution was added to each well. Plate was incubated in the dark at room temperature for 30 minutes while being mixed constantly at 500 rpm. 50 µL of stop solution was added to each well and mixed for 3 minutes at 500 rpm. Finally, the bottom of the plate was wiped dry and the optical density was read on a standard microplate reader at 450 nm within 10 minutes of adding the stop solution.

Data Analysis: Data analysis was done using IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. The main outcome variables were mean body weight, submandibular salivary gland weight, salivary lag time, flow rate, pH and salivary amylase, IgA and CRP concentrations. The mean and standard error of mean (SEM) were calculated for each data group. The mean values were compared using ANOVA and Duncan post hoc tests. Result with p-value less than 0.05 was considered significant.

RESULTS

Effect of short- and long-term caffeine administration on body and salivary glands weights: After short-term caffeine administration, the body weights were not significantly different among the groups, whereas after long-term caffeine administration, the body weight was significantly lower in the 20 and 40 mg/kg groups compared to the control (p = 0.009). After short- and long-term caffeine administration, the weights of submandibular salivary glands were not significantly different among the groups (Table 1).

Table 1:
Effect of short- and long-term caffeine treatment on body weight and weights of submandibular glands

Variable	Duration	Treatment					
		Control	3 mg	6 mg	10 mg	20 mg	40 mg
Body Weight (g)	**Short**	173.67 ± 10.18	175.83 ± 10.14	181 ± 10.81	173.5 ± 7.25	167 ± 5.91	171.33 ± 7.39
	Long	196.83 ± 12.42	215 ± 2.66	209.33 ± 11.68	214 ± 11.56	172.83 ± 9.93*	164.83 ± 14.67*
RSMG (g)	**Short**	0.17 ± 0.01	0.17 ± 0.02	0.18 ± 0.01	0.19 ± 0.02	0.21 ± 0.01	0.19 ± 0.01
	Long	0.17 ± 0.03	0.21 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.20 ± 0.01	0.19 ± 0.01
LSMG (g)	**Short**	0.17 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.18 ± 0.01	0.18 ± 0.01
	Long	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.21 ± 0.01	0.19 ± 0.02

RSMG = right submandibular gland, LSMG = left submandibular gland, *P<0.05

Effect of short- and long-term caffeine administration on salivary lag-time and flow rate: The salivary-lag time was significantly lower in the 20 mg/kg and 40 mg/kg groups compared to the control group ($p < 0.001$) after short-term caffeine administration. However, no significant difference was observed in the salivary-lag time among the groups after long term caffeine administration ($p = 0.32$). No significant difference was observed in the salivary flow rate among the groups ($p = 0.28$) after short-term caffeine administration; whereas the salivary flow rate was significantly lower in the 20 mg/kg and 40 mg/kg groups compared to the control group ($p < 0.01$) following long term caffeine administration (Table 2).

Table 2:
Effect of short- and long-term caffeine treatment on salivary lag time and flow rate

Variable	Duration	Treatment					
		Control	3 mg	6 mg	10 mg	20 mg	40 mg
Lag Time (mins)	**Short**	3.67 ± 0.21	3.83 ± 0.31	2.5 ± 0.22*	4.33 ± 0.67	1.00 ± 0.001**	1.17 ± 0.17**
	Long	3.83 ± 0.54	4.33 ± 0.21	4 ± 0.26	3.8 ± 0.37	2.8 ± 0.37	3.8 ± 0.8
Flow Rate (mls/min)	**Short**	0.05 ± 0.01	0.07 ± 0.002	0.07 ± 0.007	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
	Long	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.004	0.05 ± 0.01	0.04 ± 0.01*

*P<0.05, **P<0.01

Table 3:
Effect of short- and long-term caffeine treatment on salivary amylase, IgA and CRP

Variable	Duration	Treatment					
		Control	3 mg	6 mg	10 mg	20 mg	40 mg
Salivary amylase (x 10^2 U/ml)	**Short**	150.73 ± 47.86	132.38 ± 24.65	139.37 ± 12.95	177. 26 ± 36.45	180.21 ± 38.68	322.36 ± 24.97**
	Long	122.98 ± 11.21	62.15 ± 6.94	187.59 ± 43.11	68. 65 ± 13.08	111.08 ± 28.61	109.11 ± 43.87
Salivary IgA (U/ml)	**Short**	596.66 ± 1.04	599.02 ± 0.71	598.36 ± 0.5	595.92 ± 1.25	598.05 ± 0.84	597.17 ± 0.47
	Long	599.13 ± 0.7	599.31 ± 0.73	597.71 ± 0.53	598.65 ± 0.92	597.57 ± 0.25	601.27 ± 1.92
Salivary CRP (x 10^4 pg/ml)	**Short**	139.17 ± 15.24	100.83 ± 24.27	200 ± 63.77	79.17 ± 16.86	137.5 ± 21.09	183.33 ± 43.26
	Long	136.67 ± 18.83	152.5 ± 57.3	168.33 ± 74.58	96.67 ± 36.93	216.67 ± 45.66	164.17 ± 18.77

**P<0.01

Effect of short- and long-term caffeine administration on salivary alpha-amylase, IgA and C-reactive protein: The salivary amylase activity was significantly higher in the 40 mg/kg groups compared to the control group ($p = 0.01$) after short-term caffeine administration; whereas no significant difference was observed in the salivary amylase activity among the groups after long term caffeine administration ($p = 0.06$) (Figure 2). No significant difference was observed in the concentration of salivary IgA as well as CRP after short-term and long-term caffeine administration (Table 3).

DISCUSSION

Caffeine consumption is usually on a long-term basis as many consumers of caffeine even get addicted to it because of the stimulant effect (Farkas, 1979; Lam *et al*., 2018; Sharma *et al*., 2020). To the best of our knowledge this report is the first on the effect of long-term administration of caffeine on salivary secretion and the findings have indicated that most of the salivary parameters studied were not affected by either short- or long-term administration of different doses of caffeine up to 40 mg/kg. Importantly, only amylase activity was significantly higher in the 40 mg/kg group following short-term caffeine administration. Similarly, salivary flow rate was significantly lower in the 40 mg/kg group after long-term caffeine administration only.

Our findings showed that short-term (7 days) administration of different doses of caffeine up to 40 mg/kg body did not affect body weight as well as weight of the submandibular salivary gland.

Long term caffeine however, was associated with lower body weight in the 40 mg/kg group compared to the groups that received 3 mg/kg and 10 mg/kg. Consistent with our observation, studies have shown that long-term caffeine consumption is associated with reduced weight gain especially when taken in high doses (Dunlop and Court., 1981; Choi *et al*., 2016). Similarly, long-term caffeine ingestion in high doses has been associated with reduced serum levels of growth promoting factors such as insulin-like growth factor 1 (IGF-1), estradiol, and testosterone (Dunlop and Court, 1981). It was demonstrated that caffeine consumption can dose- and time-dependently inhibit longitudinal bone growth in immature male rats, possibly by blocking the physiologic changes in body composition and hormones relevant to bone growth (Choi *et al*., 2016). Similarly, it was reported that caffeine decreased the expression of adipogenesis-related genes in a dose-dependent manner which could contribute to the associated reduced body weight (Su *et al*., 2013).

To the best of our knowledge our study is the first to document the effect of caffeine on salivary lag-time. Salivary lag-time is important in the evaluation of salivary gland function as it indicates the threshold for salivary secretion after stimulation. Impaired salivary lag-time has been reported under different condition such as protein malnutrition, salivary gland injury due to ductal ligation and irradiation (Lasisi *et al*., 2015; Lasisi *et al*., 2019; Su *et al*., 2020). In the present study only short-term caffeine consumption was associated with lower

salivary-lag time in the 20 mg/kg and 40 mg/kg groups compared to the control. It is possible that high doses of caffeine for a short period have a synergistic effect with pilocarpine which is a parasympathomimetic drug. This effect was also noticed in the salivary flow rate which was about 60 % higher in the 40 mg/kg group compared to the control. Salivary flow rate however, was lower in the 40 mg/kg groups following long-term caffeine administration which may be explained by the reduced body weight and not necessarily the sympathomimetic effect. Reduced body weight has been associated with lower salivary secretion in rats (Lasisi *et al*, 2014; Lasisi *et al*, 2018).

Salivary amylase makes a substantial part of salivary proteins constituting approximately 50 % of the total protein produced by the salivary glands (Makinen, 1989). Recently, salivary alpha amylase has been given many considerations in the neuroendocrine-stress studies because it has shown to be a good marker of the sympathetic nervous system activity (Nater and Rohleder, 2009). In the present study, salivary amylase activity was significantly higher in the 40 mg/kg group compared to the control group after short-term caffeine administration; whereas no significant difference was observed in the salivary amylase activity among the groups after long term caffeine administration. This may imply that high dose of caffeine for a short period is necessary for the stimulatory effect on salivary amylase. The stimulating effects of caffeine on salivary alpha amylase has been attributed to the sympathomimetic effects (Nater and Rohleder, 2009), This, however has not been generally observed in previous studies (Nater *et al*., 2007; Gibson, 2012). Available data from human studies have reported conflicting results. While some studies reported that caffeine administration stimulated salivary amylase activity (Morrison *et al*. 2003; Bishop *et al*., 2006), others documented no effect (Nater *et al*., 2007; Gibson, 2012).

Salivary IgA is a key factor in the antimicrobial function of saliva and studies have reported inconsistent changes in the salivary IgA levels following caffeine consumption (Bishop *et al*., 2006; Gibson, 2012). Similar to our observation in the short-term groups, Gibson (2012) reported that salivary IgA concentrations as well as the secretion rates were unaffected by caffeine administration in human both at rest and following exercise. Thus, within the context of our study in rats, caffeine consumption at different doses up to 40 mg/kg body weight did not affect salivary IgA level regardless of whether consumption was for a short-term or long-term. Although salivary IgA in rats has been reported to be significantly affected by sympathetic stimulation (Carpenter *et al*., 1998), with increased secretion rates following increased frequencies of nerve stimulation (Proctor *et al*., 2003), findings from the present study however did not indicate that administration of caffeine (a potent stimulator of sympathetic activity agent) causes elevated salivary IgA level.

C-reactive protein (CRP) is a key marker of inflammation under different conditions. It is a pentameric 125.5 kD positive acute phase protein (APP) that mediates innate immunity. It triggers the classical pathway of the complement cascade by binding foreign pathogens and damaged cells. Intact CRP has a mass above the cut-off limit for renal excretion (43 kDa) and has been shown to be a good circulating biomarker of inflammation (Sproston *et al*., 2018). Although serum CRP is the most commonly used clinical method for assessing non-specific inflammation, salivary CRP has also been reported to be a promising alternative maker by many studies (Ouellet-Morin *et al*., 2011; Out *et al*., 2012; Pace *et al*., 2013). It has been observed that assessment of salivary CRP could offer a more accurate estimate of systemic inflammation than peripheral measures of cytokines, which are locally synthesized (Ouellet-Morin *et al*., 2011). In the present study, there was no difference in the levels of salivary CRP among the groups after both short- and long-term caffeine administration. Although, coffee consumption has been shown to be associated with reduced risk of low-grade inflammatory conditions (Paiva *et al*., 2019), the relationship between coffee consumption and inflammation is still unsettled. Clinical trials assessing the effect of coffee, caffeine or other coffee components on inflammatory markers have reported varying results (Wedick *et al*., 2011; Ohnaka *et al*., 2012; Kempf *et al*., 2015; Owoyele *et al*., 2015). Most human studies on the effects of coffee on C-reactive protein found no changes in serum C-reactive protein (CRP) (Wedick *et al*., 2011; Ohnaka *et al*., 2012). In contrast, one animal study found decreased serum CRP levels in response to caffeine (Owoyele *et al*., 2015). Fewer studies on caffeine consumption and salivary C reactive protein levels in human and animals hinder comparison of our findings.

Although caffeine generally activates the sympathetic nervous system, one of the reasons for the lack of this effect on most of the salivary parameters studied following short-term and long-term consumption could be attributed to the tolerance effect on the body system (Evans, 1992; Beaumont *et al*., 2017). Caffeine performs its stimulatory effect by blocking adenosine receptors and it has been shown that regular consumption of caffeine increases the production of adenosine receptors through negative feedback mechanism thus causing a decreasing effect of caffeine on the body system (Boulenger, 1983).

CONCLUSION

Short- and long-term administration of different doses of caffeine produced variable and minimal effects on salivary secretion in Wistar rats. Whereas short-term caffeine administration was associated with lower salivary lag time and higher salivary amylase activity at high doses, long-term caffeine administration was associated with lower salivary flow rate also at high dose.

REFERENCES

Angelucci, M.E.M., Cesário, C., Hiroi, R.H., Rosalen, P.L., and DaCunha, C. 2002. Effects of caffeine on learning and memory in rats tested in the Morris water maze. *Braz. J. Med. Biol. Res.* 35 (10): 1201-1208.

Bangsbo, J., Jacobsen, K., Nordberg, N., Christensen, N.J. and Graham, T. 1992. Acute and habitual caffeine ingestion and metabolic responses to steady-state exercise. *J. Appl. Physiol.* 72(4):1297-303.

Barasch, A. and Gordon, S. C. 2016. Effects of Caffeine on Salivation. *Oral Health Case Rep*. 1(2): 1-4 1000107.

Beaumont, R., Cordery, P., Funnell, M., Mears, S., James, L. and Watson, P. 2017. Chronic ingestion of a low dose of caffeine induces tolerance to the performance benefits of caffeine. *J. Sport Sci.* 35(19) :1920-1927

Bishop, N.C., Walker, G.J., Scanlon, G.A., Richards, S. and Rogers, E. 2006. Salivary IgA responses to prolonged

intensive exercise following caffeine ingestion. *Med. Sci. Sports Exerc*. 38 (3): 513-519.

Boulenger, J.P., Patel, J., Post, R.M., Parma, A.M., and Marangos, P.J. 1983. Chronic caffeine consumption increases the number of brain adenosine receptors. *Life Sci.* 32(10):1135-1142.

Carpenter, G.H., Garrett, J.R., Hartley, R.H. and Proctor, G. B. 1998. The influence of nerves on the secretion of immunoglobulin A into submandibular saliva in rats. *J. Physiol.* 512 :567-573.

Choi, Y.Y., Choi, Y., Kim, J., Choi, H., Shin, J. and Roh, J. 2016. Peripubertal Caffeine Exposure Impairs Longitudinal Bone Growth in Immature Male Rats in a Dose- and Time-Dependent Manner. *J. Med. Food*. 19(1):73-84.

de Almeida Pdel, V., Grégio, A. M., Machado, M.A., de Lima, A.A. and Azevedo, L.R. 2008. Saliva composition and functions: a comprehensive review. *J. Contemp. Dent. Pract.* 9(3):72-80.

Dunlop, M. and Court, J.M. 1981. Effects of maternal caffeine ingestion on neonatal growth in rats. *Biol. Neonate* 39(3-4):178-84.

Evans, S.M. and Griffiths, R. R. 1992 Caffeine tolerance and choice in humans. Psychopharmacology (Berl). 108:51–59.

Farkas, C.S. 1979. Caffeine intake and potential effect on health of a segment of northern Canadian indigenous people. *Int. J. Addict.* 14(1):27-43.

Gibson, C.A. 2012. Dose response effects of caffeine ingestion on salivary immunoglobulin A following high-intensity exercise. MSc Thesis submitted to Aukland University of Technology. Available at https://openrepository.aut.ac.nz/handle/10292/4682

Hala, Z. M. and Akram, N. 2019. Evaluation of Side Effects of Caffeine on Xerostomia. *EC Dental Sci.* 18(10): 2497-2506

Hildebrandt, G.H., Tantbirojn, D., Augustson, D.G. and Guo, H. 2013. Effect of Caffeinated Soft Drinks on Salivary Flow. *J. Caffeine Res.* 3(3):138-142.

Imamura, A., Nakamoto, T., Mukaibo, T., Munemasa, T., Kondo, Y., Kidokoro, M., Masaki, C. and Hosokawa, R. 2015. Effects of Beverage Ingredients on Salivary Fluid Secretion with an ex vivo Submandibular Gland Perfusion System: Tannic Acid as a Key Component for the Inhibition of Saliva Secretion. *Open J. Stomatol.* 5:12-18.

Iriondo-DeHond, A., Uranga, J.A., Del Castillo, M.D., and Abalo, R. 2020. Effects of coffee and its components on the gastrointestinal tract and the brain-gut axis. *Nutrients*. 13(1):88.

Johannsson, I. and Ryberg, M. 1991. The effects of moderate protein deficiency on beta-adrenoceptor density in rat parotid and submandibular salivary glands. *Arch. Oral Biol*. 36(8):591-4.

Johnson, D.A., Lopez, H. and Navia, J. M. 1995. Effects of protein deficiency and diet consistency on the parotid gland and parotid saliva of rats. *J. Dent. Res*. 74(8):1444-52.

Klein, L.C., Bennett, J.M., Whetzel, C.A., Granger, D.A. and Ritter, F.E. 2010. Caffeine and stress alter salivary alpha-amylase activity in young men. *Hum. Psychopharmacol.* 25(5):359-67.

Klein, L.C., Whetzel, C.A., Bennett, J.M., Ritter, F.E., Nater, U.M. and Schoelles, M. 2014. Caffeine administration does not alter salivary α-amylase activity in young male daily caffeine consumers. *BMC Res. Notes.* 7:30.

Lam, T., Ogeil, R.P., Allsop, S., Chikritzhs, T., Fischer, J., Midford, R., *et al*. 2018. Insomnia and Regulation of Sleep-Wake Cycle With Drugs Among Adolescent Risky Drinkers. *J. Clin. Sleep Med.*14(9):1529-1537.

Lasisi, T.J. and Alada, A.R. 2015. Effects of induced-kwashiorkor on salivary parameters in Wistar rats. *Afr. J. Med. Med. Sci.* 44(2):125-32.

Lasisi, T.J., Shittu, S.T. and Alada, A.R. 2018. Re-establishing normal diet following high fat-diet-induced obesity reverses the altered salivary composition in Wistar rats. *J. Basic Clin. Physiol. Pharmacol.* 30(1):111-120.

Lasisi, T.J., Shittu, S.T. and Alada, A.R. 2019. Switching to normal diet reverses kwashiorkor-induced salivary impairments via increased nitric oxide level and expression of aquaporin 5 in the submandibular glands of male Wistar rats. *Appl. Physiol. Nutr. Metab.* 44(4):365-372.

Lasisi, T.J., Shittu, S.T., Oguntokun, M.M. and Tiamiyu, N.A. 2014. Aging affects morphology but not stimulated secretion of saliva in rats. *Ann. Ib. Postgrad. Med.* 12(2):109-14

Leonard, T.K., Watson, R.R. and Mohs, M.E. 1987. The effects of caffeine on various body systems: a review. *J. Am. Diet Assoc.* 87(8):1048-53.

Liu, C.W., Tsai, H.C., Huang, C.C., Tsai, C.Y., Su, Y.B., Lin, M.W., *et al*. 2018. Effects and mechanisms of caffeine to improve immunological and metabolic abnormalities in diet-induced obese rats. *Am. J. Physiol. Endocrinol. Metab.* 314(5):E433-E447.

Makinen, K.K. (Ed.) 1989. Salivary enzymes. In human saliva: Clinical Chemistry and Microbiology. J. Tenovuo, editor. CRC Press, Boca Raton, Florida (USA). 93-119.

Mandel, I.D. 1989. The role of saliva in maintaining oral homeostasis. *J. Am. Dent. Assoc*.119(2):298-304.

Masoumi, S., Setoudehmaram, S., Golkari, A. and Tavana, Z. 2016. Comparison of pH and Flow Rate of Saliva After Using Black Tea, Green Tea and Coffee in Periodontal Patients and Normal Group. *J. Dent. Sch.* 34(4): 235-43.

Morrison, W.E., Haas, E.C., Shaffner, D.H., Garrett, E.S. and Fackler, J.C. 2003. Noise, stress, and annoyance in a pediatric intensive care unit. *Crit. Care Med.* 31: 113– 119.

Nater, U.M., Rohleder, N., Schlotz, W., Ehlert, U. and Kirschbaum, C. 2007. Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 32 (4), 392-401.

Nater, U.M. and Rohleder, N. 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34: 486-496.

Nehlig, A., Daval, J.L. and Debry, G. 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Rev.* 17(2):139-170.

Nehlig, A. 2022. Effects of Coffee on the Gastro-Intestinal Tract: A Narrative Review and Literature Update. *Nutrients.* 14(2):399.

Ohnaka, K., Ikeda, M., Maki, T., Okada, T., Shimazoe, T., Adachi, M., Nomura, M., Takayanagi, R. and Kono, S. 2012. Effects of 16-week consumption of caffeinated and decaffeinated instant coffee on glucose metabolism in a randomized controlled trial. *J. Nutr. Metab.* 2012:207426.

Ouellet-Morin, I., Danese, A., Williams, B. and Arseneault, L. 2011. Validation of a high-sensitivity assay for C-reactive protein in human saliva. Brain Behav. *Immun.* 25(4):640-6.

Out, D., Hall, R.J., Granger, D.A., Page, G.G. and Woods, S.J. 2012. Assessing salivary C-reactive protein: longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. *Brain Behav. Immun*. 26(4):543-551.

Owoyele, B.V., Oyewole, A.L., Biliaminu, S.A. and Alashi, Y. 2015. Effect of taurine and caffeine on plasma c-reactive protein and calcium in Wistar rats. *Afr. J. Med. Med. Sci*. 44:229-236.

Pace, T.W., Negi, L.T., Dodson-Lavelle, B., Ozawa-de Silva, B., Reddy, S.D., Cole, S.P., *et al*. 2013. Engagement with Cognitively-Based Compassion Training is associated with reduced salivary C-reactive protein from before to after training in foster care program adolescents. *Psychoneuroendocrinology* 38(2):294-9.

Paiva, C., Beserra, B., Reis, C., Dorea, J.G., Da Costa, T. and Amato, A.A. 2019. Consumption of coffee or caffeine and serum concentration of inflammatory markers: A systematic review. *Crit. Rev. Food Sci. Nutr.* 59(4):652-663.

Proctor, G.B., Garrett, J.R., Carpenter, G.H. and Ebersole, L.E. 2003. Salivary secretion of immunoglobulin A by submandibular glands in response to autonomimetic infusions in anaesthetised rats. *J. Neuroimmunol.* 136(1-2):17-24.

Proctor, G.B. 2016. The physiology of salivary secretion. *Periodontol. 2000*. 70(1):11-25.

Shah, S.A., Occiano, A., Nguyen, T.A., Chan, A., Sky, J.C., Bhattacharyya, M., O'Dell, K.M., Shek, A., and Nguyen, N.N. 2016. Electrocardiographic and blood pressure effects of energy drinks and Panax ginseng in healthy volunteers: A randomized clinical trial. *Int. J. Cardiol.* 218:318-323.

Sharma, P., Shivhare, P., Marimutthu, P., Sharma, M.K. and Murthy, P. 2020. Patterns of Caffeine Use and Validation of Assessment in Psychiatric Population: An Implication in Primary Care Setting. *J. Family Med. Prim. Care* 9(10):5252-5255.

Sproston, N.R. and Ashworth, J.J. 2018. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front. Immunol.* 9:754.

Su, S.H., Shyu, H.W., Yeh, Y.T., Chen, K.M., Yeh, H. and Su, S.J. 2013. Caffeine inhibits adipogenic differentiation of primary adipose-derived stem cells and bone marrow stromal cells. *Toxicol. In Vitro*. 27(6):1830-7.

Su, X., Liu, Y., Bakkar, M., ElKashty, O., El-Hakim, M., Seuntjens, J. and Tran, S.D. 2020. Labial Stem Cell Extract Mitigates Injury to Irradiated Salivary Glands. *J. Dent. Res.* 99(3):293-301.

Velickovic, K., Wayne, D., Leija, H.A.L., Bloor, I., Morris, D.E., Law, J., Budge, H., Sacks, H., Symonds, M.E. and Sottile, V. 2019. Caffeine exposure induces browning features in adipose tissue in vitro and in vivo. *Sci. Rep.* 9(1):9104.

Watson, R.R. and Antal, M. 1980. Effect of moderate chronic protein deficiency on rat salivary components. *J. Nutr.* 110(4):771-7.

Wedick, N.M., Brennan, A.M., Sun, Q., Hu, F.B., Mantzoros, C.S. and van Dam, R.M. 2011. Effects of caffeinated and decaffeinated coffee on biological risk factors for type 2 diabetes: A randomized controlled trial. *Nutr. J.* 10:93.

Wallenfels, K., Foldi, P., Niermann, H., Bender, H., Linder, D. 1978. The enzymic synthesis by transglucosylation of a homologous series of glycosidically substituted malto-oligosaccharides, and their use as amylase substrates. *Carbohyd. Res*. 61(1): 359-68.