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**Blood Pressure and Cardiovascular Control
in Offspring of Hypertensive and Normotensive Parents
from Large Homogeneous Omani Arab Pedigrees**

Dissertation

By

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Ao Univ.-Prof Dipl.-Ing. Dr. H. Pessenhofer
Prof. Dr. M.O. Hassan**

To

My Late Parents

Dear Bai and Baba,

You have the rightful claim to the credit of this work.

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conceived, had laid the foundation in my life.

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Offspring of normotensive (ONT), one (OHT₁) and both hypertensive (OHT₂) parents from large homogeneous Omani Arab pedigrees were subjected to ambulatory blood pressure (ABP) measurements. Beat-to-beat BP, impedance cardiography and heart rate and BP variability acquired at rest and during cold pressor (CPT), head-up tilt (HUT), word conflict and deep breathing test showed that OHT₂ have more responsive resistance vessels at a time when BP is normal. Hence, they can afford less sympathetic nervous system (SNS) (LFnuRRI) activation at rest and during HUT. This finding is corroborated by a significant correlation between change of LFnuRRI and plasma noradrenaline. If mental stress is imposed, OHT₂ respond with greater activity of HR and SNS. This is also reflected in higher HR in ABP. CPT could not differentiate offspring with positive and negative family history of hypertension.

Inconsistent cardiovascular and autonomic alterations in OHT₁ in contrast with ONT and OHT₂ showed the importance of predisposition of hypertension (HT) in one or both parents. Pathophysiological changes affecting resistance vessels and SNS are evident in OHT₂ before the onset of HT.

The cross-sectional analysis indicated that, subjects with high normal BP/preHT are likely have increased parental history of HT compared with subjects having optimum BP.

Die Nachkommen von normotensiven Eltern (ONT) sowie von Eltern mit einem (OHT₁) bzw. 2 (OHT₂) hypertensiven Elternteilen einer großen, homogenen Omani-arabischen Volksgruppe wurden einer 24-Stunden-Blutdruckmessung unterzogen (ABP). Beat-to-beat-Blutdruck, Impedanzkardiographie und Herzraten- bzw. Blutdruckvariabilität, die sowohl in Ruhe als auch während eines „cold-pressure-tests“ (CPT), eines „Kipptisch-Tests“ (HUT), eines „Wort-Konflikt-Tests“ und eines „Taktatmungs-Tests“ erhoben wurden zeigten, dass OHT₂ besser reagierende Widerstandsgefäße aufweisen, auch im Falle eines normalen Blutdrucks. Daher weisen sie sowohl in Ruhe als auch während HUT eine niedrigere Aktivierung des sympathischen Nervensystems (SNS) (LFnuRRI) auf. Dieses Ergebnis wird durch eine signifikante Korrelation zwischen Änderungen von LFnuRRI und dem Plasma-Noradrenalinspiegel bestärkt. Wenn mentaler Stress ausgeübt wird, antworten OHT₂ mit einer größeren Aktivität von Herzfrequenz (HR) und SNS. Dies drückt sich auch in einer höheren HR während der 24-Stunden-Blutdruckmessung aus. Der CPT war nicht in der Lage, zwischen Nachkommen mit positiver und negativer Familienanamnese für Hypertension zu differenzieren.

Inkonsistente Veränderungen in den kardiovaskulären und autonomen Variablen bei OHT₁ im Gegensatz zu ONT und OHT₂ weisen auf die Bedeutung einer Prädisposition für Hypertension in einem oder beiden Elternteilen hin. Pathophysiologische Veränderungen der Widerstandsgefäße und des SNS sind in OHT₂ vor dem Eintreten einer Hypertension bereits evident.

Die Querschnittsanalyse zeigte, dass Personen mit grenzwertigem Blutdruck bzw. einer Prähypertension mit höherer Wahrscheinlichkeit eine positive Familienanamnese hinsichtlich Hypertension aufweisen als Personen mit optimalem Blutdruck.

List of abbreviations

24H ABPM	24 hours ambulatory blood pressure monitoring	LF/HF	Sympatho-vagal balance
24H MBP	24 hours mean blood pressure	LFnuBPV	Low frequency normalised units from SBP
AHA	American Heart Association	LFnuRRI	Low frequency normalised units from RRI
BMI	Body mass index	LVET	Left ventricular ejection time (ms)
BP	Blood pressure	MBP	Mean blood pressure (mmHg)
BPG1, BPG2, BPG3, BPG4	Groups according to statistical quartiles	MSNA	Muscle sympathetic nerve activity
BPV	Blood Pressure Variability	OFS	Oman Family Study
BRS	Baroreceptor sensitivity	OHT1	Offspring of one hypertensive parent
CI	Cardiac index (l/min*m2)	OHT2	Offspring of both hypertensive parents
CPT	Cold pressor test	ONT	Offspring of both normotensive parents
CPT _{1min}	1 min cold pressor test	RRI	RR Interval (ms)
CPT _{3min}	3 min cold pressor test	RSA	Respiratory sinus arrhythmia
DBP	Diastolic blood pressure mmHg	SBP	Systolic blood pressure (mmHg)
DBP	Diastolic blood pressure (mmHg)	SI	Stroke index (ml/m2)
HF	High frequency	SV	Stroke volume (ml)
HFnuRRI	High frequency normalised units from RRI	TFM	Task Force Monitor
HPLC	High Performance Liquid Chromatography	TPR	Total peripheral resistance (dyne*s*/cm2)
HR	Heart rate (bpm)	TPRI	Total peripheral resistance Index (dyne*s*m2/cm2)
HRV	Heart Rate Variability	VLF	Very low frequency
HTN	Hypertension	WCT	Stroop Word Colour Conflict Test
HUT	Head up tilt test	WCT _{1min}	1 min word conflict test
HUT _{1min}	1min of head-up tilt test	WCT _{3min}	3 min word conflict test
HUT _{5min}	5min of head-up tilt test	WHO	World Health Organization
JNC1	JNC optimal BP group	WHO1	WHO optimal BP group
JNC2	JNC prehypertension group	WHO2	WHO normal BP group
JNC3	JNC hypertension group	WHO3	WHO high normal BP group
JNCVII	Joint National Commission VII	WHO4	WHO hypertensive group
LF	Low frequency		

Index

	Acknowledgements	
	Abstract English	
	Abstract German	
	List of abbreviations	7
Chapter	Title	
1	Summary	11
2	Review of literature	15
2.1	Hypertension	15
2.1.1	Pathogenesis of hypertension	15
2.1.2	Genetic and environmental factors	15
2.1.3	Prevalence of hypertension in Oman	16
2.1.4	Familial aggregation of blood pressure	16
2.2	Offspring studies	17
2.2.1	24 hour ambulatory blood pressure	18
2.2.2	Cardiovascular laboratory tests	19
	1. Cold pressor test	19
	2. Orthostatic challenge	20
	3. Word conflict test	21
	4. Respiratory sinus arrhythmia	22
	5. Plasma catecholamines	23
2.3	Prehypertension, high normal blood pressure and Genetic predisposition to hypertension	24
2.4	Research gaps	25
2.5	Aims and Objectives	26
2.5.1	<i>Part A:</i> Cardiovascular laboratory tests	26
2.5.2	<i>Part B:</i> Prehypertension, high normal blood pressure and Genetic predisposition to hypertension	27
3	Oman Family study	28
3.1	Study area and population	28
3.2	Pedigree structure of families	31
4	Methodology	33
4.1	<i>Part A</i>	
4.1.1	Subject selection	33

4.1.2	Ethics	34
4.1.3	Conduct of the study	34
4.1.4	Protocol	34
	A. Anthropometric measurements	34
	B. Phlebotomy	34
	C. Principles and procedures of cardiovascular tests	34
	1. Cold pressor test	35
	2. Orthostatic challenge	35
	3. Word conflict test	36
	4. Respiratory sinus arrhythmia	37
	5. 24H ABPM	38
	6. Plasma catecholamines	38
4.1.5	Summary of protocol for Part A	40
4.2	Part B	41
4.2.1	Subject selection	41
4.2.2	Genetic predisposition	41
4.2.3	Classification of offspring	41
	1. WHO	41
	2. JNC VII	42
	3. Statistical quartiles	42
5	Measurements and data acquisition	44
5.1	24H ABPM	44
5.2	Hemodynamic and autonomic measurements	46
5.2.1	Hemodynamic	46
	-ECG	46
	-Beat-to-beat BP	46
	-Impedance cardiography	48
5.2.2	Autonomic	49
	-Heart rate and blood pressure variability	50
	-Baroreceptor sensitivity	52
6	Statistics	54
6.1	Part A	54
6.1.1	Data extraction	54
6.1.2	Data analysis	55
6.2	Part B	56
7	Results	58
7.1	Part A	58
7.1.1	Baseline comparisons	58
7.1.2	24H ABPM	61
7.1.3	Cold pressor test	61
	-Hemodynamic parameters	61

	-Autonomic parameters	63
7.1.4	Head-up tilt test	65
	-Hemodynamic parameters	65
	-Autonomic parameters	67
	-Plasma catecholamines	69
7.1.5	Word conflict test	71
	-Hemodynamic parameters	71
	-Autonomic parameters	74
7.1.6	Respiratory sinus arrhythmia	74
7.2	Part B	75
8	Discussion	80
8.1	Part A	80
8.1.1	Summary of results	80
8.1.2	Baseline comparisons	81
8.1.3	24H ABPM	82
8.1.4	Cold pressor test	84
8.1.5	Head-up tilt test	86
	-stroke volume and total peripheral resistance	86
	-Spectral indices	88
	-Plasma noradrenaline	89
8.1.6	Word conflict test	90
8.1.7	Respiratory sinus arrhythmia	93
8.2	Part B	94
8.3	Limitations	96
8.4	Strengths	96
8.5	Conclusions	97
9	References	99

Summary

Background

The correlation of parental hypertension with the cardiovascular physiology in offspring, though well researched, has not been studied in Arab population. The available research has compared offspring of hypertensive and normotensive parents without giving importance to the predisposition to hypertension in single or both parents. The most widely used physiological stressor cold pressor test (CPT) has yielded controversial results. Differences in responses to head-tilt test (HUT) and word conflict test (WCT) in offspring of normotensive and hypertensive parents have also not been addressed in detail before.

The genetic predisposition to hypertension leads to higher blood pressure in offspring. However, there is little literature available about genetic predisposition to hypertension in subjects with prehypertension or high normal blood pressure.

Aims and Objectives

This thesis has addressed the correlation of genetic proneness to hypertension and cardiovascular and BP control in Omani Arab population by two approaches-

Part A. Cardiovascular reflex tests: by studying the cardiovascular reactivity in offspring with differential parental history of hypertension in order to understand the dynamics of pathophysiology of heritability of hypertension, and Part B. Cross section analysis: by studying subjects with prehypertension or high normal BP and their parental history of hypertension.

To my knowledge, this is the first study to be conducted in a homogeneous Arab population with high degree of consanguinity and environmental homogeneity which describes circadian changes in BP as well as changes in beat-to-beat hemodynamic,

cardiac and autonomic responses to various laboratory stresses in young (age 18-25 years) Omani Arab offspring of one and both hypertensive parents in a unique Omani Arab population.

Subjects and Methods

To study cardiovascular dynamics, offspring were selected from families which participated in the Oman Family Study. Normotensive offspring were classified into offspring of both normotensive parents (ONT; N=42), offspring of one hypertensive parent (OHT₁; N=24) and offspring of both hypertensive parents (OHT₂; N=12) groups to have maximum contrast in the genetic predisposition to hypertension. Hypertension in parents was defined as having daytime ABPM >135/85mmHg or those who are on antihypertensive treatment. All offspring were subjected to anthropometric and 24H ambulatory blood pressure measurements (24H ABPM). Beat-to-beat BP, impedance cardiography and spectral analysis for heart rate and BP variability were acquired at rest and during various physiological stressors as HUT, WCT, CPT and deep breathing for respiratory sinus arrhythmia (RSA) using Task Force Monitor. Plasma catecholamines were estimated at rest and at HUT_{5min} with HPLC.

Results

Part A

ABPM showed a trend of higher day and night BP in OHT₁ and OHT₂. However it was not statistically significant. OHT₂ had significantly higher daytime (P=0.039), night-time (P=0.0003) and total average HR (P=0.013) compared with ONT and OHT₁. The night-time systolic dip was similar in all 3 groups.

At rest, OHT₂ had significantly lower HR (P=0.04) and sympathetic drive (P=0.005) with higher LVET (P=0.05) compared with ONT. During cardiovascular stresses, OHT₂ showed contrasting responses to HUT and WCT. Compared with ONT and OHT₁, OHT₂ showed greater increases in SBP (P=0.007), DBP (P=0.001) and TPR (P=0.05), with attenuated drop in SV (P=0.047), less sympathetic activation (P=0.028) and less increase in noradrenaline with HUT while WCT invoked significantly greater increase in HR

($P=0.048$), SBP ($P=0.011$), LFnuBPV ($P=0.001$) and LF/HF ($P=0.014$) with significant decrease in SV ($P=0.0001$). The degree of RSA and cardiovascular responses to CPT were similar in all the 3 groups.

Part B

For the cross sectional analysis, all of 667 (Males 328) subjects between ages 18 and 30 years from the OFS were selected. Subjects were classified according WHO and JNC VII criteria and also based on statistical quartiles for this cohort. Genetic predisposition in these subjects was decided by identifying the status of hypertension in parents with differing factors as both normotensive parents, either of parents having isolated systolic or diastolic hypertension, only hypertensive mother, only hypertensive father and both hypertensive parents.

Logistic regression indicated that hypertension in both the parents carried maximum risk (WHO high normal group, OR 4.33, $P=0.032$; JNC prehypertension group OR 2.23; $P=0.022$; BP 3rd quartile OR 18.35; $P=0.0001$) of having higher BP in offspring while parents having isolated systolic or diastolic hypertension carry no risk. Compared to hypertensive status of mother, the hypertensive status in father had more influence on offspring BP.

Conclusion

Part A

Offspring with strong positive history of hypertension in both parents have more responsive resistance vessels at a time when BP is comparatively normal. Therefore, they can afford less activity by sympathetic nervous system (LFnuRRI) at rest and during change of posture. This finding is corroborated by highly significant correlation between change of LFnuRRI and of plasma noradrenaline with HUT. However, if mental stress is imposed, OHT₂ respond with greater activity of sympathetic nervous system (HR and LFnuBPV). This is also reflected in higher HR in 24H ABPM. The CPT is probably too unspecific stress to separate subjects with positive and negative family history of hypertension.

This study also showed the importance of realizing the predisposition of hypertension in a single parent or both parents before generalizing the observation of increased reactivity to stress in offspring of hypertensive parents. Pathophysiological changes are evident in OHT₂ even before the occurrence of hypertension as a clinical disease.

Part B

The cross-sectional analysis indicated that, subjects with high normal BP or prehypertension are likely have increased parental history of hypertension compared with subjects having optimum BP. In a clinical setting, subjects with high normal BP or prehypertension must be questioned about family history of hypertension.

Review of Literature

2.1 Hypertension

Essential hypertension or high blood pressure (BP) is characterized by systolic blood pressure of more than 140 and/or diastolic blood pressure of 90 mmHg [1,2,3]. It is the most common human cardiovascular disease, affecting approximately 1 billion people worldwide and is implicated in 7.1 million deaths each year [4].

2.1.1 Pathogenesis of Hypertension

The pathogenesis of essential hypertension is believed to be due to renal, neurogenic, vascular and genetic factors. These may be described as follows: Renal: Altered pressure-natriuresis leading to volume expansion [5]; Neurogenic: Autonomic nervous system dysfunction in cardiovascular regulation [6]; Vascular: Atherosclerosis causing arterial stiffness [7]; and genetic predisposition [8]. Hypertension in young subjects is neurogenic in origin and is characterized by hyperkinetic circulation evident as tachycardia, higher cardiac output, increased sympathetic drive and normal peripheral resistance [9]. The transition of neurogenically initiated hypertension to non-neurogenically maintained hypertension is slow [9]. The suggested mechanisms for maintenance of hypertension are compensatory structural and functional changes in heart, blood vessels and kidney. These changes could be an increase in vascular resistance, left ventricular wall thickness and threshold for pressure natriuresis, or a decrease in aortic compliance and endothelial dependant vasodilatation, or alteration in adrenergic receptor sensitivity [10,11,12].

2.1.2 Genetic and Environmental Factors

A multifactorial cause has been proposed for hypertension [13-16]. Family and epidemiological studies have demonstrated that hypertension arises from a complex interaction between genetic and environmental factors [8,17]. Loci that confer susceptibility to hypertension are now being identified by several studies in Caucasians and African heterogeneous populations [18-22].

Some of the risk factors identified include physical inactivity [23-25], sedentary life style [26], stress [27,28], obesity [29] and diet (specifically, high sodium, low potassium, and excess energy intake) [25,30]. These risk factors e.g. obesity and pathophysiologic processes e.g. hyperkinetic circulation may precede the actual manifestation of hypertension [6,31].

2.1.3 Prevalence of Hypertension in Oman

The prevalence of hypertension in Omani Arabs was estimated to be 11.8% (BP $\geq$ 140/90mmHg). The overall prevalence of high blood pressure increased to 27% when isolated systolic (7.06%; $\geq$ 140mmHg) and diastolic hypertension (8.06%; $\geq$ 90mmHg) was considered separately [32]. The prevalence of hypertension in other Arab populations in the Middle East ranges between 10 –25% [33-36]. Recently, Oman Family Study estimated a prevalence of 18% in the Interior region of Oman, the area where this study has been conducted [37].

High BP in Oman is found to be related with age, body mass index and per capita income [32,38]. The sparse research available on hypertension in urban Arabs and Bedouins has discussed the contribution of male gender, obesity, salt intake, age, literacy, and recent socioeconomic and life style changes [33-34,36,39-40].

2.1.4 Familial Aggregation of Blood Pressure

Familial aggregation of BP is a well-known phenomenon. A strong association exists between blood pressures of parents and children [41-45]. Children with positive family history of essential hypertension have increased likelihood of developing higher blood pressure in later life and eventually becoming hypertensive [41-43,46-48]. Results from twins, family and adoptive studies indicate that 50-79% of blood pressure variability in humans is attributed to genetic factors [8,47,49,50].

2.2 Offspring studies

Genetic factors contributing to the pathophysiology of EH in offspring could be identified at an early stage before established EH sets in. Various tools have been used to study early structural or functional changes in offspring. These include measurement of office/casual blood pressure [51], echocardiography [52], cardiovascular and autonomic responses to physiologic challenges [53], salt sensitivity [54], endothelial mediated vasodilatation [55] and arterial stiffness [56,57]. Cardiovascular responses and salt sensitivity have been maximally studied. Comparative studies of young offspring with and without the family history of hypertension represent a suitable research design for early physiological changes.

The various designs used to study cardiovascular responses are-

A: Differential parental history

1. Comparison of offspring of both normotensive parents (ONT) with those having both hypertensive parents (OHT₂) [51]
2. Comparison of ONT with offspring of one hypertensive parent (OHT₁) [58]
3. Comparison ONT with OHT₁ and OHT₂ [59]
4. Comparison of OHT₁ with OHT₂ [60]
5. Comparison of ONT with OHT₁ and OHT₂ considered as one group [61]
6. Influence of history of hypertension in parents only.
7. Influence of history of hypertension in two generations i.e. parents and grand parents [62,63]

B. Ethnic differences

1. Comparing of Black and White offspring [64,65]

C. Follow-up studies

1. Longitudinal 5-10 years follow-up studies in offspring of hypertensive parents [53,65]

Research available about offspring of hypertensive parents in Arabs is sparse. Only one study has described blood pressure reactivity in offspring of first cousin hypertensive parents in Oman [44]. Other studies have associated higher BP in children with their family history of hypertension [35,36,39,67].

2.2.1 Office and Ambulatory Blood Pressure in Offspring

The hypothesis of elevated resting blood pressure in offspring with differential family history of hypertension was tested either by recording casual office blood pressure or ambulatory blood pressure (24H ABPM). 24H ABPM would also reveal circadian BP pattern in offspring.

Higher resting office blood pressure [51,68,69] was found in offspring of hypertensive parents compared with ONT. This was also noted in offspring of first cousin hypertensive parents compared with those of first cousin normotensive parents in Arabs [44]. Two studies reported similar resting BP in OHT and ONT [70,71]. Parati concluded that, higher office BP in offspring with positive family history of hypertension represents an early and permanent elevation in BP secondary to predisposition to hypertension; and that it is not a stress response to BP measurements in the clinic (White-Coat effect) [51].

Studies using ABPM showed higher both daytime and night-time systolic and diastolic BP [68,69,72,73] in offspring with positive family history of hypertension. Few have reported higher daytime SBP [74-76], total 24H MBP [51] or night-time DBP [69] in offspring of hypertensive parents. One study found no differences in circadian pattern of BP between offspring of hypertensive and normotensive parents [77].

Studies on ABPM of offspring of OHT₂, OHT₁ and ONT found that OHT₂ had higher blood pressure than OHT₁ [68]. These results indicated that the risk of offspring having higher BP depended on whether one or both parents had history of hypertension. He also emphasized that the data of both parents is essential to obtain the maximum contrast in predisposition to hypertension. Goldstein [78] showed the similar observation of graded increase in BP with an increase in contrast of the parental history of hypertension. He

also mentioned the importance of ambulatory BP monitoring over office/casual BP. However some studies did not find this graded increase in BP but have reported highest BP in OHT₂ [72,79].

2.2.2 Cardiovascular Laboratory Tests

Autonomic and hemodynamic responses to dynamic laboratory challenges may be useful in understanding the pathogenesis of essential hypertension [80]. Prospective studies have indicated the predictive role of blood pressure and heart rate reactivity to physical or psychological stresses in the development of hypertension [53]. These stress responses may also help to uncover genetic and environmental contribution to the genesis of essential hypertension [8]. A recent meta-analysis proposed that modulation of responses to physiological challenges is different in subjects with and without the family history of hypertension [81]. Comparative studies of the reactive hypothesis in offspring with and without the genetic predisposition to hypertension thus reveal the expression of parental hypertension into cardiovascular physiology of offspring [81]. In the laboratory, the cardiovascular responses are elicited with physical challenges such as ice immersion test, head-up tilt or hand-grip dynamometry or with mental tasks such as mental arithmetic, Stroop word colour conflict test or mirror trace. Some of these tests are discussed below.

1. Cold Pressor Test (CPT)

Cold pressor test designed by Hynes in 1936 involves immersion of wrist or foot in ice-cold water (Temp 3-4⁰C) for 3-5 min. This induces reflex hemodynamic and autonomic responses through stimulation of cold nociceptors [82-84].

The studies which followed up young subjects over a period of 10- 36 yr, showed the association of greater SBP response to CPT with the later development of hypertension [53,85,86]. The association persisted after controlling for body mass index, age, baseline SBP and parental history of hypertension [85], which indicates the positive predictive value of CPT independent of the parental history of hypertension.

Blood pressure, HR and muscle sympathetic nerve activity (MSNA) responses to the CPT have been compared in offspring with and without the genetic predisposition to hypertension. Muldoon [87] in his review reported that 6 out of 15 studies found greater BP and HR reactivity in offspring with positive family history of hypertension. However, 4 studies published between 1989 and 1991 have not been considered in this review [51,88-90]. Some studies published since 1989 found increased BP reactivity to CPT in offspring with positive family history of hypertension [51,53,64-65,91-94], while others did not [62,73,76,80,88-90,95-97]. Lambert reported a subnormal MSNA response in hypertensive patients and also in offspring of hypertensive parents [97] compared with subjects without the family history of hypertension.

The majority of ethnicity-based studies except one [64], have reported increased BP reactivity to the CPT in Black offspring with a genetic predisposition to hypertension as compared with Whites [65,93,93,98]. Terrel [93] found that Black offspring with positive family history of hypertension responded with an increase in peripheral resistance (α adrenergic response); while White offspring with similar predisposition responded differentially with an increase in HR, cardiac output and decreased pre ejection period (β adrenergic response).

Most of the above studies have used sphygmomanometer or digital manometer to record blood pressure during the test. Ohlsson used invasive cardiography [62] while others have used impedance cardiography [65,80,93] to measure pre ejection period, stroke volume, cardiac output and total peripheral resistance during CPT. Spectral analysis has not been used to evaluate autonomic responses to CPT in any of these studies.

2. Orthostatic Tolerance Test (HUT)

Change in posture from supine position to standing deactivates cardiopulmonary and arterial baroreceptors. This elicits reflex changes in HR, BP, stroke volume and peripheral vascular resistance mediated via the autonomic nervous system. The immediate increase in HR is mediated by vagal withdrawal. The later increase in HR and BP is attributed to vagal withdrawal and stimulation of sympathetic arm of autonomic nervous system [99]. In the laboratory, the change in posture is brought about either by

active standing from supine position or using a head-up tilt (HUT) at 60-70°. Higher increase in total peripheral resistance during head-up tilt was observed in hypertensive patients compared with age-matched controls [100]. Spectral analysis showed that subjects with hypertension responded by reduced increase in normalized units of 0.1Hz low frequency band (LFnu; indicator of sympathetic activity) [101]. Hypertensive subjects also had reduced baroreceptor sensitivity.

There are few studies have been performed on offspring using HUT. Changes in HR, BP, stroke volume and peripheral resistance to active standing were not found to be different in the offspring with differential history of familial hypertension [80,102,89]. A smaller diastolic rise was noted in siblings of hypertensive subject [103]. One study with non-invasive technique for autonomic functions (Time dependant spectral analysis) [61] stated significantly less increase in LF band from SBP in offspring of hypertensive parents compared with ONT. On the contrary, Davrath LR [58] reported similar increase in HR in response to active standing; but offspring with positive family history of hypertension demonstrated significantly greater increase in 0.1 Hz low frequency band. He hypothesized that normotensive offspring with a family history of hypertension display β sympathetic over activity, or a reduced responsiveness of β receptors necessitating a greater sympathetic activity.

3. Stroop Word Colour Conflict Test (WCT)

Mental stress to study cardiovascular reactivity is given by varieties of stimuli like mental arithmetic, serial subtractions, mirror image tracing, video games etc. Stroop Word Colour Conflict Test involves sensory rejection to study the phenomenon of inhibition or interference [104]. It consists of color words written in incongruent colour e.g **Blue**. The subject is asked to state the color and not the word. He/she is pressurized for time, and encouraged to give fast responses.

This test has been reported to provoke steady increase in HR, blood pressure [105], and plasma adrenalin and noradrenaline [106-107]. Grillot [106] noted increase in low frequency band of HRV. However, this was not confirmed in another study [107]. The test also causes forearm vasodilatation through sympathetic withdrawal and β adrenergic

stimulation [108] suggesting the involvement of humoral rather than neuronal mechanism. The test is reproducible and is a good psycho-physiological indicator of stress reactivity [109]. The test has been now used as a model for defense reaction in humans [105]. The WCT produces better sympathetic activation compared with mental arithmetic test [110].

The offspring studies using WCT are limited. The most widely used mental stress is mental arithmetic. Two studies [63,111] mentioned increased BP reactivity while two studies [80,93] found no difference in reactivity to the WCT amongst offspring of hypertensive and normotensive parents. Fredrikson in 1991 [95] reported that reactivity in offspring was better differentiated with mental stress e.g. mental arithmetic, WCT than physical stress e.g. CPT and hand-grip test. The review by Muldoon [87] concludes that there is an inconsistency in differentiating HR and BP reactivity to mental stress in subjects with differences in family history of hypertension. He posed a serious question regarding replicability and magnitude of this association.

4. Respiratory Sinus arrhythmia

Changes in heart rate with respiration provide a guide to the activity of cardiac vagal efferent control. When a subject is asked to breathe slowly and deeply at the rate of 6 breathing cycles /min, there is normally a rise in heart rate with inspiration and fall in heart rate with expiration. This is the basis of respiratory sinus arrhythmia (RSA) [112]. It is quantified by calculating the difference [Δ (I-E)] between maximum heart rate during inspiration (I) and minimum heart rate during expiration (E).

There is a general agreement that the magnitude of this difference is a sensitive measure of vagal control of heart rate. The neuronal pathway of RSA is complex and involves baroreceptors [113]. The factors generating this respiratory sinus arrhythmia (RSA) are central spillover of impulses from respiratory centre to cardiovascular centers in the brain stem and reflexes evoked peripherally by respiratory movements [112,114]. RSA reduces with age and in hypertension [115].

Limited research is available on RSA in offspring of normotensive and hypertensive parents. Miller [116] studied RSA under stress conditions (Handgrip dynamometry and application cold on face) in offspring with differential parental history of hypertension. He concluded that, familial differences in heart rate responses are not mediated via parasympathetic nervous system.

5. Plasma Catecholamines

Noradrenaline is a neurotransmitter at postganglionic sympathetic nerve endings, while both adrenaline and noradrenaline (catecholamines) are released from adrenal medulla. Plasma adrenaline and noradrenaline measures are accepted biochemical markers for activation of the sympathetic nervous system [117]. High Performance Liquid Chromatography (HPLC) is the most widely used method for estimation of plasma catecholamines. Higher plasma noradrenaline is found in early phase of hypertension or in borderline hypertension, but not in established hypertensive patients [117,118].

McCrowry [103] reported higher plasma noradrenaline at rest in siblings of hypertensive patients. Increased plasma noradrenaline at rest [73,119] and increased rate of spillover of noradrenaline [119] are reported in offspring with positive history of parental hypertension. This indicates the presence of a genetic factor in activation of adrenergic sympathetic nervous system in otherwise normotensive young subjects. However, no baseline differences were found in plasma catecholamines amongst offspring with differential family history of hypertension in other studies [59,64,87,102,120]. One study reported lower catecholamines in OHT [71].

Venous plasma catecholamines have also been evaluated during active orthostasis in offspring; however, none of the studies found that the rise in adrenaline or nor-adrenaline with upright posture varies as a function of familial risk for hypertension [59,87,102,103]. Fredrikson and Noll [71,95] reported an increase in plasma catecholamine secretion during CPT, and mental stress in offspring of hypertensive parents respectively.

2.3 Genetic Predisposition to Hypertension in Offspring with High Normal BP or Prehypertension

The Joint National Commission VII [3] defined ‘Pre-hypertension’ as the range of blood pressure above optimal BP ($< 120/80$ mmHg) yet below the hypertension. A subject is said to have prehypertension when systolic BP ranges from 120-139 and diastolic BP from 80-89 mmHg. The WHO classified SBP range of 130-139 and DBP range of 85-89 mmHg as ‘High-Normal Blood Pressure’ [2].

Subjects with prehypertensive or high normal BP, compared to those with optimal blood pressure, are more prone to develop hypertension [1,121,122] and have an increased risk of developing cardiovascular diseases [1,123]. In comparison with subjects having optimal BP, prehypertensives also have higher BMI and waist circumference [124], reduced heart rate variability [125,126] and higher plasma catecholamines [127]. They also showed increased reactivity to the standing and the mental stress [126,127].

Offspring with the genetic predisposition to hypertension may have higher BP at rest compared with those without the genetic predisposition to hypertension. However, less research is available on genetic predisposition to hypertension in prehypertensive offspring or in offspring having high normal blood pressure. A 27-year follow up study found higher SBP and DBP in offspring whose parents were in the highest tertile of BP [45]. An increased probability of genetic predisposition to hypertension was noted in Black prehypertensive subjects [124].

2.4 Research Gaps

1. There are no comparative studies addressing the circadian changes in blood pressure and cardiovascular and autonomic responses to physiological challenges in offspring of hypertensive parents in homogeneous Arab population where consanguinity may potentiate the genetic abnormality.
2. It is important to study the effect of relatively less-salt and high potassium diet on cardiovascular regulation in these Arabs who are staying away from the sea under the same geographical location for more than 30 years.
3. There is limited use of beat-to-beat BP monitoring, impedance cardiography and spectral analysis in comparative studies of the reflex cardiovascular and autonomic functions in offspring of hypertensive and normotensive parents.
4. Majority of studies mentioned above have used 1 or 2 tests for the evaluation of reflex cardiovascular and autonomic functions. It is important to use a cluster of tests to investigate autonomic functions in subjects to be able to arrive at a definitive conclusion [128,129].
5. The research available on genetic predisposition to hypertension in prehypertensive offspring compared with those having optimal BP is limited.

2.5 Aims and Objectives

This thesis addresses the BP and cardiovascular control and genetic predisposition to hypertension in two parts.

2.5.1 Part A: Cardiovascular laboratory tests

Aim

To study

1. Circadian blood pressure changes and
2. Cardiovascular responses to physiologic interventions

in young offspring with differential parental history of hypertension from Omani Arab families who participated in the Oman Family Study.

Objective

1. To compare 24H ambulatory blood pressure,
2. A. To compare hemodynamic and autonomic reactivity to physiological interventions viz. deep breathing test, head-up tilt test, word colour conflict test and cold pressor test and

B. To estimate plasma catecholamines at rest and at 5 min of head-up tilt test

in young (age 18-25 yrs) normotensive offspring of both normotensive parents, with one hypertensive parent and with both hypertensive parents.

2.5.2 Part B: Genetic Predisposition to hypertension in Offspring with High Normal BP or Prehypertension

Aim

1. To study genetic predisposition to hypertension in young offspring from OFS who are prehypertensive or have high normal blood pressure.

Objective

1. To group all young (Age 18-30 yrs) offspring according to the WHO, JNC VII classification and their blood pressure quartiles.
2. To associate the status of parental hypertension in these groups.

Oman Family Study

Oman Family Study (OFS) [130] is aimed at understanding the genetics of complex disease like hypertension, diabetes and metabolic syndrome. Stringent criteria are adopted for defining the phenotypes to add precision to the genetic analysis of complex diseases. All phenotypes are dissected into its component e.g. blood pressure into heart rate, stroke volume, peripheral resistance, baroreceptor sensitivity, autonomic parameters etc. and biochemical factors that contribute to its development and eventually to hypertension. Phenotypes SBP and DBP are considered as continuous variable, instead of segregating them into hypertension and normotension [130].

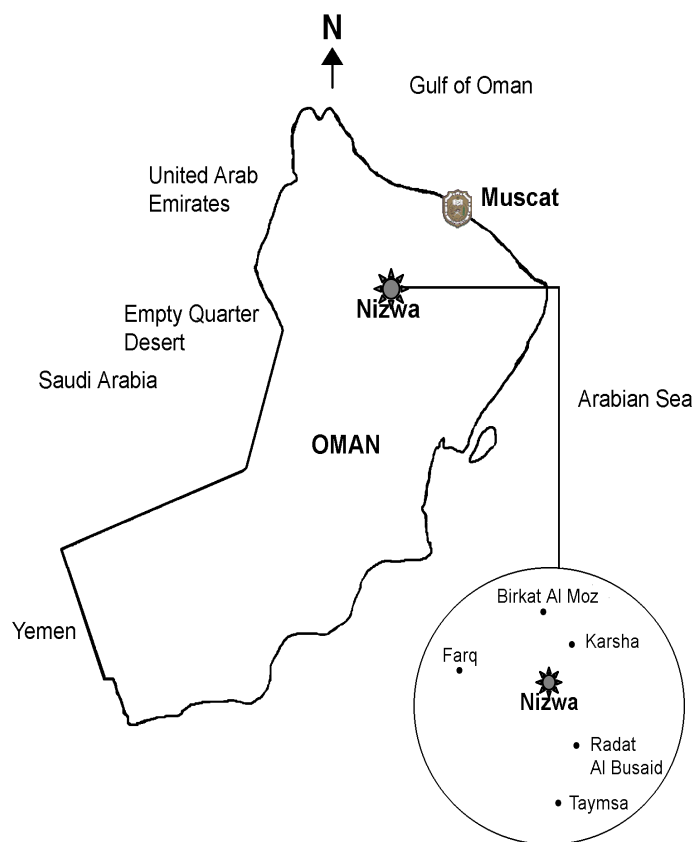
The study was started in June 2003 and is ongoing. This project is supported by a grant from HM Strategic Research Trust Fund (SR/MED/PHYS/04/01) and the Ministry of Health.

3.1 Study Area and Population

The Interior Province of Oman, the Wilayat (state) of Nizwa where the study has been conducted, is the home of very large extended Arab Muslim families. It is situated 180 km South of the capital Muscat (**Fig 1**). It is a mountainous region dotted by several oases in river beds where traditional agriculture of mainly date palm and subsistence farming, in addition to, animal breeding has been practiced by successive Arab generations. These Omani Arabs have been relatively isolated with little contact with the people of the coast who, in contrast, had close associations with the Indian subcontinent and East Africa for over 500 years.

The population size of the Interior Province (75,000) is small. It is distributed between the town of Nizwa (15,000) and several small villages averaging between 1000-5000 individuals each. While the older generations are still working in traditional agriculture and animal breeding, the younger and educated men and women have taken government

Fig 1. Map of Oman showing town Nizwa and the selected 5 villages.



or private sector jobs in Nizwa or the capital city of Muscat. Most of these jobs are office jobs and few are light manual jobs such as light vehicle drivers and labour supervisors. The 1970 renaissance of Oman, accompanying the oil boom, resulted in rapid population growth and a dramatic change in life-style.

The study population has been selected from five villages within a perimeter of 20km around Nizwa (**Fig 1**). This population is homogeneous and consists of large, highly consanguineous families and residing under similar geographical conditions. Village stations were selected within walking distance or a short shuttle bus ride for participants in the study, while the main field research centre, an annex of the Nizwa Polyclinic is situated within 20km of all five villages (**Fig 2**).

Fig 2. The Oman Family Study research center in Nizwa



Fig 3. One of the village



3.2 Pedigrees

The selected 5 large, extended and highly consanguineous families (**Table 1**), each living in a separate village (**Fig 3**) converge towards common descendents of Arab origin and founders date back to no more than 200 years. Traditionally, each individual is genealogically well defined within the family and records are well maintained by individuals and their elders. Often fathers, grandfathers and even great grandfathers are still alive. This population has maintained homogeneity by extremely high levels of inbreeding due to the tradition of encouraging consanguineous marriages mostly between first cousins. The average number of children for each marriage in this population is around six, and 60% of them are under 20 yrs of age. The relative isolation of these families in small villages is due to rough terrain. Travel, education and other modernizations of the area has developed in past 20-30 yrs only.

Table 1. Description of the families selected from 5 villages around Nizwa

Pedigree	Taimsa	BArkat-Al Mouz	Radat-Al Busaid	Firq	Karsha
Generations	9	7	9	12	8
Total closely related individuals	508	309	511	649	464
Individuals tested	327	160	230	279	281
Founders	14	3	3	17	11
Nuclear families	108	60	98	101	87
Mean Inbreeding Coefficient	0.0106	0.0092	0.0206	0.0180	0.0183

Subjects above 18 yrs of age were invited to participate in the study. The number of subjects interviewed and tested in these five pedigrees was 327, 160, 230, 279 and 281, totaling 1277. This represented approximately 10-15% of the total people in these 5 pedigrees. They all voluntarily took part in the study, appeared healthy and had no clinical complaints. On closer examination they all seemed to be related to common

ancestry and intermarriage is common between them. First cousin marriages represent > 50% of all marriages [130]. Polygamy is widely practiced with some men having up to 4 wives. Although the total number of founders ranged from 70 to 100 in each pedigree, most of these are due to marriages outside the pedigrees. The 5th –10th generation founders were usually very few and range between 3-17 individuals (**Table 1**). The rapid population growth made these fairly young isolates of 7-12 generations, each.

Family relationships were ascertained initially by local staff, volunteers and elders from each village. A questionnaire was filled-in for each subject, who is given a unique ID number that is used for creating a master file for all tests and analyses. The data collected in the questionnaire were then transferred to the master file, which contained the ID of the individual and the ID of his/her parents. The genealogy record is often cross-checked. The approved master file was fed into the SOLAR Program to generate pedigree drawings and heritability analysis.

Methodology

4.1 Part A

Cardiovascular Laboratory Tests

4.1.1 Subject Selection

Married couples fulfilling the following criteria were identified from OFS those who- 1) have undergone 24H ambulatory blood pressure measurements; 2) are non-diabetic; and 3) have normotensive offspring between 18-25yrs of age. Sixty four out of 272 couples satisfied above criteria. These couples were classified as 1) Both normotensive (n=32); 2) Only one spouse hypertensive (n=20); and 3) Both spouses hypertensive (n=12). Hypertension is defined if daytime BP measured with 24H ABPM was more than 135/85 mmHg [1] or they were on anti-hypertensive treatment.

These couples had total of 113 offspring who aged from 18-25yrs. Total 108 normotensive offspring were available for the study. Offspring of both normotensive parents (N=48), of one hypertensive parent (N=36), and both hypertensive parents (N=24) were invited to participate. Finally 83/113 subjects volunteered for the study as follows: Offspring of both normotensive parents (ONT; N=44), of one hypertensive parent (OHT₁; N=25), and of both hypertensive parents (OHT₂; N=14). Five of these offspring (2 ONT, 1 OHT₁ and 2 OHT₂) were excluded as their office BP was more than 140/90 mmHg. Cardiovascular regulation is known to be different in borderline hypertensives compared with normotensives. These 5 offspring with higher BP may confound the outcome, hence excluded. One of the ONT subjects had hypertension with hypothyroidism.

Out of 78 subjects, 25 subjects had already participated in OFS. They however were recalled to participate in the offspring protocol.

4.1.2 Ethics

The study was approved by the ethical committee of the Sultan Qaboos University, Muscat, Sultnate of Oman.

4.1.3 Conduct of the study

The study was conducted in a quiet room of the Nizwa Hypertension Research Centre in morning time at comfortable temperature (24-26⁰C). Subjects were called to the centre at 7a.m. after an overnight fast to undergo the protocol. An informed written consent was obtained from all subjects.

4.1.4 Protocol

The summary of protocol is given at the end methodology of Part A.

A. Anthropometric measurements

Anthropometric measurements (height in cm and weight in kg) were obtained. Body fat percentage was assessed using electrical impedance (Tanita, Japan). Body mass index (BMI) was estimated by dividing weight in kg with square of height in cm ($\text{Weight}[\text{Kg}]/\{\text{Height}[\text{cm}]\}^2$)

B. Phlebotomy

An indwelling catheter (BD Venflon, 22GA) was inserted in left ante-cubital vein. Blood sample for fasting glucose was drawn. The catheter was locked with normal saline. Cardiovascular measurements were started 10-15min after phlebotomy.

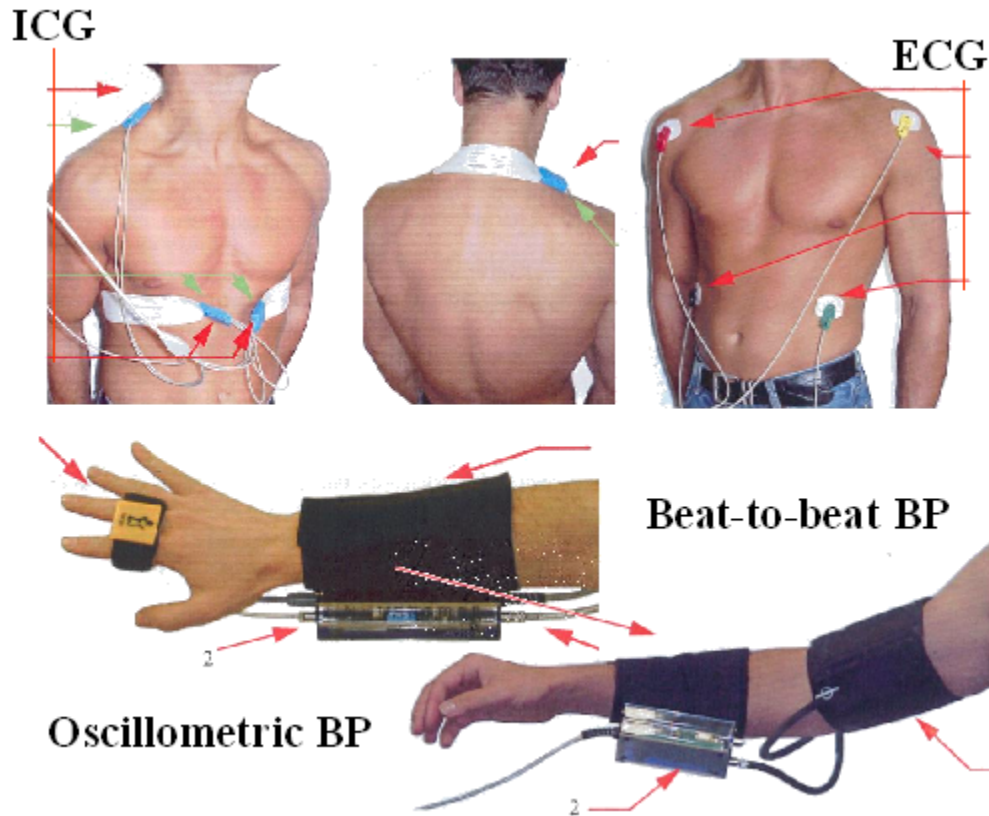
C. Cardiovascular laboratory tests

Beat-to-beat hemodynamic and autonomic parameters at rest and during various physiological challenges (CPT, HUT, WCT and RSA) were obtained using Task Force Monitor (TFM, www.cnsystems.com). The CPT was always given at the end but the remaining 3 tests were given randomly. Adequate rest was given in between tests to ensure return of cardiovascular parameters to the baseline.

Details of hemodynamic and autonomic measurements are described in the next chapter.

Subjects were connected to the TFM as shown in **fig 4**. They were secured with abdominal and waist belts to the tilt table. After initial rest of 10 min, four different types of physiologic challenges were given to assess cardiovascular and autonomic functions [128,129].

Fig 4. The figure shows positions of ECG and ICG electrodes and beat-to-beat and oscillometric BP connectins of the TFM.



1. Cold Pressor Test (CPT)

- a. **Principle:** Stimulation of cold and pain receptors induces cardiovascular reactions [82].
- b. **Procedure:** The left foot was immersed in cold water with crushed ice (temperature 2-4⁰C) up to the ankle joint for 3 minutes. The foot was wrapped in towel after the test was over while recording continued.

2. Orthostatic tolerance test (HUT)

- a. **Principle:** Orthostatic challenge through head-up tilt (HUT) test is a standard test used in many laboratories to assess cardiovascular reflex functions and in the diagnosis of syncope [131]. The shift of 300-500 ml of fluid volume towards

lower limb during HUT unloads cardiopulmonary and/or arterial baroreceptors, which increases HR, BP and vascular resistance through initial vagal inhibition and subsequent sympathetic stimulation.

- b. Procedure:** Orthostatic stress was given by head-up tilt at an angle of 70° (**Fig 5**) maintained for 5 min on electric tilt table (Huntleigh Akron). Tilt was reverted to supine if subject complained of dizziness or headache or any symptoms suggestive of presyncope. Two subjects (both ONT) precipitated syncope on HUT were not included in the final analysis.

Fig 5. The head-up tilt test



3. Stroop Word Colour Conflict test (WCT)

- a. Principle:** The Stroop Color-Word Test involves components of decision-making and time pressure. The right cerebral hemisphere recognizes colours and the left hemisphere names the word. However, the incongruence of verbal narration of the conflict of words and colours shown in this test creates hemispheric confusion and invokes cardiovascular responses through central cerebral stimulation. There is no learning effect or tolerance with repeated testing and is not biased by gender [104,105].

- b. **Procedure:** A chart (Fig 6) on which the names of colours (e.g Blue) in Arabic are written in different colours (Blue) was displayed on a monitor. One observer throughout the study selected words at a constant speed of one word/sec. The subject was asked to narrate the colour of the word and not read the word. The test was given for 3 minutes. Subjects were encouraged to respond faster and to concentrate fully throughout the test.

Fig 6. The word conflict test chart in Arabic and English



4. The Deep breathing test for respiratory sinus arrhythmia (RSA)

- a. **Principle:** Sinus arrhythmia is physiological increase in heart rate during inspiration and a decrease during expiration. There is a general agreement that the magnitude of these oscillations reflects the efferent cardiac vagal nerve activity.
- b. **Procedure:** Subjects were asked to breathe slowly and deeply at the rate of 6 breathing cycles in one min (10 sec for one breathing cycle).

5. 24 hour blood pressure measurements (24H ABPM)

- a. **Principle:** 24H ABPM is a validated method of tracking circadian changes in BP. Schillers' BR 102 BP equipment picks up the Kortokoff's sounds with a microphone at each inflation and deflation of the cuff. The monitor can automatically switch to auscultatory mode if oscillatory mode is not functioning well [132].
- b. **Procedure:** The appropriate size of cuff was fixed to the non-dominant arm. Subjects were requested to remain motionless during inflation and deflation periods and were instructed to record their daily activity in a diary. Other details of the procedure are described in the following chapter. 24H BP monitors were collected on the next day.

6. Estimation of Plasma catecholamines

Principle: Estimation of plasma adrenaline and noradrenaline are considered to be biochemical markers of sympathetic activation in the body [117]. Combination of measurements of basal catecholamines along with measurements during response to the HUT would provide useful information about sympatho-neural activation. In this study, plasma catecholamines were estimated at rest and at 5 min of HUT.

Procedure: 5 ml blood was collected with a vacutainer containing EGTA and reduced glutathione. The blood samples were immediately centrifuged at 3000 rpm for 10 min.

The separated plasma was stored in 1.5ml plastic vials and transferred to a liquid nitrogen container. Samples were then transported to the Department of Biochemistry to be stored in a refrigerator at -80°C . The plasma concentration of catecholamines was determined by high-performance liquid chromatography (HPLC) with electrochemical detection. Intra-assay coefficient of variation was 5.34% and inter-assay coefficient of variation was 5.19% for normal plasma noradrenaline at a concentration of 140pg/mL.

Special measures were taken to transport plasma catecholamine samples. Samples were transported in a liquid nitrogen (-196°C) container (**Fig 7**) considering the ultra-short half-life of catecholamine (2min), and travel distance of 200km. Adequate measures were taken for safe handling of the liquid nitrogen. A pilot study was performed for the catecholamine assay.

Fig 7. Collection of liquid nitrogen from the department of Physics, Sultan Qaboos University



4.1.5 Summary of the protocol of Part A

The overall summary of the protocol is as follows:

Day 1

- Subjects reported in fasting condition at the research centre at 7am.
- Test protocol (**Table 2**)
- Subjects will be connected to 24H blood pressure monitor (24H ABPM; BR 102, Schiller).
- Estimated total time is 1.30 hours.

Day 2

24H ABPM monitor was removed.

Table 2: Summary of the test protocol

	<u>Test</u>	<u>Time</u>
1	Obtained an informed written consent	10 min
2	Anthropometric measurements	5 min
3	Indwelling cannula positioned in left antecubital vein for blood extraction.	10 min
4	8 ml of fasting blood was collected for glucose, insulin and intracath was locked by normal saline. 75 gms of glucose was given for glucose tolerance test and blood was collected 2 hrs after.	
5	Connected to TFM	10 min
7	Baseline resting recordings were obtained in supine position.	15 min
8	Deep breathing for sinus arrhythmia (6 slow and deep breathing cycles)	1 min
9	Two minutes rest or until baseline is achieved	2 min
10	Orthostatic tolerance test: Head-up tilt at 70 ⁰ for 5 min. Blood samples for catecholamine estimation were collected pre tilt and at 5 min of tilt.	5 min
11	Subject will be reverted back to supine position.	
12	Two to five minutes rest or until baseline is achieved.	5 min
13	Stroop colour test: Read the colour and not the word	3 min
14	Rest till baseline is achieved.	5 min
15	Ice immersion test: Left foot was immersed in ice water with temp of 4 ⁰ C for 3 min.	3 min
	Total time required	~1.30 hr

4.2 Part B

Genetic Predisposition to Hypertension in Young Offspring with Prehypertension or High normal BP: Cross-sectional Analysis

4.2.1 Subject Selection

All young offspring between 18-30 yrs (N: 667; males 328 and females 339) from the OFS were considered for the analysis of genetic predisposition. These 667 subjects also included 78 subjects recruited for Part A of this thesis (Part A n=78; remaining 589). Subjects had already undergone the OFS protocol.

4.2.2 Genetic predisposition: The genetic loading of hypertension in these young subjects was defined by identifying hypertension in parents. The criteria used were daytime BP (from 24H ABPM) more than 135/85 mmHg or they were on anti-hypertension treatment. (Parents on antihypertensive treatment may have normal ABPM). The parental history of hypertension was not available for 200/589 subjects as their parents could not participate in OFS either because of very old age or had since deceased. The respective offspring were contacted personally to confirm the hypertensive status of their parents. Information about 28 parents, 52 fathers and 34 mothers could not be traced.

4.2.3 Classification: Offspring were classified based on –

1. Existing WHO classification for normal and high normal blood pressure (**WHO**)
2. Existing JNC VII classification for prehypertension (**JNC VII**)
3. The statistical quartiles of the average daytime SBP and DBP for this cohort

1. WHO classification

- a. **WHO1:** Optimal BP with BP <120/80 mmHg (N=233)
- b. **WHO2:** Normal BP with SBP between 120-129 and DBP between 80-84 (N=61)
- c. **WHO3:** High normal BP with SBP between 130-139 and DBP between 85-90 (N=20)

- d. **WHO4:** Hypertension with BP > 140/90 mmHg (N=46)

2. JNC VII for Prehypertension

- a. **JNC1:** Optimal BP with BP < 120/80 mmHg (N= 233)
b. **JNC2:** Prehypertension with SBP between 120-139 and DBP between 80-89 (N=126)
c. **JNC3:** Hypertension with BP > 140/90 mmHg (N=46)

3. Statistical Quartiles

A. Systolic BP groups

The SBP ranged from 94 to 216 mmHg and the groups based on quartiles were –

- 1) SBPG1 with systolic BP $\leq$ 113 mmHg (N=160);
- 2) SBPG2 with SBP between 114-122mmHg (N=139);
- 3) SBPG3 with BP between 123-130 mmHg and (N=172)
- 4) SBPG4 having SBP $\geq$ 131 mmHg (N=153).

B. Diastolic BP groups:

The DBP ranged from 57 to 126 mmHg and the groups based on quartiles were –

- 1) DBPG1 with diastolic BP $\leq$ 73 mmHg (N=164);
- 2) DBPG2 with DBP between 74-79 mmHg (N=163);
- 3) DBPG3 with BP between 80-85 mmHg (N=149)
- 4) DBPG4 having DBP $\leq$ 86 mmHg (N=148).

C. Systolic and Diastolic BP groups:

Subjects were further classified considering systolic and diastolic BP taken together as –

- 1) **BPG1** those who have BP $\leq$ 113/73 mmHg (n= 98);
- 2) **BPG2** whose BP is between 114/74 to 122/79 mmHg (n= 49);
- 3) **BPG3** whose BP is between 123/80 to 130/85 mmHg (n= 65);
- 4) **BPG4** whose BP is $\geq$ 131/86 (n= 98).

Summary of the above classification is shown in the following **table 3**.

Table 3: Summary of offspring groups based on WHO and JNC VII classification and statistical quartiles

WHO			JNC VII			Quartiles		
BP	Name	N	BP	Name	N	BP	Name	N
<120/80	Optimal	233	<120/80	Optimal	233	<113/73	BPG1	109
120-129/ 81-84	Normal	61	120-139/ 80-89	Pre-hypertension	126	114-122/ 74-79	BPG2	61
130-139/ 85-89	High normal	20				123-130/ 80-85	BPG3	69
>140/90	Hypertension	46	>140/90	Hypertension	46	≥ 131/86	BPG4	103
Total		360			405			342

Measurements and Data Acquisition

5.1 Ambulatory Blood Pressure Monitoring

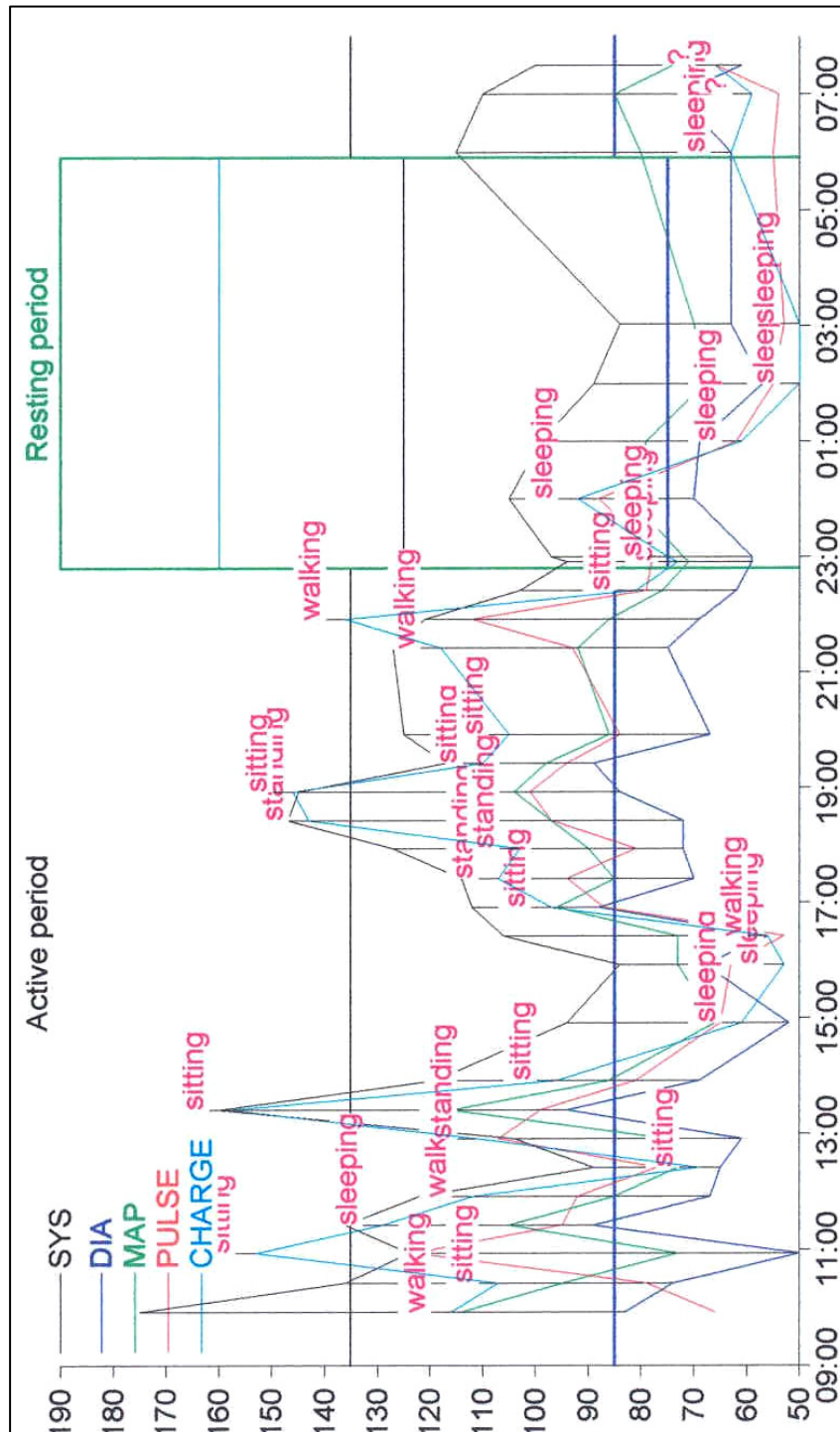
Ambulatory BP measurements were obtained using auscultatory mode of the validated Schiller BR 102 ambulatory BP monitor [132] (**Fig 8**). Microphone of the monitor picks up Korotkoff sounds with each inflation and deflation of the cuff. Schillers' BP monitor uses both auscultatory and oscillometric modes. However, the auscultatory mode was recommended for ambulatory blood pressure measurements in the clinical practice. The monitor can automatically switch to the oscillometric mode only if the device fails in the auscultatory mode. But, the oscillometric mode does not provide accurate measurements.

Procedure: The appropriate size of cuff was fixed to the non-dominant arm. The procedure was explained separately to male and female subjects. Three BP readings were also taken during the same period with a calibrated mercury sphygmomanometer. Recordings were accepted when the average of both methods of measurements did not differ by ≥ 5 mmHg.

Subjects were asked to remain as motionless as possible during the inflation and deflation periods. They were instructed to note their activity, position and location at the time of recording. They were also asked to state the time of going to bed and time of awakening in a diary. Strenuous physical activity was discouraged during the day of monitoring.

The BP monitor was programmed to record BP every 30 minutes from 11:30 AM to 9:30 PM, every 60 minutes from 9:30 PM to 6:30 AM, and again every 30 min from 6.30 am onwards to cover a total of 26 hours. The first 2 hours of monitoring were considered to be an adaptation period and were not included in the computation of BP means. Recordings were accepted as valid when the rate of artifacts was $\leq 25\%$ and when the recording lasted for at least 20 continuous hours. One technician, trained on identifying artifacts and outliers, edited all ABPM data. 24H ABPM was repeated for 5 subjects due to artifacts in the recording

Fig 8. 24H ABPM in a subject



The periods of daytime and nighttime ABPM were determined for each subject according to their actual waking and sleeping time recorded in their individual diaries. The average BP during daytime and nighttime was calculated.

Hot climate and 24H ABPM: There are chances that due to the hot climate in this area (average temperature ranging from 35-50⁰C), the sensor of the BP monitor will render insensitive due to salt deposition and copper oxide coat and the monitor will switch to oscillometric mode. Such results were not to be included in the analysis. However, this problem did not arise for these 78 subjects.

Sleep Quality: Sleep quality was recorded by each subject using a score of 0-3, according to the number of times the subject was awakened by cuff inflations: 0- sound sleep; 1- awakened once; 2, awakened twice and 3 – awakened 3 times or more. Two subjects had a score of 3 or more and their ABPM was repeated.

5.2 Hemodynamic and Autonomic Measurements

Hemodynamic and autonomic parameters at rest and in response to physiological stressors were estimated with the Task Force Monitor (TFM, CNSystems, Graz, www.cnsystems.com)

5.2.1 Hemodynamic parameters

TFM uses electrocardiogram (ECG), beat-to-beat and oscillometric BP and impedance cardiography to estimate hemodynamic parameters (**Fig 9**).

ECG

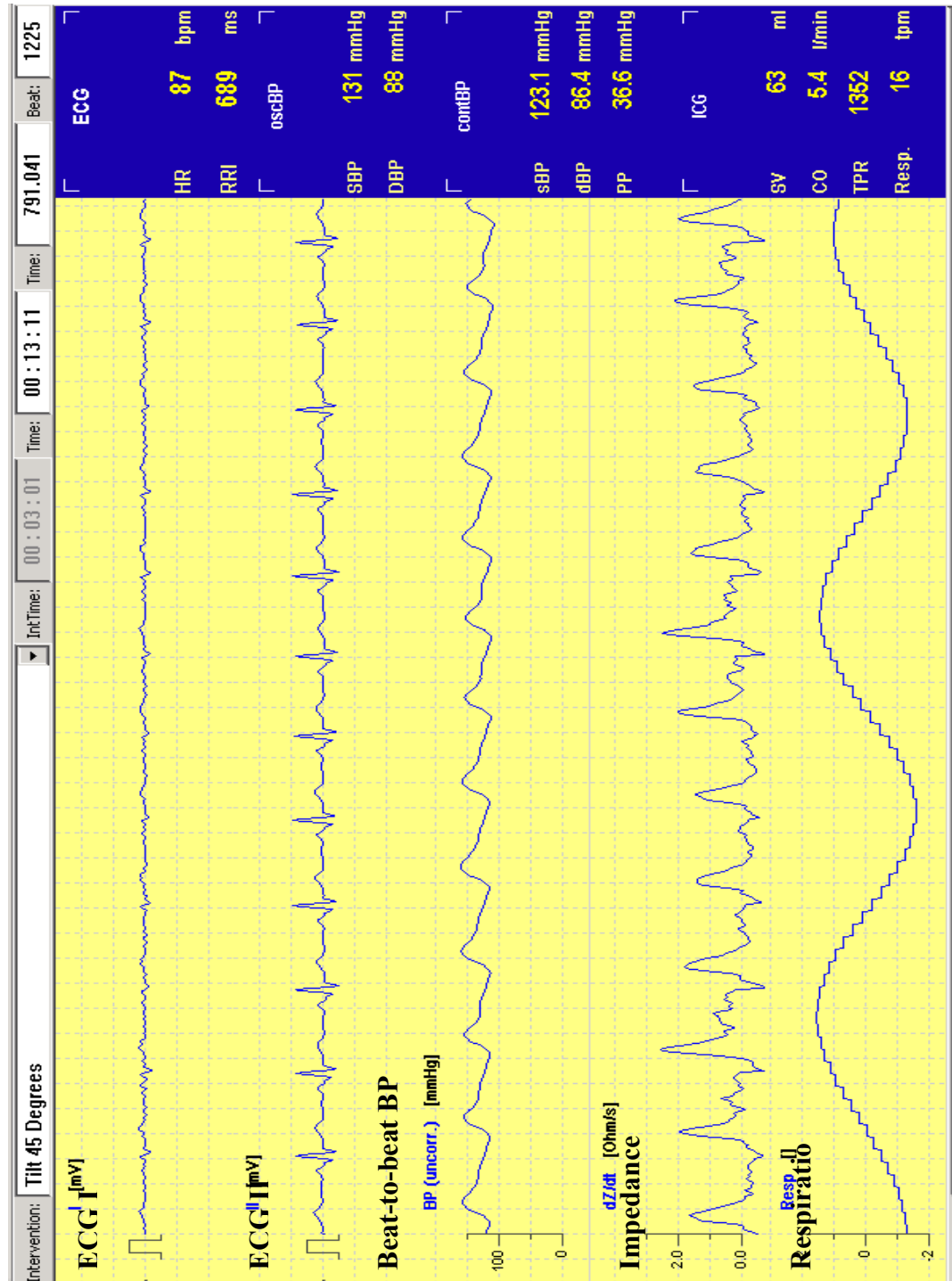
ECG in 6 leads (I, II, III, aVL, aVR and aVF) was obtained with electrodes placed at following positions- 5th left intercostal space in mid-clavicular line, retrosternal 2nd intercostals space on left and right side and a ground electrode (**Fig 4**).

Beat-to-beat Blood Pressure

Beat-to-beat BP was acquired by vascular unloading technique using finger cuffs [133]. The oscillometric BP was measured on the contralateral hand. To increase the accuracy

of BP measurements, continuous blood pressure was constantly matched to the oscillometric blood pressure during online monitoring.

Fig 9. Signals of TFM- ECG lead I and II, beat-to-beat BP, impedance and respiration



Non-derived haemodynamic and cardiac parameters were continuous R-R intervals (RRI msec), heart rate (HR; beats/min), and systolic (SBP), diastolic (DBP) and mean blood pressure (MBP).

Impedance Cardiography

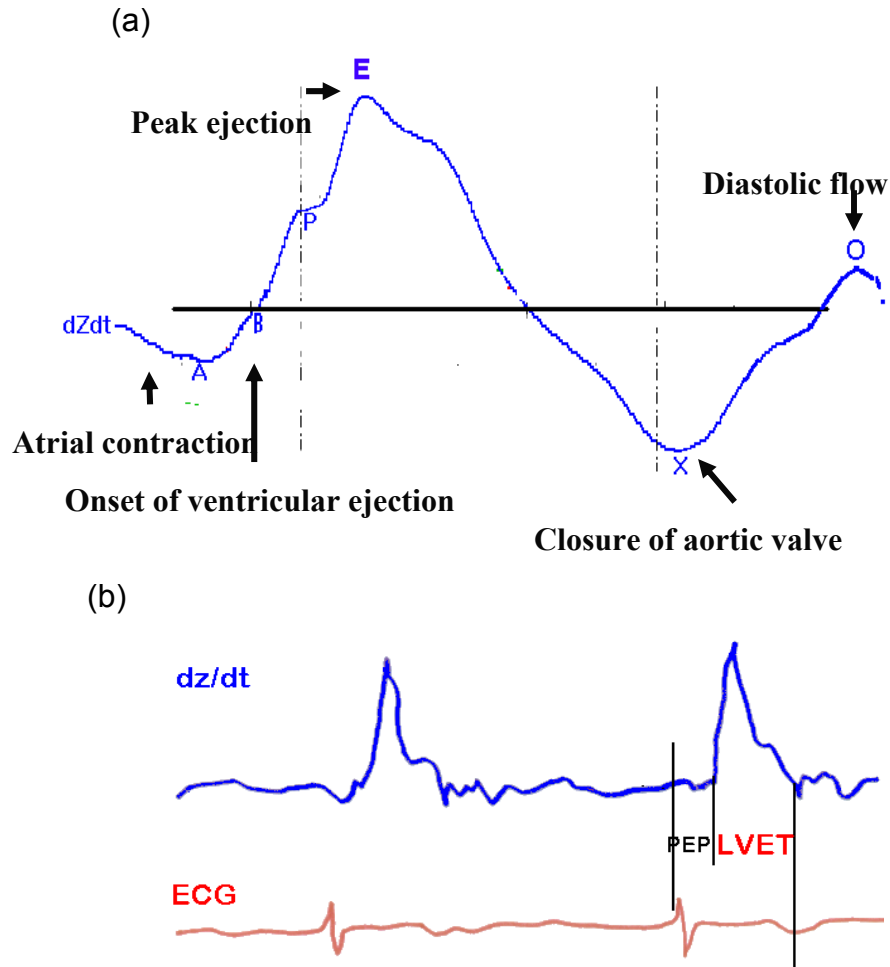
Impedance signal was acquired with a constant sinusoidal alternating current I_0 of 400 μ A and 40 kHz passing through the thorax between an electrode placed around the neck and another electrode placed around the lower thorax at xiphisternum. The voltage $u(t)$ is acquired by another electrode placed between the admitting electrodes, at a distance of 3cm from the outer electrode, in order to produce a homogeneous current field between them. The detected voltage $u(t)$ is proportional to the thorax impedance $Z(Z(t)= u(t)* I_0$.

The Z_0 normalized to inter-electrode distance represents the thoracic wetness index [134,135]. Z_0 differs with gender, thoracic muscle mass and fat content. The decrease in impedance indicates overhydration. However, the first derivative (dz/dt) of the impedance signal is a stable signal. The dz/dt along with the LVET is considered to calculate hemodynamic parameters using standard Kubisek's formula [136].

$$SV = \left(\frac{(0.17 H)^3}{4.2} \right) \frac{\left(\frac{dZ}{dt} \right)_{\max}}{Z_0} LVET$$

Left ventricular ejection time (LVET msec) is the time between 'B' (opening of aortic valve) and 'X' points (closure of aortic valve) on impedance wave. When ECG and heart functions are normal, B point occurs at or following the peak of R wave, and X point comes at the end of T wave on ECG (**Fig 10**).

**Fig 10. (a) Impedance wave form of dz/dt showing B and X point.
(b) dz/dt with ECG to denote LVET**



The following parameters were indexed for body surface area: Stroke volume (SV ml), stroke index (SI; ml/beat/m²), cardiac output (CO; L/min), cardiac index (CI; L/beat/m²), total peripheral resistance (TPR; dyne*s*/cm²) and total peripheral resistance index (TPRI; dyne*s*m²/cm²) [135,137,138].

5.2.2 Autonomic measurements

Heart Rate Variability (HRV) and Blood Pressure Variability (BPV)

Various heart rate and BP variability measures are indicative of autonomic drive of the heart rate and circulation [139,140]. Heart rate variability can be calculated using either of time domain, frequency domain or geometric methods.

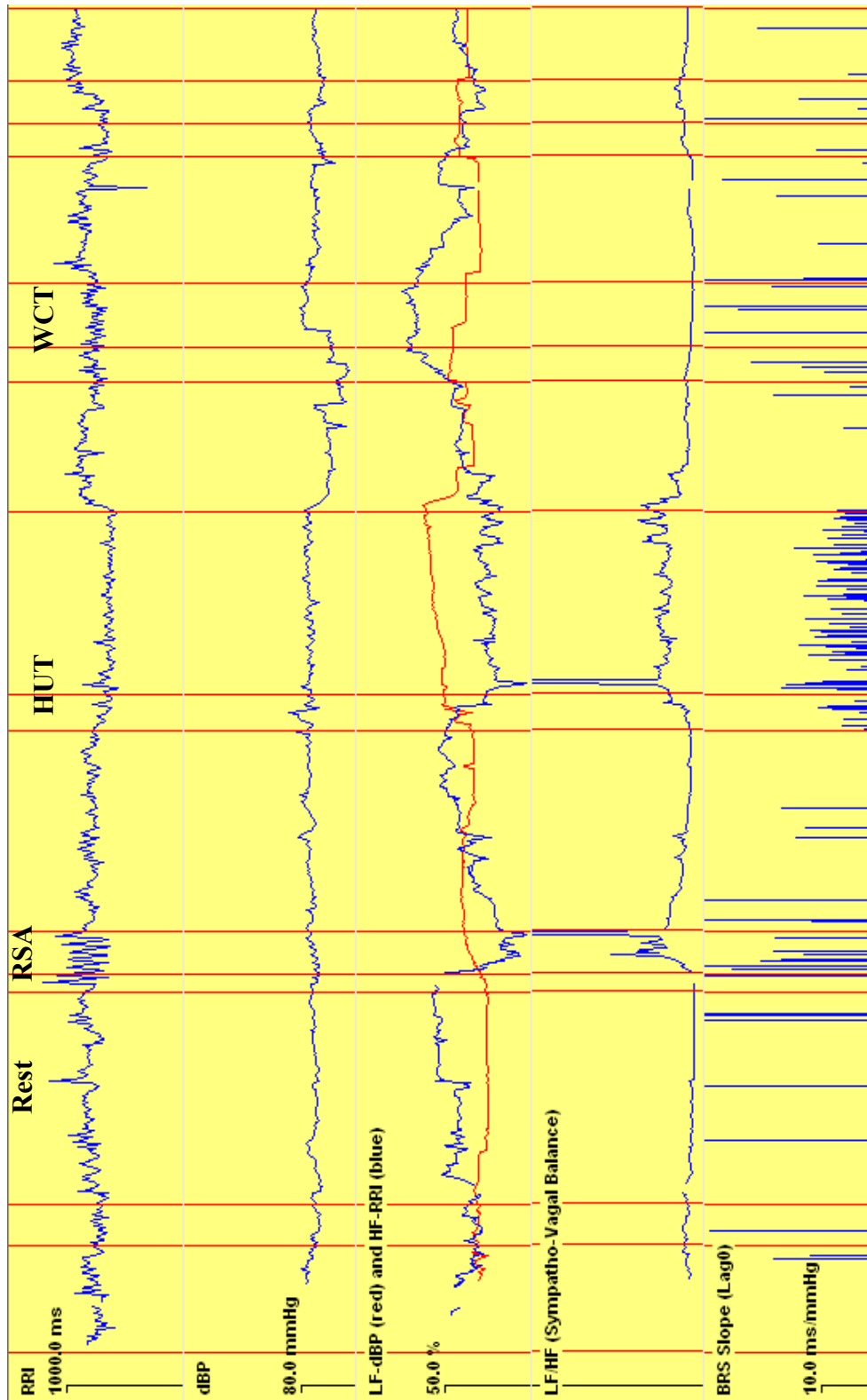
TFM uses an adaptive autoregressive model (AAR) for computing online beat-to-beat time varying spectral analysis of the heart rate (HRV) in frequency domain. It provides 0.01-0.05 Hz band (Very Low Frequency, VLFRRRI); 0.05-0.17 Hz band (Low frequency, LFRRI), and 0.17-0.5 Hz band (High Frequency, HFRRI). Absolute values for all frequency bands are calculated as area under the curve and are expressed in ms^2 . Total power spectral density (PSD) is the total area under all 3 frequency bands and provides the basic information of how variance distributes as a function of the frequency.

LF and HF were also measured in normalized units (LFnuRRI % and HFnuRRI %) (**Fig 11**). This represents the relative value of each power component in proportion to the total power minus the VLF component. The representation of LF and HF in normalized units emphasizes the controlled and balanced behavior of two branches of the autonomic nervous system. Moreover, the normalization tends to minimize the effect of changes in the total power on values of the LFRRI and HFHRV components. LF/HF ratio indicative of the sympatho-vagal balance is calculated using LF band from BPV and HF band from HRV [138,141].

TFM also provides 0.05-0.17 Hz band of systolic blood pressure variability (BPV) in absolute values (LFBPV) and in normalized units (LFnuBPV).

There is a general agreement that HFRRI and HFnuRRI reflect the parasympathetic or vagal activity, and LFRRI, LFnuRRI, LFBPV and LFnuBPV reflect the sympathetic modulation of the sinoatrial node and vasomotion [140,142,143]. Interpretation of the LFRRI band is still a matter of discussion; some authors say it is a marker of sympathetic activity [144,145] while others view LFRRI as An indicator of both sympathetic and vagal activity as it carries some information from vagal activity [139,146]. LFnuBPV mainly carries sympathetic activity. LF/HF ratio reflects the change in status of the sympathovagal balance [144] and PSD indicates overall variance in the heart rate. It is to be remembered that components of HRV provide measurement of the degree of autonomic modulations rather than the level of autonomic tone [147].

Fig 11. Figure shows TFM recording of RRI, DBP, LFnuBPV, HFnu, LF/HF and baroreceptor sequences in a subject



There are certain limitations in the interpretation of the spectral analysis in quantifying the autonomic nervous system tone by power spectral densities of these bands [148]. It is difficult to interpret values when ECG signal is recorded in the absence of standardized conditions and without the simultaneous recording of the respiratory activity. It is also difficult to extract information if ECG recording has artifacts or shows ectopics, arrhythmias or missing beats etc, or is recorded for very short period of time $< 3\text{min}$ and with less than 100Hz of sampling frequency.

Considering limitations, adequate measures were (e.g. proper fixing of electrodes to the skin) taken to avoid artifacts in the ECG recording. Only clear ECG recordings were selected for the analysis.

Baroreflex sensitivity (BRS)

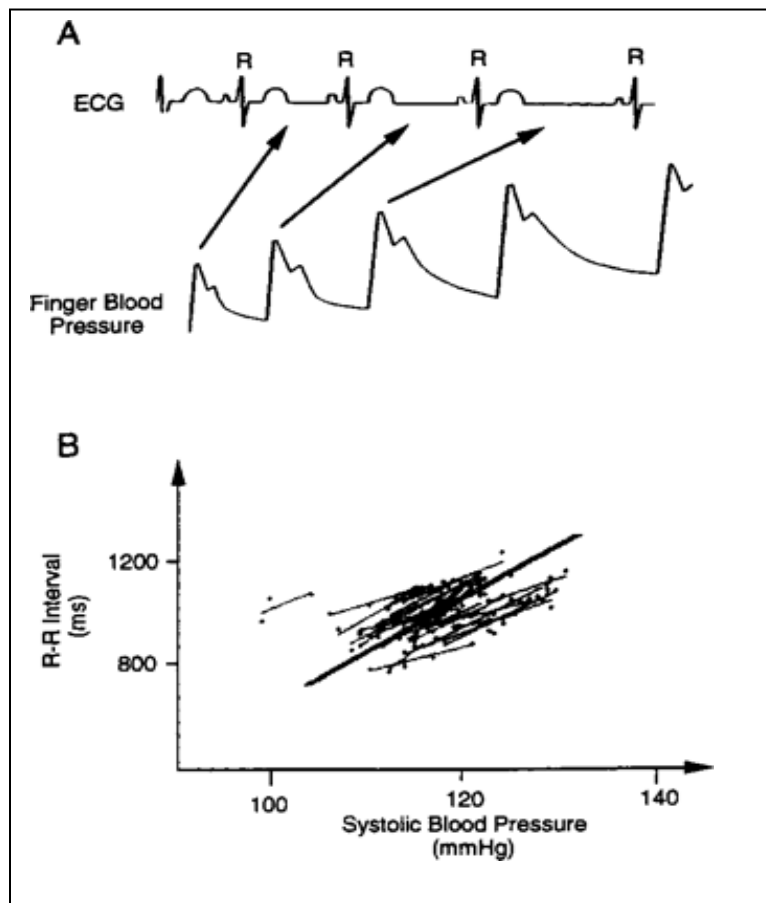
The baroreflex sensitivity indicates reflex modulation of HR with change in BP. Heart rate changes in response to changes in BP induced by bolus injection of vasopressor agent (phenylephrine) or vasodilator agent (nitroglycerine) were measured [149]. Regression line estimated for the reflex lengthening or shortening of HR represented the baroreceptor sensitivity. However, this is an invasive procedure. Non-invasive estimate of the spontaneous baroreceptor sensitivity (BRS ms/mmHg) using the ‘Sequence method’ is now possible due to beat-to-beat recording of BP and advances in computation [137,150,151].

The sequence method is based on the computer identification of spontaneously occurring sequences of four or more beats in the time domain. The sequence should have consecutive 4 or more beats showing an increase in systolic blood pressure (SBP) associated with prolongation of RR-interval (Up-events) or a fall in systolic blood pressure with shortening RR-interval (Down-events) (**Fig 12**). Sequences are considered in the analysis only when there is a consecutive 1 mmHg change in SBP with at least of 0.5 ms change in R-R interval. Slope of the regression line between systolic pressure and

R-R interval changes is taken as an index of the baroreflex sensitivity. This is same as estimated with the laboratory method of injecting phenylephrine [137,151].

Lag 0, baroreceptor activity was considered while calculating up and down events. Lag 0 means that, the RR-interval and blood pressure sequences used for calculating a baroreceptor event occurs synchronously. There is no displacement between blood pressure sequence and RR-interval sequence when an event is detected.

Fig 12. Baroreceptor sequence (Up-event); and B. Slope of regression line



Statistics

Data of Part A and part B were organized and analyzed using SPSS v.13 for windows. A P value of less than 0.05 was considered significant.

6.1 Part A: Cardiovascular Laboratory Tests

6.1.1 Data extraction

A. 24H ABPM

The periods of daytime and night-time ABPM were determined for each offspring according to the actual waking and sleeping time recorded in their individual diaries. The average BP during daytime and night-time was calculated. Subjects were defined as "dippers" when their night-time SBP fell by 10% or more of the daytime SBP. Likewise, subjects were defined as non-dippers when their SBP fell by $< 10\%$ from daytime SBP, or rose above baseline, (reverse dipping).

B. Cardiovascular tests

Beat-to-beat values of hemodynamic and autonomic measurements at rest and during various stresses obtained with TFM were extracted into windows excel format. Data were averaged for 10min of baseline (Rest), 0 to 1 min of each stress (WCT, CPT and HUT) and 1 min to end of each stress (WCT_{3min}, CPT_{3min} and HUT_{5min}). Impedance parameters Z0 and dz/dt were also extracted from TFM similarly.

The physiological challenges were given in sequence. Though adequate time was given in between challenges to ensure return of hemodynamic and autonomic parameters to the baseline, it is possible that this pre-test condition would influence response to the given challenge. Hence, in addition, pre-test averages for all parameters were also estimated.

For deep breathing test, maximum HR at 6 inspirations (I) and minimum HR at 6 expiration phases (E) of respiratory cycle were averaged. The degree of sinus arrhythmia was calculated as the difference between I and E (Δ I-E).

6.1.2 Data analysis

All variables were tested for normal distribution using Kolmogorov- Smirnov (KS) test. Variable was considered normally distributed when KS test is not significant ($P>0.05$) [152]. Non-normal variables (BRSav, BRSup and BRSdn) were log transformed. Values expressed are mean $\pm$ SD.

1. Baseline comparisons, ABPM and RSA,: Anthropometric measurements, zero and 2 hr blood glucose, 24H ABPM, resting hemodynamic and autonomic parameters and the degree of RSA were compared amongst 3 groups (ONT, OHT₁ and OHT₂) using one way analysis of variance (ANOVA). Scheffe's *post-hoc* test was used to determine group differences. Pearson's correlation test was used to correlate resting HR with resting LVET.

2. Cardiovascular tests: Hemodynamic and autonomic parameters across rest and physiological challenges (CPT, WCT and HUT) were compared in 3 groups using a repeated measures analysis of variance (Repeated MANOVA with Bonferroni adjustment) at 3 levels viz. rest, and change at 1 min and the endpoint of each test. The test was controlled for pre-test average of each variable. Repeated measure analysis was considered eligible for interpretation when overall F test was significant [153].

The significant interaction of time and group (Time x Group) indicated significant differences in reactivity of groups to the given challenge with time for that variable. Group differences and differences in reactivity with time were estimated using Scheffe's *post hoc* test.

3. Plasma noradrenaline: The change in plasma noradrenaline from pre tilt to HUT_{5min} was analyzed using repeated measure analysis at 2 levels. In addition, the magnitude of

an increase in plasma nor-adrenaline [d(noradrenaline)] levels was calculated by subtracting pre tilt value from 5 min value. Three groups were tested for this difference using ANOVA. Plasma noradrenaline at rest and at HUT_{5min} were correlated with respective autonomic parameters. In addition, the noradrenaline response was also correlated with autonomic responses.

6.2 Part B

Genetic Predisposition to Hypertension in Young Offspring with Prehypertension or High normal BP: Cross-sectional Analysis

Blood pressure of offspring was correlated with the parental BP using Pearson's correlation test. Linear regression was done separately for daytime SBP and DBP of offspring as dependant variables with daytime SBP and DBP of father and mother (predictor variables).

Parents were categorized according to their BP status as follows- 1) **Both NT**: Both parents normotensive; 2) **Isolated SBP/DBP HTN**: Either or both parents having isolated systolic or diastolic hypertension; 3) **Mother HTN**: Hypertensive mother with normotensive father; 4) **Father HTN**: Hypertensive father with normotensive mother; and 5) **Both HT**: Both hypertensive parents. Category 5 also included couples where if one had hypertension and the other partner had isolated systolic or diastolic hypertension. The difference between category 2 and 5 is that, the isolated systolic or diastolic hypertension is coupled with normotensive partner in the category 2.

Chi square was used to test the association between offspring classification and status of hypertension in parents. Chi square for trend was used to test the linear relationship of being in higher BP group of offspring with increasing contrast to the genetic predisposition to hypertension.

Odds of offspring being in higher BP group based on the parental hypertension were estimated using binary logistic regression. Univariate model contained HTN in parents as an independent variable and groups of offspring (based WHO, JNC or quartiles) as a dependant variable. Group of normotensive parents (Both NT) was taken as a reference variable. Odds for each higher BP group in WHO, JNC and quartile classification were calculated against the group 1 i.e. lowest or optimal BP group of offspring. Odds are expressed with 95% confidence intervals (CI) in tables [154].

Results

7.1 Part A: Cardiovascular Laboratory Tests

7.1.1. Baseline Comparisons

Anthropometric and biochemical measurements; and hemodynamic and autonomic parameters at rest in ONT, OHT₁ and OHT₂ groups are described in **table 4**.

1. Anthropometric and biochemical Measurements

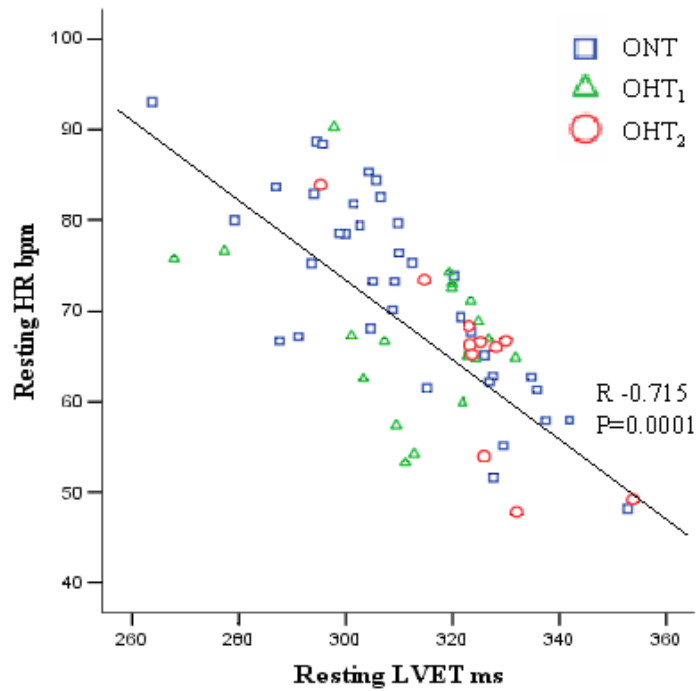
All 3 groups of offspring had similar gender distribution and were comparable for age, BMI and body fat percent. All offspring had normal glucose tolerance test; however fasting blood glucose was significantly higher in OHT₂ compared only with OHT₁ ($P=0.001$) and not with ONT ($P=0.3$). There were no differences in 2H glucose amongst 3 groups (**Table 4**).

2. Hemodynamic and Autonomic Parameters

Apart from HR ($P=0.04$) and LVET ($P=0.05$); all other hemodynamic parameters were comparable in the 3 groups (**Table 4**). OHT₂ had lower HR compared with ONT (*post hoc* OHT₂ 64.31 ± 10.52 ; vs. ONT 72.14 ± 11.04 ; $P=0.02$). LVET showed linearly increasing trend ($P=0.05$) across 3 groups and was higher in OHT₂ compared to ONT (OHT₂ 325.16 ± 13.83 vs. ONT 310.59 ± 18.84 $P=0.027$). LVET was significantly negatively correlated with HR ($r -0.716$, $P=0.0001$) (**Fig 13**).

LFnuRRI, HFnuRRI, and sympatho-vagal balance were comparable in 3 groups (**Table 4**). LFnuBPV was lowest in OHT₂ compared with ONT and OHT₁ (*post hoc* OHT₂ 34.07 ± 9.19 vs. ONT 47.66 ± 13.02 ; $P=0.018$; and OHT₂ 34.07 ± 9.19 vs. OHT₁ 50.60 ± 14.30 ; $P=0.0006$).

Fig 13. Correlation of resting HR with resting LVET.



In summary, ONT, OHT₁ and OHT₂ were comparable for age and anthropometric measurements and baseline impedance. OHT₂ had significantly higher fasting glucose, and LVET and lower LFnuBPV compared with ONT and OHT₁.

Table 4: Anthropometric and biochemical measurements and resting hemodynamic and autonomic parameters and baroreceptor sensitivity in ONT, OHT₁ and OHT₂.

	ONT (N=40; *M 19)	OHT₁ (N=23; M 13)	OHT₂ (N=11; M 5)	P**
Anthropometric measurements				
Age	21.08 (3.55)	22.91 (4.44)	20.36 (1.63)	0.08
Height	159.38 (8.07)	160.20 (7.09)	159.82 (8.57)	0.92
Weight	56.48 (10.89)	56.70 (9.50)	62.00 (12.33)	0.30
BMI	21.92 (3.64)	21.93 (3.55)	24.23 (4.19)	0.17
% fat	17.88 (8.09)	17.93 (7.45)	18.83 (9.80)	0.94
Waist	72.82 (10.40)	75.43 (11.42)	71.36 (9.27)	0.51
Hip	86.44 (8.73)	87.77 (8.55)	82.88 (17.55)	0.5
Biochemical measurements				
Glucose 0	4.81 (0.49)	4.45 (0.47)	5.24 (0.69)	0.0007
Glucose 2H	5.65 (1.13)	5.36 (1.35)	5.67 (1.46)	0.7
Hemodynamic Parameters				
ZO	35.17 (5.48)	34.50 (6.20)	32.41 (5.31)	0.41
dz/dt	2.19 (0.65)	2.38 (0.86)	1.91 (0.55)	0.23
RRI	857.76 (144.07)	919.02 (122.96)	966.39 (169.43)	0.06
HR	72.14 (11.04)	66.72 (8.95)	64.31 (10.52)	0.04
SBP	114.88 (15.23)	116.36 (13.55)	116.75 (20.73)	0.90
DBP	67.74 (9.71)	70.20 (10.54)	68.09 (14.44)	0.70
SV	75.64 (14.94)	80.68 (12.74)	80.91 (8.33)	0.29
SI	47.72 (8.27)	51.11 (8.84)	49.32 (5.23)	0.31
CO	5.36 (0.88)	5.31 (1.02)	5.19 (1.00)	0.86
CI	3.40 (0.65)	3.41 (0.77)	3.18 (0.73)	0.63
TPR	1241.11 (277.89)	1271.40 (301.73)	1343.04 (405.03)	0.63
TPRI	1972.99 (488.38)	2039.16 (599.57)	2278.82 (848.91)	0.32
LVET	310.59 (18.84)	313.20 (16.60)	325.16 (13.83)	0.05
Autonomic Parameters				
LFnuRRI	52.13 (15.35)	47.04 (15.83)	47.42 (15.91)	0.43
HFnuRRI	47.87 (15.35)	59.72 (34.92)	52.58 (15.91)	0.18
LF/HF	1.19 (0.65)	1.13 (0.49)	0.77 (0.33)	0.16
LFnu BPV	47.66 (13.02)	50.60 (14.30)	34.07 (9.19)	0.005
BRSup	24.59 (16.06)	29.52 (17.06)	22.72 (20.06)	0.55
BRSdn	23.19 (13.97)	28.93 (13.74)	26.87 (14.71)	0.39
BRS av	23.81 (13.63)	27.87 (12.07)	24.18 (16.85)	0.61

*M: Males; **P value is for ANOVA

7.1.2 24H ABPM

Table 5 describes 24H ABPM profile of 3 groups. Daytime, night-time and 24H average systolic, diastolic and mean BP were similar in ONT and OHT₁ and were higher in OHT₂, however differences were not statistically significant.

Daytime and 24H average HR was higher in OHT₂ compared with OHT₁ (*post hoc* P=0.05; and P=0.02 respectively). Night-time HR was also significantly higher in OHT₂ compared to ONT (80.30±9.75 vs. 70.97±9.14; P=0.03) and OHT₁ (80.30±9.75 vs. 66.33±9.06; P=0.0001). Ambulatory HR was similar in ONT and OHT₁ groups. All 3 groups had similar night-time systolic dip.

Table 5: 24H ABPM in ONT, OHT₁ and OHT₂

	ONT	OHT ₁	OHT ₂	P
Daytime SBP	115.47 (9.47)	115.30 (11.71)	121.80 (6.39)	0.18
Daytime DBP	73.91 (8.01)	75.90 (10.63)	77.10 (8.13)	0.54
Daytime MBP	88.27 (7.42)	92.58 (10.16)	92.20 (6.84)	0.18
Daytime HR	82.15 (10.83)	75.42 (6.56)	86.40 (10.79)	0.039
Night-time SBP	100.47 (8.94)	100.33 (9.44)	107.20 (11.62)	0.14
Night-time DBP	62.10 (8.02)	59.25 (9.10)	66.70 (9.81)	0.14
Night-time MBP	65.86 (7.69)	59.29 (4.11)	69.30 (12.12)	0.07
Night-time HR	70.97 (9.14)	66.33 (9.06)	80.30 (9.75)	0.0003
24H SBP	111.76 (9.37)	111.55 (9.14)	118.30 (7.39)	0.11
24H DBP	71.00 (7.57)	71.95 (7.81)	74.70 (8.49)	0.42
24H MBP	84.67 (7.56)	86.00 (6.94)	89.10 (7.17)	0.26
24H HR	78.24 (10.55)	70.25 (5.83)	82.40 (10.31)	0.013
Systolic DIP	-12.98 (4.91)	-15.15 (9.11)	-12.02 (8.19)	0.52

*P value is for ANOVA

7.1.3 Cold Pressor Test (CPT)

1. Hemodynamic responses

There was an increase in HR, SBP and DBP from rest to CPT_{1min} to CPT_{3min}; however there were no group differences. There was no significant change in impedance in response to the CPT (**Table 6**).

Table 6: Hemodynamic responses of ONT, OHT₁ and OHT₂ to CPT

	Groups	Rest	1 min	3 min	P*
Z0	ONT	35.60 (5.64)	35.13 (5.59)	35.22 (5.63)	0.39
	OHT₁	33.81 (5.93)	33.39 (5.93)	33.18 (5.78)	
	OHT₂	33.76 (5.04)	32.53 (6.08)	32.42 (5.87)	
dz/dt	ONT	2.30 (0.69)	2.12 (0.66)	2.18 (0.71)	0.59
	OHT₁	2.26 (0.80)	2.24 (0.92)	2.16 (0.86)	
	OHT₂	2.08 (0.45)	1.88 (0.52)	1.94 (0.49)	
RRI	ONT	879.80 (150.46)	716.24 (126.52)	754.99 (22.06)	0.2
	OHT₁	924.64 (127.77)	774.62 (105.74)	799.97(19.53)	
	OHT₂	966.39 (169.43)	723.17 (74.72)	817.84 (17.83)	
HR	ONT	70.37 (10.96)	86.81 (14.24)	81.94 (12.89)	0.3
	OHT₁	66.40 (9.33)	79.53 (11.35)	75.88 (10.76)	
	OHT₂	64.31 (10.52)	84.47 (8.85)	75.04 (10.87)	
SBP	ONT	110.09 (10.52)	114.22 (16.69)	122.53 (20.91)	0.69
	OHT₁	118.51 (12.18)	126.38 (19.07)	133.70 (22.13)	
	OHT₂	116.75 (20.73)	110.72 (17.67)	122.03 (12.43)	
DBP	ONT	66.33 (9.15)	73.83 (13.55)	79.40 (17.29)	0.68
	OHT₁	70.91 (10.81)	80.93 (13.61)	86.62 (13.90)	
	OHT₂	68.09 (14.44)	71.94 (10.11)	77.30 (9.21)	
PP	ONT	43.75 (6.44)	40.38 (9.80)	43.12 (8.31)	0.79
	OHT₁	47.60 (9.67)	45.45 (11.71)	47.07 (14.13)	
	OHT₂	48.66 (18.74)	38.78 (9.53)	44.73 (6.19)	
SV	ONT	77.41 (15.85)	70.37 (15.42)	71.87 (15.39)	0.69
	OHT₁	80.34 (13.21)	75.69 (16.09)	74.68 (15.39)	
	OHT₂	80.91 (8.33)	71.92 (6.77)	74.73 (10.45)	
SI	ONT	49.29 (8.51)	44.98 (8.38)	46.01 (8.63)	0.74
	OHT₁	50.44 (9.04)	47.36 (9.59)	46.79 (9.54)	
	OHT₂	49.32 (5.23)	43.97 (4.78)	45.60 (5.99)	
CO	ONT	5.33 (0.75)	5.96 (1.01)	5.79 (1.10)	0.87
	OHT₁	5.30 (1.03)	5.91 (0.97)	5.61 (0.94)	
	OHT₂	5.19 (1.00)	6.06 (0.82)	5.57 (0.90)	
CI	ONT	3.43 (0.64)	3.85 (0.76)	3.74 (0.79)	0.86
	OHT₁	3.32 (0.79)	3.66 (0.68)	3.49 (0.68)	
	OHT₂	3.18 (0.73)	3.72 (0.65)	3.43 (0.75)	
TPR	ONT	1185.31 (190.16)	1159.98 (231.27)	1294.81 (254.08)	0.51
	OHT₁	1319.29 (299.34)	1330.94 (319.09)	1458.88 (391.78)	
	OHT₂	1343.04 (405.03)	1101.97 (255.86)	1329.69 (320.66)	
TPRI	ONT	1868.31 (378.01)	1643.52 (757.68)	2039.77 (528.64)	0.56
	OHT₁	2106.50 (590.91)	2105.68 (606.05)	2349.77 (647.36)	
	OHT₂	2278.82 (848.91)	1811.20 (455.47)	2204.14 (628.24)	
LVET	ONT	314.43 (17.58)	259.37 (86.99)	298.29 (20.28)	0.07
	OHT₁	312.12 (17.09)	293.32 (22.49)	299.93 (23.41)	
	OHT₂	325.16 (13.83)	295.61 (14.81)	308.14 (15.01)	

*P value is for group vs time interaction in the repeated measure analysis

2. Autonomic responses

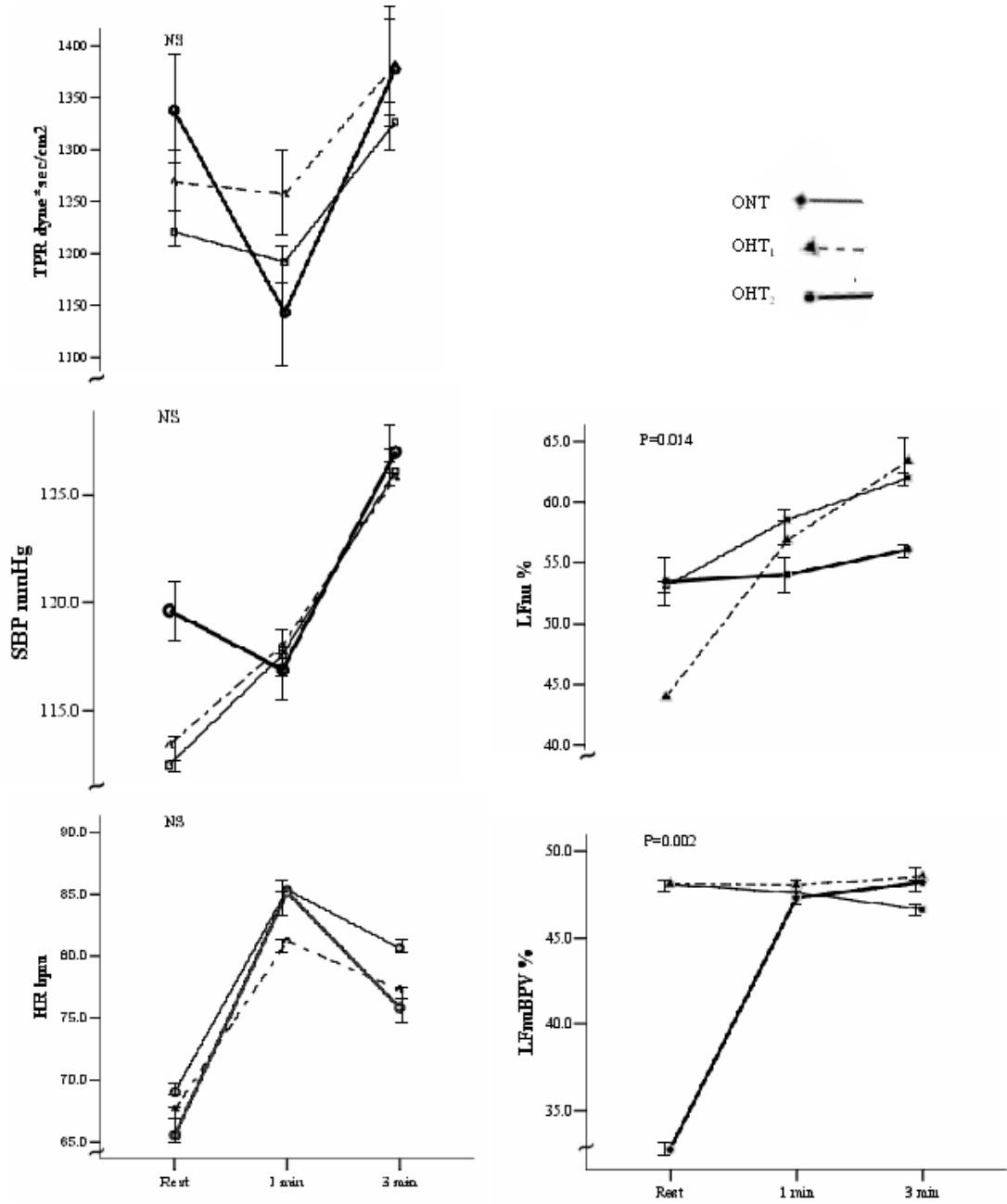
There was less increase in LFnuRRI ($P=0.014$) and less decrease in HFnuRRI ($P=0.017$) in OHT₂ compared with ONT and OHT₁ from rest to CPT_{1min} to CPT_{3min} (Table 7, Fig 14). However, OHT₂ responded with greater increase in LFnuBPV at CPT_{1min} compared with the other 2 groups ($P=0.002$). Baroreceptor sensitivity decreased from rest to CPT_{1min}, but there were no group differences in the reaction.

Table 7: Autonomic responses to CPT in ONT, OHT₁ and OHT₂

	Group	Rest	1 min	3 min	P*
LFnuRRI	ONT	51.29 (15.27)	55.02 (13.03)	60.16 (13.34)	0.014
	OHT₁	47.19 (16.27)	59.54 (10.72)	65.55 (10.10)	
	OHT₂	47.42 (15.91)	50.46 (13.82)	53.22 (8.17)	
HFnuRRI	ONT	48.71 (15.27)	44.98 (13.03)	39.84 (13.34)	0.017
	OHT₁	52.77 (16.75)	40.43 (11.03)	34.49 (10.39)	
	OHT₂	52.58 (15.91)	49.54 (13.82)	46.78 (8.17)	
LFnuBPV	ONT	46.81 (13.09)	46.63 (10.65)	45.51 (11.11)	0.002
	OHT₁	48.46 (14.30)	48.79 (9.28)	49.23 (9.97)	
	OHT₂	34.07 (9.19)	50.51 (12.72)	50.89 (14.06)	
LF/HF	ONT	1.15 (0.66)	1.20 (0.66)	1.64 (1.64)	0.9
	OHT₁	1.11 (0.53)	1.42 (0.55)	1.83 (1.13)	
	OHT₂	0.77 (0.33)	1.32 (0.75)	1.63 (1.42)	
BRS Total	ONT	24.96 (14.48)	16.86 (15.18)	16.49 (15.10)	0.54
	OHT₁	26.33 (11.05)	15.95 (7.93)	17.77 (7.49)	
	OHT₂	27.70 (16.15)	17.08 (13.16)	13.35 (5.28)	
BRS up	ONT	26.23 (16.11)	17.51 (16.62)	17.28 (24.82)	0.3
	OHT₁	29.25 (18.95)	14.01 (5.07)	20.23 (8.22)	
	OHT₂	19.86 (3.49)	15.53 (8.93)	12.03 (4.32)	
BRS dn	ONT	24.98 (14.21)	12.72 (15.17)	15.65 (12.58)	0.8
	OHT₁	27.40 (15.04)	14.73 (10.66)	13.15 (8.27)	
	OHT₂	25.31 (14.86)	7.19 (0.86)	9.69 (4.16)	

*P value is for groupXtime interaction in the repeated measure analysis

Fig 14: 0.1 Hz band of HRV and BPV during CPT in ONT, OHT₁ and, OHT₂ groups. P value is for group X time interaction.



7.1.4 Head-UP Tilt Test (HUT)

1. Hemodynamic responses

The hemodynamic responses to HUT are given in **table 8**. All 3 groups showed similar increase in HR (**Fig 15**) and decrease in dz/dt from rest to HUT_{1min} to HUT_{5min}.

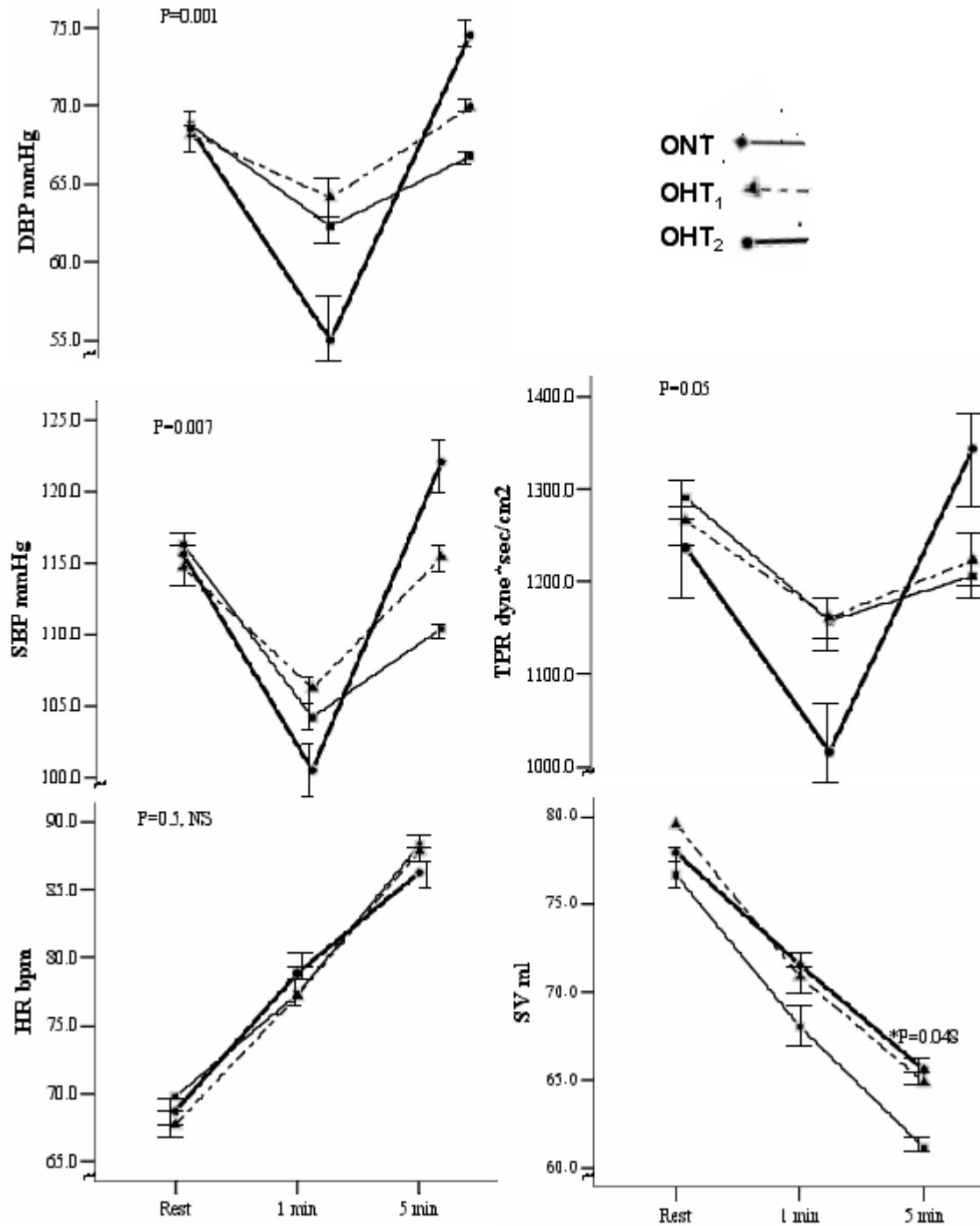
Blood pressure and vascular resistance exhibited biphasic response- drop from rest to HUT_{1min} and increase from HUT_{1min} to HUT_{5min} in all the 3 groups. However, OHT₂ had greater decrease in SBP, DBP and TPR at HUT_{1min} compared with other 2 groups. These parameters also showed greater reflex increase at HUT_{5min} in OHT₂ compared with ONT and OHT₁ (**Fig 14**). Stroke volume and index decreased consistently during HUT (From rest to HUT_{1min} to HUT_{5min}) in all groups; however SV decreased to lesser extent in OHT₂ compared with ONT (P=0.047) (**Fig 14**). This probably has resulted in the attenuated increase in CO in OHT₂ though statistically not significant.

Table 8: Hemodynamic responses of ONT, OHT₁ and OHT₂ to HUT

	Groups	Rest	1 min	5 min	P*
Zo	ONT	35.41 (5.59)	36.98(5.86)	37.26(5.83)	0.20
	OHT₁	35.86 (6.47)	37.42(7.05)	37.81(7.22)	
	OHT₂	32.69 (5.11)	34.85(6.72)	35.54(7.45)	
dz/dt	ONT	2.20 (0.65)	2.09 (0.65)	1.93 (0.69)	0.90
	OHT₁	2.24 (0.82)	2.14 (0.75)	2.02 (0.76)	
	OHT₂	1.92 (0.52)	1.91 (0.57)	1.86 (0.58)	
RRI	ONT	854.68(140.88)	773.54(138.26)	674.50(108.82)	0.18
	OHT₁	919.02(122.96)	807.57 (97.91)	703.88 (84.61)	
	OHT₂	1009.58(153.60)	911.52(160.12)	826.36(133.95)	
HR	ONT	72.33 (10.83)	80.71 (14.30)	91.83 (14.36)	0.5
	OHT₁	66.72 (8.95)	76.09 (9.34)	86.79 (10.67)	
	OHT₂	61.13 (8.29)	68.15 (11.33)	74.81 (12.11)	
SBP	ONT	115.38 (15.17)	102.89 (16.47)	109.28 (18.23)	0.007
	OHT₁	116.36 (13.55)	108.03 (17.63)	117.37 (18.37)	
	OHT₂	115.25 (20.62)	101.40 (22.44)	121.90 (29.12)	
DBP	ONT	68.29 (9.36)	61.48 (11.06)	66.45 (12.03)	0.001
	OHT₁	70.20 (10.54)	66.20 (13.30)	71.77 (11.56)	
	OHT₂	65.79 (14.69)	53.29 (15.90)	71.51 (10.38)	
PP	ONT	46.60 (13.03)	41.29 (13.30)	42.62 (11.43)	0.35
	OHT₁	47.32 (11.28)	44.98 (17.21)	47.74 (19.65)	
	OHT₂	48.46 (18.97)	47.58 (18.99)	50.29 (23.05)	
SV	ONT	75.47 (15.30)	67.00 (10.90)	60.10 (9.68)	0.8
	OHT₁	80.68 (12.74)	71.48 (8.62)	65.29 (9.79)	
	OHT₂	80.23 (9.15)	74.28 (9.52)	68.73 (9.13)	
SI	ONT	47.88 (8.48)	42.57 (6.24)	38.23 (5.86)	0.9
	OHT₁	51.11 (8.84)	45.27 (6.46)	41.24 (5.98)	
	OHT₂	48.87 (5.54)	45.02 (2.96)	41.63 (3.03)	
CO	ONT	5.35 (0.84)	5.27 (0.70)	5.44 (0.85)	0.9
	OHT₁	5.35 (1.02)	5.37 (0.69)	5.63 (0.87)	
	OHT₂	4.88 (0.80)	4.96 (0.55)	5.06 (0.57)	
CI	ONT	3.42 (0.65)	3.38 (0.57)	3.49 (0.67)	0.8
	OHT₁	3.41 (0.77)	3.42 (0.61)	3.57 (0.61)	
	OHT₂	2.99 (0.61)	3.05 (0.51)	3.11 (0.52)	
TPR	ONT	1248.94(283.43)	1120.35(261.65)	1180.93(278.81)	0.05
	OHT₁	1271.40(301.73)	1165.40(270.10)	1227.43(282.64)	
	OHT₂	1387.16(428.55)	1153.06(326.31)	1431.41(407.23)	
TPRI	ONT	1975.72(501.98)	1780.09(496.81)	1848.38(553.28)	0.069
	OHT₁	2039.16(599.57)	1874.70(572.05)	1969.88(609.50)	
	OHT₂	2362.27(897.98)	1953.62(761.78)	2417.91(939.40)	
LVET	ONT	310.15 (19.09)	282.59 (27.62)	263.01 (26.39)	0.4
	OHT₁	313.20 (16.60)	291.46 (19.98)	270.59 (22.74)	
	OHT₂	329.62 (9.60)	299.10 (22.45)	279.63 (19.49)	

*P value is for groupXtime interaction in the repeated measure analysis

Fig 15: Hemodynamic responses at HUT_{1min} and HUT_{5min} in ONT, OHT₁ and OHT₂. P value is for groupXtime interaction. *P value is for the difference between ONT and OHT₂ at HUT_{5min}.



2. Autonomic responses

Autonomic responses to HUT are given in **table 9**. There was an increase in LFnu in all 3 groups from rest to HUT_{1min} to HUT_{5min}. However, OHT₂ showed less increase in LFnu

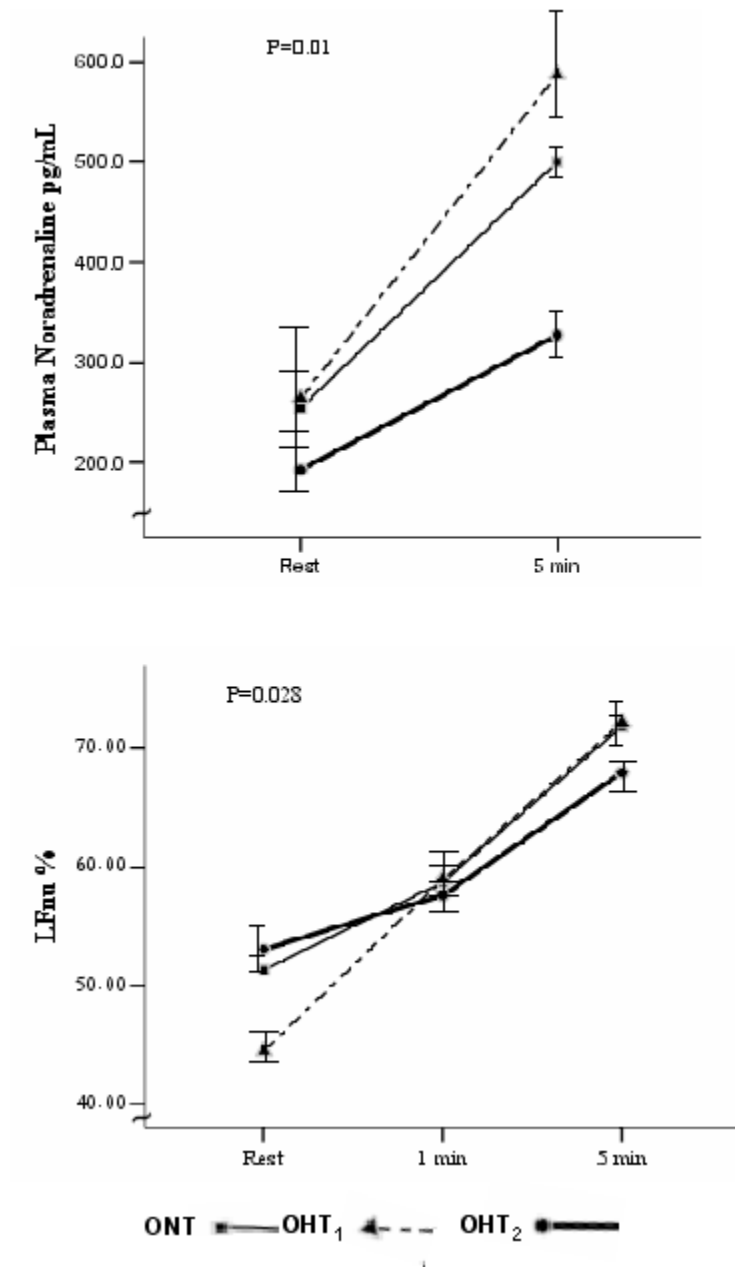
($P=0.028$) and complimentary less decrease in HFnuRRI ($P=0.035$) during HUT compared with other 2 groups (**Table 9 and Fig 16**). LFnuBPV was lower in OHT₂ compared to OHT₁ and it also showed less increase during HUT. The increase in LFnuRRI and LFnuBPV was greater in OHT₁. The decrease in baroreceptor sensitivity from rest to HUT_{1min} was similar in 3 groups.

Table 9: Autonomic and noradrenaline responses to HUT in ONT, OHT₁ and OHT₂

	Group	Rest	1 min	5 min	P*
LFnuRRI	ONT	52.13 (15.35)	59.33(14.17)	72.44 (13.12)	0.028
	OHT₁	47.04 (15.83)	61.86 (13.63)	74.79 (14.21)	
	OHT₂	47.42 (15.91)	52.65 (13.29)	64.36 (12.12)	
HFnuRRI	ONT	47.87 (15.35)	40.67 (14.17)	27.57 (13.12)	0.035
	OHT₁	52.93 (16.24)	38.10 (13.98)	25.25 (14.58)	
	OHT₂	52.58 (15.91)	47.35 (13.29)	35.64 (12.12)	
LFnuBPV	ONT	47.12 (12.81)	45.40 (12.33)	51.75 (12.33)	0.7
	OHT₁	50.60 (14.30)	52.56 (10.41)	57.82 (8.51)	
	OHT₂	33.37 (9.46)	34.93 (5.34)	42.38 (7.22)	
LF/HF	ONT	1.17 (0.61)	1.25 (0.56)	2.72 (1.89)	0.4
	OHT₁	1.05 (0.48)	1.47 (0.57)	2.94 (1.91)	
	OHT₂	0.67 (0.27)	0.80 (0.24)	1.48 (0.87)	
BRS Total	ONT	23.36 (13.69)	13.00 (10.78)	10.43 (7.71)	0.7
	OHT₁	26.95 (10.14)	13.27 (6.34)	9.09 (3.46)	
	OHT₂	32.34 (20.98)	18.12 (5.59)	14.23 (2.82)	
BRS up	ONT	24.90 (15.63)	17.20 (19.01)	11.06 (8.45)	0.6
	OHT₁	30.58 (18.17)	14.20 (8.34)	10.06 (3.64)	
	OHT₂	32.28 (28.52)	18.88 (6.69)	14.96 (1.59)	
BRS dn	ONT	22.94 (13.64)	12.09 (8.65)	9.79 (7.30)	0.2
	OHT₁	28.82 (12.61)	12.91 (7.94)	8.10 (3.19)	
	OHT₂	34.17 (17.63)	14.94 (6.50)	11.61 (3.78)	
Noradrenaline	ONT	254.56 (108.38)	-	500.27 (178.17)	0.01
	OHT₁	264.47 (141.81)	-	587.47 (269.05)	
	OHT₂	192.88 (66.66)	-	327.38 (93.05)	

*P value is for groupXtime interaction in the repeated measure analysis

Fig 16: Autonomic and noradrenaline response to HUT in ONT, OHT₁ and OHT₂. P value is for groupXtime interaction.



3. Catecholamine response

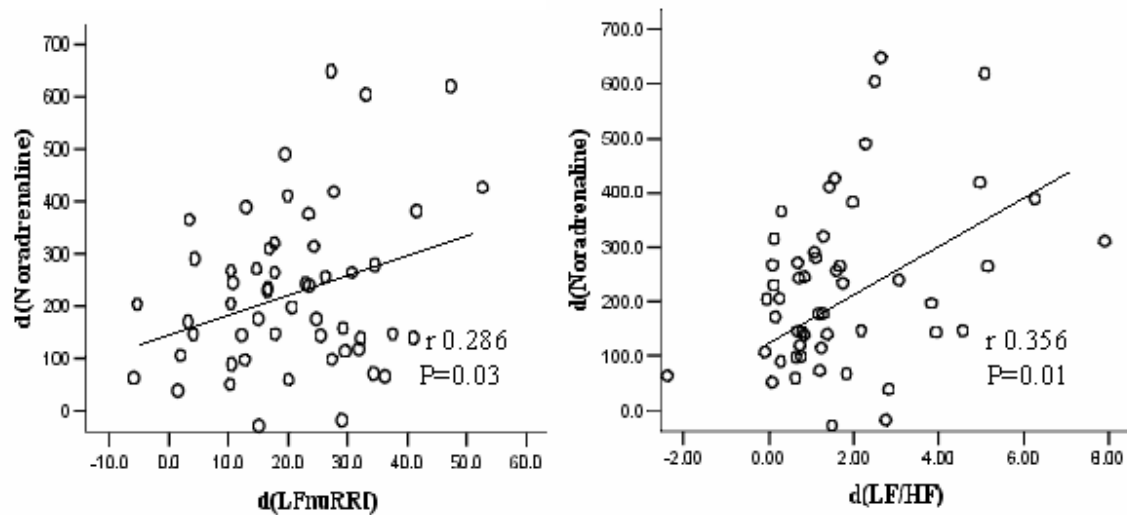
OHT₂ showed significantly less increase in plasma noradrenaline in response to HUT compared with the other two groups (P=0.011) (**Table 9, Fig 16**). The increase in noradrenaline levels (pg/mL) at HUT_{5min} was 134.50±60.05 in OHT₂, 323.00±160.70 in OHT₁ and 245.71±136.91 in ONT (OHT₂ vs. OHT₁; P=0.011).

Correlations of noradrenaline levels with autonomic parameters during HUT are shown in **table 10**. Only 5 min noradrenaline was correlated with autonomic parameters at HUT_{5min}. It showed significant positive correlation with LFnu (r 0.432; P=0.003) and LF/HF (r 0.417; P= 0.004) and negative correlation with HFnu (r -0.432; 0.003). The noradrenaline response to HUT [d(noradrenaline)] was also significantly correlated with responses of LFnuRRI [d(LFnuRRI); r 0.286, P=0.03] and LF/HF [d(LF/HF); r 0.356, P=0.01] (**Fig 17**).

Table 10: Correlations of noradrenaline with autonomic parameters at pre-tilt and 5 min of the HUT

	Noradrenaline			
	HUT _{0 min}		HUT _{5 min}	
	r	P	r	P
HUT_{0 min}				
LFnuRRI	0.1	0.49	0.112	0.45
HFnuRRI	-0.1	0.49	-0.112	0.45
LFnuBPV	0.049	0.72	0.039	0.79
LF/HF	-0.028	0.85	0.021	0.89
HUT_{5min}				
LFnuRRI	0.233	0.12	0.432	0.003
HFnuRRI	-0.233	0.12	-0.432	0.003
LFnuBPV	0.247	0.09	0.06	0.69
LF/HF	0.302	0.04	0.417	0.004

Fig 17. Correlation of noradrenaline response with responses of LFnuRRI and LF/HF during HUT



5. Word Colour Conflict Test (WCT)

1. Hemodynamic responses: Changes in impedance and hemodynamic parameters in response to WCT are shown in **Table 11**. There were significant differences in reactivity of dz/dt , HR, SBP, SV and SI amongst 3 groups.

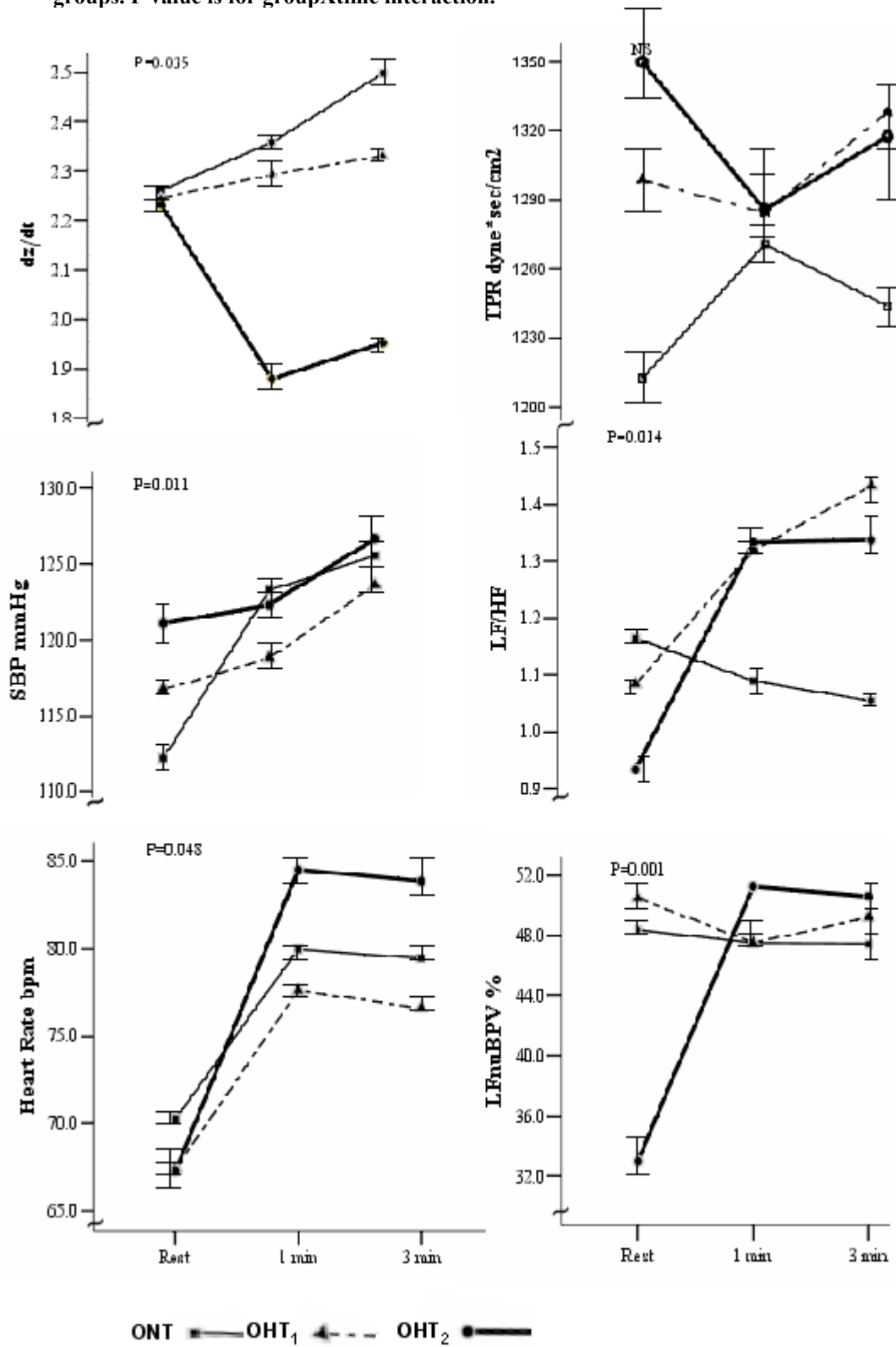
OHT₂ showed significant increase in HR and SBP ($P = 0.048$ and 0.011 respectively) (**Fig 18**) and decrease in SV and SI ($P = 0.001$ and $P = 0.004$ respectively) from rest to WCT_{1min} compared with ONT and OHT₁. The dz/dt decreased significantly ($P = 0.035$) from rest to CPT_{1min} only in OHT₂ compared with the other two groups. Drop in SV and SI observed could be a function of significant drop in dz/dt (**Fig 18**) as the analysis repeated for SV and SI including dz/dt as a covariate nullified this difference. Other hemodynamic parameters though showed expected physiological changes during the test; responses were parallel in 3 groups (**Table 11**).

Table 11: Hemodynamic responses of ONT, OHT₁ and OHT₂ to WCT

	Groups	Rest	1 min	3 min	P*
Z0	ONT	34.80 (5.33)	35.20 (5.21)	35.34 (5.21)	0.038
	OHT₁	34.50 (6.20)	35.24 (6.01)	35.20 (6.03)	
	OHT₂	33.76 (5.04)	32.86 (6.02)	32.86 (6.12)	
dz/dt	ONT	2.21 (0.64)	2.28 (0.71)	2.42 (0.84)	0.035
	OHT₁	2.38 (0.86)	2.43 (1.06)	2.47 (1.06)	
	OHT₂	2.08 (0.45)	1.85 (0.55)	1.92 (0.61)	
RRI	ONT	879.80 (150.46)	775.66 (152.82)	778.53 (136.26)	0.035
	OHT₁	919.02 (122.96)	799.27 (110.08)	810.93 (107.63)	
	OHT₂	966.39 (169.43)	771.61 (110.36)	798.83 (146.93)	
HR	ONT	70.37 (10.96)	80.96 (15.08)	79.67 (13.47)	0.048
	OHT₁	66.72 (8.95)	76.80 (10.76)	75.72 (10.41)	
	OHT₂	64.31 (10.52)	79.49 (11.53)	77.69 (14.43)	
SBP	ONT	110.09 (10.52)	121.69 (11.41)	123.02(12.94)	0.011
	OHT₁	116.36 (13.55)	118.40 (15.58)	123.20 (14.79)	
	OHT₂	116.75 (20.73)	114.36 (16.93)	118.79 (16.73)	
DBP	ONT	66.33 (9.15)	76.94 (10.73)	77.36 (11.50)	0.24
	OHT₁	70.20 (10.54)	74.44 (16.92)	78.51 (10.50)	
	OHT₂	68.09 (14.44)	73.50(12.45)	73.19(9.91)	
PP	ONT	43.75 (6.44)	44.75 (9.54)	45.96 (8.76)	0.21
	OHT₁	46.16 (10.29)	43.96 (10.88)	44.69 (10.18)	
	OHT₂	48.66 (18.74)	40.86 (10.89)	45.60 (9.54)	
SV	ONT	77.41 (15.85)	76.02 (16.46)	78.62 (16.63)	0.001
	OHT₁	80.68 (12.74)	77.11 (14.23)	77.91 (13.60)	
	OHT₂	80.91 (8.33)	70.98 (7.33)	72.75 (8.34)	
SI	ONT	49.29 (8.51)	48.54 (8.97)	50.28 (9.44)	0.004
	OHT₁	51.11 (8.84)	49.02 (10.62)	49.53 (10.30)	
	OHT₂	49.32 (5.23)	43.35 (5.34)	44.58 (7.04)	
CO	ONT	5.33 (0.75)	6.02 (1.15)	6.15 (1.18)	0.57
	OHT₁	5.35 (1.02)	5.87 (1.14)	5.84 (1.06)	
	OHT₂	5.19 (1.00)	5.62 (0.87)	5.63 (1.19)	
CI	ONT	3.43 (0.64)	3.88 (0.84)	3.97 (0.86)	0.63
	OHT₁	3.41 (0.77)	3.75 (0.94)	3.73 (0.88)	
	OHT₂	3.18 (0.73)	3.45 (0.73)	3.47 (0.94)	
TPR	ONT	1185.31 (190.16)	1248.59 (281.91)	1213.21 (228.50)	0.29
	OHT₁	1271.40 (301.73)	1250.64 (424.10)	1300.87 (358.36)	
	OHT₂	1343.04 (405.03)	1228.58 (239.21)	1260.72 (276.99)	
TPRI	ONT	1868.31 (378.01)	1952.82 (529.90)	1908.34 (452.07)	0.39
	OHT₁	2039.16 (599.57)	2019.02 (789.90)	2072.14 (735.81)	
	OHT₂	2278.82 (848.91)	2031.66 (483.11)	2102.33 (603.09)	
LVET	ONT	314.43 (17.58)	298.00 (20.18)	300.31 (19.56)	0.39
	OHT₁	313.20 (16.60)	298.62 (21.17)	297.23 (22.53)	
	OHT₂	325.16 (13.83)	302.07 (15.55)	304.05 (20.22)	

*P value is for groupXtime interaction in the repeated measure analysis

Fig 18: Hemodynamic and autonomic responses to WCT_{1min} and WCT_{3min} in ONT, OHT₁ and, OHT₂ groups. P value is for groupXtime interaction.



2. Autonomic responses

Group OHT₂ and OHT₁ showed significant increase in sympatho-vagal balance (LF/HF) from rest to WCT_{1min} compared with ONT (P=0.014) (**Table 12**). Significant increase in LFnuBPV was observed only in OHT₂ (**Fig 18**). The increase in LFnu, LFnuBPV and LF/HF and corresponding fall in HFnu noted at WCT_{1min} in all 3 groups signified sympathetic activation in response to the test. The decrease in baroreceptor sensitivity in response to the test was similar in 3 groups (**Table 12**).

Table 12: Autonomic responses of ONT, OHT₁ and OHT₂ to WCT

	Groups	Rest	1 min	3 min	P*
LFnuRRI	ONT	51.29 (15.27)	53.98 (12.17)	52.31 (11.92)	0.062
	OHT ₁	47.04 (15.83)	59.02 (9.97)	59.61 (13.21)	
	OHT ₂	47.42 (15.91)	52.92 (12.43)	50.75 (13.77)	
HFnuRRI	ONT	46.95 (14.82)	44.84(12.47)	46.42(11.90)	0.010
	OHT ₁	52.93 (16.24)	40.13 (9.41)	39.01 (11.90)	
	OHT ₂	52.58 (15.91)	47.08 (12.43)	49.25(13.77)	
LFnuBPV	ONT	47.51 (13.30)	45.12 (13.32)	45.71 (12.18)	0.001
	OHT ₁	51.32 (14.26)	49.56 (12.60)	51.02 (11.48)	
	OHT ₂	34.07 (9.19)	54.83 (12.29)	52.48(11.61)	
LF/HF	ONT	1.15 (0.67)	1.07 (0.52)	1.06(0.49)	0.014
	OHT ₁	1.10 (0.48)	1.33 (0.48)	1.44 (0.70)	
	OHT ₂	0.77 (0.33)	1.31 (0.55)	1.28 (0.64)	
BRS	ONT	24.44 (14.74)	21.83 (13.01)	19.37 (11.97)	0.98
	OHT ₁	27.98 (10.66)	27.70(17.46)	23.77 (9.14)	
	OHT ₂	24.70 (17.67)	17.32 (15.17)	17.59 (12.09)	
BRS up	ONT	24.60 (16.06)	29.82 (16.81)	18.81 (11.76)	0.68
	OHT ₁	29.52 (17.06)	25.98 (21.10)	23.84 (14.57)	
	OHT ₂	25.56 (20.10)	24.80 (17.43)	16.88 (14.81)	
BRS dn	ONT	23.19 (13.97)	19.04 (11.90)	19.74 (10.63)	0.87
	OHT ₁	28.93 (13.74)	22.49 (12.50)	17.38 (7.91)	
	OHT ₂	26.87 (14.71)	12.17 (6.94)	15.00 (8.99)	

*P value is for groupXtime interaction in the repeated measure analysis

7.1.6 Deep Breathing test

The degree of sinus arrhythmia was similar in 3 groups (OHT₂ 25.04±6.60; OHT₁: 22.02±6.23 and ONT: 24.74±5.25; P=0.24).

7.2 Part B

Genetic Predisposition to Hypertension in young Offspring with High Normal BP or Prehypertension: Cross-sectional Analysis

Daytime SBP and DBP of offspring between 18 and 30 years (N=667) was correlated with ambulatory daytime SBP and DBP of mother and father (**Table 13**). Daytime SBP of offspring was correlated significantly with daytime SBP (r^2 0.225, $P=0.0001$) and DBP (r^2 0.257, $P=0.0001$) only of father. However, Daytime DBP of offspring was significantly correlated with daytime SBP (r^2 0.284, $P=0.0001$) and DBP (r^2 0.324, $P=0.0001$) of father and also of mother (SBP r^2 0.148, $P=0.0001$; DBP r^2 0.131, $P=0.0001$) (**Table 13**).

Table 13: Correlation and β coefficient for BP of offspring and BP father and mother

	Offspring			
	Daytime SBP		Daytime DBP	
Parents	R^2	β	R^2	β
Father				
Daytime SBP	0.225**	0.061	0.284**	0.044
Daytime DBP	0.257**	0.372*	0.324**	0.338**
Mother				
Daytime SBP	0.061	0.133	0.148**	0.157*
Daytime DBP	0.051	-0.120	0.131**	-0.072

* $P=0.01$; ** $P=0.0001$

Linear regression of SBP and DBP of offspring over parental BP showed that daytime DBP of father was a significant positive predictor of daytime SBP (β 0.372, $P=0.01$) and DBP (β 0.338, $P=0.01$) of offspring. There was weak association between daytime SBP in mother and daytime DBP of offspring (β 0.157, $P=0.01$). Neither SBP nor DBP in offspring were associated with the SBP of father and the DBP of mother (**Table 13**).

Offspring classified according to WHO criteria, JNC VII criteria, and quartiles were cross-tabulated against parental status of hypertension (**Table 14**). In this cohort, the prevalence of prehypertension according to JNC criteria was 18.8%. According to WHO criteria, the prevalence of normal BP (WHO1) was 9.1% and prevalence of high normal BP (WHO2) was 2.9%. In statistical quartiles, the BPG3 prevalence was 10.33% while BPG4 was 15.44 %.

Table 14: Cross-tabulation of offspring groups and hypertension status of parents

Offspring Groups	Hypertension in parents				
	Both NT	Isolated SBP/DBP HTN	Mother HTN	Father HTN	Both HTN
WHO					
WHO1	78	55	13	18	23
WHO2	22	9	4	9	6
WHO3	5	2	-	5	6
WHO4	6	11	5	8	11
Total	111	77	22	40	46
JNC VII					
JNC1	78	55	13	18	23
JNC2	38	16	7	16	25
JNC3	6	11	5	8	11
Total	122	82	25	42	59
Quartile					
BPG1	39	28	5	4	3
BPG2	19	14	4	6	11
BPG3	24	7	5	10	9
BPG4	17	21	11	18	24
Total	99	70	25	38	47

The chi square analysis for association of offspring groups with parental status of hypertension was significant for all classifications (WHO χ^2 29.23, df 12, P=0.004; JNC VII χ^2 24.27, df 8, P=0.002 and Quartiles χ^2 42.37, df 12, P=0.0001). The chi square trend

analysis also showed a significant linear trend of being in higher BP group with the increasing predisposition to hypertension (WHO: χ^2 for trend 18.04, df 1, P=0.0001; JNC VII: χ^2 for trend 17.09, df 1, P=0.0001 and Quartiles: χ^2 for trend 30.70 df 1, P=0.0001).

The odds of having higher BP in offspring with respect to hypertensive status in parents are shown in **Table 15**. Optimal BP group (WHO1 and JNC1) or lowest quartile (BPG1) groups were considered as reference groups.

Table 15: Odds ratios for being in higher BP group with hypertension status in parents

Offspring	Isolated SBP/DBP HTN		Mother HTN		Father HTN		Both HTN	
	*Odds	P	*Odds	P	*Odds	P	*Odds	P
WHO								
WHO2	0.58 (0.25,1.36)	0.2	1.09 (0.32,3.68)	0.8	1.77 (0.70,4.49)	0.2	0.93 (0.34,2.55)	0.8
WHO3	0.57 (0.11,3.03)	0.5	0.002 (0.00,0.050)	0.8	4.33 (1.13,16.57)	0.032	4.07 (1.14,14.56)	0.031
WHO4	2.60 (0.91,7.45)	0.07	4.99 (1.33,18.79)	0.017	5.77 (1.78,18.73)	0.003	6.22 (2.07,18.64)	0.001
JNC VII								
JNC2	0.59 (0.30,1.18)	0.1	1.11 (0.41,2.99)	0.8	1.82 (0.84,3.97)	0.1	2.23 (1.12,4.43)	0.022
JNC3	2.60 (0.91,7.45)	0.07	4.99 (1.33,18.79)	0.017	5.77 (1.78,18.73)	0.003	6.22 (2.07,18.64)	0.001
Quartile								
BPG2	1.03 (0.44,2.39)	0.9	1.64 (0.39,6.23)	0.5	3.08 (0.78,12.23)	0.1	7.53 (1.88,30.19)	0.004
BPG3	0.41 (0.15,1.07)	0.06	1.63 (0.43,6.21)	0.5	4.06 (1.15,14.41)	0.03	4.88 (1.20,19.81)	0.027
BPG4	1.72 (0.77,3.84)	0.2	5.04 (1.52,16.77)	0.008	10.32 (3.04,35.11)	0.0001	18.35 (4.86,69.29)	0.0001

*Odds (95% confidence intervals)

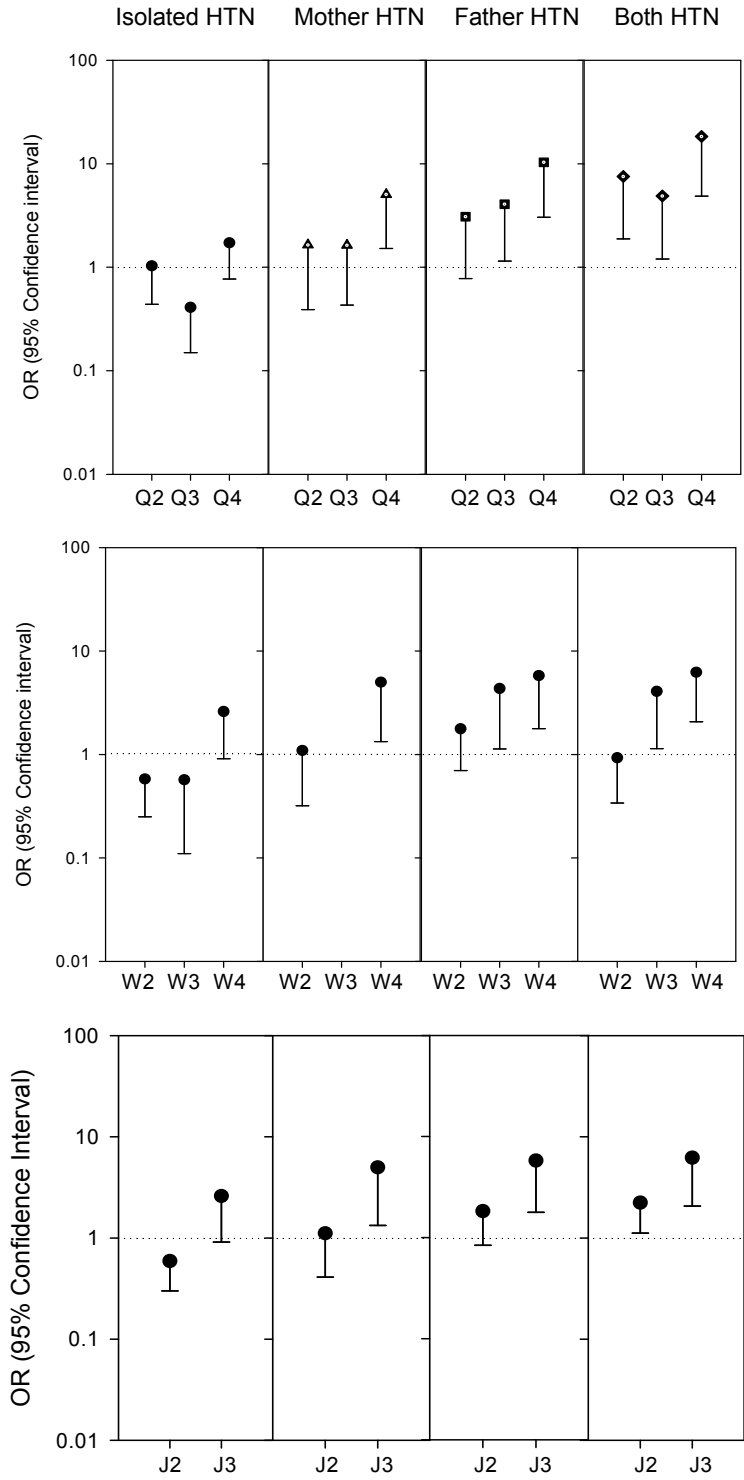
The odds of offspring having high normal BP (WHO3) was 4 times if either father was hypertensive or if both parents were hypertensive (OR 4.33, P=0.032 and OR 4.07, P=0.031 respectively). There was no risk noted in the normal BP groups according to WHO criteria (WHO2). The odds of being in the pre-hypertension group (JNC2 group according to JNC VII criteria), was 2 times when both parents were hypertensive (OR

2.23; $P=0.022$). There was 5 to 6 fold odds of being in hypertensive group (WHO4 and JNC3) when one had either a hypertensive mother (OR 4.99; $P=0.017$), or a hypertensive father (OR 5.77; $P=0.003$) or both hypertensive parents (OR 6.22; $P=0.001$).

The odds of the offspring to be in the lower quartile (BPG2) was 7 times when both parents were hypertensive (OR 7.53; $P=0.004$). Having a hypertensive father or both hypertensive parents raised the odds to 4 times (OR 4.06 and OR 4.88 respectively) for the offspring to be in BPG3. Offspring in the group BPG4 (BP > 131/86 mmHg), showed an interesting finding. The odds of being in BPG4 were 18 fold if both parents were hypertensive (OR 18.35; $P=0.0001$) and they reduced if only father was hypertensive (OR 10.32; $P=0.0001$) or if only mother was hypertensive (OR 5.04; $P=0.008$). It showed linearly increasing odds with hypertensive mother and father and the highest odds when both parents were hypertensive (**Fig 19**). It is important to note that, there were no significant odds associated with an isolated SBP or DBP hypertension in parents in any of the classification.

In conclusion, linear regression showed that BP in offspring was significantly associated with daytime DBP of father and daytime SBP of mother. Logistic regression indicated that parents having isolated systolic or diastolic hypertension carry no risk. Hypertension in both the parents carried maximum risk of having higher BP in offspring. However, the hypertensive status in father had more influence than that of mother.

Fig 19. Odds ratios for offspring grouped according to WHO, JNC VII classification and statistical quartiles with status of hypertension in parents.



Q2: BPG2, Q3: BPG3, Q4: BPG4; W2: WHO2, W3: WHO3, W4: WHO4; J2: JNC2, J3: JNC3

Discussion

This dissertation compares circadian changes in BP as well as changes in beat-to-beat hemodynamic, cardiac and autonomic responses to various laboratory stresses in young (age 18-25 years) Omani Arab offspring of one or both hypertensive parents in a unique Omani Arab population. To my knowledge, this is the first study to be conducted in such a homogeneous Arab population with a high degree of consanguinity and environmental homogeneity. This study has classified offspring into ONT, OHT₁ and OHT₂ groups to have maximum contrast in the genetic predisposition to hypertension.

Hemodynamic and autonomic changes were studied in response to a cluster of tests - physical stress (HUT and CPT), mental stress test (WCT) and deep breathing test. This study used detailed measurements based on beat-to-beat BP, impedance cardiography and spectral analysis of HR and BP variability, with the intention of a more accurate conclusion on controversial results in the literature.

This study in its second part investigated the genetic predisposition to the hypertension in young Arab offspring (age 18 to 30 years) from OFS having high normal BP or prehypertension.

8.1 Part A: Cardiovascular Laboratory Tests

8.1.1 Summary of Results

The important findings of this study are summarized below.

All three groups had comparable age, BMI and % body fat. OHT₂ had significantly lower baseline hemodynamic and autonomic measurements such as resting HR and LFnuBPV (**Table 4**) but higher LVET compared with ONT and OHT₁. LVET was significantly inversely correlated with HR (**Fig 13**).

ABPM showed similar day and night BP and systolic dip in 3 groups. OHT₂ had significantly higher day, night and total average HR compared with ONT and OHT₁ (**Table 5**).

The CPT showed no differences in hemodynamic responses in 3 groups (**Table 6**); however OHT₂ showed significantly less increase in LFnu and greater increase in LFnuBPV compared with ONT (**Fig 14**).

At HUT_{5min}, OHT₂ had significantly greater increase in SBP, DBP and TPR (**Table 8**) compared with the other 2 groups. This response was associated with the attenuated drop in SV and less sympathetic activation (less increase in LFnuRRI, LFnuBPV and catecholamine responses) (**Table 9**).

There was significantly greater increase in HR, SBP and sympatho-vagal balance in OHT₂ from rest to WCT_{1min} compared with the other 2 groups (**Table 11, Fig 18**). Hemodynamic and autonomic changes from WCT_{1min} to WCT_{3min} were similar in the 3 groups. Overall, OHT₂ showed contrasting responses by having more increases in SBP, DBP and TPR and less sympathetic activation with HUT (**Fig 15,16**) but significantly greater HR and SBP increase and greater sympathetic activation with the mental stress (**Fig 18**).

The degree of Sinus arrhythmia, an indicator of vagal activation was similar in 3 groups.

8.1.2 Baseline comparisons

Anthropometric, age and gender distribution were similar amongst 3 groups (**Table 4**). No differences were noted in the baseline SBP and DBP recorded with beat-to-beat BP monitoring using TFM in all three groups. Davrath [58] who used beat-to-beat BP has reported similar BP in offspring of hypertensive and normotensive parents. However, consecutive three BP recordings at 10 minutes interval in Omani offspring of first cousin marriages showed significantly higher baseline BP in offspring of hypertensive parents compared with normotensive parents [44].

A significantly lower HR and sympathetic drive with higher LVET at rest (**Table 4, Fig 13**) in OHT₂ indicate alterations in cardiac morphology. There is a consistent evidence of increased left ventricular mass index in offspring with genetic history of hypertension [87]. The increased left ventricular mass index is said to be secondary to higher baseline BP in offspring with history of parental hypertension [87, 155]. Even though baseline BP is similar in this study, the heightened BP responses to stresses (as discussed later) may contribute to the above. The finding of higher LVET in OHT₂ does not corroborate with other studies [62,80]. This study observed a non-significant trend of successively higher TPR in OHT₁ and OHT₂ compared with ONT (**Table 4**). Similar non-significant trend was also reported earlier [62]. Other hemodynamic and autonomic parameters at rest were parallel in three groups. The sympathetic drive at rest measured either with MSNA, plasma catecholamine or HRV show inconsistent results. At rest, the offspring of hypertensive parents were shown to have either similar MSNA [71,97], similar catecholamines [80,95,120], increased catecholamines [73], or lower catecholamines [71] and similar HRV [58].

Lower sympathetic drive and similar hemodynamic variables at rest coupled with increased sympathetic activation during stress (discussed later) in OHT₂ found in the present study reveals the complicated heritability of hypertension as a disease. It also emphasizes the requirement of stress to uncover the differences in inherited cardiovascular reflexes.

8.1.3 24H ABPM

The ambulatory BP recording has an advantage over clinic BP because the ABPM is devoid of the White Coat effect of BP measurements in the physician's office (BP recording outside the physician's office) [78].

OHT₂ in this study had higher daytime and night-time SBP and DBP (**Table 5**) compared with ONT and OHT₁; however the differences were not statistically significant. ABPM was shown to be significantly higher in offspring of hypertensive parents [51,69,72-76]; but offspring of all these studies were older than 35 years. Offspring who participated in

the present study are younger (mean age 23.4 ± 4). Muiesan and Noll who studied young offspring mean age 25 and 27 years respectively also did not find any differences in the ABPM between offspring of hypertensive and normotensive parents [70,71]. The present study shows similar results. The only explanation that could be offered is that, BP differences due to the genetic predisposition has probably not yet manifested at this younger age. Apriori selection criteria of 'normotensive subjects' could also reflect in having similar BP in this cohort.

OHT₂ had significantly higher ambulatory heart rate (daytime $P=0.039$; night-time $P=0.0003$ and total 24H $P=0.013$) than ONT and OHT¹ (**Table 5**). Ambulatory heart rate measurements differed according the status of hypertension in one or both parents. Higher daytime and night-time HR in OHT₂ has been reported by Lopez in 2001 [73]. Increased HR in OHT₂ could be attributed to higher sympathetic tone in OHT₂ or difference in hemodynamic responses to daily physical activities in OHT₂.

The significantly higher HR may suggest the hyperkinetic state in OHT₂ which is known to precede the development of essential hypertension [9]. Increased HR is associated with the increased baseline sympathetic tone in the hyperkinetic phase of hypertension. However, in this study, the baseline sympathetic tone (0.1Hz LFnuRRI) (**Table 4**) and noradrenaline (**Table 9**) were same in all three groups. Parents of subjects with the hyperkinetic state were also shown to have higher HR when they were young [9]. This familial component is corroborated in the present study.

Genetic factors not only influence the physiology at rest but also account for some responses to the stress. Recent twin and family studies designed to uncover genetic variance during stress suggest that there is an increased heritability of HR and BP reactivity to stress, and there is little variance due to shared environment [156,157]. In OHT₂, a trend of higher BP and significantly higher daytime and total 24H HR may reflect their increased reactivity to physical activities during daytime.

Significantly higher night-time HR in OHT₂ (**Table 5**) compared with the other 2 groups in the present study could be due to the higher sympathetic drive at night. Sympathetic nervous system contributes significantly to the diurnal variation in BP, HR as also to the night-time systolic dip [158]. Higher sympathetic drive is associated with higher night-time BP, HR and less dip [158,159]. However, night-time autonomic profile was not done in this study so it is not possible to confirm this. Sleep quality is also likely to affect night-time HR and dip [160]. However, all subjects had good sleep quality with median score of 2 (Refer to measurements chapter).

This study did not find any differences in systolic dipping amongst 3 groups. One study reported that non-dippers are more likely to have family history of hypertension [159]. However, non-dipping or less dipping in offspring of hypertensive parents compared with ONT was not confirmed in any of these studies [69,73,77,78,155]. Non-dipping of SBP at night is associated with higher incidence of cardiovascular target organ damage and poor prognosis for cardiovascular diseases when compared with dippers [161,162]. Non-dipping has though been observed in normotensives [163]; its magnitude increases with an increase in daytime BP and decreases with increase in nighttime BP [164]. Non-dipping also increases with the age. All subjects who participated in this study were normotensive with an average BP of 116/74 mmHg and showed an average of 13% dip. The similar night-time SBP dipping could be due to their young age and optimum BP level. Differential parental history of hypertension does not contribute to non-dipping in this young age sample. This study, therefore, proposes that non-dipping may be a later onset phenomenon.

8.1.4 Cold Pressor test

Immersion of hand / foot in ice cold water stimulates local cold sensation which turns into pain sensation within 30 sec. Muscle sympathetic nerve activity (MSNA) and impedance cardiography during CPT suggested that an increase in CO elevates BP in the initial period (0-30 sec) of the test; while MSNA plays an essential role in elevating the BP at the later period (90-120 sec) of the CPT [83]. He also showed a significant

correlation between grade of painful sensation and total MSNA confirming that it is the sensation of pain that is responsible for the activation of the sympathetic nervous system.

All three groups in the present study showed similar responses of HR, BP and CO to CPT (**Table 6, Fig 14**). CPT has been used widely to differentiate physiological responses in offspring of hypertensive and normotensive parents. However, the review of literature revealed inconsistent outcomes of the CPT in offspring of hypertensive parents. The spectrum of results varies from increased HR, BP and MSNA during CPT [51,53,64-65,91-94], to no differences in the response [62,73,76,80,88-90,95-97] and also subnormal response [97]. It was noted by Muldoon in his review that CPT yields equivocal results when examines in relation to family history of hypertension [87].

Autonomic responses however showed an interesting and contrasting findings which differed according to genetic predisposition to hypertension. The significantly greater increase in LFnuBPV was observed in OHT₂ compared with other two groups. However, the same group showed an attenuated increase in LFnuRRI (**Table 7, Fig 14**). The increase in LFnuRRI was higher in OHT₁. There are no studies reporting non-invasive measurements of HRV and BPV responses to CPT in offspring with differential parental history of hypertension. Lambert et.al [87] reported lower MSNA in response to CPT in hypertensive subjects and in offspring of hypertensive parents. Such phenomenon of differential response of 0.1Hz band from HR and BP are reported in the literature. Piccirillo [61] has reported greater increase in LFnu but lesser increase in LFnuBPV in offspring of hypertensive parents in response to the HUT.

This study suggests increased autonomic activity during CPT as indicated by the increase in 0.1 Hz band of LFnuBPV. LFnuRRI being mixture of sympathetic and parasympathetic activity probably is a poor indicator of sympathetic activity. The present study also suggests that, CPT is not as strong as HUT or WCT in differentiating hemodynamic and autonomic reactivity in offspring of hypertensive parents compared with normotensive parents.

8.1.5 Orthostatic tolerance test (HUT)

In humans posture change from supine to standing caused shift of fluid from cardiopulmonary region to gravity dependant lower body parts. The upright posture also decreases the venous return to the heart. This offloads cardiopulmonary and arterial baroreceptors resulting in a reflex increase in HR, SBP, DBP, with vasoconstriction, venoconstriction, decrease in SV, and an increase in plasma noradrenaline [99]. In this study, OHT₂ showed a significant drop in SBP, DBP and TPR at HUT_{1min} followed by the significant increase in SBP, DBP and TPR at HUT_{5min} compared with ONT and OHT₁ (**Table 8, Fig 15**). Manuck [80] found an attenuated increase in DBP during the tilt. Other studies did not find HR and BP changes during tilt as a function of parental hypertension [61,89,102,103]. A study [58] using active standing has shown an initial drop (though statistically insignificant) in HR, SBP and DBP at 15-45 sec of standing followed by stabilization of these parameters with continuation of standing for 5 min.

Stroke volume and total peripheral resistance response to HUT

To my knowledge, there are no hemodynamic studies showing OHT₂ response to HUT as found in this study (less decrease in SV and stronger TPR compared with ONT) (**Table 8, Fig 15**). The closest relevant study to this is one by de Visser et al [165]. He also reported stronger TPR response and an attenuated increase in the CO in offspring with history of hypertension in **both** parents using bicycle ergometry (dynamic exercise) as a stress. Though stresses used are different, HUT in this study and dynamic exercise used by him, the common denominator in both stressors is the activation of sympathetic nervous system and in both studies is the comparison of offspring of both hypertensive parents with offspring of both normotensive parents.

The significantly higher SV at HUT_{5min} in OHT₂ compared with ONT in this study could be due to the possibility of increased left ventricular mass index which may increase the myocardial contractility. M-mode echocardiography has revealed increased posterior wall thickness and diastolic functional changes indicative of cardiac hypertrophy in normotensive offspring of hypertensive parents [72,155].

The cardiopulmonary changes during upright posture are induced by decrease in activity of the arterial mechanoreceptors secondary to decrease in central blood volume during standing. The magnitude of change is related to the amount of fluid shift [166] and sensitivity of baroreceptors [167]. All 3 groups had similar baroreceptors sensitivity at rest (**Table 4**). The decrease in BRS with HUT was also identical in 3 groups (**Table 9**).

In this study, the central blood volume was measured indirectly with the impedance cardiography (Z_0 and dz/dt). Differences in Z_0 and dz/dt at rest and following HUT were found to be identical in 3 groups. Also, the magnitude of change in dz/dt was related to the pre-test value and not the group differences. However, it is possible that compliance of capacitance vessels in OHT₂ is reduced which may help to maintain venous return and so the stroke output.

In conclusion, the higher in SV in OHT₂ compared with ONT in the present study is definitely not due to differences in baroreceptor sensitivity. However, echocardiography not being done in this study, the role of increased left ventricular mass contributing to myocardial contractility can not be commented upon.

Significantly increased TPR response in OHT₂ observed in this study is similar to the increased TPR response to tilt found in subjects with an established hypertension [100]. It indicates an enhanced vasoconstriction. Alterations in blood vessel characteristics like vascular hypertrophy or decreased arterial compliance may lead to an increased vasoconstriction in OHT₂. Arterial stiffness is present in established hypertensives [9].

Normotensive offspring with the genetic proneness to hypertension are shown to have decreased arterial elasticity [168], decreased effective vascular compliance [111] and augmented arterial stiffness indices [56,57]. The indices of arterial stiffness measured with pulse wave velocity technique [57] were found to be altered in offspring of hypertensive parents. These changes in arterial stiffness were independent of age, BMI, lipid profile, and smoking habits, all of which are known to affect arterial stiffness.

Increased vasoconstriction could also be influenced by α and β adrenergic receptor density in blood vessels. An increase in the operative alpha/beta adrenoceptor ratio with a high salt intake results in vasoconstriction and reduced vasodilatation at resistance vessels, as well as increased renal proximal tubular sodium reabsorption [169]. This effect could be genetically mediated. Platelet α 2 receptor density which is related to the salt sensitivity [54] was reported to be higher in offspring of one hypertensive parent [165,170]. However, salt sensitivity and platelet α 2 receptor density have not been carried out in Arabs and also in this study.

The significant contribution of this study is the finding of a maintained SV with an increased TPR in response to the HUT in OHT₂ compared with ONT in this sample. This finding has been documented only in established hypertensives till now.

Spectral indices during HUT

This study showed significant parasympathetic withdrawal (decrease in HFnuRRI) and sympathetic activation (increase in LFnuRRI) (**Table 9, Fig 16**) during HUT. The sympathetic activation was greatest in the OHT₁ (Δ LFnuRRI 27%) and was followed by that in ONT (Δ LFnuRRI 20%). However, in OHT₂, sympathetic activation was significantly reduced (Δ LFnuRRI 14%) compared with the other 2 groups. This response in OHT₂ is similar to LFnuRRI response reported in hypertensive subjects [101].

Out of two studies which have used HRV and BPV measurements during orthostatic stress in offspring Piccirillo et.al 2000 [61] has reported a greater increase in LFnu but lesser increase in LFnuBPV while Davrath 2003 [58] has reported a greater increase in LFnu in offspring of hypertensive parent. However, the differences in the study group characteristics in 2 studies and the present one require a special mention. Offspring in the study by Piccirillo et al [61] had one or both hypertensive parents while offspring in the study by Davrath [58] had history of hypertension in one parent only. In this regard, this study finding of greatest LFnuRRI response in OHT₁ (Δ 27%) corroborates with above studies. In this study, the difference in genetic predisposition in single or both parents is

shown to influence autonomic responses. Similar influence was observed in SV and TPR responses to HUT in OHT₂ and OHT₁ in this study.

The physiologic significance of 0.1 Hz LF band obtained from R-R interval and SBP variability is controversial. The simultaneous recording of HRV and BPV at rest in healthy subjects showed the positive correlation between LF HRV and LF BPV [171]. This indicates that LF BPV is also a measure of cardiac sympathetic activity. This study did not find any difference in spectral indices from SBP in 3 groups. But, the limited increase in LFnuRRI in OHT₂ indicates less sympathetic activation in this group in response to HUT. The similar finding of less increase in LFnu from SBP variability in study by Piccirillo [61] could be due to offspring of single or both hypertensive parents considered together. It was possible in this study to differentiate the autonomic responses in ONT, OHT₁ and OHT₂ due to the strong design of having three groups. This study proposes that, it would not be appropriate to generalize the observation of increased sympathetic response in offspring of hypertensive parents without giving due weightage to the history of hypertension in single or both parents.

Plasma noradrenaline during HUT

The less increase in LFnu in OHT₂ compared with ONT in this study is supported by the biochemical evidence of significantly less increase in plasma noradrenaline in response to HUT in OHT₂ (**Table 9, Fig 16**). There was also significant positive correlation of plasma noradrenaline at HUT_{5min} with autonomic parameters at HUT_{5min} (**Table 10**). Furthermore, magnitude of response of noradrenaline was also significantly correlated with the responses of LFnuRRI and LFnuBPV (**Fig 17**). Venous plasma catecholamine measured during active orthostasis was not found to be varying with familial risk for hypertension [80,87,102]. However, similar observation of limited increase in noradrenaline has been documented in subjects with the arterial hypertension [172,101] and in siblings of hypertensive subjects [103]. Sympathetic nervous system affects vascular growth independent of its hemodynamic effects. In the disease process of hypertension, the adaptive structural changes may lead to an increase in sensitivity of

vascular smooth muscles to noradrenaline [10,11]. Could there be a genetic transmission of this hypersensitivity from hypertensive parents to their offspring? Vascular muscles of spontaneously hypertensive rats have an increased passive permeability to sodium resulting into augmented vasoconstrictor response to noradrenaline [173]. Authors attributed this response to the genetically determined trophic influence of the sympathetic nervous system. This may suggest that genetically determined sympathetic influences acting during the development may cause long-term alterations in membrane properties that could contribute to the hypertension [173]. The Framingham Offspring Study reported that, 13-23% of variance in heritability of HRV was due to genetic factors [174]. In the Oman Family Study, the heritability of LFnu and HFnu from HRV was found to be 25% and 27% respectively (unpublished). It needs further studies to confirm the genetic effect of sympathetic system on developmental changes in smooth muscles in humans.

In summary, this study showed that, compared with ONT and OHT₁, OHT₂ responded with- 1. significantly greater increase in SBP, DBP and TPR with 2. an attenuated drop in SV and 3. less increase in sympathetic activation and noradrenaline release during HUT. This response in OHT₂ is similar to HUT responses in hypertensive subjects. Pathophysiologic changes observed in established hypertensives in literature have been demonstrated in these young OHT₂ in the present study (though they are normotensive at the baseline). This study suggests role of interaction of stress and genetic susceptibility in understanding the disease process of hypertension. Response of OHT₁ is statistically different than that of OHT₂. It is essential to define history of hypertension in one or both parents as that affects the response to the HUT.

8.1.6 Word Conflict Test

Word conflict test involves sensory rejection and it produces sympathetic activation [106,107,110] as result leads to increase in HR and BP [105] and forearm vasodilatation [108]. OHT₂ responded to WCT with significantly greater increase in HR, SBP, LFnuBPV and LF/HF ratio compared with ONT and OHT₁ (**Table 11, Table 12 and Fig 18**). As against OHT₂, the ONT showed minimal sympathetic activation. There are very

few studies which used WCT to differentiate responses in offspring of hypertensive and normotensive parents. One study reported an increased BP but not HR in response to WCT in offspring of hypertensive parents [111], while other three studies found no correlation between responses to WCT and family history of hypertension [63,80,93]. The differing results could be due to differences in the subject selection and methodology. Widgren et al [111] selected offspring with only hypertensive fathers while Wright et al selected offspring with history of hypertension in any one amongst parents or grandparents. The limitation of the above mentioned studies having not used beat-to-beat BP monitoring, impedance cardiography and spectral analysis has been overcome in the present study. Also, the strong design of three offspring groups has contributed in differentiating responses to WCT according to the genetic history of hypertension.

Drop in vascular resistance with WCT was observed in OHT₂ and OHT₁ groups and not in ONT; though the difference in the response was not statistically significant (**Fig 18**). Reduced vascular resistance with an increased HR and SBP in response to WCT has been observed in healthy volunteers [174]. WCT induced greater increase in circulatory adrenaline than venous noradrenaline suggested a predominant humoral response rather than a neuronal response [110,174]. This vasodilatory response could be blocked by regional β blockers [108,175]. Hypertensive patients on long term treatment with β adrenoceptor antagonists showed attenuated TPR response to WCT [176]. Thus WCT evokes a “defense reaction” characterized by cardiac and sympathetic activation and unchanged or reduced peripheral resistance. OHT₂ exhibited this reaction predominantly compared with the other two groups.

There was significant drop in SV during WCT in OHT₂ compared with other two groups. This decrease in SV was associated with significant drop in dz/dt in OHT₂ compared with other two groups (**Table 11, Fig 18**). The differences in SV response were abolished when analysis was repeated after controlling for the effect of dz/dt . The dz/dt reflects volumetric and velocity changes during various systolic and diastolic phases of the cardiac cycle, and magnitude of dz/dt dictates calculated SV [134,137]. This indicates a change in stroke output of heart during WCT in OHT₂. In spite of the drop in SV, the CO

was found to be increased in OHT₂ (**Table 11**) and this is attributed to the significant increase in HR in OHT₂. The greater increase in HR reduced the time available for ventricular filling which resulted in decrease in SV. In this study, the use of beat-to-beat estimates of the impedance cardiography has made it possible to detect the subtle changes in hemodynamics.

The role of chronic mental stress operating through sympathoadrenal system leading to development of essential hypertension is well known [177]. Chronic real-life stress in human appears to be associated to an increased arterial BP and to impaired autonomic functions. Sympathetic predominance and blunted baroreflex sensitivity are thought to be treatable links between psychological factors and future incidence of the hypertension [28]. Compared with normotensive controls, subjects with the borderline hypertension and also offspring of hypertensive parents were found to be characterized by internalized aggression, submissive nature, a desire for self control, and a high level of situation-specific anxiety. They also reported episodes of palpitations and hand tremors more frequently compared with normotensive controls [178]. These behavioral characteristics have been related to the hyperdynamic cardiovascular regulation in borderline hypertensives and offspring of hypertensive parents [178]. The 10 year follow-up study in offspring of hypertensive parents showed that high daily life stress augments BP effects due to the family history [53]. Psychometric evaluation of offspring to substantiate the above findings was beyond the scope of this thesis.

In the present study, the increased HR and sympathetic activity in OHT₂ in response to WCT may indicate the presence of hyperkinetic circulation and features of the neurogenic mechanisms of an early hypertension as proposed by Julius S [6].

8.1.7 Respiratory Sinus Arrhythmia

The respiratory sinus arrhythmia (RSA) is increase in HR with inspiration and fall in heart rate with expiration when a subject is asked to breathe slowly and deeply [112,114]. The magnitude of RSA is a sensitive measure of efferent vagal control of heart rate.

The variation in sympathetic nervous system activation has always been thought to be the underlying mechanism of differential responses of offspring of hypertensive and normotensive parents. Parasympathetic nervous system has largely been ignored in this research. It was speculated that compared to offspring of normotensive parents, the offspring of hypertensive parents may show smaller heart rate changes in response to deep breathing. However, the finding of similar degree of RSA in the present thesis refutes this contention. In the view of significantly higher HR, SBP and 0.1Hz band of LFnuBPV responses to HUT and WCT in OHT₂ in the present study, it might also suggest that familial differences are mediated by sympathetic and not by parasympathetic nervous system. The present study did not find any differences in the cardiac vagal control between ONT, OHT₁ and OHT₂. Miller et al [116] who investigated RSA at rest and in response to application of cold pack on forehead also did not find differences among offspring of hypertensive and normotensive parents.

Piepoli [113] mentioned the role of baroreceptors in generating RSA in normal healthy conscious humans. RSA is suggested to be a relatively simple index of the arterial baroreflex gain. Age has strong negative effect on RSA [112,115]. Similar RSA in all three offspring can be explained by their young age and normal baroreflex sensitivity at rest (**Table 4**). Alterations in the cardiac vagal activity have not become evident in offspring of hypertensive parents at this young age and it could be a later phenomenon.

The RSA is reduced in hypertensive subjects. Studies on hypertension and RSA suggest impaired cardiac vagal tone exists before the onset of overt disease [115]. Masi et al in his review on RSA and diseases also suggests that vascular or hemodynamic changes prior to the onset of clinical hypertension lead to an impaired vagal tone which get manifested as reduced RSA. RSA was also found to be a significant negative predictor of hypertension [115].

8.2 Part B

Genetic Predisposition to Hypertension in Young Offspring with High Normal Blood Pressure and Prehypertension: Cross Sectional Analysis

Young subjects between 18-30 years from OFS were divided into subgroups based on available WHO [2] and JNC [3] classification and statistical quartiles of BP for this cohort. Main results of the analysis were as follows- A) Linear regression indicated that BP in offspring was significantly associated with daytime DBP of father and daytime SBP of mother. B) 1. Logistic regression indicated that parents having isolated systolic or diastolic hypertension carry no risk. 2. Hypertension in both the parents carried maximum risk of having higher BP in offspring. 3. Compared to hypertensive status of mother, the hypertensive status in father had more influence on offspring BP.

These results emphasize the importance of genetic predisposition to hypertension in high normal and prehypertensive subjects. However the differential risk seen when subjects are classified according to different criteria is discussed. JNC VII considers subjects with SBP from 120-139 mmHg and diastolic BP from 80-89 mmHg as having prehypertension. WHO however further stratifies this above range into having normal (SBP 120-130 mmHg and DBP 80-85 mmHg) and high normal BP (SBP range of 130-139 mmHg and DBP range of 85-89 mmHg) (**Table 3**). The WHO3 group had 4.33 odds ($P=0.032$) when Father HTN and 4.07 odds ($P=0.031$) when both parents had hypertension. The WHO2 group did not have any odds associated with parental hypertension. The narrow WHO classification has helped to surface the risk in high normal BP WHO3group (**Table 15**). On the contrary, the JNC2 category having a comparatively wider range has shown the risk to be only 2 times which this study feels is diluted due to further non stratification.

Results obtained with quartiles classification are similar to WHO classification. The BPG3 (BP 123-130/80-85mmHg) extends over the WHO2 and WHO3 groups but becomes a subset of JNC2 and hence includes both high normal and prehypertensives

based on comparative WHO and JNC2 classification. It showed 4.6 odds ($P=0.03$) when Father HTN, 4.9 odds ($P=0.027$) when both parents are hypertensive (**Table 15, Fig 19**). The BPG4 group ($\geq 131/86$ mmHg; overlaps with WHO3 and 4 and JNC2 and 3) showed odds of Mother HTN (OR 5.04, $P=0.008$) which doubles when Father HTN (OR 10.32, $p=0.0001$). Hypertension in both parents further increased the odds to 18.35, ($P=0.0001$). No maternal influence was noted in BPG2 and 3, WHO2 and 3 and JNC2 groups.

One differing finding of increased odds of both parents HT (OR 7.53, $p=0.004$) in BPG2 group (114-122 SBP and 74-79 DBP) was noted. This group overlaps with WHO1 and 2 and JNC 1 and 2. The only rational explanation this study could offer was that this was due to the classification of 5 offspring in BPG2 but not in WHO 2 due to different cut offs.

Polygamy in this culture could have potentiated paternal influence on offspring BP in this study. In the study sample of 667 offspring were borne by 222 couples and only 2 husbands had 2 wives each. So, we have more than 99% of couples in monogamic alliance. This study thus proposes that, offspring BP in this cohort has more paternal influence.

The earlier part of this thesis showed difference in reactivity of OHT₂ compared with OHT₁ and ONT. The Part B of the thesis showed that the risk of having high normal BP or prehypertension is increased with the hypertension in both the parents. Both the findings emphasize the importance of hypertension in both parents.

The comparison of metabolic, hemodynamic and autonomic measurements in this cohort (N=667) of Omani Arab having high normal BP/prehypertension with optimal BP was beyond the scope of this thesis. However presence of risk factors in prehypertensive/high normal BP subjects as documented in the literature [124-127] coupled with the genetic predisposition may make prehypertensive/high normal BP subjects more vulnerable to the development of an essential hypertension.

The present study proposes that, in a clinical setting, family history of hypertension must be asked to a subject who has high normal BP or is in prehypertensive phase. Subjects with a positive history of hypertension in both parents should be targeted for the primary prevention. If high BP is a common denominator, then, subject with positive genetic history will require more attention.

8.3 Limitations

1. The strength that, offspring in this study belong to pedigrees where consanguinity is common becomes a limitation in generalizability of the results. Results need to be interpreted with caution for a general population.
2. Psychometric evaluation of offspring to corroborate the study findings was beyond the scope of this thesis.

8.4 Strengths

1. This is the first offspring study in an unique homogeneous Arab population with a high degree of consanguinity and environmental homogeneity comparing circadian BP and the cardiovascular and autonomic dynamics in offspring with differential parental history of hypertension
2. The three group design strengthens the interpretation of findings. Most studies in the literature restrict to the two group design. They combine offspring with single and both parents as one group.
3. Complete non-invasive measurements with the state of art equipment (24H ABPM, beat-to-beat BP, impedance cardiography and spectral analysis of HR and BP) helped to understand subtle differences in reflex responses in offspring, perhaps for the first time.
4. Simultaneous study of the close loop, 'BP-baroreceptors-autonomic activity and catecholamine-HR, SV-BP' gave better insight into cardiovascular reflex function operating in OHT₂ compared with OHT₁ and ONT.

5. Cluster of cardiovascular reflex tests with 24H ABPM helped better to correlate findings and draw conclusions.
6. The cross-sectional analysis of a large sample of young subjects with prehypertension or high normal BP for their genetic predisposition to the hypertension.

8.5 Conclusions

This dissertation addressed the correlation of genetic proneness to hypertension and cardiovascular physiology by two approaches- 1. Study of the cardiovascular reactivity in offspring with differential parental history of hypertension, and 2. The cross sectional analysis of the family history of hypertension in subjects with prehypertension or high normal BP.

Offspring with strong positive history of hypertension in both parents have more responsive resistance vessels at a time when BP is comparatively normal. Therefore, they can afford less activity by sympathetic nervous system (LFnuRRI) at rest and during change of posture. This finding is corroborated by highly significant correlation between change of LFnuRRI and of plasma noradrenaline with HUT. However, if mental stress is imposed, OHT₂ respond with greater activity of sympathetic nervous system (HR and LFnuBPV). This is also reflected in higher HR in 24H ABPM. The CPT is probably too unspecific stress to differentiate subjects with positive and negative family history of hypertension. OHT₂ but not OHT₁ showed cardiovascular and autonomic responses to change of posture and mental stress similar to those documented for established hypertensives.

This study showed that, knowing the status of hypertension in a single parent or both parents is important before generalizing the observation of increased reactivity to any stress in offspring of hypertensive parents. Pathophysiological changes in OHT₂ predate the occurrence of hypertension as a clinical disease. Results of simultaneous mental stress (WCT) and physical stress (especially HUT) generated better conclusive evidence.

It is possible that, in offspring with genetic predisposition to hypertension, increased cardiovascular and autonomic reactivity to repetitive mental and physical stress in real life situations may eventually precipitate the hypertension.

The cross-sectional analysis concluded that, subjects with high normal BP or prehypertension are likely have increased family history of hypertension in both parents compared with subjects having optimum BP. In a clinical setting, family history of hypertension must be asked to subjects with high normal BP or prehypertension. Early intervention in these subjects may be helpful to prevent the progress of prehypertension to overt hypertension.

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