J. Afr. Ass. Physiol. Sci. 3 (2): 218 – 224, December, 2015.

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

http://www.jaaps.aapsnet.org

Research Article

Induced-hypercholesterolemia as a probable cause of alterations in pulse pressure in wistar kyoto rats

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Keywords:
hypercholesterolemia, remodeling, vascular, pharmaceutical, dysfunctions, cardiovascular, telemetry, WKY rats

ABSTRACT

Background: The involvement of hypercholesterolemia in cardiovascular disorders has been widely researched but the impact on the specific cardiovascular (CV) indices following remodeling and cardiac malfunction remain to be fully elucidated. The aim this research is intended to further the understanding of cardiovascular function under hypercholesterolemic condition in mammals and serve as a guide to pharmaceutical formulation and medical interventions. **Methods:** The telemetry technique was used to investigate the cardiovascular dysfunctions in induced hypercholesterolemia in Wistar Kyoto (WKY) rats. Methods for this investigation include: inducing hypercholesterolemic condition in Wistar Kyoto rats through diet; measuring the blood cholesterol levels of the experimental animals; measuring cardiovascular indices in conscious rats to establish vascular dysfunction and/or cardiac malfunction. **Results:** Our study showed that pulse pressure decreases in experimental WKY rats with increasing cholesterol content in the diet. It also shows that diet related pulse pressure decrease occurs in both low and high animal activities. The pulse pressure was reduced at both low and high animal activities in the 2% cholesterol diet (N=6) when compared to control (N=4) and 1% cholesterol diet (N=7). All results presented were statistically significant at a P value < 0.05. Our study has shown that pulse pressure (PP) declined significantly in the 2% cholesterol loaded diet, but not in the 1% diet. We also observed that in overall, the 1% diet group maintained close to normal cardiovascular indices compared to the control and 2%. **Conclusion:** Our results show that a high cholesterol diet may have negatively impacted the cardiac function more than the vascular function.

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INTRODUCTION

Hypercholesterolemia is a form of hyperlipidemia - excessive level of lipids in blood. Cholesterol is one of the most dreaded classes of lipids, principally because of its implication in atherosclerosis, coronary artery disease (CAD), and other cardiovascular disorders (Balakumar et al., 2007). In spite of this unsavory reputation for cholesterol, it is also acclaimed for its usefulness as a primary component of the most valued steroid hormones. Hypercholesterolemia is a metabolic derangement that can be secondary to many diseases and can contribute to many disease forms of most notably, cardiovascular diseases. This may be related to diet, genetic factors *(familial hypercholesterolemia*) and the presence of other diseases such as diabetes and under-active thyroid (Mozaffarian et al., 2006). The disorders associated with high levels of blood cholesterol (hypercholesterolemia - > 240 mg/dL or > 6.2 mmol/L) have been widely researched, and their physiological/biochemical relevance have also been moderately reported. The initial focus of researchers was to fully establish its involvement in many cardiovascular diseases.

hypercholesterolemia often occurs in conjunction with other metabolic risk factors, including glucose intolerance, obesity, diabetes and metabolic syndromes (Rothad et al., 2011). Clinical trials show that lowering lipids reduce the morbidity and mortality associated with cardiovascular complication (Amundsen et al., 2002). Consequently, several pharmaceutical compounds such as Lipitor, Crestor, Zocor, pravastatin

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etc. have been formulated to control excess blood cholesterol. Several researchers have equally reported on compounds of plant origins that showed hypolipidemic activity, including *Punica granatum* (flower extract) Mazhar Igbal et al., 2011; *Bengal gram (cicer arietinum)*, Sharma 1999 Camphene, a plant-derived Monoterpene, Villianou et al., 2011, and more.

Currently, the focus has shifted to the effect of hypercholesterolemia in vascular dysfunction and the mechanistic pathways involved in the dysfunction. Hypercholesterolemia is a common risk factor for early atherosclerosis prior to the appearance of over atherosclerotic changes in the vascular wall; it induces vascular functional changes that may lead to local ischemia and vascular remodeling (Bentley et al., 2002). Steinberg and Witztum (1999) reported that LDL oxidation, endothelial dysfunction and inflammation are involved in the pathogenesis of atherosclerosis. Therefore, the role of nitric oxide (NO/EDRF) and the free radicals in vascular health cannot be overemphasized. Free radicals have been reported to induce oxidation of lipids controlled by a wide spectrum of enzymatic antioxidants and non-enzymatic antioxidants such as superoxide dismutase and glutathione peroxides (GSHPx), vitamin E and glutathione (Valko et al., 2007). Nitric oxide has also been widely reported as a central modulator of vascular damage. A number of intracellular effects leading to vascular relaxation, endothelial regeneration, reduction of oxidative mechanism, inhibition of leukocyte chemotaxis and platelet adhesion have been attributed to nitric oxide (Napoli et al. 2001). In understanding the mechanisms of vascular wall remodeling, it has been suggested that Protein Kinase N1 (PKN1) plays a critical role in vascular wall remodeling, and therefore, could be a promising new target for the next generation of drugs for vascular diseases, particularly restenosis following angioplasty, stent implantation or vein grafting (Nikhlesh et al., 2012).

In light of the above overwhelming evidence, this study was aimed to investigate the possibility of identifying some dynamics in cardiovascular indices emanating from induced-hypercholesterolemia in Wistar Kyoto (WKY) rats. It has been reported that pulse pressure (PP) has evidently risen after the fifth decade of life, due to arterial stiffening with increasing age (Franklin et. al. 1997; Kelly et. al. 1989). Conversely, other studies involving patients with advanced heart failure, low PP was associated with high CV mortality rates (Aronson and Burger 2004; Petrie et al. 2009). Due to this controversy, the present study was designed to examine if the high cholesterol diet is capable of triggering low or high PP.

MATERIALS AND METHODS

Animals and experimental Design

Animal selection and diet inducing hyper-cholesterolemia

The experiment was conducted, using animals that had the same date of birth and weaned on the same date, but grown to be a twelve-week-old or more before implant. The 36 females (or male) Wistar Kyoto rats (WKY) were acquired from the department of biological sciences, animal facility at Minnesota State University (MSU), Mankato, Minnesota, USA. The animals were divided into three groups. The group I was designated as a control group and groups II and III were fed on high cholesterol diet [*diet item number TD.02331 and TD.82312 – manufactured by Halan Laboratories Inc., Indianapolis, USA*]. The characteristics of the experimental diet Cholesterol-fed rats (groups II & III) were chow enriched with 1% and 2% (w/w) cholesterol for 12-week diet period respectively. The control (normal) group was fed on standard chow. The rats were maintained in groups in their individual cages, under controlled temperature, humidity, and illumination conditions, with water and diet *ad libitum* for twelve weeks (Matos et al., 2005). All animal protocols were performed in accordance with the relevant guidelines and regulations approved by the Institutional Animal Care and Use Committee (IACUC) of the Minnesota State University, Mankato, Minnesota, USA.

Establishing the condition of elevated blood cholesterol level (hypercholesterolemia) – At the end of the 12-week diet period (Csont et al., 2002), blood cholesterol levels were measured in the control and cholesterol-fed groups to ascertain that hypercholesterolemia condition does exist in the experimental animals. Abcam's HDL and LDL/VLDL Cholesterol Quantification Assay Kit (ab65390) was used for the measurement. The HDL and LDL/VLDL Cholesterol Quantification Kit provide a simple quantification method of HDL and LDL/VLDL after a convenient separation of HDL from LDL and VLDL (very low-density lipoprotein) in serum samples. In the assay, cholesterol oxidase specifically recognizes free cholesterol and produces products which react with probe to generate color (570 nm) and fluorescence (Ex/Em = 538/587 nm). Cholesterol esterase hydrolyzes cholesteryl ester into free cholesterol, therefore, cholesterol ester and free cholesterol can be detected separately in the presence and absence of cholesterol esterase in the reactions. Reacts with**:** Mouse, Rat, Rabbit, and Human and predicted to work with: all Mammals (Hu P. et. al., 2013 and Kuhla A. et. al., 2013)

Measuring Cardiovascular indices in conscious rats

Various CV indices were measured in conscious hypercholestrolemic rats and a control group for vascular dysfunction. A smaller and lighter telemetry device was implanted through the femoral artery and extended into the abdominal aorta of rats, leading to the acquisition of stable and high-quality data, similar to those obtained by using a larger telemetry device developed for rats. The use of smaller transmitters represents an alternative telemetry technique, especially for those cases in which space in the abdominal cavity is particularly limited such as during pregnancy have been reported (Braga and Prabhakar 2009). Each implant was carried out under a standardized sterilization procedure, including hand washing with cholorohexidine and autoclaving of surgical equipment before each surgery. All animals were sedated with a mixture of isofluorane and oxygen gases in a ratio of 3:1. The relatively tiny rat femoral artery was dilated with a perfusion of Lidocaine Hydrochloride Injectable *(Lot # 7070512 - Phoenix pharmaceutical, Inc. USA).* The animals that survived the surgery (after a shot of Rimadyl *(carprofen) NADA # 141-199- Pfizer Animal Health, NY, New York, USA)* are allowed recovery time of at least one week before data collection commences. The telemetry device is capable of measuring multiple parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), heart rate (HR), and activity level of the rats simultaneously. During recording, animals are allowed to feed and move freely in a cage that is placed on a receiver plate connected to a computer. Each telemetry device is assigned a code and hence could be used for different animals at a given time.

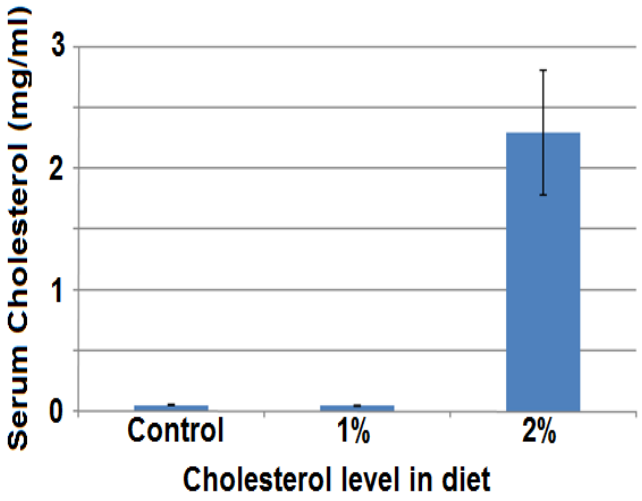

Fig. 1: Total serum cholesterol is unchanged in control (N=5) and 1% diet (N=5) but there was significantly increased rats fed on 2% diet (N=5) compared to control and 1% diet groups. The result is significant at $p < 0.05$. (For control and 2% diet); T = 9.729893; P-Value is 1E-05. The result is significant at $p < 0.05$ for 1% and 2% diet.

Statistics

All experiments repeated several times with similar results would have their data presented as the means $\pm$ S.D. The control *versus* treatment effects (hypercholesterolemic group) were compared using two-tailed independent Student's *t* test, and *p* values < 0.05 was considered to be statistically significant.

RESULTS

Table 1 Shows low activity pulse pressure in control, 1%, and 2% cholesterol diet (hypercholesterolemic) in WKY rats. The result is significant at $p < .05$ among the control and 2% diet animals, but not between control and 1%, or between 1% and 2% diet animals at week 2 only. * Indicates significant difference among the groups

Table 1

Pulse Pressure (mm Hg)	Control (N=4) (Regular Chow) *	1% Cholesterol Diet (N=6)	2% Cholesterol Diet (N=6) *
	32.10	34.04	28.01
	37.73	39.42	25.38
	34.44	35.85	13.58
	31.78	23.88	30.58
	-	40.20	15.33
	-	20.46	30.58
Mean	34.013	32.308	23.91
StDev	2.7474	8.244	7.5931
SEM	1.374	3.366	3.1

Table 2 Shows low activity pulse pressure mean analyses for weeks 1 and 2 in 1% and 2% cholesterol diet (hypercholesterolemic) variables in WKY rats. Pulse pressure in animals fed with 2% cholesterol diet was significantly lower than values for 1% cholesterol diet; T-value is 2.267594, P= .046761. The result is significant at $p < .05$; but not between control and 1% or 2% groups. * Indicates statistical significance among the groups.

Table 2

Pressure (mm Hg)	Control (N=4) (Regular Chow)	1% Cholesterol Diet (N=6) *	2% Cholesterol Diet (N=6) *
	30.78	34.54	27.22
	35.79	39.65	27.23
	34.50	32.17	23.27
	30.49	37.59	31.51
	-	39.94	16.47
	-	22.12	31.51
Mean	32.89	34.13	26.20
StDev	2.6592	6.6962	5.6877
SEM	1.33	2.734	2.322

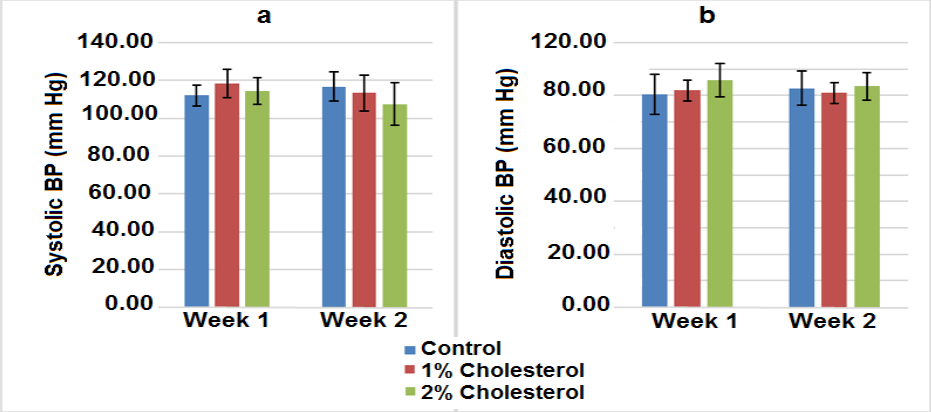

Fig. 2: Low activity systolic (a) and diastolic (b) pressures in WKY rats fed on diet with different levels of cholesterol. There was no statistically significant difference in systolic and diastolic pressure values between control and experimental groups.

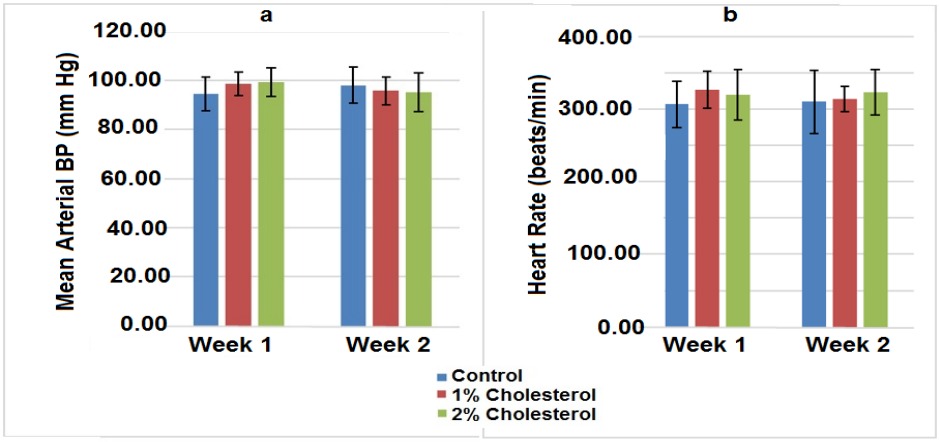

Fig. 3: Low activity mean arterial pressure (a) and heart rate (b) in WKY rats fed on diet with different levels of cholesterol. There was no statistically significant difference in heart rate and mean arterial pressure between controls and experimental groups

Table 3 Shows low activity pulse pressure mean analyses for weeks 1 in 1% and 2% cholesterol diet (hypercholesterolemic) variables in WKY rats. Pulse pressure in animals fed with 2% cholesterol diet was significantly lower than values for rats fed on 1% cholesterol diet. T-value is 2.247212; P-Value = 0.048408. The result is significant at $p < 0.05$; but not between control and 1% or 2% groups. *Indicates statistical significance among the groups.

Table 3

Pulse Pressure (mm Hg)	Control (N=4) (Regular Chow)	1% Cholesterol Diet (N=7) *	2% Cholesterol Diet (N=6) *
	29.45	35.03	26.43
	33.84	39.87	29.07
	34.55	39.33	32.95
	29.20	40.46	32.44
	-	39.67	17.60
	-	37.39	32.44
	-	23.77	-
Mean	31.76	36.503	28.488
StDev	2.8284	5.9175	5.9037
SE Mean	1.414	2.237	2.41

Table 4: Shows high activity pulse pressure mean analyses for week1 in WKY rats fed with 1% and 2% cholesterol diets Pulse pressure in animals fed with 2% cholesterol diet was significantly lower than values for rats fed on 1% cholesterol diet. T-value is 2.371242; P= 0.03707. The result is significant at $p < 0.05$. * Indicates statistical significance among the groups

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Table 4

Pulse Pressure (mm Hg)	Control (N=4) (Regular Chow)	1% Cholesterol Diet (N=7) *	2% Cholesterol Diet (N=6) *
	29.45	35.03	26.43
	33.84	39.87	29.07
	34.55	39.33	32.95
	29.20	40.46	32.44
	-	39.67	17.60
	-	37.39	32.44
	-	23.77	-
Mean	31.76	36.503	28.488
StDev	2.8284	5.9175	5.9037
SE Mean	1.414	2.237	2.41

Discussion

Our study shows that pulse pressure decreases in experimental WKY rats with increasing cholesterol content in the diet. It also shows that diet-related pulse pressure decrease occurs in both low and high animal activities. The telemetry device is capable of measuring multiple parameters such as a systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse pressure (PP), mean arterial pressure (MAP), heart rate (HR), and activity level of the rats simultaneously A statistically significant difference was only observed in pulse pressure between the various experimental animals and control. Pulse pressure as a blood pressure parameter is an important mortality predictor (Assman et al. 2005; Asmar et al. 2003), and among hemodialysis (HD) patients, pulse pressure appears to be a stronger independent predictor of morbidity and mortality than other BP parameters such as systolic (SBP), diastolic (DBP), and mean arterial BP (Foley et. al. 2002). The pulse pressure is a function of SBP and DBP and is dependent on stroke volume and arterial wall elastic properties (Kelly et. al 1992; Alfie et. al. 1999). Our findings show significant change in pulse pressure in the 2% cholesterol fed group compared to the 1% cholesterol fed group and control group. One would expect a corresponding significant difference in systolic pressure and diastolic pressure since the PP is a function of SBP and DBP. Although some differences did exist among the SBP and DBP between the groups, they were however not statistically significant. While it is widely acknowledged that high SBP and DBP (hypertension) correspond to high PP in hypertension, a few findings have equally reported low PP to be associated with the high CV mortality rate (Tansel et al 2010). In mild heart failure, a high PP is probably the result of vascular stiffening or decreased aortic elasticity, which indicates atherosclerosis, whereas in advanced heart failure, low PP chiefly indicates decreased cardiac function and an associated adverse prognosis (Tansel Y, et. al 2010). Tansel et al. 2010 also observed low PP in patients who were experiencing advanced heart failure. An independent association between low PP and CV mortality rate, not just in patients with advanced heart failure, but in the entire study population have been reported. In addition, low PP independently predicts death in ischemic heart failure - a novel finding (Tansel et al 2010). Other investigators who used PP quartiles or tertiles and regression analysis reported that patients in low PP quartile (< 35 mm Hg) experienced higher CV mortality rates, and the cutoff value of 30 mmHg was highly predictive of CV death (Domanski et. al. 1999; Aronson & Burger 2004; Voors et. al. 2005; Petrie et. al. 2009).

In some cases during our study, PP < 23mmHg were recorded. Although our group does not know of any previous report on investigation of cardiac function following induced-hypercholesterolemia in experimental animals, our results are indicative of deteriorated cardiac function. Since our findings did not show a significant increase in SBP and DBP, it is reasonable to speculate that the high cholesterol diet in this investigation negatively impacted heart function rather than the vascular function. As the cardiac and vascular function closely influence each other, the overall CV well-being of an individual heavily depends on both.

One interesting observation of this study is that the 1% coloesterol diet-fed rats showed indices close to normal cardiac function in some cases compared to the control and the 2% groups. Therefore, it remains to be elucidated if elevated cholesterol to a certain degree confers some advantages on cardiovascular function.

The entire process involved in the small animal surgery and post surgery events are very delicate and constitutes an impediment to generating high sample size; we therefore consider sample size a major weakness of our study. The telemetry technique employed is this study allows the animals to remain free while the data were remotely collected; this is considered unique and produces data that are reliable for cardiovascular investigations.

In conclusion, we report that a decline in pulse pressure in induced-hypercholesterolemic animals is indicative of poor cardiovascular function. Based on our findings, it would be safe to assert that a high cholesterol diet may negatively impact the cardiac function more than the vascular function. A further investigation on changes in CV that would involve either human subjects that have been diagnosed with hypercholesterolemia or a more robust sample size of the experimental animals may shed more light on the effect of hypercholesterolemia on specific CV indices such as the pulse pressure.

Acknowledgement

This research work was jointly funded by the Minnesota State University, Mankato Faculty Research Grant (FRG) and the Department of Biological Sciences (MSU). I wish to thank Dr. Penny Knoblich for her support and also making her laboratory available to me for this investigation. My thanks also go to Dr. Michael Bentley, Dr. Sharlin David, Dr. Timothy Secott, and Messrs Brent Pearson and Kaleb Short for their contributions.

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