

SLEEP DEPRIVATION AND COFFEE CONSUMPTION INDUCED CHANGES IN BLOOD PRESSURE, BODY MASS INDEX AND BLOOD GLUCOSE IN MALE WISTAR ALBINO RATS

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Abstract

Intentional restriction of sleep is progressively high and common among those experiencing environmental/psychological stress due to work demands, abnormal working hours and psychiatric/physical disorders in developing and developed industrialized societies. Deficiency of sleep have its several concerns, amongst which include increased prevalence of disease risks and mortality. 30 adult rats were randomly divided into six groups with sleep deprivation (SD) (using multiple platform method) and coffee administration for 30 days after 2 weeks of acclimatization: A (control), B (SD only), C (416.75ml/kg coffee), D (833.50ml/kg coffee), E (SD + 416.75ml/kg coffee) and F (SD + 833.50ml/kg coffee). Blood pressure was determined by cannulation of carotid artery using pressure transducer and a polygraph. Glucose concentration was determined after enzymatic oxidation and BMI calculated using rat weights and lengths. Mean arterial pressure (MAP) was significantly increased in groups B, D, E and F compared to control. Blood glucose demonstrated a significant reduction in groups B, C, D, E and F compared to control. SD rats had a significant decrease in BMI compared to control while groups C, D, E and F were significantly increased compared to B. Reduction in glucose across the treatment groups could indicate an improved glucose tolerance, no insulin resistance. Also, increased energy expenditure, may explain the reduction in BMI in high and low doses of coffee + SD groups and increase in MAP. SD + coffee induced stress may aid in the prevention of obesity, type 2 diabetics although the significant increase in MAP.

INTRODUCTION

Sleep deprivation (SD), which can either be chronic or acute, is a condition of not having enough sleep. A chronic sleep-deprived state can cause fatigue, daytime sleepiness, clumsiness and weight loss or weight gain.¹ It adversely affects the brain and cognitive function.² SD can, paradoxically, lead to increased energy consumption, alertness and enhanced mood. It has been used as a treatment for depression.^{3,4}

KEYWORDS: Sleep Deprivation, Coffee, Plasma glucose, Blood Pressure.

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In modern industrialized societies, voluntary restriction of sleep is increasingly common due to increasing work demands with increasing working hours.⁵ Partial loss of sleep is common among people who experience environmental or psychological stress, people who have psychiatric or physical disorders or who participate in shift work.⁶

The cumulative long-term effects of sleep loss and sleep disorders have been associated with a wide range of deleterious health consequences including an increased risk of hypertension, diabetes, obesity, depression, heart attack, stroke, and even increasing mortality.⁶ Sufficient sleep is a key component in the regulation of energy metabolism. Sleep is a restorative process with beneficial effects on body systems (digestive, cardiovascular and the immune system).

It is consequently important to understand and elucidate the mechanisms through which sleep and health are related if we are to find ways to manage people with chronically restricted sleep.

Epidemiological studies showed that habitual short sleep duration is correlated with an increased risk of developing obesity and diabetes.^{7,8} There was impairment in glucose tolerance (factor for developing type II diabetes) after six days of SD (4 hours/night), in some participants when compared to other participants who were allowed 12 hours in bed per night for six days.⁹ Likewise, SD resulted in reduction of the satiety hormone leptin, accompanied by increased hunger and increased serum concentrations of the orexigenic factor ghrelin,¹⁰ which may upturn the risk of developing obesity.

Coffee is a beverage consumed commonly as psychoactive substances by billions of people in most countries of the world. Traditionally, high consumption of coffee has been considered to have negative health consequences which is attributed to the stimulant effects of caffeine.¹¹ However, coffee is also one of the largest sources of antioxidants and it contains various compounds with potential beneficial effects on glucose metabolism, inflammation and blood vessel function.¹² The numerous health benefits of coffee consumption have received considerable scientific attention. Moderate consumption of coffee is correlated with a lower incidence of gallstones, Alzheimer's disease, dementia and Parkinson's disease.¹³⁻¹⁷ Coffee consumption improves short term memory¹⁸ while the tannins in coffee may reduce the cariogenic potential of foods by reducing plaque formation.¹⁹

Epidemiological studies indicates that consumption of coffee is consistently associated with a lower risk of Type 2 diabetic mellitus (T2DM).²⁰⁻²² An inverse relationship between consumption of coffee and insulin insensitivity and glucose intolerance has been reported.²³ Also, Ding et al., (2014)²⁴ after conducting a meta-analysis indicated an inverse relationship between regular consumption of coffee and the risk of T2DM. Oxidative stress is a fundamental factor in the pathogenesis of type 2 diabetes mellitus,²⁵ interestingly, coffee is a rich source of antioxidant polyphenols. Thus, the antioxidant properties of coffee may contribute to its anti-diabetic effects. Since coffee is consumed for its taste and as a stimulant, consumption of coffee beverages and their circumstantial intake during stressful situations (SD) was considered. This will increase our knowledge on possible changes that coffee consumption vis-a-vis SD may have on body mass index (BMI), blood pressure and blood glucose.

MATERIALS AND METHOD

Preparation and administration of coffee drink

Experimental Design and Animal grouping

Thirty (30) male Wistar rats of Albino strain weighing between 180-200g were obtained from a reputable animal house in Ibadan. They were brought to the animal house of Department of Physiology, Olabisi Onabanjo University, Ogun state, Nigeria. They were housed in standard plastic cages at room temperature. They were allowed to acclimatize for about two (2) weeks before the commencement of the study. They were fed with rat pellet (Caps Feed Ibadan), and allowed free access to drinking water.

They were divided into six (6) groups with five rats per group, labeled A, B, C, D, E, and F. The rats' weights were monitored twice a week using a manual weighing balance. Group A which served as the control, received distilled water. Group B contained rats deprived from sleep only. Group C rats were administered with 416.75ml/kg dose of coffee only. Group D rats were administered with 833.50ml/kg dose of coffee only. Group E rats were deprived of sleep and administered with 416.75ml/kg dose of coffee. Group F rats were deprived of sleep and administered with 833.50ml/kg of coffee.

All animal handling and experimental protocols implemented in this study was in conformation with the international principles for laboratory animals as obtained in the Helsinki's Declaration.²⁶

Experimental Sleep deprivation method
The modified multiple platform method as described by Oh et al., (2012),²⁷ was adopted to deprive the rats of sleep. Plastic cages used measured 1050 x 550 x 270mm and cylindrical mug cups used measured 8.5cm high, 6.5cm in diameter. Six (6) Mug cups were placed in the plastic cage to form the platform. The plastic cage was filled with water up to 2cm from the platform tops. The rats were able to move around the cage by jumping from one platform to another. Throughout the experiment, the experimental room was maintained at the normal available atmosphere condition.

Body Mass Index (BMI) Determination

The weights of the rats were determined with the use of a weighing balance and the lengths of the rats were determined with the use of tread and a ruler. Rat's lengths

were measured from head to its tail end. BMI was calculated by dividing the weights of the rats in grams with the squares of the length in centimeters.²⁸

$$\text{BMI} = \text{weight (g)} / \text{length (cm}^2\text{)}$$

3.4 Blood Glucose Determination

After 14 days of administration, the blood glucose was determined using the Accu-Chek active glucometer. Rats were picked at random from each group. The tail of each rat was sterilized with methylated Spirit and cotton wool. The rats was held at the mid-region with left hand, the tip of rats tail was cut with a pair of scissor, blood were collected from the tail vein to the test strip of Accu-Chek active glucometer to commence reading.

3.5 Blood Pressure (BP) Determination

Invasive BP was determined by the use of a pressure transducer connected to a polygraph, after the rats were anaesthetized by intra-peritoneal injection of urethane at a dose of 0.5mL/100g body weight, followed by cannulation of carotid artery. This was as described by Parasuraman and Raveendran (2012)²⁹. Heparin (500 IU/kg-body weight) was infused into the cannulated artery immediately to prevent intravascular coagulation. The systolic and diastolic BP were obtained through the graphic records of the polygraph and mean arterial BP was determined by using the formula: $(\text{SBP} + 2 \times \text{DBP}) / 3$.

STATISTICAL ANALYSIS

Results are presented as means \pm SEM and comparison of the means was done using the one way analysis of variance, followed by Student's Newman-Keuls post hoc test, using the GraphPad software. A p-value < 0.05 was considered statistically significant.

RESULT

Table 1 displays the effect of SD and coffee (separately and combined) on pulse and blood pressure. Low dose coffee raised SBP (compared to control) and pulse rate (compared to sleep deprived group) ($p < 0.05$). While high dose coffee raised both SBP and DBP, as well as MABP (compared to both control and sleep deprived group) ($p < 0.05$). Coffee and SD combined raised MABP increased significantly (except in the group administered low dose coffee), along with pulse rate in all groups when compared with control and sleep deprived group. The increase was significantly higher for SBP and MABP in the high dose coffee +

sleep deprived group, taking a deviation from the thread of increase in other groups. Also the pulse reading from this group showed a statistical significance compared to sleep deprived group only.

Blood glucose reduced significantly in both groups administered doses of coffee as well as sleep deprived group when compared with the control. While the groups treated with the combination of doses of coffee and SD exhibited reduced blood glucose level when compared with control and sleep deprived group ($p < 0.05$) (Figure 1). High dose coffee and SD group also exhibited reduced organ sizes (Liver, kidney) when compared with sleep deprived group ($p < 0.05$).

Table 1: Effects of Sleep deprivation and Coffee consumption on the Blood Pressure

Group	Treatment	Blood Pressure			
		Systolic	Diastolic	Pulse	MABP
A	Control	54.45 ± 0.64	21.97 ± 1.94	32.52 ± 1.29	32.77 ± 1.51
B	Sleep deprived	57.41 ± 0.51	33.24 ^a ± 3.07	24.19 ± 3.56	41.29 ^a ± 1.88
C	Low dose of coffee (0.5mL/kg-body weight)	61.08 ^a ± 0.18	27.23 ± 5.42	33.85 ^b ± 5.39	38.51 ± 3.62
D	High dose of coffee (1.0mL/kg-body weight)	73.35 ^{abc} ± 0.57	47.46 ^{abc} ± 1.29	25.89 ± 0.83	56.09 ^{abc} ± 1.04
E	Sleep deprived with low dose of coffee (0.5mL/kg-body weight)	76.47 ^{abc} ± 0.79	35.39 ^a ± 1.39	41.08 ^{ab} ± 0.60	49.08 ^{abc} ± 1.17
F	Sleep deprived with high dose of coffee (1.0mL/kg-body weight)	91.84 ^{abc} ± 5.28	52.84 ^{abc} ± 3.07	39.00 ^b ± 2.50	66.44 ^{abc} ± 3.44

a - show significance when compared with Control

b - show significance when compared with Sleep Deprived

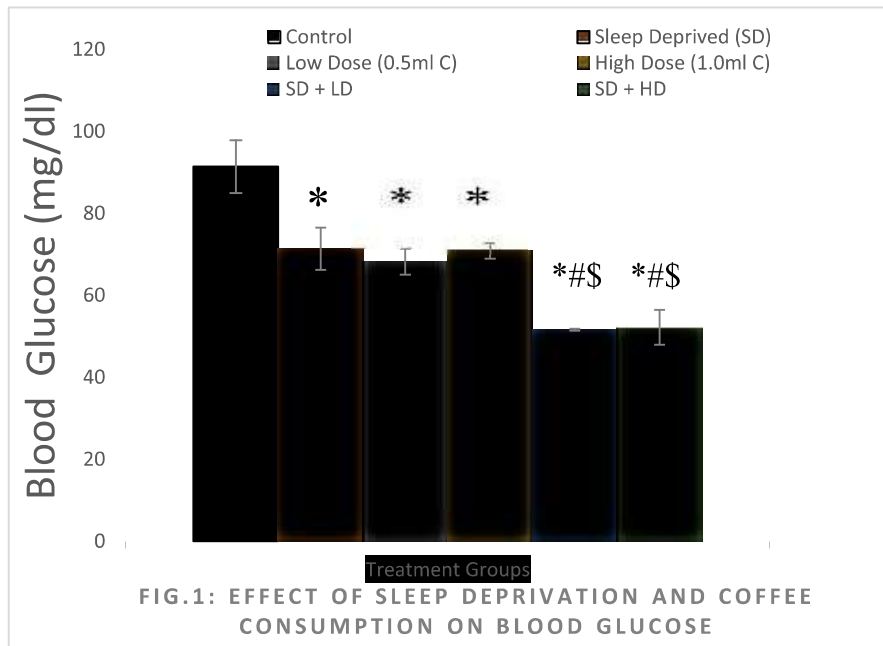
c - show significance when compared with Low dose coffee

$p < 0.05$

Table 2: Effects of Sleep deprivation and Coffee consumption on Body mass Index

Group	Treatments	% Change in Body mass index
A	Control	7.87 ±1.36
B	Sleep deprived animals only	-25.40 ±9.89 ^{ac}
C	Rats administered with low dose of coffee (0.5mL/kg-body weight)	7.77 ±0.52 ^b
D	Rats administered with high dose of coffee (1.0mL/kg-body weight)	0.22 ±0.81 ^{abc}
E	Sleep deprived rats, administered with low dose of coffee (0.5mL/kg-body weight)	0.51 ±0.39 ^{ab}
F	Sleep deprived rats, administered with high dose of coffee (1.0mL/kg-body weight)	-2.88 ±0.83 ^{ab}

a show significance when compared with Control
 b show significance when compared with Sleep Deprived
 c show significance when compared with Low dose coffee
 p < 0.05



* show significance when compared with Control
 # show significance when compared with Sleep Deprived
 \$ show significance when compared with Low dose coffee
 p < 0.05

DISCUSSION

To our knowledge, this is the first deliberate examination of the pressor effect, glucose metabolic effect and obesity risk of both coffee and sleep deprivation (SD) combination. The present study demonstrates that coffee and SD collectively upsets systolic and diastolic BP. It further demonstrates that the higher the number of hours of SD and increased doses of coffee, the more likely a progressive increase in mean arterial BP after two weeks of treatment in rats. Jee et al. (1999),³⁰ reported a positive relationship between cups of coffee consumed on a daily basis and elevated systolic blood pressure (SBP), after conducting a meta-analysis of controlled clinical trials without respect to age.

The present study shows that chronic elevations in BP which could pose serious risk for the development of hypertension are accompanied by increased BP response to high dose of coffee compared to control, SD alone and low dose of coffee. The increase in BPs were further intensified with the combination of both doses of coffee + SD. These findings suggest that higher dose of coffee may exert greater BP effects when compared to SD but combination of both have the tendency to poses a greater risk of hypertension.

Even though existing evidence does not clearly point fingers at habitual coffee drinking as a related risk of hypertension,³¹ it does not rule out the fact that caffeinated coffee can causes at least an acute rise in BP soon after exposure.³² This may be similar to other lifestyle factors like physical activity or talking, which possess mostly transient physiological responses. But it is possible that psychosocial factors may perhaps contribute to either beneficial or adverse

effects on BP.³³ In the present study, interaction of coffee with SD (mental stress) progressively increased BP. A dose-response relationship between doses of coffee consumed + SD and change in BP was exhibited. Both coffee and sleep deprived stress could be proposed to have caused increasing significant raise in BP through sympathetic activation, antagonism of adenosine receptors, increased norepinephrine release and/or activation of the Renin Angiotensin Aldosterone System (RAAS). The possibility of these mechanisms have been discussed in detail by Myers³³ and Nurminen et al.,³². Although Umemura et al., (2006)³⁴ reported an enhanced endothelium-dependent vasodilatation after acute caffeine administration in young healthy men, a mechanism by which caffeine could lower BP. The synergetic effect of SD + coffee consumption could lead to increase in C-reactive protein (CRP).^{35,36} CRP is a major concern for the development of hypertension, which cannot be completely ruled out in this case.

Increasing epidemiological and experimental evidence proposed that SD (alteration in physiological sleep need) can modifies metabolism in a manner that promotes weight gain.^{10, 1} Taheri et al,¹ cited that reports have established a link between routinely short sleep time and increased body mass index (BMI) in large population samples. This was clearly stated to be connected to a decrease and increase in leptin and ghrelin levels respectively. These hormones, which are believed to alter appetite, may only contribute to the BMI changes that occurs with SD. Also animal studies have demonstrated a connection between sleep and metabolism.^{37, 38} Rats subjected to prolonged, complete SD showed increase in both food intake and energy expenditure. But the net effect was weight

loss and eventually, death.³⁹ Although leptin and ghrelin hormonal levels were not determined in the present study, the drastic BMI reduction observed in the sleep deprived group (compared to control) correlates with the findings of Rechtschaffen and Bergmann.³⁹ This can be presumed to be as a result of a stressful state as postulated by Everson and Wehr (1993)⁴⁰ and Everson (1995)⁴¹.

Coffee has been reportedly used for weight loss, although its effectiveness has not been proven.⁴² This was not evident in this study, as the coffee + SD combination even though slightly reduced BMI (compared to control group), exhibited increased BMI compared to the sleep deprived group. After SD, weight loss was reported in obese individuals.^{1, 43} Therefore, the role of hormonal changes (leptin and ghrelin) can only be said to contribute but not compensate for the association between SD and BMI. In same vein, the wide range variation between coffee only groups, coffee + SD groups compared to the control can be accounted for, although the role of fat producing hormone, cortisol cannot be disregarded.

Hence, increased cortisol activity could also account for the significant BMI increase in the coffee + SD groups compared to the sleep deprived group.

Watson et al. (2010)⁴⁴ has described weight gain as a positive energy balance; energy intake greater than energy expenditure. Natural processes (such as glucose metabolism and an upregulation of appetite) related to energy balance, as well as external factors (such as food choice and increased time available to eat) are largely disturbed by SD. This, according to Watson et al.,⁴⁴ cannot be said to have occurred in the present study with significant and slight reductions in BMI across the groups (compared to control). The assumed increased energy

expenditure in the present study can be related to the increased mental and physical activities engaged in by the rats due to lack of sleep. This can also explain significantly reduction observed in blood glucose level of the coffee treated groups (compared to control) and coffee + SD groups (compared to control and sleep deprived group). Although previous studies have reported reduced glucose tolerance and insulin resistance (due to increase in the level of evening cortisol) associated with SD.^{45,9,46} The present study showed an improved glucose tolerance (which could indicate adequate insulin sensitivity) across the treatment groups during the early hours of the morning. This is quite contradictory to the findings of Knutson.⁴⁵ Possibly, there were also no serious disturbances to the secretory profiles of the counter regulatory hormones, cortisol and growth hormone (GH) and as such no alterations in glucose regulation in the treatment groups as reported during SD. This effect may further be attributed to the influence of coffee on glucose metabolism. As reports have stated that habitual coffee consumption is associated with insulin sensitivity,⁴⁷ although there are a few contrasting views.

In conclusion, the present findings show progressively higher BP responses to coffee and SD combination treatment, relatively reduced blood glucose level and reduced BMI. The direct effect of coffee + SD after two weeks of study may suggest that alteration in sleep time and moderate consumption of coffee may perhaps prove to be an important aide to preventing obesity and possible occurrence of type 2 diabetics. Our findings seem to be at variance with the most established inverse association between coffee, SD, increase in BMI and the possible impairment in glucose metabolism. At

this point, we cannot ignore the possibility that a higher coffee dosage and consumption could probably exhibit insulin sensitivity lowering effect. Also a longer duration of this study may have proven otherwise. Long-term trials of coffee consumption and SD that include detailed measures of insulin sensitivity, glucose metabolism, energy expenditure and total amount of food intake are justified to elucidate the apparent discrepancy with studies like this that observed an inverse association between habitual coffee consumption, SD and risk for obesity and type 2 diabetes.

REFERENCES

1. Taheri S, Lin L, Austin D, Young T, Mignot E (2004) Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med.* 2004;1(3): e62. DOI: 10.1371/journal.pmed.0010062
2. Aihola P and Polo-Kantola P. Sleep deprivation: impact on cognitive performance. *Neuropsychiatr Dis Treat.* 2007; 3(5): 553-567. Vol 3 (553-567).
3. Nykamp Keith, Rosenthal Leon, Folkerts Mike, Roehrs Timothy, Guido Peter, and Roth Thomas. The Effects of REM Sleep Deprivation on the Level of Sleepiness /Alertness SLEEP, 1998; Vol. 21, No. 6, (609 - 614)
4. Riemann D, Berger M, Voderholzer U. Sleep and depression--results from psychobiological studies: an overview. *Biol Psychol.* 2001; 57:67-103.
5. Kronholm E., Partonen T., Laatikainen T., et al., "Trends in self-reported sleep duration and insomnia-related symptoms in Finland from 1972 to 2005: a comparative review and re-analysis of Finnish population samples," *Journal of Sleep Research*, 2008; vol. 17, no. 1, pp. 54-62.
6. Rajaratnam S. M.W. and Arendt J., "Health in a 24-h society," *Lancet*, 2001; vol. 358, no. 9286, pp. 999-1005.
7. Patel S. R. and Hu F. B., "Short sleep duration and weight gain: a systematic review," *Obesity*, 2008; vol. 16, no. 3, pp. 643-653.
8. Schulte PA. Characterizing the burden of occupational injury and disease. *J Occup Environ Med.* 2005; 47:607-622.
9. Spiegel K., Leproult R., and Van Cauter E., "Impact of sleep debt on metabolic and endocrine function," 1999; *Lancet*, vol. 354, no. 9188, pp. 1435-1439.
10. Spiegel K., Tasali E., Penev P., and Van Cauter E., "Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite," *Annals of Internal Medicine*, 2004; vol. 141, no. 11, pp. 846-850.
11. Wierzejska R. Caffeine - common ingredient in a diet and its influence on human health. *Rocz Panstw Zaki Hig.* 2012; 63(2):141-7.
12. Bhupathiraju SN, Pan A, Malik VS, Manson JE, Willett WC, van Dam RM, Hu FB. Caffeinated and caffeine-free beverages and risk of type 2 diabetes. *Am J Clin Nutr.* 2013; 97(1):155-66. doi: 10.3945/ajcn.112.048603.
13. Leitzmann MF, Stampfer MJ, Willett WC, Spiegelman D, Colditz GA, Giovannucci EL. Coffee intake is associated with lower risk of symptomatic gallstone disease in women. *Gastroenterology.* 2002 Dec;123(6):1823-30.
14. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, and Feeley M. Effects of caffeine on human health. *Food Addit. Contam.* 2003; 20:1-30.
15. Ranheim T and Halvorsen B. Coffee consumption and human health - beneficial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and Type 2 diabetes mellitus. *Mol. Nutr. Food. Res.* 2005; 49:274-284.
16. Lindsay Joan, Laurin Danielle, Verreault René, Hébert Réjean, Helliwell Barbara, Hill B. Gerry and McDowell Ian (2002). Risk Factors for Alzheimer's Disease: A Prospective Analysis from the Canadian Study of Health and Aging, *American Journal of Epidemiology* 2002; Vol. 156, No. 5: 445-453

17. Jaslów Ryan. "Three Cups of Coffee per Day Might Prevent Alzheimer's in Older Adults". CBS News. 2012. Accessed 12 October, 2015
18. Leitzmann MF, Willett WC, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E. A prospective study of coffee consumption and the risk of symptomatic gallstone disease in men. *JAMA*. 1999 Jun 9; 281(22):2106-12.
19. Armstrong LE, Pumerantz AC, Roti MW, Judelson DA, Watson G, Dias JC, Sokmen B, Casa DJ, Maresh CM, Lieberman H, Kellogg M. Int J Sport Nutr Exerc Metab. Fluid, electrolyte, and renal indices of hydration during 11 days of controlled caffeine consumption. 2005; Jun; 15(3):252-65.
20. Van Dam R.M., Dekker J.M., Nijpels G., Stehouwer C.D., Bouter L.M. and Heine R.J. Coffee consumption and incidence of impaired fasting glucose, impaired glucose tolerance and type 2 diabetes: the Hoorn Study. *Diabetologia*. 2004; 47:2152-2159
21. Agardh E.E., Carlsson S. and Ahlborn A. Coffee consumption, type 2 diabetes and impaired glucose tolerance in Swedish men and women. *J. Intern. Med.* 2004; 255:645-652.
22. Bidel S., Hu G. & Qiao Q. Coffee consumption and risk of total cardiovascular mortality among patients with type 2 diabetes. *Diabetologia*. 2006; 49:2618-2626.
23. Natella F, Nardini M. and Beletti F. Coffee drinking induces incorporation of phenolic acids into LDL and increases the resistance of LDL to ex vivo oxidation in humans. *Am. J. Clin. Nutr.* 2007; 86:604-609.
24. Ding M, Shilpa N, Bhupathiraju N.S, Chen M, van Dam R.M, Hu B.F. Caffeinated and Decaffeinated Coffee Consumption and Risk of Type 2 Diabetes: A Systematic Review and a Dose-Response Meta-analysis. *Diabetes Care* 2014; 37(2): 569-586. doi: 10.2337/dc13-1203.
25. Maritim A.C., Sanders R.A. and Watkins J.B. Diabetes oxidative stress and antioxidants; A Review. *J. Biochem Mol. Toxicol.* 2003; 17:24-38
26. NIH (1985). Guide for the Use of Laboratory Animals DHHS, PHS. NIH Publication No. 85-23.
27. Oh M.M, Kim J.W, Jin H.M, Kim J.J, Moon G.D. Influence of paradoxical sleep deprivation and sleep recovery on testosterone level in rats of different ages *Asian J Androl*. 2012 Mar; 14(2): 330-334.
28. Engelbregt MJT, van Weissenbruch MM, Popp-Snijders C, Lips P, Delemarre-van de Waal HA. Body Mass Index, body composition, and leptin at onset of puberty in male and female rats after intrauterine growth retardation and after early postnatal food restriction. *Pediatric Research* 2001; 50:474-478.
29. Parasuraman S, Raveendran R. Measurement of invasive blood pressure in rats. *J Pharmacol Pharmacother* 2012; 3:172-177.
30. Jee SH, He J, Whelton PK, Suh I, Klag MJ. The effect of chronic coffee drinking on blood pressure: a meta-analysis of controlled clinical trials. *Hypertension*. 1999; 33:647-652.
31. Geleijnse JM. Habitual coffee consumption and blood pressure: An epidemiological perspective. *Vascular Health and Risk Management* 2008; 4(5) 963-970
32. Nurminen ML, Niittynen L, Korpela R, et al.. Coffee, caffeine and blood pressure: a critical review. *Eur J Clin Nutr*, 1999; 53:831-9.
33. Myers MG. Effects of caffeine on blood pressure. *Arch Intern Med*, 1988; 148:1189-93.
34. Umemura T, Ueda K, Nishioka K, et al. Effects of acute administration of caffeine on vascular function. *Am J Cardiol*, 2006; 98:1538-41
35. Chang JJ, Pien GW, Duntley SP, Macones GA. Sleep deprivation during pregnancy and maternal and fetal outcomes: is there a relationship? *Sleep Med Rev*. 2010; 14(2):107-114
36. Micheli K, Komninos I, Bagkeris E, Roumeliotaki T, Koutis A, Kogevas M, et al. Sleep patterns in late pregnancy and risk of preterm birth and fetal growth restriction. *Epidemiology*. 2011; 22(5):738-744.

37. Berger RJ, Phillips NH. Energy conservation and sleep. *Behav Brain Res.* 1995; 69: 65-73.
38. Dinges D, Chugh DK. (1997) Physiologic correlates of sleep deprivation. In: Kinney JM, Tucker HN, editors. *Physiology, stress, and malnutrition: Functional correlates, nutritional intervention.* New York: Lippincott-Raven. pp. 1-27.
39. Rechtschaffen A and Bergmann BM. Sleep deprivation in the rat: An update of the 1989 paper. *Sleep* 2002; 25: 18-24.
40. Everson CA and Wehr TA. Nutritional and metabolic adaptations to prolonged sleep deprivation in the rat. *Am J Physiol* 1993; 264:R376-R387
41. Everson CA. Functional consequences of sustained sleep deprivation in the rat. *Behav. Brain Res* 1995; 69: 43-54.
42. Tamm AL, Šott N, Jürimäe J, Lätt E, Orav A, Parm Ü. *International Journal of Nutrition and Food Sciences* 2015; Vol: 2, No: 9.
43. Hansen TK, Dall R, Hosoda H, et al. Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol (Oxf)* 2002; 56:203-6.
44. Watson NF; Buchwald D; Vitiello MV; Noonan C; Goldberg J. A twin study of sleep duration and body mass index. *J Clin Sleep Med* 2010; 6(1):11-17.
45. Knutson KL. Impact of sleep and sleep loss on glucose homeostasis and appetite regulation. *Sleep Med Clin.* 2007; 2(2): 187-197.
46. Scheen AJ, Byrne MM, Plat L, Leproult R, Van Cauter E. Relationships between sleep quality and glucose regulation in normal humans. *Am J Physiol* 1996; 271:E261-70.
47. Arnlov J, Vessby B, Riserus U: Coffee consumption and insulin sensitivity. *JAMA* 2004; 291:1199-1201,