Niger. J. Physiol. Sci. 38 (December 2023): 195 – 200
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Effect of Acute Caffeine Exposure on Blood Glucose and Hepatic Glycogen Content in Normal and Thyroidectomized Male Wistar Rats

***Shittu S.T., Ogiogio H.A., Isehunwa G.O. and Alada A.R.A**
Endocrinology and Metabolism Unit, Department of Physiology, University of Ibadan. Ibadan, Nigeria

Summary: Acute caffeine exposure had been shown to induce hyperglycemia however; the influence of thyroid hormones on the caffeine-induced hyperglycemia is yet to be established. The present study was therefore designed to investigate the effect of caffeine exposure on blood glucose and hepatic glycogen content in thyroidectomized rats. Sixty adult male Wistar rats were randomly divided into 10 groups as I-X (n=6). Rats in groups I, III, V, VII and IX were given normal saline, caffeine, prazosin + caffeine, propranolol +caffeine, combined prazosin+ propranolol+caffeine injections respectively while rats in groups II, IV, VI, VIII and X were thyroidectomized and treated in similar manner as the normal rats respectively. Surgical removal of the thyroid gland was done in the thyroidectomised groups while sham-operation was done in Normal group to serve as control. After healing and following an overnight fast, the rats were anaesthetized and the femoral vein and carotid artery were cannulated for drug administration and blood glucose measurement respectively. After stabilization, following basal measurements, rats from each group were injected normal saline or caffeine (6mg/kg) while another sets were pre-treated prazosin (0.2 mg/kg), propanolol (0.5 mg/kg) or their combination before caffeine administration. Blood glucose was then monitored for 60 minutes post-injection of caffeine at 5 minutes interval. Liver samples were collected at the end of the observation period for glycogen content determination. Caffeine caused significant increased blood glucose levels in both normal and thyroidectomized rats which were up to 210% and 180% respectively at the peak of their responses. Liver glycogen content of the thyroidectomized rats (3.11 ± 0.20 mg/100g tissue weight) was significantly higher than the normal rats (1.91 ± 0.43 mg/100g tissue weight). These glycogen contents were significantly reduced by caffeine in both normal (0.25 ± 0.04 mg/100g tissue weight) and thyroidectomized rats (1.65 ± 0.16 mg/100g tissue weight) when compared with their controls. The caffeine effects on blood glucose and hepatic glycogen content were abolished by pretreatment with propanolol or a combination of prazosin and propanolol pretreatment in both normal and thyroidectomized rats but pretreatment with prazosin caused only significant reduction in hyperglycemic response to caffeine. The findings of this study suggest that caffeine-induced hyperglycemia in both normal and thyroidectomized rats are mediated through both alpha and beta adrenoceptors.

Keywords*: Caffeine, Hyperglycemia, Hepatic glycogen, Hypothyroid, Rat*

**Authors for correspondence: st.shittu@ui.edu.ng , Tel: +2348052644680*

Manuscript received- September 2023; Accepted: November 2023

DOI: https://doi.org/10.54548/njps.v38i2.8

©Physiological Society of Nigeria

INTRODUCTION

Caffeine is a mildly psycho-active commonly consumed substance with an average daily intake of approximately 300 mg from dietary sources such as coffee, tea, soft drinks, chocolate and energy drinks (Shi *et al*., 2016). It is also contained in kola nut, a common masticatory in West Africa (Salahdeen *et al*., 2015). Consumption above 600 mg per day may be addictive and negatively impact the body (Czarniecka-Skubina *et al*., 2021). In fact, consumption of coffee, the major source of caffeine in excess of 5 cups per day has been shown to be a risk factor for several diseases and mortality (Grosso *et al*., 2016; Park *et al*., 2017; Loftfield *et al*., 2018; Abe *et al*., 2019).

Acute exposure to caffeine increased blood glucose, hampered insulin sensitivity, induced adrenaline and cortisol stimulated hepatic glucose production and reduced glucose uptake and utilization by peripheral tissues (Dekker *et al*., 2007; Salahdeen and Alada, 2009; Alagbonsi *et al*., 2016; Shi *et al*., 2016; Reis *et al*., 2018). Physiologically, increased blood glucose is channeled into increased glucose utilization and storage as glycogen. Glycogen, a multi-branched polysaccharide of glucose is stored primarily in the liver to modulate blood glucose between prandial states; it is stored during hyperglycemia and mobilized during hypoglycemia through the activities of glycogen synthase and glycogen phosphorylase, respectively (Han *et al*., 2016). These enzymes, involved in glycogen metabolism have been shown to be influenced by hormones of the thyroid gland (Chu *et al*., 1985; Bollen and Stalmans, 1988).

Thyroid hormones regulate energy metabolism and metabolic processes essential for normal growth and development therefore, their status correlates with body weight and energy expenditure, thus hyperthyroidism is associated with hypermetabolic state characterized by increased basal energy expenditure while hypothyroidism is characterized by declined resting energy expenditure (Mullur *et al*., 2014). Basically, they influence key metabolic pathways that regulate energy storage and expenditure. For instance, they promote insulin secretion and glucose uptake in the gastrointestinal tract, liver, skeletal muscles, and adipose tissue with differences in the manifestation of their effect across the different tissues (Nishi, 2018) therefore their absence may inhibit glucose uptake by these tissues.

Data on the effect of acute caffeine-induced high blood glucose on hepatic glycogen content are lacking and the likely impact of thyroid hormone on the acute effect is yet to be elucidated. The present study was therefore designed to investigate the effect of acute caffeine exposure on hepatic glycogen content in thyroidectomized male Wistar rats. The involvement of the adrenergic receptor was also examined.

MATERIALS AND METHODS

Adult male Wistar rats, fasted for 12 hours were used for this study. They were anesthetized by intraperitoneal injection of 50 mg/kg Sodium thiopental (Roche®, Germany) and firmly secured on a dissecting board to exposed the carotid artery and femoral vein. Cannulas, previously flushed with heparinized saline (100 IU/mL), were inserted into these blood vessels for blood sampling and drug administration, respectively. To ensure unobstructed airflow, the trachea was intubated, and sodium heparin, 300 IU per/kg body weight was administered intravenously to prevent blood clotting. Following the surgical procedures, a stabilization period of approximately 30 minutes to one hour was observed. The drugs of interest (caffeine 6mg/kg, prazosin 0.2mg/kg, propranolol 0.5mg/kg) were injected through the femoral vein, while blood samples were collected from the carotid artery for glucose measurements in each group.

Group I (Control): These rats were given a bolus injection of normal saline through the femoral vein, and blood samples were collected from the carotid artery at specific time intervals of 5min, 10min, 15min, 30min, 45min, and 60min post injection for glucose measurement.
Group II (Control-Thyroidectomized): Similar to Group I, these rats underwent thyroidectomy and received a bolus injection of normal saline. Blood samples were collected as in Group I.
Group III (Caffeine): Rats in this group were given a bolus injection of caffeine dissolved in normal saline. Blood samples were collected as in Groups I and II.
Group IV (Caffeine - thyroidectomized): Similar to Group III, these rats underwent thyroidectomy and received a bolus injection of caffeine. Blood samples were collected as in Groups I and II.
Group V (Prazosin + Caffeine): Rats in this group were first injected with prazosin dissolved in acidified water, followed by a basal blood glucose measurement. Then, they received a bolus injection of caffeine. Blood samples were collected as in Groups I and II.
Group VI (Prazosin + Caffeine - thyroidectomized): Similar to Group V, these rats underwent thyroidectomy and received prazosin followed by caffeine. Blood samples were collected as in Groups I and II.
Group VII (Propranolol + Caffeine): Rats in this group were first injected with propranolol dissolved in normal saline, followed by a basal blood glucose measurement. Then, they received a bolus injection of caffeine. Blood samples were collected as in Groups I and II.
Group VIII (Propranolol + Caffeine - thyroidectomized): Similar to Group VII, these rats underwent thyroidectomy and received propranolol followed by caffeine. Blood samples were collected as in Groups I and II.
Group IX (Prazosin + Propranolol + Caffeine): Rats in this group were first injected with prazosin and propranolol, followed by a basal blood glucose measurement. Then, they received a bolus injection of caffeine. Blood samples were collected as in Groups I and II.
Group X (Prazosin + Propranolol + Caffeine - thyroidectomized): Similar to Group IX, these rats underwent thyroidectomy and received prazosin, propranolol, and caffeine. Blood samples were collected as in Groups I and II.
In each group, the basal blood glucose levels were measured before the respective injections, and subsequent blood samples were collected at 5min, 10min, 15min, 30min, 45min, and 60min post injection of drugs to assess the effects of the given treatments on blood glucose levels.

Procedure for thyroidectomy: Total thyroidectomy was carried out as described by Jin and Sugitani (2021). Briefly, anaesthetized [100 mg/kg Ketamine, i.p. (Ketanir®, Aculife Healthcare Pvt Ltd, India) and 5 mg/kg Xylazine, i.m. (Xylased®, Bioveta, Czech Republic)] rat was firmly secured on a dissecting board, the neck regions was shaved and a longitudinal incision (2.0-2.5 cm) of the neck skin and subcutaneous connective tissue was made. The sternohyoid muscle of the trachea was bluntly separated along the mid line to expose the white trachea and the thyroid glands, which appeared reddish-brown and were attached to the thyroid cartilage on both sides. By ligating the superior thyroid arteries and cutting the thyroid isthmus, one side of the thyroid gland was carefully separated from the trachea, allowing visualization of the recurrent laryngeal nerve running between them. The nerve was cautiously stripped, and one side of the thyroid gland and the isthmus were resected. The same procedure was then repeated on the other side until all thyroid tissue below the thyroid cartilage was removed. Throughout the surgery, measures were taken to prevent bleeding, and any bleeding that occurred was promptly addressed using sterile cotton swabs or gauze. After confirming no further bleeding, the clamp in the neck was removed, and the sternohyoid muscle of the trachea was reset. The incision was sutured layer by layer using catgut, a type of absorbable suture. The site of the suture was covered with penicillin (DBT fortified Procaine Penicillin®, Azhui Chengshi Pharmaceutical Co. Ltd, China) for disinfection and the animals were kept in a warm and well illuminated environment until recovery.

Blood glucose measurement: The blood glucose level was determined using an ACCU-CHEK glucometer ® which is based on the glucose-oxidase method (Trinder, 1969). Test strips were inserted into the glucometer, and small blood sample (approximately 0.05 ml) was applied to the test spot. Within seconds, the glucometer displayed the blood glucose value. Previous studies have shown a high correlation between this method and standard laboratory methods for blood glucose measurement.

Determination of liver glycogen content: After blood glucose monitoring, the liver was quickly removed from each animal 60 minutes after injection under anesthesia. Adherent tissues and blood were removed, and the liver was weighed. A separate one-gram sample of the liver was taken to determine its glycogen content using the anthrone reagents method (Seifter *et al*., 1950; Jermyn, 1975).

Isolation and purification of glycogen: 1 g of the liver was placed in pre-heated Erlnmeyer flasks containing 10 ml of 30% KOH solution. The liver was digested by heating the flasks for 20 min over light flame with occasional shaking until the tissues dissolved. The solution was allowed to cool. Then, 4 ml of the aliquot from the flasks was taken and placed in a 15 ml centrifuge tube. 5 ml of 95% ethanol was added to the sample, mixed and centrifuged for 5 min; it was then decanted and drained for 5 min. The glycogen precipitated from each sample was dissolved in 0.5 ml distilled water and mixed thoroughly. This was reprecipitated with 5 ml of 95% ethanol and recovered by centrifugation. The centrifugation was repeated four times until a white precipitate was obtained. The final glycogen precipitate was dissolved in 0.5 ml of distilled water. 0.5 ml aliquot was taken from the unknown glycogen solution obtained from above. Then, 0.5 ml of concentrated HCl, followed by 0.5 ml formic acid (88%) and 4 ml of anthrone reagent were added in a stepwise manner. The anthrone reagent was added slowly and mixed thoroughly. 0.5 ml of distilled water was treated as above and used as a blank. Several dilutions of the glycogen standard (0.2 mg/ml) were prepared. The dilutions used were 0.1, 0.2, 0.3, 0.4 ml of standard glycogen solution with enough distilled water to make 0.5 ml. These dilutions of glycogen standard were then treated as above. A standard curve was prepared from this.

All the tubes containing the solutions were heated in boiling water for ten minutes and allowed to cool. A portion of the contents from each tube was poured into a cuvette, bubbles were allowed to disperse and the absorbance was read. The absorbance was read at 630 nm against the blank. Calculation of glycogen was done using Equation 1:

Mg glycogen/100g fresh liver =
Mg glycogen/ml × 10/4 × 2/0.5 × 100/total liver weight

Statistical analysis:
All values were Mean ± S.E.M of the variables measured. Values between two groups were compared using student t-test while analysis of variance (ANOVA) was used to compare mean values in multiple groups. P-values≤0.05was taken as statistical significant.

RESULTS

Effect of Thyroidectomy on serum thyroid hormones in male Wistar rats: The effect of thyroidectomy on circulating thyroid hormone is shown in figure 1, significant reductions were observed in both triiodothyronine (24.45 ± 1.26 Vs 36.55 ± 1.43 pg/ml) and thyroxine (49.28 ± 2.63 Vs 107.6 ± 2.62 pg/ml) in the thyroidectomized rats when compared with the normal rats.

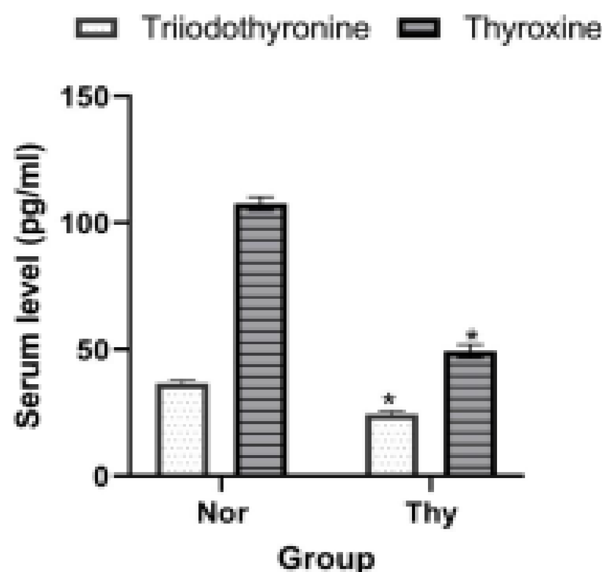

Figure 1:
Effect of thyroidectomy on serum triiodothyronine and thyroxine level in male Wistar rats. *P<0.05 Vs Normal. Nor – Normal, Thy -thyroidectomized

Effect of caffeine on blood glucose in normal and thyroidectomized male Wistar rats: Caffeine caused a significant increase in the blood glucose of both the normal and thyroidectomized rats which was sustained throughout the 60 minutes observation period. The increased blood glucose produced by caffeine in the normal and thyroidectomized rats was however significantly different, for instance, at the peak of their responses, caffeine caused 210 % increase in blood glucose in normal rats while it caused 180 % increase in the thyroidectomized rats (figure 2).

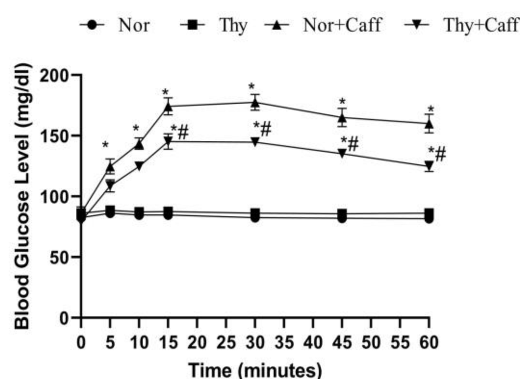

Figure 2:
Effect of caffeine on blood glucose level in normal and thyroidectomized male Wistar rats. *P<0.05 Vs Normal, #P<0.05 Vs Thyroidectomized. Nor – Normal, Thy – thyroidectomized, Nor+Caff – Normal+Caffeine, Thy+Caff – Thyroidectomised+ Caffeine

Effect of adrenergic blockers on caffeine-induced high blood glucose in normal and thyroidectomized male Wistar rats: As shown in figure 3, pretreatment with alpha or beta adrenergic blocker caused significant reduction in the increased blood glucose produced by caffeine in normal (fig 3A) and thyroidectomized (fig 3B) rats. The effect was however abolished when the rats were pretreated with a combination of the 2 adrenergic receptor blockers.

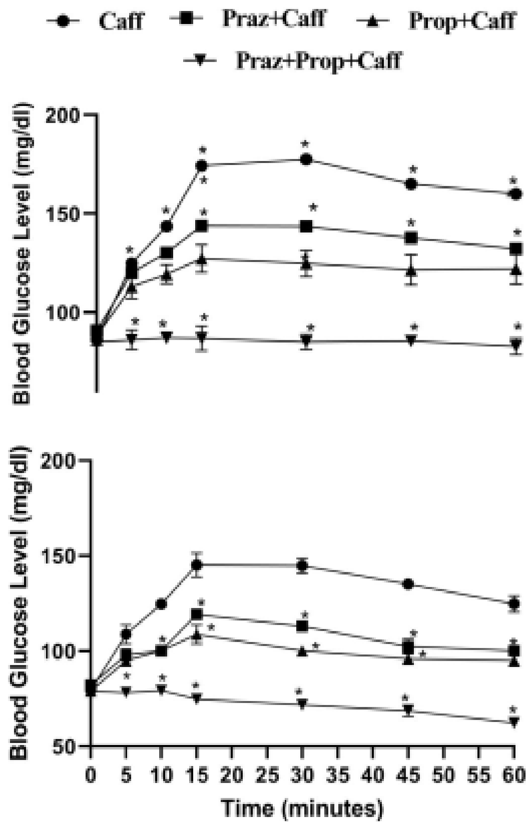

Figure 3:
Effect of adrenergic blockers on caffeine-induced increase blood glucose in (A) normal and (B) thyroidectomized rats. *P<0.05 Vs Caffeine. Caff – Caffeine, Praz+Caff –Prazosin + Caffeine, Prop+Caff –Propanolol+Caffeine, Praz+Prop+Caff – Prazosin+ Propanolol+Caffeine

Effect of caffeine on liver glycogen content in normal and thyroidectomized male Wistar rats: Liver glycogen content of the thyroidectomized rats (3.11 ± 0.20 mg/100g tissue weight) was significantly higher than the normal rats (1.91 ± 0.43 mg/100g tissue weight). These glycogen contents were significantly reduced by caffeine in both normal (0.25 ± 0.04 mg/100g tissue weight) and thyroidectomized rats (1.65 ± 0.16 mg/100g tissue weight) when compared with their respective controls. While pretreatment with prazosin had no effect on the caffeine-induced glycogen depletion, the effect was abolished by propanolol and a combination of prazosin and propanolol, figure 4.

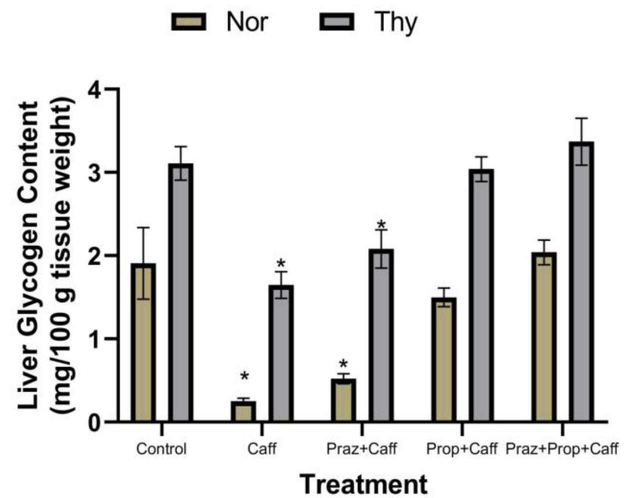

Figure 4:
Effect of caffeine on liver glycogen contents in normal and thyroidectomized male Wistar rats. *P<0.05 Vs Control.

DISCUSSION

The role of thyroid hormones on the effect of acute caffeine exposure on blood glucose and hepatic glycogen was examined. Thyroidectomy caused significant decrease in circulating thyroxine and triiodothyronine level in this study. These decreased hormonal levels are consistence with the levels reported in the method adopted in this study (Jin and Sugitani *et al*., 2021). Caffeine caused significant increased blood glucose level in normal and thyroidectomized rats of this study. This effect is in consonance with the well documented effect of acute caffeine exposure (Oyebola and Alada, 1993; Dekker *et al*., 2007; Salahdeen and Alada, 2009; Alagbonsi *et al*., 2016; Shi *et al*., 2016; Reis *et al*., 2018) on blood glucose in normal rats. Caffeine increases blood glucose through its stimulation of adrenal gland release of adrenaline (Battram *et al*., 2005), hence studies have linked acute caffeine consumption, increased plasma adrenaline and decreased insulin sensitivity (Dekker *et al*., 2007). Caffeine acts directly on the adrenal medulla by stimulating the caffeine-sensitive calcium ion store (Usachev *et al*., 1993) which is coupled with exocytotic release of stored adrenaline by the chromaffin cells of the adrenal medulla (García *et al*., 2006). The increased adrenaline mediates increase blood glucose by promoting hepatic glycogenolysis and gluconeogenesis while it inhibits insulin-dependent glucose disposal (Sherwin and Saccà, 1984). Several studies have documented that intracellular calcium mobilization is dependent on thyroid hormone (Dho *et al*., 1989; Marino *et al*., 2006) and thyroidectomy had been shown to suppress intracellular calcium release (Amadi *et al*., 2005), it conceivable that caffeine-mediated calcium release which is associated with increased adrenaline release will be hindered in the thyroidectomized rats, it is therefore not surprising that caffeine-elicited increase blood glucose was reduced from 210 % to 180 % in the thyroidectomized rats of this study.

The lack of total abolishment of the caffeine-induced increased blood glucose in the thyroidectomized rats of the current study may be linked with the interaction of caffeine

with adenosine receptor, caffeine is a potent non-selective adenosine receptor antagonist and exerts most of its biological activities by blocking all types of adenosine receptors (Ribeiro and Sebastião, 2010). Agonist of adenosine receptors have been shown to decrease blood glucose and their blockade increases blood glucose (Alagbonsi *et al*., 2016) therefore, caffeine may utilize the adenosine receptor blockade pathway independently of its direct stimulation of adrenaline release to increase blood glucose level. Earlier reports have shown that adenosine receptor expression is greatly increased in hypothyroid rats (Baños *et al*., 2002) thus, their blood glucose lowering effect may be effectively blocked by caffeine administration in the thyroidectomized rats hence providing additional pathway for caffeine induction of high blood glucose level in the thyroidectomized rats.

The significant depletion in hepatic glycogen content induced by caffeine in normal and thyroidectomized rats in this study is consistent with the effect of caffeine on hepatic glycogen content reported by Martin *et al*. (2004). Although, the effect of caffeine on hepatic glycogen is conflicting, some studies have reported that it inhibits glycogenolysis by competitive binding of glycogen phosphorylase a (Ercan-Fang and Nuttall, 1997; Tsitsanou *et al*., 2000) while others reported that it potentiates glycogenolysis secondary to its stimulation of adrenaline release (Knapik *et al*., 1983; García *et al*., 2006). These conflicting reports appear to be settled by the findings of Jarrar and Obeid (2014) that the time of caffeine administration determines if it will be glycogenolytic or glycogen-sparing. Administration of caffeine to fasted animals 1 hour before meal ingestion caused significant glycogenolysis while co-administration with meal or 1 hour after meal shifted the effect to glycogen-sparing (Jarrar and Obeid, 2014), the glycogenolytic effect of caffeine observed in the current study is in tandem with their former observation given that caffeine was administered to fasted animals. In addition to the glycogenolytic effect of adrenaline secondary to caffeine stimulation, the antagonistic effects of caffeine on adenosine receptors could synergistically activate glycogenolysis (Davis *et al*., 2003). Hence, the observed glycogen depletion in the thyroidectomized rats of this study may not be surprising given that blockade of adenosine receptors by caffeine stimulates glycogen depletion (Davis *et al*., 2003) and hypothyroidism in rats is associated with increased expression of adenosine receptor (Baños *et al*., 2002) thereby providing more binding sites to be blocked in the thyroidectomized rats. It is worth noting that, glycogen content of the thyroidectomized rats was significantly higher than the normal rats, this aligns with the association of hypothyroidism with decreased gluconeogenesis and glycogenolysis resulting in increased hepatic glycogen content (Storm *et al*., 1984; Chakrabarti *et al*., 2007) as well as reduction in glycogen phosphorylase activity secondary to reduced responsiveness to adrenergic stimulation in the thyroidectomized rats (Chu *et al*., 1985).

The abolishment of the caffeine-induced increased blood glucose and hepatic glycogen depletion observed in this study underscores the role of adrenergic receptors in mediating the caffeine effect and the postulation that induction of adrenaline release is a primary mechanism by which caffeine mediates its effect on glucose metabolism. In conclusion, caffeine elicits hyperglycemic and glycogenolytic effects in both normal and thyroidectomized rats through mechanism that involves adrenergic stimulation.

REFERENCES

Abe S.K., Saito E., Sawada N., Tsugane S., Ito H., Lin Y., Tamakoshi A., Sado J., Kitamura Y., Sugawara Y., et al. (2019). Coffee consumption and mortality in Japanese men and women: A pooled analysis of eight population-based cohort studies in Japan (Japan Cohort Consortium). Preventive Medicine, 123:270–277

Alagbonsi, A.I., Salman, T.M., Salahdeen, H.M. and Alada, A.A. (2016).

Effects of adenosine and caffeine on blood glucose levels in rats. Niger J

Exp Clin Biosci 4:35-41.

Amadi, K., Sabo, M.A,, Adelaiye, A.B. and Sagay, A.S. (2005). Dependence of calcium on thyroid hormone for the regulation of cellular functions. Nigerian Journal of Physiological Sciences. 20(1-2):95-100.

Baños, G., Martínez, F., Grimaldo, J. I. and Franco, M. (2002). Adenosine participates in regulation of smooth muscle relaxation in aortas from rats with experimental hypothyroidism. Canadian Journal of Physiology and Pharmacology, 80(6): 507-514.

Battram, D.S., Graham, T.E., Richter, E.A. and Dela, F. (2005). The effect of caffeine on glucose kinetics in humans – influence of adrenaline. Journal of Physiology, 569, 347–355.

Bollen, M. and Stalmans, W. (1988). The effect of the thyroid status on the activation of glycogen synthase in liver cells. Endocrinology, 122(6):2915-9.

Chakrabarti, S., Guria, S., Samanta, I. and Das, M. (2007). Thyroid dysfunction modulates glucoregulatory mechanism in rat. Indian Journal of Experimental Biology, 45, 549-553

Chu, D.T., Shikamat, H., Khatra, B.S. and Exton, J.H. (1985). Effects of Altered Thyroid Status on & Adrenergic Actions on Skeletal Muscle Glycogen Metabolism. Journal of Biological Chemistry, 260(18), 9994-1000.

Czarniecka-Skubina, E., Pielak, M., Sałek, P., Korzeniowska-Ginter, R. and Owczarek, T. (2021). Consumer Choices and Habits Related to Coffee Consumption by Poles. International Journal of Environmental Research and Public Health, 18(8):3948.

Davis, J.M., Zhao, Z., Stock, H.S., Mehl, K.A., Buggy, J. and Hand, G.A. (2003). Central nervous system effects of caffeine and adenosine on fatigue. American Journal of Physiology Regulation Integration and Comparative physiology, 284, R399-R404.

Dekker, M., Gusba, J., Robinson, L., and Graham, T (2007). Glucose homeostasis remains altered by acute caffeine ingestion following 2 weeks of daily caffeine consumption in previously non-caffeine-consuming males. British Journal of Nutrition, 98(3), 556-562.

Dho, S., Ansah, T.A. and Case, R.M (1989). Influence of thyroid status on Ca2+ mobilization and amylase secretion in rat pancreatic acini. Cell Calcium, 10(8), 551-560.

Ercan-Fang, N, and Nuttall, F.Q. (1997). The effect of caffeine and caffeine analogs on rat liver phosphorylase-a activity. Journal of Pharmacology, 280:1312–8.

García, A.G., García-De-Diego, A.M., Gandía, L., Borges, R., and García-Sancho, J. (2006). Calcium signaling and exocytosis in adrenal chromaffin cells. Physiological Review, 86(4):1093-131.

Grosso, G., Micek, A., Godos, J., Sciacca, S., Pajak, A., Martínez-González, M.A., Giovannucci, E.L. and Galvano, F. (2016). Coffee con-sumption and risk of all-cause, cardiovascular, and cancer mortality in smokers and non-smokers: A dose-response

me-ta-analysis. European Journal of Epidemiology, 31:1191–1205.

Han, H.S., Kang, G., Kim, J., Choi, B. and Koo, S. (2016). Regulation of glucose metabolism from a liver-centric perspective. Experimental and Molecular Medicine, 48, e218.

Jarrar, S.F. and Obeid, O.A. (2014).Timing of caffeine ingestion alters postprandial metabolism in rats. Nutrition, 30(1): 107-111.

Jermyn, M.A. (1975). Increasing the sensitivity of the anthrone method for carbohydrate. Analytical Biochemistry 68, 322-335.

Jin, S. and Sugitani, I. (2021). TSH Suppression was induced in rat model after total thyroidectomy. Journal of Nippon Medical School 88: 311-318

Knapik, J.J., Jones, B.H., Toner, M.M., Daniels, W.L. and Evans, W.J. (1983) Influence of caffeine on serum substrate changes during running in trained and untrained individuals. Biochemistry of Exercise, 13:514–519.

Loftfield, E., Cornelis, M.C., Caporaso, N., Yu, K., Sinha, R., Freedman N. (2018). Association of coffee drinking with mortality by genetic variation in caffeine metabolism: Findings from the UK biobank. JAMA Internal Medicine, 178:1086–1097.

Marino, F., Guasti, L., Cosentino, M., De Piazza, D., Simoni, C., Bianchi, V., Piantanida, E., Saporiti, F., Cimpanelli, M.G., Crespi, C., Vanoli, P., De Palma, D., Klersy, C., Frigo, G.M., Bartalena, L., Venco, A., Lecchini, S. (2006). Thyroid hormone and thyrotropin regulate intracellular free calcium concentrations in human polymorphonuclear leukocytes: In vivo and in vitro studies 2006 International Journal of Immunopathology and Pharmacology, 19(1), 149-160.

Martin, J.V., Nolan, B., Wagner, G.C. and Fisher, H. (2004). Effects of dietary caffeine and alcohol on Liver carbohydrate and fat metabolism in rats. Medical Science Monitor, 10(12):BR455-61.

Mullur, R., Liu, Y.Y. and Brent, G.A. (2014). Thyroid hormone regulation of metabolism. Physiol Rev. 94(2):355-382.

Nishi, M. (2018). Diabetes mellitus and thyroid diseases. Diabetol International, 9:108–12.

Park, S.Y., Freedman, N.D., Haiman, C.A., Le Marchand, L., Wilkens, L.R., Setiawan, V.W. (2017) Association of coffee consumption with total and cause-specific mortality among nonwhite populations. Annals of Internal Medicine, 167:228–235.

Reis, C.E.G., Dórea, J.G., da Costa, T.H.M. (2018). Effects of coffee consumption on glucose metabolism: A systematic review of clinical trials. Journal of Traditional Complement Medicine, 9(3):184-191.

Ribeiro, J.A. and Sebastião, A.M. (2010). Caffeine and adenosine. Journal of Alzheimers Disease, 20 (1):S3-15.

Salahdeen, H.M. and Alada, A.R.A. (2009). Role of adrenergic receptors in the caffeine induced increase in glucose uptake by the canine hindlimb. Niger J Physiol Sci 24 (2):141 7

Salahdeen, H.M., Omoaghe, A.O., Isehunwa, G.O., Murtala, B.A., and Alada, A.R.A. (2015). Gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extracts of kolanut (Cola nitida) (vent) and its toxicity studies in rats. Journal of Medicinal Plants Research, 9, 56-70.

Seifter, S., Dayton, S., Novic, B. and Muntwryler, E. 1950). The estimation of glycogen with anthrone reagent. Archives of Biochemistry 25,191-199

Sherwin, R.S. and Saccà, L. (1984). Effect of epinephrine on glucose metabolism in humans: contribution of the liver. American Journal of Physiological, 247(2 Pt 1):E157-65.

Shi, X., Xue, W., Liang, S. et al (2016). Acute caffeine ingestion reduces insulin sensitivity in healthy subjects: a systematic review and meta-analysis. Nutrition Journal, 15, 103.

Storm, H., Van Hardeveld, C. and Kassenaar, A. (1984). The influence of hypothyroidism on the adrenergic stimulation of glycogenolysis in perfused rat liver, Biochimica Biophysica Acta, 798-350.

Tsitsanou, K.E., Skamnaki, V.T. and Oikonomakos, N.G. (2000). Structural basis of the synergistic inhibition of glycogen phosphorylase a by caffeine and a potential antidiabetic drug. Archives of Biochemistry and Biophysics, 384:245–54.

Usachev, Y., Shmigol, A., Pronchuk, N., Kostyuk, P. and Verkhratsky, A. (1993). Caffeine-induced calcium release from internal stores in cultured rat sensory neurons. Neuroscience, 57(3):845-59.