

Minireview

Autonomic Regulation of Cardiovascular Function in Obese Rat versus Obese Human

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ABSTRACT

Among the various health problems associated with obesity, cardiac autonomic neuropathy is one of the serious and relatively under-investigated problems. The authors have previously showed, in 2008, that vagal dysfunction is the main underlying factor in cardiac autonomic neuropathy in a rat model of congenital obesity. The aim of this article is to review the published findings in this topic since then and to compare the data in rats with those in human subjects.

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INTRODUCTION

Obesity has emerged world-wide as a major public health concern, and Africa is not an exception. It has been estimated that obesity/overweight is increasing by an average of 5% annually in sub-Saharan countries (Ziraba et al., 2009). Epidemiological and experimental studies show that obesity is a causative factor in the development of cardiovascular disease. In this case, the deleterious effects of obesity upon autonomic function are seen, at least in part, as a probable contributor to ultimate cardiovascular dysfunction. Many of the earlier studies that outlined the relationship(s) between obesity and circulatory dysfunction were conducted using animals, especially rats or mice fed calorie rich diets or strains genetically biased to become obese. The results of many of these rodent experiments have only subsequently been followed up with corollary observations in man. Recent human studies reported strong association of waist-hip ratio and low heart rate variability in humans (Yadav et al., 2017). Eleven years ago (2008) our study in Zucker^{fa/fa} obese rats vs. a control strain was among the earlier observations of diminished

Parasympathetically-mediated bradycardia during an acute behavioral challenge (i.e., classical aversive conditioning) (El-Wazir, et al., 2008). In the sense of 'translational science' this earlier study is primarily of interest to the degree that the findings are applicable to human obesity. The purpose of the present account is to summarize our earlier work and then to examine the subsequent decade's findings in human studies that are either congruent or inconsistent with our earlier rat findings. As such, it should provide objective validation or refutation of an important aspect – autonomic control - of the use of rats to model human obesity.

Review of our obese rat cardiovascular 'stress response' and its autonomic control

The Zucker obese rats used in our study (El-Wazir et al., 2008) were 9- to 11-weeks of age. The obese animals (n=10) weighed 452 ±45 g (mean ± SD) while the age-matched control rats (n=13) weighed 280 ± 46 g. We found no significant differences in mean arterial blood pressure (mBP_a via indwelling femoral artery catheter) while the rats were unanesthetized and at rest between obese (111.7 ± 5.6 mm Hg) vs. lean (113.1 ± 7.0 mm Hg). Likewise, resting heart rate (HR) was similar in obese (422 ± 22 /min) and lean (413 ± 43 /min.). The major novel finding of the study was that the bradycardia observed in a fully trained rat during a 'stressful stimulus' (a 15 sec. 'conditional stimulus' (CS) tone followed by 0.5 sec. tail shock) was

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significantly ($p < 0.05$) smaller in the obese (-17.8 ± 21.7 /min) as compared to the lean group (-46.0 ± 21.5 /min). Fig.1 is a composite analysis (i.e., each recording is an ensemble average across the lean (black) and obese (red) animals). Note particularly the significantly smaller bradycardia during the CS-induced stress in the obese (red) animals (i.e., vs. pre-tone control) ($p < 0.05$). This HR conditional response was essentially eliminated following delivery of atropine (not shown, Fig.1); thus, the HR slowing may be attributed largely to increased cardiac parasympathetic activity. The conditioned increase in mBP_a (not shown, Fig.1) was not significantly different between groups. Finally, the reflex change in HR divided by the change in mBP_a (HR/mBP_a) produced by a bolus iv infusion of phenylephrine, an index of baroreceptor function, was significantly smaller in obese ($n=6$; -1.36 ± 0.60) vs. lean ($n=5$; -2.80 ± 0.92). Conversely, the reflex tachycardia following iv nitroprusside did not differ between groups.

Our prior work (e.g., Randall 1994) with the conditioning paradigm helps interpret these findings. First, the pattern of changes in SNA relative to pre-tone control consists of an initial 'sudden burst' followed immediately by a momentary decrease which is next replaced during the majority of the CS by a modest increase over control. This patterned, learned cardiovascular response 'evolves' as the animal acquires the association between the tone and shock (El-Wazir, 2005). Second, the accompanying increases in mBP_a are reliably predicted by these changes in SNA (Burgess 1997). If so, our finding that the changes in blood pressure during the CS are similar in the two groups of rats implies that the underlying changes in SNA must be similar between the two groups. Finally, though we have not recorded changes in parasympathetic activity, the bradycardia shown in Figure 1 apparently results from baroreflex activity secondary to the conditional increase in BP (Randall, et al., 1994).

Age-matching of rats and humans

Our present intention is to compare these findings with the closest possible match to subsequent human studies. Perhaps the first issue, therefore, concerns the equivalent human developmental age as compared to 9-11-week old rats: how many rat days are equivalent to one human year? A recent review (Andreollo, et al., 2012) of the relevant literature matching rat development to human development explains that rats become sexually mature at 6 weeks while humans don't experience puberty until between about 12 to 13 years. The 'non-linear' differences in rate of-maturity

between the two makes comparison difficult, but over the total lifespan Andreollo and colleagues (2012) suggest that during the 'adult phase' 11.8 rat days is equivalent to 1 human year, while across the entire lifespan 13.8 rat days is equivalent to one human year. Using Andreollo's equivalents and accepting 6 weeks / 12 years as sexual maturity in rat / human, we estimate that our animals were roughly equivalent to humans of 23-24 years of age. Given the relative paucity of published human studies we have accepted a somewhat wider human age range for consideration in our comparisons.

Assessments of autonomic function in humans

Autonomic function in humans is often evaluated using heart rate variability (HRV) as assessed either in the time or frequency domain. Moreover, as in our rat study, the physiological response to a challenge can be particularly telling in human tests; challenges to homeostasis have often included standing-up from supine or lying down (e.g., Makary et al., 1999). Parasympathetic influences on heart rate are often evaluated in terms of 'high frequency power' (i.e., power in the HR 'signal' as revealed by Fourier analysis within the frequency range typically, in the human, between 0.15 to 0.40 Hz.) We agree, in fact, since in resting dog – an animal with significant cardiac parasympathetic activity while at rest – selective surgical interruption of the parasympathetic fibers projecting to the SA-node essentially eliminates the high frequency peak (Randall 1991). The low frequency peak (human: 0.04 to 0.15 Hz) is often taken as an approximate index of cardiac sympathetic nervous activity (SNA). In dog, selective SA-nodal parasympathectomy significantly decreases this power, but does not eliminate it; addition of \exists -adrenergic blockade (i.e., administration of propranolol) further, and significantly, decreases this power in the low-frequency range of HRV (Randall 1991). Direct recording of muscle sympathetic nerve activity has also been reported in lean vs. overweight or obese humans (Lambert et al., 2010).

Identification of comparable studies of 'young humans' conducted during the ensuing decade

We identified 5 studies of autonomic function in human obese vs. lean subjects generally within the prescribed age range that were published over the 10 years since our 2008 paper. The average age and weight of Rossi, et al.'s (2015) obese individuals (24 men / 20 women) were 20.45 ± 1.57 years and 102.3 ± 20.82 kg, respectively, while their 'eutrophic' (24 men/ 24 women) averaged 20.7 ± 1.39 and 62.89 ± 10.47 kg.

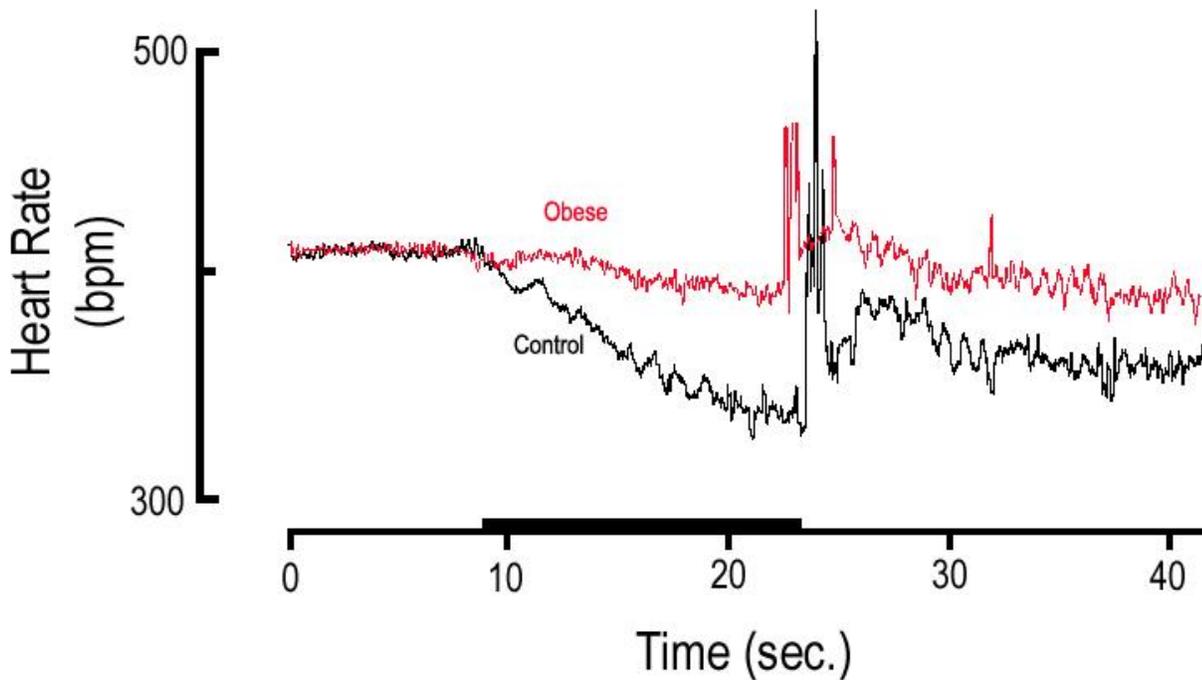


Fig. 1. The rats were un-disturbed while in a comfortable restraining ‘sock’ for first 9 seconds (i.e., pre-tone control, left portion of time base); the 15 sec. long tone was presented during the dark bar on the X-axis, and the short shock was presented at the end of the dark bar; the irregular fluctuations in the recordings during shock delivery are unreliable because of the rats’ flinching during the shock. The heart rate slowing during the stressful stimulus (i.e., 15 sec. tone) was significantly smaller in the obese group vs. the lean animals.

Lee, *et al.* (2014), who included an orthostatic challenge (lower body negative pressure, LBNP) in their work, studied obese individuals who were 27.5 ± 9.3 years of age and 100.8 ± 14.9 Kg. while their non-obese individuals were aged 26.0 ± 7.3 yrs and 66.3 ± 11.9 kg. Muralikrishnan, *et al.* (2013) studied 31 obese people (25.42 ± 7.86 yrs; BMI = 26.84 ± 2.47 kg/m² (weight was not provided)) and 31 ‘normal’ (25.38 ± 4.61 years and 21.71 ± 2.99 kg/m²). Park, *et al.* (2012) reported recordings of muscle sympathetic nerve activity (mSNA) responses to the challenge of the cold pressor test (CPT) and static hand grip (SHG) in 12 overweight subjects (32.3 ± 2.2 yrs; 182.7 ± 7.0 lbs) and in 12 lean individuals (28.9 ± 1.4 yrs (SEM); 138.4 ± 5.0 lbs); although these individuals were older than our estimated rat-human comparison, because the study recorded mSNA and also included a sympatho-excitatory challenge renders it of particular interest. In addition, Indumathy, *et al.* (2015) examined response to standing, deep breathing and isometric handgrip in their obese (29.84 ± 5.88 year; 68.06 ± 7.66 kg) group and their 28.33 ± 7.22 year old controls (54.44 ± 6.40 kg).

Comparisons of effects of obesity on autonomic function in age-matched humans and Zucker rats

We observed no between-group differences in our rats’ mBP_a during the pre-tone control. Likewise, Park, *et al.* (2012), Lee, *et al.* (2014) and Muralikrishnan, *et al.* (2013) report similar control mBP_a across their groups, while Indumathy, *et al.* (2015) and Rossi, *et al.* (2015) reported higher arterial pressure in their obese individuals compared to controls. We also reported no difference in pre-tone HR; while Park, *et al.*’s findings in this regard agree with ours, the overweight or obese subjects for the other studies (Lee, Rossi, Indumathy, Muralikrishnan) had elevated resting HR. HR, in our experience, is quite responsive to the subject’s status, emotional and otherwise; we adapted our rats to the sock restraint such that, we believe, their HR was close to ‘resting’. In any case, it would be difficult on the basis of control HR alone to draw any firm conclusions on any effects of obesity on autonomic balance.

The HR power spectra from the obese individuals studied by Rossi, *et al.* (2015), as well as Indumathy, *et al.* (2015) had significantly lower HF power than observed in their lean, or control, subjects; such

findings would typically be interpreted to have resulted from decreased parasympathetic control of HR. Absolute LF power in Rossi's study did not differ between groups, but normalized LF power was higher in the obese; Indumathy (2015) reported both a higher total and normalized LF value in his obese subjects. The LF/HF ratio, often regarded as an index of the relative balance of sympathetic and parasympathetic tone, was significantly higher in the obese as compared to the 'eutropic' people in both studies. These investigators conclude that, at rest, obesity was associated with a reduction in parasympathetic and a relative predominance of sympathetic activity. Muralikrishnan, *et al.* (2013) used Poincare Plot analysis to come to the same conclusion. Finally, Park, *et al.* (2012) recorded mSNA via microneurography; baseline bursts/min. in the lean (20.5 ± 2.2) tended toward fewer per minute than observed in the overweight (27.8 /min.), but the difference fell short of statistical significance ($p = 0.08$).

The study referenced immediately above by Park, *et al.* (2012), is particularly reminiscent of our overall rat work (Randall, *et al.*, 1994) in that they examined changes in mSNA during sympathoexcitatory challenges: the cold pressor test (CPT) and static handgrip exercise at 30% of maximum (SHG 30%). They reported no significant between group differences in the increases (vs. rest) in mBP_a or in HR during the moderate hand grip, though systolic pressure increase in the overweight subjects ($+16.6 \pm 1.6$ mm Hg) tended ($p = 0.17$) to be blunted compared to the lean ($+22.4 \pm 3.5$ mm Hg); there was no difference in the magnitude of the increase in mSNA in the overweight ($+13.0 \pm 1.6$ /min) as compared to the lean ($+11.9 \pm 1.3$ /min.). Recall that we (El-Wazir *et al.*, 2008) saw no difference (i.e., obese vs. lean rats) in the change in mBP_a during the stress tone which, because changes in arterial pressure 'map' (i.e., are closely related to) changes in SNA (Burgess, 1997), implies that underlying changes in SNA were similar. Conversely, while the changes in pressure and HR were similar between groups during the cold pressor test, the overweight individuals increased mSNA bursts/min more ($+18.1 \pm 2.8$ /min) than the lean ($+10.8 \pm 1.2$ /min; $p = 0.03$).

The major focus of Indumathy, *et al.*'s (2015) study was to investigate the relationship of their indices of sympathovagal imbalance (SVI) to anthropometric indices (e.g., waist or hip circumference), but they also reported comparisons of the "30:15 ratio (ratio of maximum RR interval at 30th beat to minimum RR interval at 15th beat following standing," i.e., orthostatic challenge) and determined the arterial pressure at the first minute and 2nd minute of contraction () DBP_{IHG} or

maximum rise in diastolic blood pressure above baseline) during an isometric handgrip test at 30% of maximum voluntary contraction. On the bases of these "classical autonomic function tests" in the obese vs. control group they reported decreased vagal reactivity in their obese subjects during the orthostatic challenge and, during the SHG, they found increased sympathetic reactivity in the obese subjects. As a result of these findings they conclude that differences between the spectral LF:HF ratio in the control (0.69 ± 0.39 ; $n=43$) and obese group (1.41 ± 0.73 ; $n=45$, $p < 0.001$) are attributable to "concomitant increase in sympathetic activity and reactivity as well as to the decrease in parasympathetic activity and reactivity" (quoted from p. 62). Lee, *et al.* (2014) also reported reduced orthostatic tolerance in obese humans which, on the basis of previously established alterations in autonomic function in humans, "support the contention that autonomic nervous system activity is altered with weight gain and obesity," but they do not offer a more specific analysis.

The subjects in the study by Yadav, *et al.* (2017) were somewhat older (average: 30 – 32 years), and the age range (18 – 75 years) within the normal weight and within the obese individuals was large. Irrespective, we note parenthetically that they report significantly lower HRV HF power in the obese (216 ms²) as compared to the normal weight (640.5 ms²; $p = 0.014$); LF power was lower (obese: 248 ms²; normal weight: 480 ms²; $p = 0.063$) while LF/HF was higher (obese: 1.2 ; normal weight: 0.79 ; $p = 0.045$). In short, they state that variables which reflect the cardiac parasympathetic nerve activity were lower in obese persons than in normal weight persons while the sympathetic marker LF/HF ratio was increased in obese subjects. It may be noteworthy to state that this study was conducted on Asian subjects (Nepal), which may highlight the trans-ethnicity of the obesity-associated cardiac autonomic dysfunction.

Baroreflex function in lean vs. obese individuals

None of the five studies we identified assessed baroreflex function directly in obese vs. lean subjects, so we looked at studies with subjects beyond the optimal age-range and/or published other than within the most recent decade. Using a sequence technique (Randall & Brown, 2014), Skrapari, *et al.* (2007) compared baroreflex sensitivity in obese (BME > 30 kg/m²) vs. lean (BMI < 25 kg/m²) women; though aged (42 yrs) somewhat older than our range, the data are worth our noticing. They found a severe reduction in baroreflex sensitivity by the sequence technique in obese (9.18 ± 3.77 ms/mm Hg), but otherwise healthy,

subjects as opposed to their lean women (19.63 ± 9.16 ms/mm Hg, $p < 0.001$). These differences were closely associated with the high frequency component (i.e., parasympathetic component) of HRV. Jaju, *et al.* (2016) compared spectral HRV assessments for mental (word conflict test; WCT) and physical (cold pressor test) challenges for large ($n=1149$) groups of lean (average age: 32.8 yrs; 56.6 kg), overweight (38.0 years; 68.5 kg) and obese (38.1 yrs; 83.4 kg) individuals from 5 large families from an interior province of Oman. Although the average age ranges are near our range, the direct applicability of their findings to ours is limited because of the wide range of the ages of their subjects within any category. Nonetheless, we note that they report no differences in the HR or mBP_a changes (vs. baseline) during WCT or CPT in their autonomic parameters or in their index of baroreflex function.

Interpretations of comparative rat vs. human findings

Stables, *et al* (2013) point out that “it is crucial to be able to determine in what ways the animal models are similar to the human disease, and in what ways they are different” (quoted from p. 75) in their consideration of the applicability of animal (primarily rat) models of diabetes vs. human diabetic cardiac autonomic neuropathy. We attempt such a comparison here of human and rodent (i.e., rat) obesity, where, in our case, the rodent obesity is attributable to a genetically controlled lack of the leptin receptor in the Zucker^{fa/fa} rat: this animal harbors a missense mutation in the leptin receptor gene. Stables, *et al* (2013) point out that such comparison requires a combination of several suggestive tests. In our rat study the primary novel finding depended upon use of a classical aversive conditioning paradigm which is sympatho-excitatory with, thereby, an increase in mBP_a that, in turn, slows HR via activation of the baroreflex with resultant elevated cardiac PNS activity. It is difficult, perhaps impractical, to identify a corresponding human paradigm, which thereby limits our ability to compare strictly our animal with human work. Not unexpectedly, multiple inferences are required.

The human studies are nearly unanimous in positing an overall diminution of parasympathetic nervous activity in obesity, at least as inferred by HRV or other indirect assessments. It is more difficult to convincingly compare findings in these human studies to our major conclusion – decreased parasympathetically mediated bradycardia in the obese rats attributable to differences in baroreflex function – but the findings of Indumathy, *et al*'s (2015) “classical autonomic function tests” certainly come close to such a confirmation. Findings

are less unanimous as regards differences in sympathetic nervous activity. This is, at least in part, attributable to the weaker linking of changes in LF power (i.e., both sympathetic and parasympathetic activity contribute to LF power) and ambiguity in the LF/HF ratio (i.e., both increases in LF power, or decreases in HF power might contribute to an increased ratio) in assessing ‘sympathovagal imbalance’ (SVI). Moreover, our conclusions in this regard are inferential as based upon our prior assessment of the relationship between the coupling of changes in SNA and changes in arterial pressure. Nonetheless, our rat findings are generally consistent with a relatively unchanged, or only modest increase in sympathetic arousal with acute challenge (e.g., orthostatic challenge). In sum, it appears from our perusal of the relevant literature that our specific conclusion – diminution of parasympathetic autonomic control of HR early in the development of obesity with only modest, if any, changes in sympathetic activation – is a faithful reflection of the human situation.

What of the underlying mechanism(s) of the lessened bradycardia during stress? Since the bradycardia we observed occurs during an elevation in SNA we believe the HR slowing is secondary to baroreflex activation induced by the arterial pressure increase during the stress. Since the magnitude of the stress-induced pressor response was similar in the obese vs. lean rats, the input to the baroreceptors should have been similar and, at least inferentially, the reflex response of the baroreflex was, therefore, similar. If so, the reduced HR slowing is attributable to diminished parasympathetic control of the SA-node (i.e., rather than diminished sympathetic control). Our finding that the reflex HR slowing to phenylephrine, but not the reflex HR acceleration to nitroprusside, was attenuated is consistent with this interpretation. In sum, our retrospective examination of the age-appropriate human literature published during the last decade indicates that our findings in Zucker rat were in an important and significant sense ‘translational’ with respect to autonomic function in obese humans as compared to lean individuals.

REFERENCES

- Andreollo NA, dos Santos EF, Arađjo MR, Lopes LR. Rat's age versus human's age: what is the relationship? *Arq Bras Cir Dig* 25: 49-51, 2012.
- Burgess DE, Hundley JC, Li S, Randall DC, Brown DR. Multifiber renal sympathetic nerve activity recordings predict mean arterial blood pressure in unanesthetized rat. *Am. J. Physiol., 273 (Regulatory Integrative Comp. Physiol. 36):* R851 - R857, 1997.

- El-Wazir YM, Li SG, Williams DT, Sprinkle AG, Brown DR and Randall DC. Differential Acquisition of Specific Components of a Classically Conditioned Arterial Blood Pressure Response in Rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 289: R784 - R788, 2005.
- El-Wazir YM, Li S-G, Smith R, Silcox DL, Brown DR Randall DC. Parasympathetic response to acute stress is attenuated I young Zucker obese rats. *Auton Neurosci: Basic Clin* 143: 33-39, 2008.
- Indumathy J, Pal GK, Pal P, Ananthanarayanan PH, Parija SC, Balachander J, Dutta TK. Association of sympathovagal imbalance with obesity indices, and abnormal metabolic biomarkers and cardiovascular parameters. *Obes Res Clin Prac* 9: 55-66, 2015.
- Jaju D, Al Abri M, Hassan MO, Alvarenga JL, Comuzzie A, Albarwani S, Al Yahysee S, Bayoumi R, Al Hashmi K. Hemodynamic and autonomic reactivity to mental and physical stress in lean, overweight and obese subjects. *J Obes Overweight* 2(1), 2016 (doi: 10.15744/2455-7633.2.105)
- Lambert Elisabeth, Sari Carolina Ika, Dawood Tye, Nguyen Julie, McGrane Mariee, Eikelis Nina, Chopra Reena, Wong Chiew, Chatzivlastou Kanella, Head Geoff, Straznicki Nora, Esler Murray, Schlaich Markus, Lambert Gavin, 2010. Sympathetic Nervous System Activity Is Associated With Obesity-Induced Subclinical Organ Damage in Young Adults. *Hypertension* 56, 351–358. <https://doi.org/10.1161/HYPERTENSIONAHA.110.155663>
- Lee JF, Harrison ML, Christmas KM, Kim K, Hurr C, Brothers RM. Elevated resting heart rate and reduced orthostatic tolerance in obese humans. *Clin Auton Res* 24: 39-46, 2014.
- Makary SM, Selim HA, El-Wazir YMN, Mahmoud A E-A. Physiological assessment of cardiac autonomic reflexes in diabetes. *Suez Canal Univ Med J* 2: 61-72, 1999.
- Muralikrishnan K, Balasubramanian K, Ali SMJ, Rao BV. Poincare plot of heart rate variability: an approach towards explaining the cardiovascular autonomic function in obesity. *Indian J Physiol Pharmacol* 57: 31-37, 2013.
- Park J, Middlekauff HR, Campese VM. Abnormal sympathetic reactivity to the cold pressor test in overweight humans. *Am J Hypertension* 25: 1236-1241, 2012 (<https://doi.org/10.1038/ajh.2012.115>).
- Randall DC, Brown DR, Raisch RM, Randall WC. SA-nodal parasympathectomy delineates autonomic contributions to heart rate power spectrum. *Am. J. Physiol.* 260 (*Heart Circ. Physiol.* 29): H985-H988, 1991.
- Randall, DC and Brown DR. Closed and open loop mechanisms controlling arterial blood pressure in waking state in human and experimental animal. *Bulletin of the Egyptian Society of Physiol Sci* 34(1): 1-6, 2014.
- Rossi RC, Vanderlei LCM, GonHalves ACCR, Vanderlei FM, Bernardo AFB, Yamada KMH, da Silva NT, de Abreu LC. Impact of obesity on autonomic modulation, heart rate and blood pressure in obese young people. *Auton Neurosci: Basic Clin* 193: 138-141, 2015.
- Skrapari I, Tentolouris N, Perrea D, Bakoyiannis C, Papazafiropoulou A, Katsilambros N. Baroreflex sensitivity in obesity: relationship with cardiac autonomic nervous system activity. *Obes* 15: 1685 – 1693, 2007.
- Stables CL, Glasser RL, Feldman EL. Diabetic cardiac autonomic neuropathy: insights from animal models. *Auton Neurosci: Basic Clin* 177: 74-80, 2013.
- Yadav RL, Yadav PK, Yadav LK, Agrawal K, Sah SK, Islam MN. Association between obesity and heart rate variability indices: in intuition toward cardiac autonomic alteration –a risk of CVD. *Diab Met Syndr Obes: Targets Therapy* 10: 57- 64, 2017.
- Yokoi N, Hosino M, Hidaka S, Yoshida E, Beppu M, Hoshikawa R, Sudo K, Kawada A, Takagi S, Seino S. A novel rat model of type 2 diabetes: the Zucker fatty diabetes mellitus ZFDM rat. *J Diab Res* , 2013.
- Ziraba AK¹, Fotso JC, Ochako R. Overweight and obesity in urban Africa: A problem of the rich or the poor? *BMC Public Health.* 15;9:465, 2009.