BioBacta

Journal of Bioscience and Applied Research

www.jbaar.org

Comparing the level of some stress biomarkers among smoking and non-smoking healthy adults in Egypt

Asmaa Fathi Galal[1*], Mai Sabry Saleh[2], Nagat Mohamed Amer[2], Amal Saad-Hussein[2]

[1]Narcotics, Ergogenics and Poisons department, Medical Research Division, National Research Centre, Dokki, Giza, Egypt

[2]Environmental and Occupational Medicine Department, Environmental Research Division, National Research Centre, Dokki, Giza, Egypt

**Correspondence: asma.galal.79@gmail.com*

DOI: 10.21608/jbaar.2019.147675

Abstract

Objective: The causal effect relationship between smoking and stress is a subject that invites continuous research. Hypothetically, investigation of stress biomarkers that are reported to be affected by tobacco intake may give us some explanation of the association between stress and smoking as a habit**.** Consequently, the present study aimed to assess the serum level of some stress biomarkers and compare them among smokers and non-smokers in a sample of Egyptian male healthy volunteers. **Methods:** Fifty-nine subjects were enrolled in the study (29 smokers and 30 non-smokers of matched age and gender). We measured serum levels of cortisol and interleukin-6 (IL-6) using the ELISA technique, and serum levels of α-amylase, triglycerides (TG), and total cholesterol (TC) using colorimetric methods**. Results:** Serum cortisol levels were decreased in smokers, and IL-6, TG, and TC were significantly higher in smokers than non-smokers, whereas, serum α-amylase did not show a significant difference. Serum cortisol showed to be negatively correlated with serum IL-6 in smokers. **Conclusions:** The present study assumes that smokers suffer from a state of chronic stress as evidenced by the observed decrease in serum cortisol due to negative feedback effects and increase in levels of serum IL-6, TG, and TC. This in turn enhances the craving to smoke to face stressors and leads to a vicious circuit that smokers fail to quit smoking.

Keywords: Smoking; stress; cortisol; IL-6; triglycerides; cholesterol

---

Received date: May 25, 2019. Accepted: August 10, 2019, Published: August 18, 2019

1. Introduction

Smoking is believed by many smokers and health care professionals to reduce stress and improve symptoms related to poor mental health (Taylor and Munafò, 2019). Many studies ensure that exposure to acute stress is often followed by an increase in cigarette craving (Buchmann et al., 2010). Smokers declare that stress relief and relaxation are their main driving force for smoking (Filder and West, 2009).

Controversially, research states that nicotine found in tobacco cigarettes increases biomarkers of stress and affects the hypothalamic-pituitary-adrenal (HPA) axis (Kapoor and Jones, 2005). The HPA is known to mediate the body response towards physical and mental stress (Miller and O'Callaghan, 2002) and is responsible for the release of glucocorticoids like cortisol (Edwards C, 2012). Peripheral cortisol levels could also be increased through the direct activation of nicotine for the central nicotinic receptors (Chiodera et al., 1997).

Interleukin-6 (IL-6) is another stress biomarkers positively associated with smoking. Many empirical studies show increased levels of IL-6 in smokers compared to non-smokers (Arnson et al., 2010; Moretti et al., 2014; Helmersson et al., 2005). Similarly, serum alpha-amylase is a biomarker of stress that is commonly affected by nicotine. Complex pharmacological actions of nicotine pressure were found to produce injury to a pancreatic cell at subclinical levels with subsequent elevation of alpha-amylase in the blood (Azab and Dawood, 2012). According to Dubick et al. (1987), the basal serum amylase activity was found to be 100% higher in cigarette smokers than non-smokers. More recent research emphasized the same findings where serum alpha-amylase level was higher in smokers than non-smokers (Hasan and Jabir, 2017; Weiner et al., 2009).

Smoking is strongly associated with the malfunction of lipid metabolism and thus predisposes to cardiovascular diseases (Hallit et al., 2017). Lipid metabolism is also modulated by stressors (Maduka et al., 2015). Previous research postulated a causal relationship between increased stress and alterations in lipid profile (Najee and Miller, 1989).

In this context, the present work aimed to assess the serum level of the aforementioned stress biomarkers and compare them among smokers and non-smokers in a sample of Egyptian male healthy volunteers.

2. Subjects and Methods

Study Participants

The study is a case-control study performed on 29 adult male smokers and 30 controls of matched age and gender. A medical sheet was completed for all participants about respiratory complaints, blood pressure, and body mass index (BMI). Exclusion criteria were the presence of diabetes mellitus, malignancy, history of corticosteroid exposure, and also the presence of any disease that could affect the HPA axis. Consents were collected from all participants declaring their willingness to participate and ethical approval was taken from the ethical committee of the National Research Centre for study procedures.

Biochemical assessment

Five mls blood were collected in a plain red top venipuncture tube for serum samples. Blood was left to clot then centrifuged at 3000 rpm for 10 minutes and serum was separated. The serum was kept at -20 °C for five days until analysis. Serum cortisol and serum IL-6 were measured by enzyme-linked immune sorbent assay (ELISA) technique using a kit manufactured by Elabscience, USA. Serum α-amylase, triglycerides (TG), and total cholesterol (TC) were determined using a colorimetric kit (Biodiagnostic, Egypt).

Statistical Analysis

Statistical analysis was done using Graph pad Prism 7. Descriptive analysis for mean and standard deviation,

student t-test, and Pearson correlation was performed. A *p*-value of less than 0.05 was considered significant.

3. Results

Both smokers and non-smokers showed mean values for systolic and diastolic blood pleasures within the normal range with a very low percentage of each suffering from a respiratory complaint (22% in smokers and 18 % in non-smokers). Mean values for BMI showed both groups to be overweight (27.33 in smokers and 27.24 in non-smokers). Smokers showed a mean age of 41.7 years ranging from 25 to 60. Non-smokers showed a mean age of 40.06 years ranging from 23 to 58.

Table 1 illustrated the measured parameters in the serum of smokers and non-smokers. Serum cortisol levels were decreased by 52.8% in the smoker's group with a significantly lower mean value of 164.4 ng/ml at $p < 0.001$ compared to the corresponding non-smoker's group (234.9 ng/ml). Serum IL-6 was increased by nearly 25% in smokers compared to non-smokers counterparts with a significant difference at $p < 0.001$. Significant increases in serum TG and TC levels were also detected in smokers as compared to non-smokers while α-amylase was lower in smokers (887.2 mg/dl versus 933.3 mg/dl) yet non significantly.

Pearson correlation showed a significant negative correlation between serum cortisol and serum IL-6 (r= -0.4374, *P*= 0.0255) as shown in figure 1. No other significant results were detected yet a negative correlation appeared between serum cortisol and serum α-amylase, serum cortisol and serum TG, and serum cortisol and serum TC.

Table 1. Serum levels of cortisol, α-amylase, IL-6, TG, and TC in smokers and non-smokers

	Non-smokers (30)				**Smokers (29)**			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Cortisol (ng/ml)	234.9	99.84	46.74	410.9	164.4*	100.4	35.67	400
α-amylase (U/L)	933.3	261.3	551.6	1324	887.2	262.2	478	1287
IL-6 (pg /ml)	0.1257	0.1162	0.01	0.479	0.2026*	0.1639	0.023	0.554
TG (mg/dl)	101.7	53.1	42.5	264.3	143*	85.89	45.63	329.5
TC (mg/dl)	129	63.63	36.46	328.6	167.5*	81.3	104.3	357.1

**Significantly different from non-smokers group at p<0.001. Cortisol (normal range (8-10 a.m.): 50-230 ng/ml), α -amylase (normal range: 70-340 U/L), IL-6: Interleukin-6, TG: Triglycerides (normal range: 60-165 mg/dL), TC: Total cholesterol (normal range: 150-225 mg/dL)*

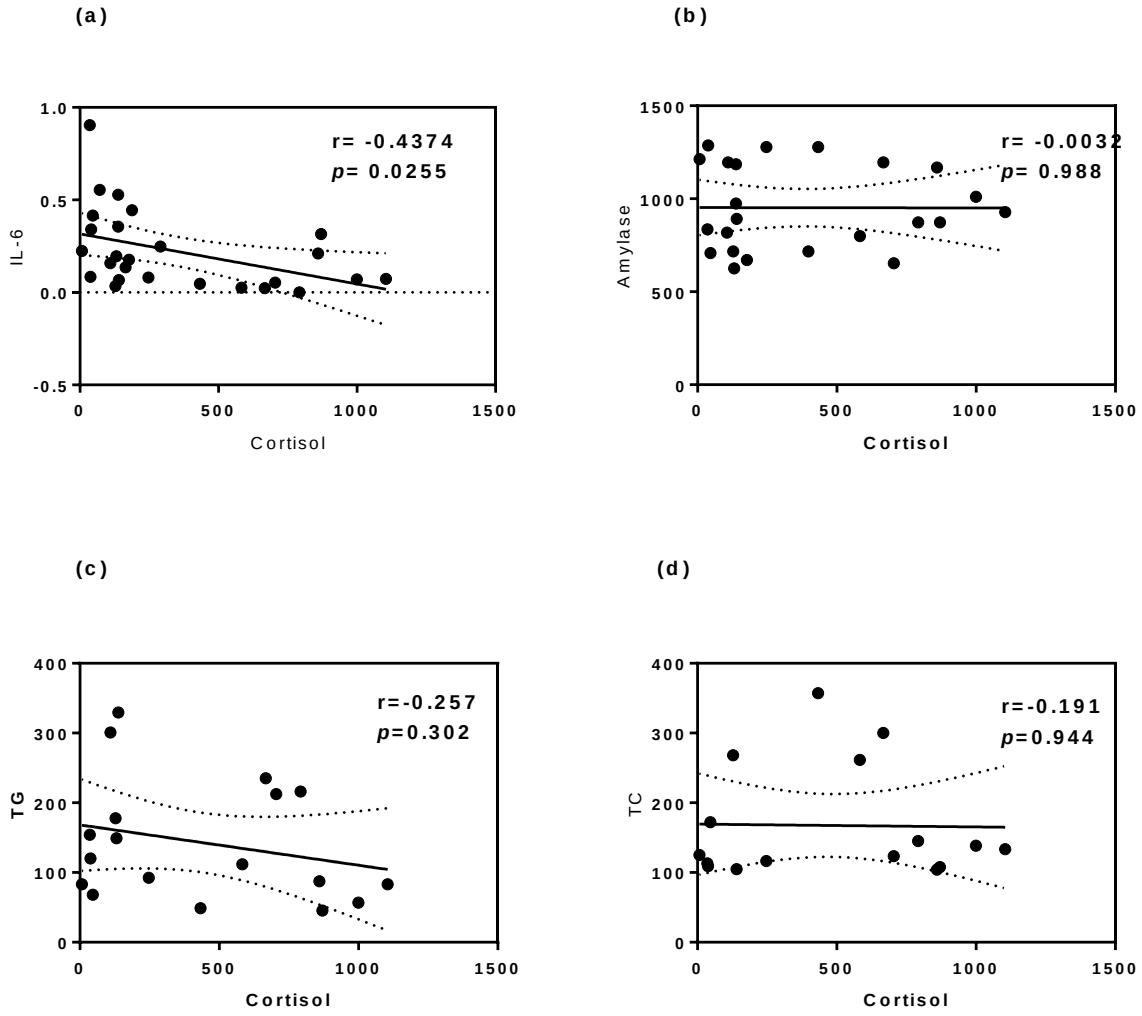

Figure 1. Pearson correlation between serum cortisol and serum α-amylase, IL-6, TG and TC in smokers, (a) cortisol and IL-6, (b) cortisol and α-amylase, (c) cortisol and TG, (d) cortisol and TC

4. Discussion

In the present study, serum cortisol was decreased significantly in smokers compared to non-smokers counterparts. Similar results were reported by literature (Handa et al., 1994; Elbuken et al., 2015), nevertheless, the level of cortisol as compared between smokers and non-smokers is still controversial (Steptoe and Ussher, 2006).

According to Neves et al. (2017), salivary cortisol level was higher in heavy smokers compared to both light smokers and non-smokers. The author introduced some explanations including the increased activation of the mesolimbic system due to the increase in nicotine exposure resulting in modifying the dopaminergic activity and lowering cortisol levels. Another explanation stated that frequent and prolonged activation of the HPA axis by tobacco smoking could have lead to downregulation of corticotrophic hormone receptors and reduced responsiveness of the adrenal cortex to a psychological challenge (al'Absi et al., 2006). As evident, low HPA stress responsiveness

usually predate increased cigarette craving (Moss et al., 1999). It was also stated by Kirschbaum and colleagues (1993) who compared the response of habitual smokers and non-smokers to a standardized public speaking task and mental arithmetic task in front of an audience that an attenuated cortisol response to such stressors was detected among smokers. Similar findings were reported by Childs and de Wit (2010) who illustrated that Smokers exhibited a blunted cortisol response to the Trier Social Stress Test and prolonged subjective scores of agitation in response to stressors as compared to non-smokers, suggesting a possible link between hypoactive HPA axis reactivity to stress and a prolonged time to recovery from the subjective mood-altering effects of stress exposure. Moreover, such an explanation could clarify why acute physical or psychological stress and behavioral challenges enhance craving to smoke, smoking rate, and nicotine intake (al'Absi et al., 2002; al'Absi et al., 2003; Buchmann et al., 2010).

According to the literature, the level of both serum and salivary α-amylase is higher in smokers compared to non-smokers (Nater et al., 2007; Dubick et al., 1987; Onyesom et al., 2012). Controversially, in the present study, serum α-amylase level was lower in smokers yet not significant. Strikingly, the level of serum α-amylase as detected by the present work in both smokers and non-smokers highly exceeded the normal range that could indicate a pathological state. Such rise could reflect renal insufficiency, nephrectomy (Onyesom et al., 2012), hepatic necrosis, or cirrhosis (Rueff and Bentamou, 1973).

Another marker in the present study was the pro-inflammatory cytokine; IL-6. IL-6 is produced by macrophages in the bronchial epithelium in response to infections as well as other stimuli such as smoking (Rodrigues et al., 2014). Serum IL-6 level was markedly increased in the smoker's group in our investigation similar to other studies (Tibuakuu et al., 2017; Daloee et al., 2017). Despite IL-6 is believed to stimulate HPA (Kunz-Ebrecht et al., 2003) and hence cortisol secretion, a significant negative correlation was detected in the present study between the two biomarkers. This result could augment the assumption of negative feedback for cortisol secretion previously mentioned.

In the present study, smokers showed unfavorable lipid profiles, represented in a significant rise in serum TC and TG levels. Previous studies proposed smoking as a strong risk factor for dyslipidemia. Smoking increase TC, TG, and LDL cholesterol and lowers HDL cholesterol, while smoking cessation improves lipid metabolism (Lee et al., 1998; Hallit et al., 2017; Li et al., 2018). Mjos (1988) assumed the reason to be the stimulating effect of nicotine on the sympathetic activity leading to the release of catecholamines, that mediate lipolysis and increases plasma levels of TG and free fatty acids. In their study, Maduka et al. (2015) suggested a relation between cortisol level and lipid profile under stressful conditions. Their results proved a significant increase in both cortisol and TC but not in TG. Generally speaking, both TG and TC are supposed to increase under stress (Le Fur et al., 1999). As represented by results obtained by the present study, both TG and TC indeed showed a significant increase in smokers compared to non-smokers, yet no significant correlation was distinguished between TC and cortisol, and TG was negatively correlated with cortisol but non-significantly.

In conclusion, findings from the present study suppose that smokers are suffering from a state of chronic stress that is manifested by a significant decrease in cortisol due to negative feedback effect, increased levels beyond normal of α-amylase, IL-6, TC, and TG. Such findings can explain the vicious circuit in which chronic smokers are trapped in and that could be explained as follows. When smokers are faced with stressful conditions, cortisol levels increase in their

blood. In such a situation their craving for smoking increases since nicotine stimulates the secretion of cortisol that results from a final decrease in cortisol level by a feedback mechanism. More investigations are highly recommended concerning having more credible evidence to the concluded hypothesis. A larger sample size, using psychometric measurements for stress, degree of the smoking habit, and surveying smoker habits and explanations for their desire to keep smoking despite its harmful effect on health. Stress is to be measured using the aforementioned biomarkers in addition to the Allostatic load index that can give an indication of stress level attained by smokers to be correlated with their smoking habits.

Conflict of interest

The authors declare no conflict of interest.

5. References

al'Absi, M. (2006). Hypothalamic-pituitary-adrenocortical responses to psychological stress and risk for smoking relapse. Int. J. Psychophysiol., 59(3):218-27.

al'Absi, M., Amunrud, T., Wittmers, L.E. (2002). Psychophysiological effects of nicotine abstinence and behavioral challenges in habitual smokers. Pharmacol. Biochem. Behav., 72(3):707-16.

al'Absi, M., Wittmers, L.E., Erickson, J., Hatsukami, D., Crouse, B. (2003). Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers. Pharmacol. Biochem. Behav., 4(2):401-10.

Arnson, Y., Shoenfeld, Y., Amital, H. (2010). Effects of tobacco smoke on immunity, inflammation, and autoimmunity. J. Autoimmun., 34:J258-65.

Azab, NY., Dawood, A. (2012). Effect of Smoking on Serum Amylase and Lipase Enzymes. J. Am. Sci., 8(11): 406-410.

Buchmann, A.F., Laucht, M., Schmid, B., Wiedemann, K., Mann, K., Zimmermann, U.S. (2010). Cigarette craving increases after a psychosocial stress test and is related to cortisol stress responses but not to dependence scores in daily smokers. J. Psychopharmacol., 24:247–55.

Childs, E., de Wit, H. (2010). Effects of acute psychosocial stress on cigarette craving and smoking. Nicotine Tob. Res., 12 (4): 449–453

Chiodera, P., Volpi, R., Capretti, L., Speroni, G., Necchi-Ghiri, S., Caffarri, G., Colla, R., Coiro, V. (1997). Abnormal effect of cigarette smoking on pituitary hormone secretions in insulin-dependent diabetes mellitus. Clin. Endocrinol (Oxf)., 46:351–357.

Daloee, MH., Avan, A., Mirhafez, S.R., Kavousi, E., Hasanian-Mehr, M., Darroudi, S., Tajfard, M., Tayefi, M., Qazizade, H., Mohammadi, A., Ferydouni, N., Ebrahimi, M., Ghayour-Mobarhan, M. (2017). Impact of Cigarette Smoking on Serum Pro- and Anti-Inflammatory Cytokines and Growth Factors. Am. J. Mens Health., 11(4):1169-1173.

Dubick, M.A., Conteas, C.N., Billy, H.T., Majumdar, A.P., Geokas, M.C. (1987). Raised serum concentration of pancreatic enzymes in cigarette smokers. Gut, 28(3): 330-350.

Edwards, C. (2012). Sixty tears after hench-Corticosteroids and chronic inflammatory disease. J. Clin. Endocrinol. Metab., 97:1443-1451.

Elbuken, G., Karaca, Z., Tanriverdi, F., Unluhizarci, K., Yasin Simsek, Y., Kelestimur, F. (2015). Does cigarette smoking affect serum total cortisol and salivary cortisol levels?. Endocrine Abstracts, 37: EP729.

Filder, J.A., West, R. (2009). Self-perceived smoking motives and their correlates in a general population sample. Nicotine Tob. Res., 11:1182–8.

Hallit, S., Zoghbi, M., Hallit, R., Youssef, L., Costantine, R., Kheir, N., Salameh, P. (2017). Effect of exclusive cigarette smoking and in combination with waterpipe smoking on lipoproteins. J. Epidemiol. Glob. Health, 7(4):269-275.

Handa, K., Kono, S., Ishii, H., Shinchi, K., Imanishi, K., Arakawa, K.. (1994). Relationship of alcohol consumption and smoking to plasma cortisol and blood pressure. J. Hum. Hypertens., 8: 891 – 894.

Hasan, H.R., Jabir, R.J. (2017). Salivary and Serum α-Amylase Activity and their correlations with Oxidative Stress Index Among Different Iraqi Smokers Groups. MOJ. Addict. Med. Ther., 4(1): 00071.

Helmersson, J., Larsson, A., Vessby, B., Basu, S. (2005). Active smoking and a history of smoking are associated with enhanced prostaglandin F(2alpha), interleukin-6, and F2-isoprostane formation in elderly men. Atherosclerosis, 181: 201- 7.

Kapoor, D., Jones, T.H. (2005). Smoking and hormones in health and endocrine disorders. Eur. J. Endocrinol., 152:491–499.

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H. (1993). The 'Trier Social Stress Test'--a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiol., 28(1-2):76-81.

Kunz-Ebrecht, S.R., Mohamed-Ali, V., Feldman, P.J., Kirschbaum, C., Steptoe, A. (2003). Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines. Brain Behav. Immun., 17:373–83.

Le Fur, C., Romon, M., Lebel, P., Devos, P., Lancry, A., GuédonMoreau, L., et al. (1999). Influence of mental stress and circadian cycle on postprandial lipemia. Am. J. Clin. Nutr., 70:213–20.

Lee, K.S., Park, C.Y., Meng, K.H., Bush, A., Lee, S.H., Lee, W.C., Koo, J.W., Chung, C.K. (1998). The association of cigarette smoking and alcohol consumption with other cardiovascular risk factors in men from Seoul, Korea. Ann. Epidemiol., 8(1):31-8.

Li, X.X., Zhao, Y., Huang, L.X., Xu, H.X., Liu, X.Y., Yang, J.J., Zhang, P.J., Zhang, Y.H. (2018). Effects of smoking and alcohol consumption on lipid profile in male adults in northwest rural China. Public Health, 157:7-13.

Maduka, I.C., Neboh, E.E., Ufelle, S.A. (2015). The relationship between serum cortisol, adrenaline, blood glucose, and lipid profile of undergraduate students under examination stress. Afr. Health Sci., 15(1):131-6.

Miller, D.B., O'Callaghan, J.P. (2002). Neuroendocrine aspects of the response to stress. Metabolism, 51:5–10.

Mjos, O.D. (1988). Lipid effects of smoking. Am. Heart J., 115:272–275.

Moretti, E., Collodel, G., Mazzi, L., Campagna, M., Iacoponi, F., Figura, N. (2014). Resistin, interleukin-6, tumor necrosis factor-alpha, and human semen parameters in the presence of leukocytospermia, smoking habit, and varicocele. Fertil. Steril., 102:354-60.

Moss, H.B., Vanyukov. M., Yao, J.K., Kirillova. G.P. (1999). Salivary cortisol responses in prepubertal boys: the effects of parental substance abuse and association with drug use behavior during adolescence. Biol. Psychiatry, 45(10):1293-9.

Nanjee, M.N., Miller, N.E. (1989). Plasma lipoproteins and adrenocortical hormones in men - positive association of low-density lipoprotein cholesterol with plasma cortisol concentration. Clin. Chim. Acta, 180(2):113-120.

Nater, U.M., Rohleder, N., Schlotz, W., Ehlert, U., Kiv-schbaum, C. (2007). Determinants of the diurnal

course of salivary alpha amylase. Psychoneuro endocrine, 32 (4): 392-401.

Neves, C.D.C., Lacerda, A.C.R., Lima, L.P., Lage, V.K.S., Balthazar, C.H., Leite, H.R., Mendonça, V.A. (2017). Different levels of brain-derived neurotrophic factor and cortisol in healthy heavy smokers. Braz. J. Med. Biol. Res., 50(12):e6424.

Onyesom, I., Osioma, E., Ifie, E. J., Oweh, O. T. (2012). Activities of Alpha Amylase in Serum and Saliva of Some Nigerian Cigarette Smokers. Adv. Life Sci., 2(1): 28-30.

Rodrigues, F.M., Ramos, D., Xavier, R.F., Ito, J.T., Souza, A.P., Fernandes, R.A., Cecchini, R., Rossi e Silva, R.C., Macchione, M., Toledo-Arruda, A.C., Guarnier, F.A., Ramos, E.M. (2014). Nasal and systemic inflammatory profile after short term smoking cessation. Respir. Med., 108(7):999-1006.

Rueff, B., Bentamou, J.P. (1973). Acute hepatic necrosis and fulminant hepatic failures. Gut, 10: 805-815.

Steptoe, A., Ussher, M. (2006). Smoking, cortisol, and nicotine. Int. J. Psychophysiol., 59(3):228-35.

Taylor, G.M.J., Munafò, M.R. (2019). Does smoking cause poor mental health? Lancet Psychiatry, 6(1):2-3.

Tibuakuu, M., Kamimura, D., Kianoush, S., DeFilippis, A.P., Al Rifai, M., Reynolds, L.M., et al. (2017).
The association between cigarette smoking and inflammation: The Genetic Epidemiology Network of Arteriopathy (GENOA) study. PLoS ONE, 12(9): e0184914.

Weiner, D., Khankin, E.V., Levy, Y., Reznick, A.Z. (2009). Effects of cigarette smoke borne reactive nitrogen species on salivary alpha-amylase activity and protein modifications. J. Physiol. Pharmacol., 60: 127-132.