EVALUATION OF CARDIOVASCULAR RESPONSES TO SHORT TERM HEAD DOWN TILT IN HEALTHY SUBJECTS AND DIABETIC PATIENTS

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(PHYSIOLOGY)

JULY 2020

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CERTIFICATE

This is to certify that this thesis titled "**EVALUATION OF CARDIOVASCULAR RESPONSES TO SHORT TERM HEAD DOWN TILT IN HEALTHY SUBJECTS AND DIABETIC PATIENTS**" is an original work of **Dr. MAMTA SHOBHAWAT** carried out under our direct supervision and guidance at Department of Physiology, All India Institute of Medical Sciences, Jodhpur.

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DECLARATION

I, hereby declare that the work reported in the thesis titled "**EVALUATION OF CARDIOVASCULAR RESPONSES TO SHORT TERM HEAD DOWN TILT IN HEALTHY SUBJECTS AND DIABETIC PATIENTS**" embodies the result of original research work carried out by me in the Departments of Physiology, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other Institution/ University.

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ACKNOWLEDGEMENT

The success and final outcome of this dissertation project required a lot of guidance and assistance from many people and I am extremely fortunate to have got this all along the completion of my dissertation work. Whatever I have done is only due to such guidance and assistance and I would not forget to thank them

First and foremost, I would like to thank the supreme power the Almighty God for giving me the strength, knowledge, and ability to undertake this research study. Words cannot express my deep sense of gratitude and appreciation to him whose blessing and love have been always with me throughout my endeavour, whose support enabled me to complete it.

Any amount of gratitude shall be less for the contribution of my parents, Late Mr Ramesh Kumar Shobhawat and Late Mrs Shanti Chouhan and my brother Mr Hitesh Shobhawat for their faith in me. Their moral support, encouragement and unconditional love were undeniably the bedrock upon which my life has been built.

I am thankful to Prof Dr (Col) CDS Katoch, Executive Director, AIIMS Jodhpur for thesis approval and permitting me to avail all the facilities available in the institute for conducting this study.

I owe deep sense of gratitude and sincere thanks to my guide, my advisor, my mentor Dr. Shival Srivastav, Associate Professor, Department of physiology, AIIMS Jodhpur who provided me an opportunity to work under his guidance. Also, thanks for his continuous support of my dissertation, for his patience, motivation, enthusiasm and immense knowledge. His guidance helped me in all the time of research and writing of the thesis. I am greatly in debt to him for devoting valuable time out of his busy schedule and being concerned for completion of this project. I could not have imagined a better advisor and mentor for my whole post-graduation.

I express my sincere gratitude to my co-guide Dr. Om Lata Bhagat, Additional Professor, Department of Physiology, AIIMS Jodhpur for her invaluable support, encouragement, guidance and help throughout my work. I am also thankful to her for her mentorship throughout the course of the work. Her vast knowledge, experience and suggestions has been a boon for completion of my study.

Besides my mentor, I owe great debt of gratitude to my co-guide, great advisor and truly wonderful person Dr. Ravindra Kumar Shukla, Additional Professor, Department of Endocrinology, AIIMS Jodhpur for providing me with the opportunity to work in his guidance. Words are not sufficient to express my grateful thanks for constant support, encouragement, guidance and help throughout my work. I shall remain grateful to him forever.

I want to take a moment to express my sincere thanks and gratitude to our faculty members Dr. Abhinav Dixit, Professor and Head, Dr. Prasunpriya Nayak, Professor, Dr. Bharati Mehta, Professor, Dr. Pooja Ojha, Associate Professor, and Dr. Mahesh Gadhvi, Assistant Professor, Department of Physiology, AIIMS Jodhpur for their invaluable advice, guidance, inspiration and encouragement from time to time.

A special note of thanks to my seniors, Dr. Annu Ankita, Dr. Neelesh Gehlot, Dr.Anshika Baranwal and Dr.Jagdish Seoran for their help, encouragement and guidance during the course of my study.

I want to thank my friends and colleagues Dr. Annie S. Jose, Dr. Ashwat Reddy, Dr Ritu Kumari, Dr Shreeparvathy, Dr. Vakode Vani, Ms Tripti, Ms Deepshikha, Ms Priyanka for their support and co-operation throughout my tenure. I got lucky to get such great friends who made this journey, a smooth ride.

I am thankful to all the other staff of Department of Physiology, Mr Shiv Kumar, Mr Amit Kumar, Mr Ajay Kumar, Mr Martin Silvester, Mr Gaje Singh Parmar, Mr Surendra Kumar and Mr Babu Ram for their technical support.

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LIST OF ABBREVIATIONS

ANS	:	Autonomic Nervous System
BP	:	Blood Pressure
CAN	:	Cardiac Autonomic Neuropathy
CARTs	:	Cardiac autonomic reactivity tests
CFT	:	Cold face test
CPT	:	Cold Pressor Test
CVLM	:	Caudal Ventrolateral Medulla
DBP	:	Diastolic blood pressure
DBT	:	Deep Breathing Test
DM	:	Diabetes Mellitus
ECG	:	Electrocardiography
HDT	:	Head Down Tilt
HGT	:	Hand Grip Exercise Test
HRV	:	Heart Rate Variability
IBIs	:	Interbeat Intervals
IOL	:	Intraocular lens implantation
LST	:	Lying to Standing test
MHR	:	Mean heart rate
NTS	:	Nucleus of Tractus Solitarius
OGTT	:	Oral glucose tolerance test
PNS	:	Parasympathetic Nervous System
PPG	:	Photoplethysmography
PTT	:	Pulse transit time
RVLM	:	Rostral Ventrolateral Medulla
SAS	:	Survey of Autonomic Symptoms
SBP	:	Systolic blood pressure
SNS	:	Sympathetic Nervous System
VM	:	Valsalva Manoeuvre

1. Introduction

The autonomic nervous system (ANS) consists of two mutually antagonistic limbs – sympathetic and parasympathetic systems. These act in harmony to maintain cardiovascular homeostasis. Various maneuvers such as deep breathing, head up/head down tilt, isometric exercise, cold stimulation and Valsalva maneuver can be used to stimulate the ANS.

In addition of its use in autonomic function stimulation, Head down tilt (HDT) is also commonly used to simulate microgravity conditions on earth. The maneuver involves passively tilting the subject used a motorized tilt table to induce cephalad shift of blood. When administered in the long term, HDT induces various physiological changes mimicking microgravity exposure. These include changes in bone mineral density, muscle atrophy, diuresis and structural and functional adaptations in the heart and vessels. Other maneuvers to simulate similar changes are head out thermoneutral water immersion and dry immersion. (1,2).

HDT is commonly administered at 6 degrees of inclination (3–6) to simulate the effects of weightlessness in human subjects. This maneuver leads to shifting of blood from caudal to cephalic end of the body, resulting in increased preload (5). Stimulation of cardiopulmonary baroreceptors occurs as a result of HDT. This is consequent to the receptor loading without change in total blood volume (7). Increased heart rate, cardiac output and with increased sympathetic outflow may result due to this engagement (8). In addition, release of various vasoactive substances such as Atrial natriuretic peptide and inhibition of release of vasopressin, norepinephrine and renin have been reported.

While the long term effects of 6 degree HDT are well studied (9–12), acute effects of HDT are not well established. The acute shift of blood to varying degrees of HDT are likely to provide interesting clues to cardiovascular regulatory mechanisms. In addition, comparison of the responses between healthy subjects and those with underlying autonomic neuropathy will provide additional information regarding these mechanisms.

2. Review of Literature

2.1 Autonomic nervous system: overview

The autonomic nervous system is integral to the regulation of the cardiovascular system. Two mutually antagonistic limbs – sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) – exert their effects on the heart and the vasculature in maintaining homeostasis.

The parasympathetic supply is primarily by means of the Vagus nerve (Cranial nerve X) and the fibers originating from the sacral portion of the spinal cord. The sympathetic nervous system originates from the thoracic and lumbar segments of the spinal cord and provides innervation to different viscera. The arrangement is in the form of pre- and post- ganglionic fibers with Acetylcholine being released at the preganglionic terminal and Acetylcholine, Epinephrine and Norepinephrine being released at the post ganglionic end depending on the SNS or PNS ending (13). In addition to aforementioned neurotransmitters, non-adrenergic and non-cholinergic neurotransmission has also been identified.

While sympathetic nervous system is associated with 'fight or flight' response, parasympathetic system is responsible for 'rest and digest' behavior. SNS stimulation has a positive ionotropic and chronotropic effect on the heart and causes increased tone of the peripheral vasculature. PNS primarily acts to reduce heart rate and conduction of impulse in the heart and does not have much effect on the contractility.

The Nucleus of tractus solitarius (NTS) is the primary integrating center for autonomic control of the cardiovascular system located at the level of the brainstem. It receives afferent information from the baroreceptors, pressure sensors located in the large vessels (carotid sinus and aortic arch) by means of glutaminergic endings. The neurons of the NTS excite caudal ventrolateral medulla (CVLM) which extend GABA-nergic fibers to the rostral ventrolateral medulla (RVLM). The RVLM provides excitatory projections to the sympathetic efferent supply to various organs. In addition, NTS also sends projections to the Nucleus ambiguus and Dorsal motor nucleus of the Vagus nerve thus regulating parasympathetic efferent supply to the cardiovascular system.

Assessment of Autonomic function integrity

Assessment of integrity of the ANS can be performed by multiple tests, each pertaining to a particular organ system supplied by the ANS. These tests include pupillometry and pupil cycling time, heart rate variability and Ewing's battery of tests, thermoregulatory sweat tests and Electrogastrography. In addition imaging studies can also be performed to look at the cardiovascular regulatory centers at the level of brain and brainstem (14, 15). Cardiac autonomic function is primarily assessed by a combination of Heart rate variability (HRV) and a battery of tests standardized by Ewing and colleagues (16–18) referred to as Ewing's battery of tests.

2.2 Assessment of autonomic tone: Heart rate variability

Heart rate variability is beat to beat variation in heart rate or interbeat intervals under resting condition. The interbeat intervals (IBIs) are commonly derived using RR intervals computed from a Lead II ECG record (19). Reduced heart rate variability is associated with adverse prognosis in various heart diseases such as heart failure and myocardial infarction (20,21). HRV analysis is commonly done by recording of Lead II ECG. The window of recording may extend from 5 minutes, indicative of short term HRV to 24 hour recordings, suggestive of long term HRV (22).

Heart rate variability measurement is performed by means of following indices

1. Time Domain Measures
2. Frequency Domain Measures
3. Geometric Measures

Time Domain measures

Time domain indices measure the variation in RR interval over the time. The metrics was proposed by European society of Cardiology and North American Society of Pacing and Electrophysiology (22). Common time domain measures include SDNN, RMSSD, SDSD, nn50 and pNN50.

Frequency domain measures

Once the RR interval time series has been obtained, the series is subjected to Fast Fourier transformation. The data thus obtained is expressed in term of power of the

individual frequency bands (ms^2) which is proportional to the energy of the bands. The graph thus obtained is known as Power spectrum plot (22). Common spectral measures include Total Power, Low frequency power, High frequency power and ratio of low frequency and high frequency power referred to as LF:HF ratio.

Geometric measures

In this method, a Poincare Plot is most commonly used for quantification. Each RR interval is plotted against a preceding RR interval in a Cartesian plane. Analysis of the plot thus obtained may be done visually or using the following parameters. SD1 and SD2 are commonly reported metrics in this category.

Heart rate variability measurement is conventionally performed by means of following indices

1. Time Domain Measures
2. Frequency Domain Measures
3. Geometric Measures

1-Time Domain measures

Time domain indices measure the variation in RR interval over the time. The metrics proposed by European society of Cardiology and North American Society of Pacing and Electrophysiology (22).

Table 2.1: Time domain parameters of Heart rate variability

Variable	Description
SDNN	Standard deviation of all NN interval
SDANN	Standard deviation of the averages of NN intervals in all 5 minute segments of the entire recording
RMSSD	The square root of the mean of the sum of the squares of differences between adjacent NN intervals
SDNN index	Mean of the standard deviations of all NN intervals for all 5 minute segments of the entire recording
SDSD	Standard deviation of differences between adjacent NN intervals

NN50 count	Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording. Three variants are possible counting all such NN intervals pairs or only pairs in which the first or the second interval is longer
pNN50	NN50 count divided by the total number of all NN intervals

Source: Task Force Guidelines (22)

2-Frequency domain measures

Once the RR interval time series has been obtained, the series is subjected to Fast Fourier transformation. The data thus obtained is expressed in term of power of the individual frequency bands (ms^2) which is proportional to the energy of the bands. The graph thus obtained is known as Power spectrum plot.

Table 2.2: Frequency domain parameters of Heart rate variability

Parameter	**Frequency band**	**Significance**
VLF	≤ 0.04 Hz	Power in very low frequency range, represents influence of respiration, hormones and thermoregulatory mechanisms on the heart rate
LF	0.04-0.15 Hz	Power in low frequency range, due to sympathetic influences with a small contribution from parasympathetic influences
HF	0.15-0.4 Hz	Power in high frequency range, represents parasympathetic influence on the heart rate
LF (n.u.)	0.04-0.15 Hz	Computed as LF/ (Total Power-HF) × 100. Power in normalized units. Represents pure sympathetic influence
HF (n.u)	0.15-0.4 Hz	Computed as HF/ (Total Power- HF) × 100. Power in normalized units. Represents pure parasympathetic influence

Total Power	Approximately less than 0.4 Hz	Variance of all NN intervals
LF/HF ratio	-	Represents sympatho-vagal balance

Source: Task Force Guidelines (22)

3- Geometric measures- Geometric measure is for quantification of autonomic tone. In this Poincare Plot is most commonly used for quantification. Each RR interval is plotted against a preceding RR interval in a Cartesian plane. Analysis of the plot thus obtained may be done visually or using the following parameters.

Table 2.3: Geometric measures of Heart rate variability

Parameter	**Definition**	**Represents**
SD1	Dispersion of points perpendicular to the line of identity	Represents instant beat to beat variability; parasympathetic influence
SD2	Dispersion of points along the line of identity	Represents long term or slow variations in the RR intervals; sympathetic influence
SD1/SD2	Ratio of SD1 and SD2	Represents the ratio of short term versus long term influences

Source: Task force guidelines (22)

We explored literature to document the aforementioned parameters in healthy subjects as well as patients with diabetes mellitus. Table 2.4 and 2.5 provide summary of HRV parameters in healthy subjects and patients with Diabetes mellitus.

Table 2.4: Summary of heart rate variability indices in healthy subjects (23–31)

	Mirza et.al (2012)	**Kumar et.al (2021)**		**Mural et.al (2022),**	**Kunikullaya U et.al (2021)**		**Woo kim et.al (2021)**	**John et.al (2022)**	**Javorka et.al (2005)**	**Jensen-urstad et..al (1998)**	**Koskinen et.al (2009) ***
Population	n= 200, Both male and female	n= 74, male, age= 18-30 years	n= 37, female, age= 18-30 years	n=50, Both male and female	n= 125, male, age= 18-30 years	n=163, female, age= 18-30 years	n=75, male=27, female=48, age= 33.44 ± 13.11	n=40	n=17	n=123, male= 63, female=70	n=1780, age-24-39 yesrs, males-831, females- 949
SDNN (ms)	-	-	-	38.4 ± 22.6	64.44 ± 2.05	64.42 ± 2.44	50.11 ± 109.39	42.34	72 ± 7	-	3.86±0.42 males) 3.84±0.44, (females)
RMSSD (ms)	-	42.93 ± 23.76	47.68 ± 24.65	32.9 ± 35.8	57.36 ± 2.56	64.93 ± 3.60	37.76 ± 20.11	30.05	59 ± 9	-	3.70±0.58 (males), 3.74±0.66 (females)
SDSD (ms)	-	-	-	32, .9 ± 35.8	-	-	-	-	-	-	
NN50	-	-	-	-	207.19 ± 13.14	258.44 ± 14.59	58.12 ± 57.14	28	-	-	
pNN50	-	21.78 ± 17.71	30.74 ± 20.62	61.5 ± 335.7	30.42 ± 1.89	32.38 ± 1.83	17.75 ± 17.97	8.08	0.28 ± 0.05	-	
TP (ms^2)	2011.86 ± 2107.24	2364.57 ± 1960.69	2377.95 ± 2023.51	1894.6 ± 2321	4423.07 ± 277.04	4935.08 ± 429.48	1668.64 ± 1506.37	1661.3	-	3081±1238 (males), 2377±1095 (females)	7.40±0.86 (males), 7.34±0.91 (females)
LF (ms^2)	327.63 ± 165.35	619.48 ± 562.73	630.44 ± 515.97	448.9 ± 590.7	1314.84 ± 94.36	1160.40 ± 110.92	848.42 ± 907.53	312.7	705 ± 218	1422±549 (males), 1014±522 (female)	5.97±0.89 (males), 5.52±0.93 (females)
HF (ms^2)	187.4 ± 79.93	963.34 ± 1037.25	1130.58 ± 1033.60	709.9 ± 1155.1	1471.35 ± 116.15	2155.20 ± 245.93	609.95 ± 584.44	355.50	516 ± 140	423±234 (males), 412±298 (females)	6.18±1.10 (males), 6.44±1.17 (females)
LF/HF	1.83 ± 0.64	0.92 ± 0.78	0.82 ± 0.71	-	1.34 ± 0.10	0.88 ± 0.05	-	1.12	-	4.47±1.21 (males), 3.52±1.06 (female)	

*All values are log transformed

Table 2.5: Summary of heart rate variability indices in patients with Diabetes mellitus (23,25,28,29,32–36)

	Mirza et.al (2012)	**Mural et.al (2022)**	**John et.al (2022)**	**Metelka et.al (2018)**	**Jayachandra et.al (2022)**	**Ramanathan et.al (2020)**	**Javorka et.al (2005)**	**Bhati et.al (2019)**	**Tarvainen et.al (2014)**
Population	n= 200	n= 50	n= 40	n=15	n=35	n=30	n=17	n=27	n=199
SDNN (ms)	-	20.9 ± 16.04	29.29	-	100.2±46.2	27.84±15.97	40 ± 6	37.1 ± 15.08	20.3(14.2-27.7)
RMSSD (ms)	-	16.37 ± 17.95	19.41	-	19.4±8.6	20.61±16.81	32 ± 6	24.6 ± 11.20	19.1(12.5-28)
SDSD (ms)	-	15.54 ± 17.56	-	-	-	-	-	-	-
NN50	-	-	5.5	-	-	-	-	-	-
pNN50	-	4.5 ± 10.35	1.49	-	3.1±2.3	-	0.10 ± 0.04	1.6 ± 1.20	2.02(0.42-5.18)
TP (ms^2)	813.3 ± 1456.49	407.3 ± 667.4	748.2	483.2 ± 479.3	22.4±13.2	-	-	716.8 ± 285.73	25.9(22.8-28.9)
LF (ms^2)	189.1 ± 114.93	139.6 ± 433.1	228.76	158 ± 124.9	13.2±7.9	15.95±13.07	231 ± 74	207.2 ± 116.81	23.1(19.8-26.2)
HF (ms^2)	97.2 ± 71.56	229.6 ± 937.8	92.61	197.6 ± 371.4	7.0±4.0	5.74±6.95	171 ± 69	170.9 ± 85.21	21.2(16.6-24.6)
LF/HF	2.13 ± 0.54	-	1.3	1.50 ± 1.27	1.82±0.4	3.64±1.6	-	1.53 ± 0.71	-

2.3 Assessment of Autonomic reactivity: Ewing's battery

Autonomic reactivity is assessed by administering maneuvers that lead to change in heart rate and blood pressure. These changes can be quantified and compared with standard values to determine the degree of autonomic function. Autonomic reactivity is most commonly assessed by Ewing's battery of tests (18).

DJ Ewing and colleagues formulated the Ewing's battery of tests (18). They described a battery of five tests.

1- Lying to standing test (LST)
2- Deep breathing test (DBT)
3- Valsalva maneuver (VM)
4- Hand grip exercise test (HGT)

Cold pressor test was not a part of initial battery given by Ewing et al. It has been included as a part of the battery of tests and has been validated in many studies (37,38). So, we included Cold pressor test in our autonomic function assessment protocol. We excluded Valsalva maneuver from our battery of tests due to COVID-19 pandemic, since it was not possible to disinfect the sphygmomanometer used for estimation of expiratory pressure after each use. We also included an additional validated test, Cold face test in our protocol (39). Blood pressure and heart rate were measured at rest and on performance of these tests.

We explored literature to document the aforementioned parameters in healthy subjects as well as patients with diabetes mellitus. Table 2.6 and 2.7 provide summary of autonomic reactivity parameters in healthy subjects and patients with Diabetes mellitus.

Table 2.6: Summary of studies of autonomic reactivity assessment in healthy subjects (34,40–51)**.**

	Meena et. al. (2020)	**Ganguly et.al. (2020)**	**Ramanathan et.al (2020)**	**Kulshreshtha et.al. (2021)**	**Shalimar et.al. (2006)**	**Sakhuja et.al. (2007)**	**Jain et.al. (2016)**	**Shobana et.al. (2022)**	**Matei et.al. (2013)**	**Sucharita et.al. (2011)**	**Rosengard-Barlund et.al. (2009)**	**Agrawal et.al. (2019)**	**Solanki et.al. (2020)**	**Shobana et.al. (2022)**
Population	n=30	n=30	n=30	n=17	n=30	n=25	n=30	n=35	n=23	n=23	n=36	n=60	n=120	n=35
30:15 Ratio (LST)	1.35± 0.25	1.19± 0.193	-	1.31± 0.17	1.17±0.17	1.28± 0.17	1.31 (0.28)	1.13± 0.26	1.2± 0.2	1.36± 0.19	1.65± 0.03	1.08± 0.041	1.28± 0.40	1.13± 0.26
ΔSBP LST	5.77± 4.59	7.23± 1.28	-	7.09± 4.93	-4.33± 4.84	-3.92± 4.92	-	6.30± 0.86	29.9± 2.65	4.07± 11.1	3.0± 1.4	5.6± 2.38	3.43± 1.56	6.30± 0.86
E:I Ratio (DBT)	1.43± 0.23	1.22± 0.10	1.25±0.11	1.34± 0.13	-	1.39±0.15	1.58 (0.33)	1.47± 0.79	-	-	1.40± 0.03	-	1.46± 0.40	1.47± 0.79
ΔHR (DBT)	-	14.37± 6.54	-	24.76± 9.9	24.50± 7.81	24.01±8.26	-	-	9±1.58	14.8± 7.5	-	18.48± 3.21	-	-
ΔDBP CPT	-	10.81± 2.13	-	16.59± 8.33	13.66± 3.86	16.32±9.09	-	-	-	-	-	-	-	-
ΔDBP HGT	11.70± 4.48	19.14± 2.11	10.13± 1.89	12.12± 9.12	14.86± 4.32	19.44± 9.74	20.0 (16.0)	16.30± 4.53	25.3±5	8.9±11.1	-	18.87± 3.08	14.07± 2.60	16.30±4.53
CFT Ratio	-	-	-	-	-	1.04±0.07	-	-	-		-	-	-	-

LST: lying to standing test, DBT: deep breathing test, CPT: cold pressor test, HGT: hand grip test, CFT: cold face test

Table 2.7: Summary of studies of autonomic reactivity assessment in in patients with diabetes mellitus (34,35,47–52)**.**

	Bhati et. al. (2019)	**Ramanathan et. al. (2020)**	**Matei et. al. (2013)**	**Sucharita et. al. (2011)**	**Rosengard-Barlund et. al. (2009)**	**Agrawal et. al. (2019)**	**Solanki et. al. (2020)**	**Sumaswi et. al. (2016)**
Population	n=27	n=30	n=30	n=23	n=116	n=30	n=120	n=50
30:15 Ratio (LST)	1.2 ± 0.23	-	1.07±0.04	1.17±0.12	1.67±0.03	0.99±0.130	1.15±0.35	1.07±0.08
E:I Ratio (DBT)	1.1±0.15	1.09±0.05	-	-	1.40±0.01	-	1.25±0.32	-
ΔHR (DBT)	11.7 ± 9.31	-	7.6±1.32	10.2±4.3	-	13.66±5.66	-	13.77±5.60
ΔDBP CPT	-	-	-	-	-	-	-	-
ΔDBP HGT	14.11 ± 5.96	3.20±1.54	21.56±3.34	1.9±8.4	-	15.8±4.24	5.6±3.54	14.40±5.27
ΔSBP LST	-2.0 (-30-22)	-	38.16±3.75	-9.7±14.9	5.0±0.7	9.8±7.8	7.06±5.00	10.80±6.94
CFT Ratio	-	-	-	-	-	-	-	-

LST: lying to standing test, DBT: deep breathing test, CPT: cold pressor test, HGT: hand grip test, CFT: cold face test

2.4 Cardiac autonomic neuropathy in Diabetes mellitus

Diabetes mellitus is the most common cause of autonomic neuropathy in world (53). It involves cardiovascular, gastrointestinal, genitourinary, sudomotor, and neuroendocrine system and responsible for diverse manifestation of diabetes (54).

Prevalence of cardiac autonomic neuropathy reported in different study is different due to difference in diagnostic criteria used in different studies or due to difference in patient selection for study. As per a study done by Indian Council of Medical Research-India Diabetes study group (55), prevalence of diabetes mellitus in India is approximately 7.3%. The number is expected to grow in the years to come. Multiple adverse prognostic factors are associated with development of cardiac autonomic neuropathy (CAN) in diabetes mellitus. These consist of age, poor sugar control, associated co morbidities and obesity (56). In addition, Metabolic syndrome and insulin resistance plays a key role in development of cardiac autonomic neuropathy. Both Type I and II DM are associated with CAN.

The symptoms of CAN are variable – they may be as benign as resting tachycardia and as dangerous as myocardial infarction and arrythmias leading to sudden cardiac death (56). Orthostatic hypotension is another common cardiovascular manifestation of CAN in DM. Appearance of CAN in DM may be associated with high morbidity and mortality, with 5 year mortality going all the way up to 50% (57). Therefore it makes logical sense to ensure early screening for CAN in this patient population to reduce long term morbidity and mortality.

Different cellular processes are associated with the development of CAN. These include Metabolic insult and molecular pathways such as polyol pathway, Protein kinase C, advanced glycosylation end products and hexosamine pathway (38).

Institution of good glycemic control along with lifestyle and dietary modifications with weight loss can help in the reversal of early stages of CAN (57). It has been observed that parasympathetic loss along with intact sympathetic modulation is responsible for resting tachycardia. It may also be the causative factor behind the loss of circadian variability in blood pressure, that is seen early in the course of the disease.

In early stage of cardiac autonomic neuropathy reduction of parasympathetic autonomic tone is developed with intact sympathetic supply, so in early stage there is sympathetic predominance which is reflected by higher resting heart rate and loss of circadian rhythm of blood pressure. Late in course of the disease, both limbs may be involved leading to myriad symptoms involving the cardiovascular and other symptoms.

Clinical scoring based on questionnaires can be used to objectively grade CAN in DM. Composite Autonomic Symptom Score 31 questionnaire has been validated against cardiovascular reflex tests-based diagnosis of cardiac autonomic neuropathy (58). In addition, the presence of autonomic symptoms in early diabetic neuropathy can be assessed by the Survey of Autonomic Symptoms (SAS), which is a validated and easily administered tool to measure autonomic symptoms that is sensitive enough to detect mild autonomic neuropathy (59).

Early screening of CAN may be done using combination of HRV and Ewing's battery of tests, which are well established tools for screening as well as identification of patients at risk of adverse events.

2.5 Head Down Tilt: cardiovascular effects

Head down position leads to a shift blood from lower extremities to central circulation and is widely used to treat hypotension and shock (60). Trendelenburg positioning used in the operating room is a variant of head down tilt used to mitigate the effects of hypovolemia and hypotension using surgical interventions.

During Head down tilt blood and fluid are redistributed from the caudal to cephalic portion of the body, leading to an increased venous return. This increased venous return induces distension of the heart and adjacent vessels, which leads to stimulation of cardiopulmonary baroreceptors. Activated cardiopulmonary baroreceptors lead to increase in heart rate and cardiac output to relieve increased pressure in atria (13). Atrial stretch due to increased venous return leads to increase release of atrial natriuretic peptide and the plasma level of vasopressin, norepinephrine, renin and aldosterone have been reported to be decreased (5).

2.5.1 Long term Head down tilt

Long term head down tilt, also known as prolonged head down bed rest (pHDBR) is used as intervention ranging from weeks to months to simulate the effects of microgravity condition in human subjects. A number of studies have investigated human adaptations to microgravity exposure by long term 6° head down tilt.

Millet et.al (2000) enrolled 16 healthy subjects (6 male subjects, 6 female subjects) and gave 6° HDT for 7 days. Blood pressure, heart rate and vasoactive hormonal response was measured before head down tilt and after completion of 7 days of head down tilt. They found no change in total body water and body mass in either gender, decrease in plasma volume was similar in both male and female subjects, urinary normetanephrine was decreased during head down tilt in both male and female subjects, urinary metanephrine and plasma catecholamines level were unchanged, renin and aldosterone level in plasma was increased. They also observed impaired orthostatic tolerance after head down tilt in both male and female subjects (9).

Iwasaki et.al (2000) enrolled 9 healthy subjects and gave 6° HDT for 2 weeks. They found reduction in plasma volume after head down tilt, heart rate was increased significantly after head down tilt, they observed no significant change in both systolic blood pressure and diastolic blood pressure (10).

Shiraishi et.al. (2003) enrolled 6 healthy male subjects and administered 6° HDT for 120 days. Continuous BP, ECG was measures. They observed no significant difference in heart rate before bed rest, after 60 days and 120 days of bed rest but after 120 days of bed rest heart rate was significantly increased at daytime compared with the other measuring time. Systolic blood pressure did not show any significant difference at any of the time points in the study (61).

2.5.2 Short term Head down tilt

Short term head down tilt is usually administered for a period of few minutes, ranging from 5-30 minutes. Common inclinations used are 6, 10 and 30 degrees. The observations of hemodynamic parameters have been performed at varying time points.

In one of the earliest studies of cardiovascular parameters post HDT, London et.al (1983) enrolled 29 male subjects with hypertension and 29 age matched healthy male subjects. They administered 10° HDT for 30 minutes and measured different

cardiovascular and hormonal parameters after this period. They found that blood pressure, heart rate and arterial baroreflex sensitivity did not change during the tilt. Cardiopulmonary blood volume was also increased in all subjects whereas increase in central venous pressure, cardiac output and forearm blood flow was higher in hypertensive subjects. Forearm venous tone was decreased in healthy subjects but remain unchanged in hypertensive subjects. There was decrease in plasma renin and catecholamines levels in all subjects (62).

In another study, Goldsmith et.al. (1985) enrolled 6 healthy subjects and administered 30° HDT for 60 minutes. They measured central venous pressure by intrathoracic catheterization, blood pressure, heart rate at baseline and at every 15 minutes during the tilt. Intermittent saline infusions were given to maintain central venous pressure at the 15^{th} minute value. Central venous blood samples were taken for plasma renin and norepinephrine level measurement. They observed that central venous pressure increased at 15^{th} minute (and maintained thereafter, by infusion of saline) whereas heart rate and mean arterial pressure did not changed during the tilt. Plasma renin and norepinephrine levels were also not changed during tilt (63).

Weise et.al. (1995) enrolled 12 healthy male volunteers and administered supine rest followed by 10° HDT for 10 minutes. Continuous BP, ECG, and forearm vascular resistance was measured. They observed reduced variability in BP and ECG, predominantly in low frequency component, suggestive of sympathetic withdrawal. In addition, reduced forearm vascular resistance and increase forearm blood flow was also observed. This was accompanied by decreased plasma noradrenaline and renin levels suggestive of sympatho-inhibitory response to 10° HDT for 10 minutes. The authors concluded that acute short term HDT leads to stimulation of cardiopulmonary baroreceptors leading to a "buffering effect on sympathetic vasomotor control" (7).

Nagaya et.al (1995) enrolled 12 healthy male subjects and administered 15° and 30° tilt for 10 minute each with 10 minutes of rest between the maneuvers. They measured heart rate, blood pressure, stroke volume, muscle sympathetic nerve activity continuously throughout the procedure. They observed that heart rate and blood pressure remain unchanged during each degree of tilt. Stroke volume and central venous pressure and forearm blood flow increased during each degree of tilt, but magnitude was more in 30° of tilt (64).

Fu et.al (2000) enrolled 10 healthy male subjects and administered lower body positive pressure at 10 mmHg, 20 mmHg, 30mmHg and 6° HDT, each for 6 minute duration. They compared autonomic response of these two simulation models of weightlessness. They measured muscle sympathetic nerve activity, mean arterial pressure, heart rate, cardiac output, stroke volume continuously throughout the procedure. hey observed that muscle sympathetic nerve activity decreased similarly in 6° HDT and low level (10 and 20 mmHg) of lower body positive pressure but at high level (30 mmHg) of lower body positive pressure it increased. Mean arterial pressure was unchanged at 6° HDT and at low level (10 and 20 mmHg) of lower body positive pressure but at high level (30 mm Hg) of lower body positive pressure it increased significantly. Heart rate did not show any change in 6° HDT or at any level of lower body positive pressure. Total peripheral resistance decreased by 6° HDT but it increased by 30 mmHg lower body positive pressure. Stroke volume and cardiac output increased by 6° HDT but decreased by 30 mmHg lower body positive pressure. They concluded that both HDT and lower body positive pressure causes shifting of fluid from lower body to thoracic compartment but autonomic responses were not similar. HDT activated only cardiopulmonary baroreflex, and lower body positive pressure of greater than 20 mmHg activated both cardiopulmonary baroreflex and intramuscular mechanoreflexes (4).

A study was conducted by Vijaylakshmi et.al. (2006) wherein 20 healthy subjects (all males) were exposed to 30°, 60°, 80° HDT for five minutes each after a supine rest of 10 minutes. Five minutes of supine rest was administered between the different maneuvers to allow the parameters to return to baseline before the next HDT test. Blood pressure and heart rate (derived from Lead II ECG) were measured every minute for each maneuver. They did not observe any significant change in SBP and HR in 30°, 60°, 80° HDT. However, increase in DBP was observed with 60° and 80° HDT, when compared to baseline values. This increase in DBP was directly proportional to angle of tilt. They proposed that sympathetic stimulation may be responsible for increased DBP response to 60°and 80° HDT. They also observed reduction in pulse pressure at 60°and 80° HDT. This was also proportional to the angle of tilt. The authors attributed the reduction in pulse pressure to probable excessive preloading (65).

Galanis et.al. (2013) enrolled 33 healthy adult males in their study. Supine rest was provided for 15 minutes. Thereafter, head down tilt was administered at 15° and 30° HDT posture for 7.5 minutes each in succession. Blood pressure and heart rate were

monitored during supine rest and head down tilt using mercury sphygmomanometer and Lead II ECG. Parameters were measured at 1st minute, 2nd minute and 7th minute of HDT. They did not observe any significant change in heart rate in 15° HDT when compared to supine value. However, significant fall in heart rate was observed between 15° HDT and 30° HDT and supine values and 30° HDT. SBP was increased significantly with respect to supine values at 15° HDT and 30° HDT. Similar rise was observed in SBP between 15° HDT and 30° HDT. DBP values also followed a similar trend as SBP. Their findings were contradictory to previous literature where in only DBP was increased/DBP was decreased. They proposed that head down tilt could be utilised as a test to study cardiopulmonary adaptations in healthy and diseased subjects (66).

Kadono et.al (2013) performed study on 35 males undergoing robot assisted laproscopic radical prostatectomy, surgery was done in 20°, 25°, 30° head down position, degree of tilt was assigned to each patient in random manner, Heart rate, mean arterial pressure, respiratory rate, end tidal CO_2 pressure, tidal volume, peak inspiratory pressure, and dynamic compliance were recorded throughout the procedure, they found that degree of head down tilt affected the cardiovascular and respiratory parameters and these effects were stronger with more degree of tilt. They found that mean arterial pressure, respiratory rate, peak inspiratory pressure, end tidal CO_2 pressure increased with increasing angle of head down tilt and dynamic compliance decreased with increasing angle of tilt (67).

A study was conducted by Medala et.al. (2016), 200 healthy subjects were enrolled (100 males and 100 females). Lead II ECG was done to estimate heart rate. BP was measured using mercury sphygmomanometer. Blood pressure was measured within 20 second of giving each degree of tilt. Supine rest was provided followed by HDT of 30°, 60° and 80° HDT. A gap of 5 minutes was provided between individual HDTs. They observed reduced heart rate when compared to supine values for all degrees of HDT. They also observed an increased SBP, reduced DBP and widened pulse pressure for all degrees of tilt. These findings were attributed to sympathetic withdrawal (68).

Chouchou et.al. (2019) enrolled 10 healthy males in their study. 6° HDT was administered for 30 minutes after supine rest of 15 minutes. A recovery period of 15 minutes was provided. ECG and blood pressure was recorded continuously throughout

the procedure. Spectral analysis of blood pressure and heart rate was performed to study autonomic modulation of blood pressure and heart rate. They observed decrease in both systolic and diastolic blood pressure during tilt (2).

Shankhwar et.al (2021) enrolled 30 healthy male subjects and administered 4°, 6°, 8° HDT each for 7 minute with 10 minute rest between each degree of tilt. They recorded ECG, blood pressure, cardiac output and stroke volume continuously throughout the procedure. They observed no significant change in systolic blood pressure, diastolic blood pressure and mean arterial pressure at 4° HDT in comparison to baseline. In 6° HDT diastolic blood pressure and mean arterial pressure increased significantly in comparison to baseline, systolic blood pressure increased significantly in comparison to 4° HDT. In 8° HDT systolic blood pressure increased significantly in comparison to 4° HDT and diastolic blood pressure increased significantly in comparison to baseline and 4° HDT. Cardiac output and stroke volume at 4° HDT shows no change in comparison to baseline. In 6° HDT cardiac output and stroke volume increased significantly in comparison to baseline and 4° HDT. In 8° HDT cardiac output and stroke volume increased significantly in comparison to baseline, 4° HDT and 6° HDT (6).

To summarize, the effects of short term HDT are variable because of the different degrees and durations of tilts administered. In addition, cardiovascular parameters have been measured at different time points leading to heterogeneity in the results.

2.6 Assessment of vascular function: Pulse Transit Time

Vascular function assessment can be done with a variety of methods. These techniques include recording of the peripheral pulse waveforms using tonometry and photoplethysmography. These waveforms provide information about the condition of the vascular tree. When simultaneously coupled with ECG estimation, the finger pulse waveform can provide information about the stiffness of the vasculature. This technique is known as Pulse transit time (PTT).

To estimate PTT, Lead II ECG and finger pulse are simultaneously acquired. The time lag between the peak of R wave and the foot of finger pulse is used to quantify PTT. Some groups also advocate the use of time taken to reach 1/3rd of pulse amplitude.

However, the foot of the finger pulse is a reliable and reproducible time point for PTT analysis.

Different studies have utilized PTT estimation to assess sympathetic neural tone of the vasculature. Jaryal et. al. (2009) measured PTT in 11 subjects using the delay between the peak of R wave and the foot of the finger pulse. They reported the PTT to be 187.1±3.9 ms in healthy subjects and 192.9±5.6 ms in patients with DM (n=10) (69). Similar methodology was used by Chandran et. al. (2011) and Khare et. al. (2016) who reported the PTT to be 230.80±23.22 and 199.1±0.86 ms respectively in healthy subjects (70,71).

Thus, PTT can provide an objective measure of vascular tone. We did not find any literature that has reported PTT in conjunction with HDT.

3. Aim and objectives

Aim

To evaluate the cardiovascular responses to short term head down tilt (HDT) in healthy subjects and patients with diabetes mellitus

Objectives

1. To study the effect of administration of short term 6°, 15° and 30° HDT in healthy subjects and patients with diabetes mellitus on
    (a) Blood Pressure
    (b) Heart Rate
    (c) Pulse Transit Time
2. To compare the responses between the two groups

4. Materials and Methods

The present study was conducted at the Autonomic function laboratory, Department of Physiology, All India Institute of Medical Sciences, Jodhpur.

4.1 Ethical clearance

The study was approved by Institute Ethics Committee (IEC), All India Institute of Medical Sciences, Jodhpur vide letter number AIIMS/IEC/2021/3551 dated 12/03/2021. We started the study after obtaining ethical clearance from the institute's ethics committee. Written informed consent was taken from all subjects before their participation in the study.

4.2 Inclusion and Exclusion criteria

The following inclusion and exclusion criteria were followed for enrollment of subjects in the study

Inclusion criteria

1. Healthy subjects: Apparently healthy subjects of age group 18-45 years
2. Diabetic subjects: Diabetic patients of age group 18-45 years (Clinically diagnosed Type-1 and Type-2 diabetes mellitus patients were enrolled in the study).

Exclusion criteria

1. Subjects with pre-existing neurological and cardiovascular disease (other than Diabetes Mellitus)
2. Previous complaints of vertigo/dizziness
3. Any disease of cervical spine
4. Subjects with retinopathy/Intraocular lens implantation (IOL) / other disease of retina
5. Yoga practitioners
6. Patients with severe autonomic neuropathy

4.3 Study Design

The study was cross sectional observational in nature. Study was conducted in the Department of Physiology, All India Institute of Medical Sciences Jodhpur. Fifty

healthy subjects (32 males, 18 females, mean age = 30.32 ± 6.64 years) and fifty diabetic subjects (32 males, 18 females, mean age = 36.16 ± 8.59 years) participated in the study. The subjects were recruited from the Department of Endocrinology of our hospital and were diagnosed as per the following criteria:

(a) Fasting Plasma Glucose ≥126mg/dl. Fasting is defined as no caloric intake for at least 8 hour or

(b) 2-h Plasma Glucose ≥200mg/dl during Oral Glucose Tolerance Test (OGTT) or

(c) A1C ≥6.5% or

(d) In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dl

Source: American Diabetes Association, 2010 (72).

4.4 Methodology

Study procedure was explained in detail to all subjects and informed written consent was taken from all subjects before their enrollment in the study. We examined all subjects and medical history was taken to ensure that subjects fit our inclusion criteria. The subjects reported to the Autonomic function laboratory, Department of Physiology, All India Institute of Medical Sciences Jodhpur. They were requested to report two hours after a light meal. They were requested to refrain from alcohol, tea, coffee, tobacco or any other caffeinated beverage on the day of the test. Detailed medical history was taken using the case record form of Autonomic function laboratory, Department of Physiology, All India Institute of Medical Sciences Jodhpur. Cardiac autonomic tone was assessed using Heart rate variability (HRV). Autonomic reactivity was assessed using a battery of Cardiac autonomic reactivity tests (CARTs) as per standard protocol (18,37,38). Heart rate variability and cardiac autonomic reactivity tests were performed for all subjects at the baseline. Subjects with severe autonomic neuropathy were excluded from study. Valsalva maneuver was excluded from the study protocol in view of COVID-19 pandemic.

All recordings were done in noise free and temperature-controlled environment in the Autonomic function laboratory at our department in forenoon session. Disposable adhesive ECG electrodes were applied to the skin in Lead II configuration, after

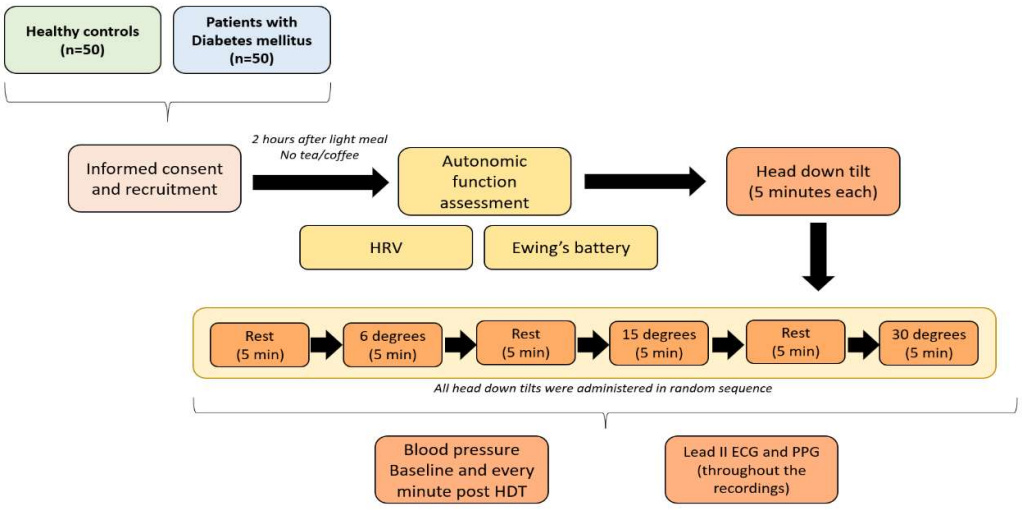
cleaning the area with spirit swabs. ECG and respiratory movement signals were acquired using the Bionomadix® wireless module (Biopac Systems Inc. United States of America Figure no- 4.8). Respiratory movements were acquired by the module attached to an elastic Velcro based belt tied around the 4th intercostal space around the chest. The Bionomadix® module transmitted the ECG and respiratory movement signals to the Biopac MP150® system (Biopac System Inc. United States of America). Data was acquired and visualized in real time using AcqKnowledge® software version (Biopac systems Inc., United States of America). A band pass filter (0.5 and 35 Hz) was applied to the ECG signal in real time. Blood pressure was measured using Omron model no- HEM-8712 digital oscillometric blood pressure device.

We could get reasonable signal quality with PPG probe in the Biopac MP150® system (Biopac systems Inc., United States of America). Therefore, we simultaneously recorded finger pulse waveform and ECG on PowerLab 26T® hardware system (AD Instruments, Australia) for the assessment of Pulse transit time. Finger pulse (Infrared, reflection type photoelectric transducer-MLT1020 PPG) signal acquisition was done using Power Lab 26T® system (AD Instruments, Australia). The finger PPG signal was acquired along with Lead II ECG (band pass 0.5 and 35 Hz) and visualized in the LabChart Pro® software (version 8, AD Instruments, Australia).

Lead II ECG, respiratory movements and finger pulse were recorded throughout the study. Blood pressure was taken intermittently during autonomic function test as per standard protocol. During tilt blood pressure was recorded as baseline and then every minute during tilt.

The testing protocol is summarized in Figure: 4.1

Figure 4.1: Summary of work plan



4.4.1 Assessment of autonomic tone using HRV

Autonomic tone provides information about the basal activity of the autonomic nervous system under resting conditions. Evaluation of autonomic tone is done by Heart rate variability (HRV). Heart rate variability assesses the variation in RR intervals using different statistical measures. The duration of ECG signal for the purpose of HRV estimation varies from 5 minutes to 24 hours. A recording of 5 minutes has been shown to provide sufficient information regarding short term Heart rate variability (22). The same was used in our study. Supine rest of 5 minutes was provided before data acquisition. This was followed by recording of Lead II ECG and respiration signal for 5 minutes. Subjects were instructed to closed eyes during recording and to breathe as normally as possible and to avoid body movements.

Data analysis was performed using LabChart Pro® software version 8 (AD Instruments, Australia). HRV toolbox of the software was used to perform the analysis. The signal was visualized to ensure optimum selection of all R peaks in the five-minute window. Following peak selection, different indices were computed and tabulated, as described ahead.

Heart rate variability indices: Time domain and frequency domain

Time domain

These indices were derived using mathematical computations of interbeat intervals (RR intervals) derived from the 5 minute window.

(1) SDNN- Standard deviation of NN intervals
(2) SDSD- Standard deviation of differences between adjacent NN intervals
(3) RMSSD- Square root of the mean of sum of squares of differences between adjacent NN intervals
(4) pNN50- Percentage of NN intervals differing by more than 50 ms

Frequency domain

Once the RR interval time series was obtained, the series was subjected to Fast fourier transformation. The data thus obtained is expressed in terms of power of the individual frequency bands which is proportional to the energy of the bands. The graph thus obtained is known as Power spectrum plot.

The following indices were computed and tabulated for frequency domain analysis.

(1) LF power- power in the Low frequency band
(2) HF power- power in the High frequency band
(3) Total power- power in the complete frequency bands
(4) LF/HF ratio- Ratio of LF power and HF power

The classification of frequency bands has been based of the classification proposed by Task force guidelines (22).

Figure 4.2: Representative record of Tachogram of Heart rate variability (HRV) of Healthy subject

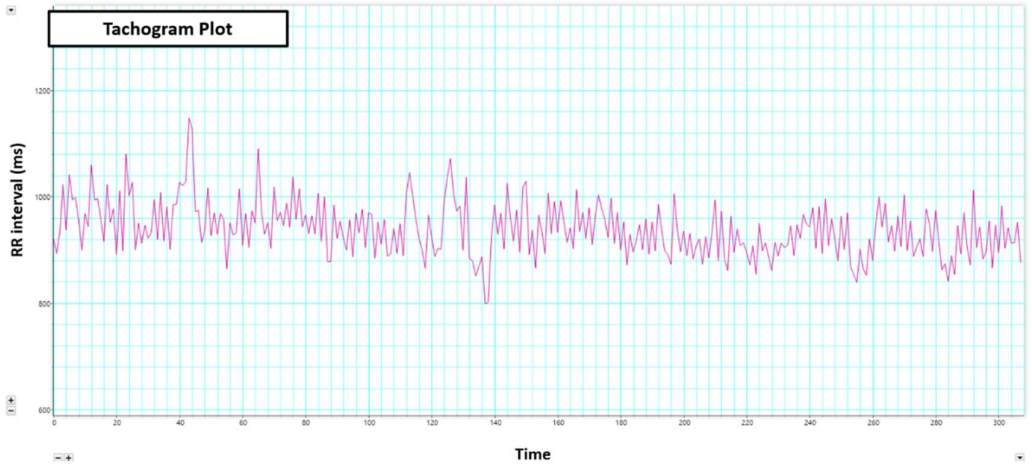


Figure 4.3: Representative record of Poincare of Heart rate variability (HRV) of Healthy subject

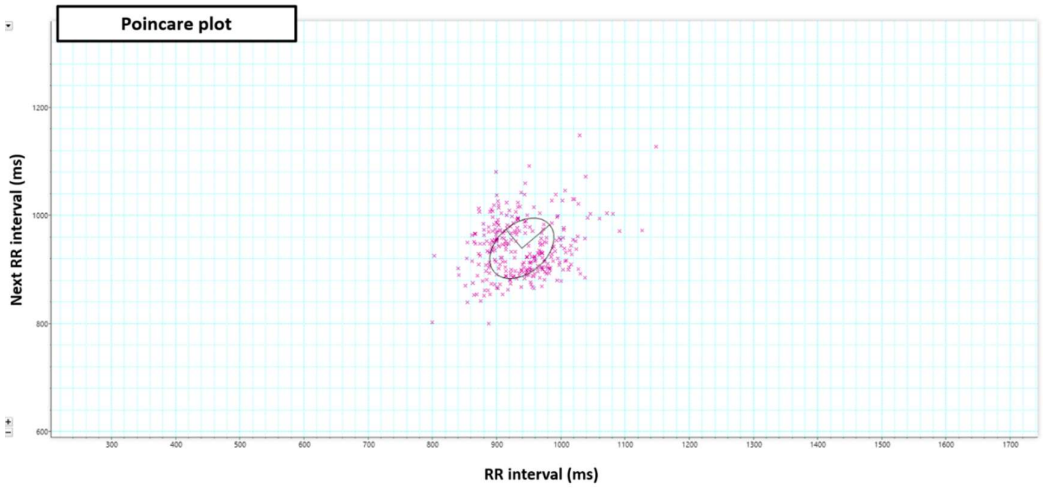
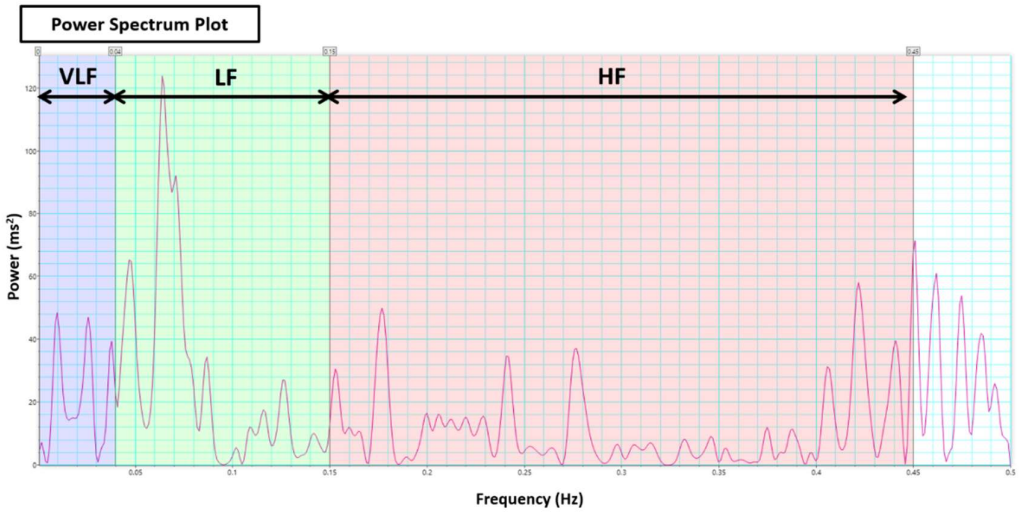


Figure 4.4: Representative record of Power spectrum of Heart rate variability (HRV) of Healthy subject



4.4.2 Assessment of autonomic reactivity using Ewing's battery of tests

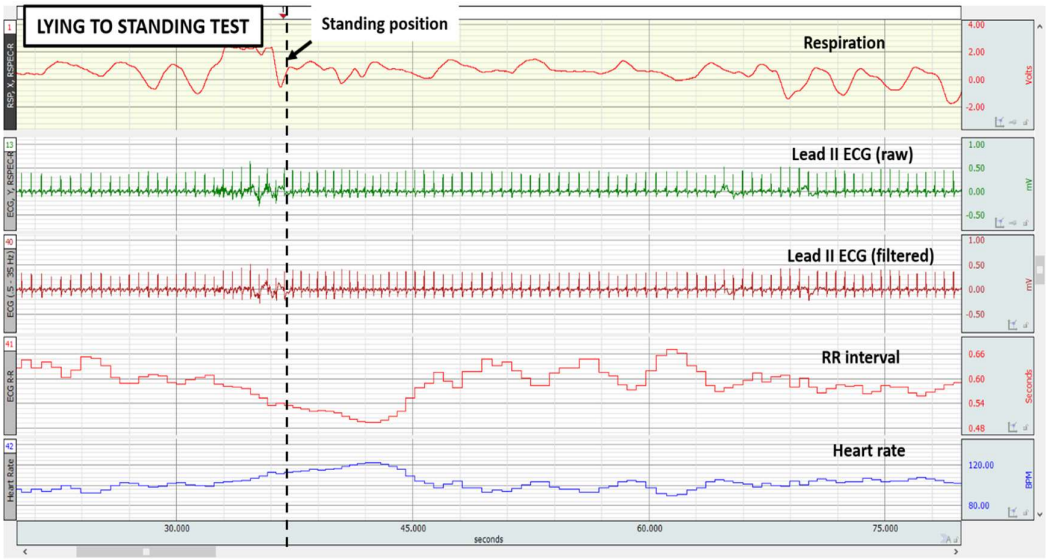
Autonomic reactivity was assessed using the standard battery of tests given by Ewing et al (2). The battery consists of five tests: Lying to standing test, Deep breathing test, Cold pressor test, Hand grip test and Cold face test. Base line blood pressure and heart rate was taken before every maneuver. Gap of 5 minutes was given between each test to allow cardiovascular parameters to come to the baseline.

(a) Lying to standing test (LST)

Blood pressure was recorded after 5 minutes of supine rest. Then Subjects were instructed to change their posture from supine to standing within 3 seconds. The subjects were instructed to stand for 5 minutes. Then we measured their blood pressure at 0.5^{th}, 1^{st}, 2^{nd}, 2.5^{th} and 5^{th} minute post standing. Both systolic blood pressure and diastolic blood pressure values were noted. Subject was instructed to sit on couch after completion of 5 minute. Fall in systolic blood pressure (ΔSBP) was calculated by subtracting the systolic blood pressure values at 0.5^{th} minute, 1^{st} minute, 2^{nd} minute, 2.5^{th} minute and 5^{th} minute from baseline value. Maximum fall of systolic blood pressure was calculated and documented as ΔSBP. We recorded Lead II ECG and respiration throughout the procedure. 30:15 ratio was calculated using lead II ECG,

30:15 ratio was calculated as the ratio of longest RR interval taken between 25th to 35th beat and shortest RR interval taken between 10th to 20th beat.

Figure 4.5: Representative record of Lying to standing test (LST) of Healthy subject

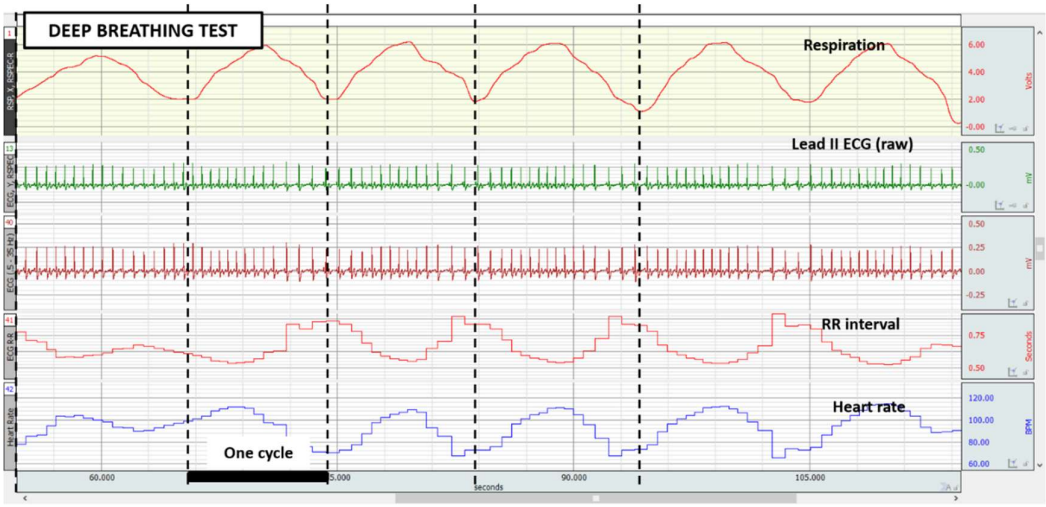


After LST all test for assessment of autonomic reactivity were done in sitting position.

(b) Deep Breathing test (DBT)

Rest of 5 minutes was provided and procedure was explained to subjects. They were instructed to take deep and slow inspiration for 5 second followed by deep and slow expiration for 5 second, this 10 second of inspiration and expiration constituted one cycle. We recorded 8 cycles of deep breathing. We recorded Lead II ECG and respiration throughout the test. E:I Ratio and ΔHR was calculated using this test. Last 6 cycles were used for calculation. Longest RR interval and shortest RR interval was selected from each cycle and average of longest RR interval and shortest RR interval was calculated, E:I ratio was calculated as ratio of average of longest RR interval to shortest RR interval. Maximum heart rate and minimum heart rate was selected from each cycle and average of maximum heart rate and minimum heart rate was calculated. ΔHR was calculated as difference between average maximum heart rate and average minimum heart rate.

Figure 4.6: Representative record of Deep Breathing test (DBT) of Healthy subject



(c) Hand grip test (HGT)

A rest of 5 minute was given to subjects. Baseline blood pressure was recorded at the end of 5 minute rest. Then Subjects were instructed to grip dynamometer using their maximum force with their dominant hand. Maximum voluntary contraction (MVC) was determined as the maximum grip force exerted by the subject. A mark was made on the dynamometer at 30% of MVC of subject and then subjects were instructed to sustain the grip for 4 minutes. Blood pressure was measured at 1st, 2nd, and 4th minutes during exercise. We recorded Lead II ECG and respiration throughout the procedure. ΔDBP was calculated by this test, ΔDBP was calculated as difference of maximum diastolic blood pressure during exercise to baseline diastolic blood pressure.

(d) Cold Pressor Test (CPT)

Baseline blood pressure was recorded at the end of 5-minute rest. Then subjects were instructed to immerse his/her hand in cold water (10°C) up to the wrist for 1 minute. Subject was requested to avoid touching the base of the container so as to expose maximum surface area of the hand in contact with cold water. Blood pressure was measured at 1st, 2.5th, and 5th minute post immersion of hand in cold water. We recorded Lead II ECG and respiration throughout the procedure. ΔDBP was calculated by this test. ΔDBP was calculated as difference of maximum diastolic blood pressure during the test to baseline diastolic blood pressure.

(e) Cold Face Test (CFT)

Patient was request to assume supine posture for Cold face test. After a rest period of 5 minutes, we put cold compresses (0-1°C) on subject's forehead for 30 sec. We recorded Lead II ECG and respiration throughout the procedure. CFT ratio was calculated as ratio of average RR interval of one minute recording just before putting the cold compresses on subject's forehead to maximum RR interval of 30 second duration of placement of cold compresses on subject's forehead (39).

The following parameters were assessed and included for statistical analysis:

Autonomic reactivity parameters

(1) Lying to standing test (LST)- Δ Systolic blood pressure and 30:15 ratio
(2) Deep breathing test (DBT)- E:I ratio and Δ HR
(3) Hand grip test (HGT)- Δ Diastolic blood pressure
(4) Cold pressor test (CPT)- Δ Diastolic blood pressure
(5) Cold face test (CFT)- CFT ratio

4.5 Administration of Head Down Tilt

Short term (5 minute) head down tilt of 6°, 15°, 30° each was given to subjects in supine position by tilt table. Tilt was given using a motorized tilt table. Subjects were instructed to lie supine on tilt table. A pillow was placed between subject's head and tilt table to provide cushion effect during the tilt. Three degree of tilt 6°, 15°, 30° were given each for 5 minute each with 5-minute supine rest between each degree of tilt. The order of the tilt administration was randomized. Sham tilt was provided before actual data acquisition to familiarize subjects with the procedure and allay apprehension, if any, associated with HDT.

Parameters calculated in Head down tilt

(1) Blood Pressure- Systolic blood pressure and Diastolic blood pressure were measured at baseline and at each minute during 5 minute duration of each degree of tilt. Blood pressure was measured using Omron model no- HEM-8712 digital Oscillometric blood pressure device.

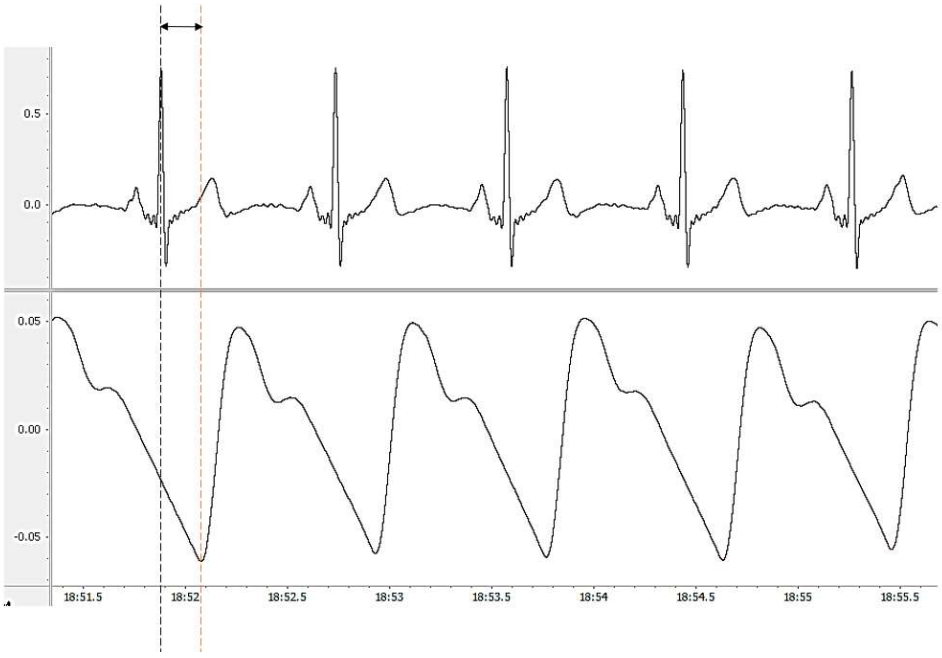
(2) Mean Heart Rate- Mean heart rate was calculated for baseline and for each minute during 5-minute duration of each degree of tilt. To calculate mean heart rate average of heart rate for each minute during 5 minute of tilt was taken. For baseline mean heart rate average of heart rate for 5 minute was taken. Mean heart was calculated from lead II ECG through LabChart Pro® Software.

(3) Pulse Transit Time- Calculated using finger pulse photoplethysmography and Lead II ECG, baseline pulse transit time was calculated for 5 minute of supine rest of each degree of tilt, then during each degree of tilt pulse transit time was calculated for 1st minute, 2nd minute, 3rd minute, 4th minute and 5th minute of tilt.

Calculation of Pulse transit time (PTT)

Pulse transit time was calculated as interval between R peak of ECG waveform to foot of successive PPG wave form as described previously (69–71,73). PTT was calculated for baseline and every minute post administration of HDT.

Figure 4.7: Calculation of Pulse transit time



Representative record of a healthy male subject aged 29 years. Top Panel shows lead II ECG and bottom panel shows Finger PPG signal. The time interval between two dotted lines (Corresponding to peak of R wave in lead II ECG and foot of finger PPG) represents pulse transit time (PTT).

4.6 Statistical analysis

The aforementioned parameters were tabulated in a spreadsheet program. Gaussian fit of parameters was assessed by Shapiro Wilk test. Values were expressed as Mean SD or Median (Interquartile range) depending on Gaussian fit. Unpaired t test or Mann Whitney U test were used for comparison. For repeated measures comparison, repeated measures ANOVA (RMANOVA) or Friedman's test was used. Appropriate post hoc tests were used for further comparison. A p value of <0.05 was considered as statistically significant.

Figure 4.8: Biopac MP 150® system

Biopac MP150® system was used to capture biological signals and transmit to the desktop system using LAN cable. The system used RESPEC-R module to obtain data wirelessly from Bionomadix® module.

Figure 4.9: Bionomadix® module

Bionomadix® module was strapped around the chest using the Velcro belt shown. The module acquired and transmitted ECG and respiratory movements data to the Biopac MP150 system.

Figure 4.10: Photoplethysmography Probe

Finger pulse (Infrared, reflection type photoelectric transducer-MLT1020 PPG)

Figure 4.11: Power Lab 26T® system used for PTT acquisition and analysis

Figure 4.12: Head down tilt administered using a motorized tilt table

5. Results

The present study was conducted in autonomic function lab, Department of physiology, AIIMS, Jodhpur, in temperature controlled and noise free environment. All the tests were performed between 9 am to 1 pm.

We enrolled 50 healthy subjects (mean age= 30.32±6.64, male=32, female=18) and 50 subjects with diabetes mellitus (mean age=36.16±8.59, male=32, female=18). Subjects' characteristics are shown in table 5.1

Table 5.1: Characteristics of study participants

	Healthy Subjects	**Patients with Diabetes mellitus**
Number	n=50, male=32, female=18	n=50, male=32, female=18
BMI (kg/m^2)	24.20±3.74	24.23±4.62
Age (years)	30.32±6.64	34.16±8.59
Height (cm)	165.06±10.19	164.36±10.21
Weight (kg)	66.51±15.18	65.42±13.36
Mean duration of diabetes	--	4.8 years

Comparison of autonomic function parameters between healthy subjects and patients with diabetes mellitus

Following parameters were assessed for autonomic function test.

1- Autonomic tone- Assessed by Heart rate variability (Time domain and frequency domain parameters are shown in table no- 5.2)
2- Autonomic Reactivity- Assessed by Cardiac autonomic reactivity tests (30:15 ratio, ΔSBP, E:I ratio, ΔHR, ΔDBP-HGT, ΔDBP-CPT, CFT ratio are shown in table: 5.3)

Following parameters were assessed during Head down tilt.

1- Systolic Blood Pressure
2- Diastolic Blood Pressure
3- Mean Heart Rate
4- Pulse Transit Time

Table 5.2: Summary of Autonomic tone parameters in healthy subjects and patients with diabetes mellitus

	Healthy	Patients with Diabetes Mellitus	*P* value
Population	n=50, male=32, female=18	n=50, male=32, female=18	
SDNN (ms)	42.98 (35.74-55.06)	27.48(21.15-42.20)	<0.0001*
SDSD (ms)	37.58(27.95)	22.34(14.42-35.64)	<0.0001*
RMSSD (ms)	38.62(28.15-55.61)	22.50(13.04-35.92)	<0.0001*
pNN50	16.29(6.79-32.95)	1.76(0.21-14.97)	<0.0001*
Total Power(ms^2)	1588(1144-2982)	737.1(396.8-1583)	<0.0001*
LF (ms^2)	475.8(268.3-851.1)	186.7(79.99-449.4)	<0.0001*
HF (ms^2)	723.9(360.9-1274)	166.8(65.82-602.1)	<0.0001*
LF/HF	0.79(0.34-1.37)	1.04(0.60-2.12)	0.07

Values expressed as Mean ± SD or Median (Interquartile range) based on fit. A p value of <0.05 was considered significant.

Table 5.3: Summary of Autonomic reactivity test parameters in healthy subjects and patients with diabetes mellitus

	Healthy	Patients with Diabetes Mellitus	*P* value
Population	n=50, male=32, female=18	n=50, male=32, female=18	
30:15 Ratio (LST)	1.23(1.10-1.51)	1.26(1.07-1.43)	0.79
ΔSBP (LST)	-2(-6.5-12.50)	-2(-6-12)	0.70
E:I Ratio (DBT)	1.33±0.11	1.27±0.14	0.04*
ΔHR (DBT)	22(18-25.25)	18(12.75-24)	0.01*
ΔDBP (HGT)	16(12-22.50)	18(12-24.50)	0.38
ΔDBP (CPT)	12(10-16.50)	10(10-14)	0.21
CFT Ratio	0.84(0.76-0.88)	0.86(0.80-0.92)	0.04*

Values expressed as Mean ± SD or Median (Interquartile range) based on fit. A p value of <0.05 was considered significant.

Cardiovascular parameters following HDT in healthy subjects

Systolic Blood Pressure response after 6° HDT

The systolic blood pressure values at 1st minute (117(105-124) mmHg), 2nd minute (115(102-122) mmHg), 3rd minute (115 (106-122) mmHg), 4th minute (114 (107.5-122) mmHg) and 5th minute (114 (106-122) mmHg) did not show any significant change in comparison to baseline (114 (103.5-122) mmHg) or in comparison to each other. (Friedman test, p= 0.27). The results are summarized in figure 5.1

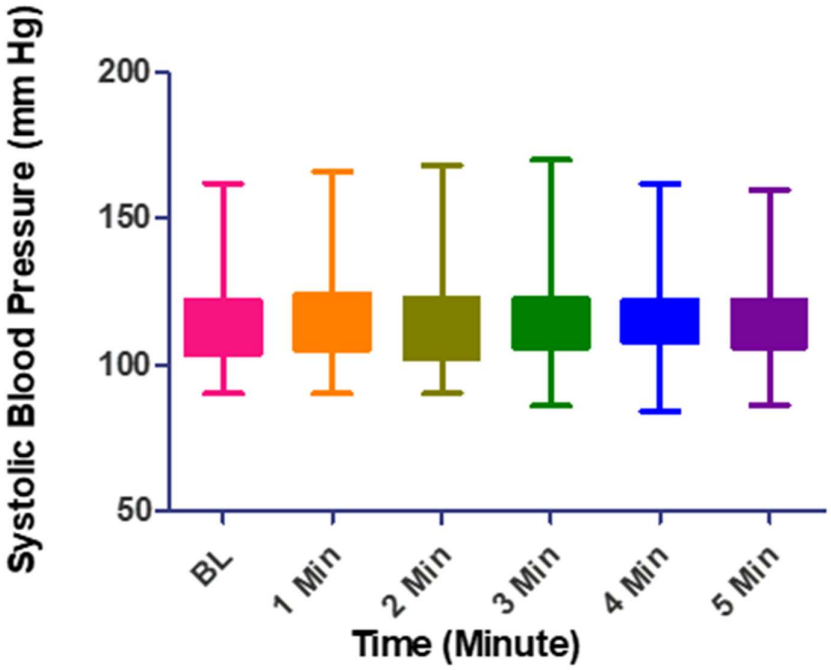


Figure 5.1: Response of systolic blood pressure after 6° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Diastolic Blood Pressure response after 6° HDT

The diastolic blood pressure values at 1st minute (72(66-80 mmHg), 2nd minute (72(66-78) mmHg) and at 3rd minute (72(66-78) mmHg) did not shows any significant change in comparison to baseline baseline (74 (68-78.50) mmHg).

The diastolic blood pressure values at 4th minute (71 (64-76) mmHg) and at 5th minute (70 (64-76.50) mmHg) were significantly lower in comparison to baseline (74 (68-78.50) mmHg). (Friedman test, *p=<0.001).*

The diastolic blood pressure values at 4th minute (71 (64-76) mmHg) and at 5th minute (70 (64-76.50) mmHg) were significantly lower in comparison to 1st minute (72(66-80 mmHg). The results are summarized in Figure 5.2

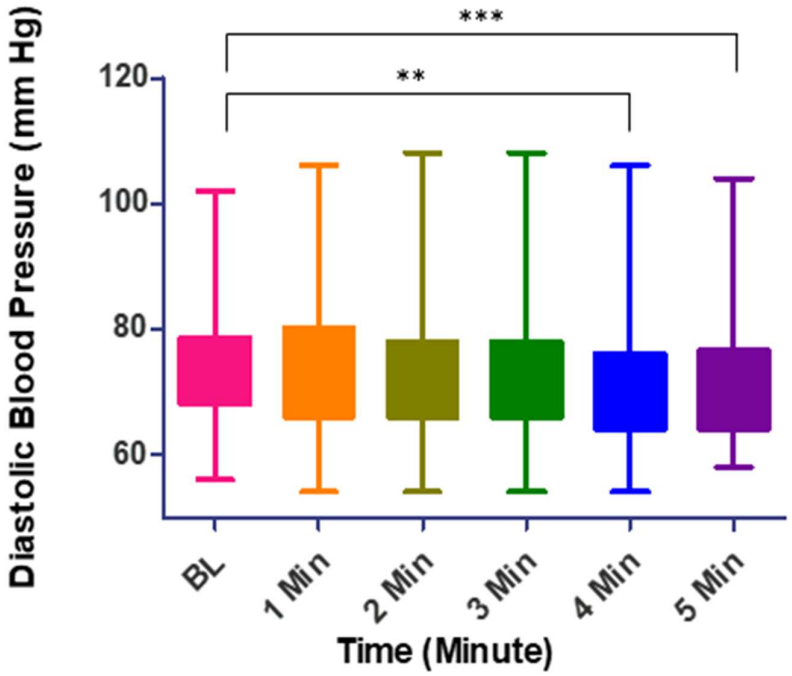


Figure 5.2: Response of Diastolic blood pressure after 6° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Mean Heart Rate response after 6° HDT

The mean heart rate values at 1st minute(67.34±8.30), 2nd minute(67.55±8.09) and 3rd minute(67.36±8.30) were significantly lower than baseline (68.73±8.74). (Repeated Measures ANOVA, p= 0.003). The results are summarized in Figure 5.3.

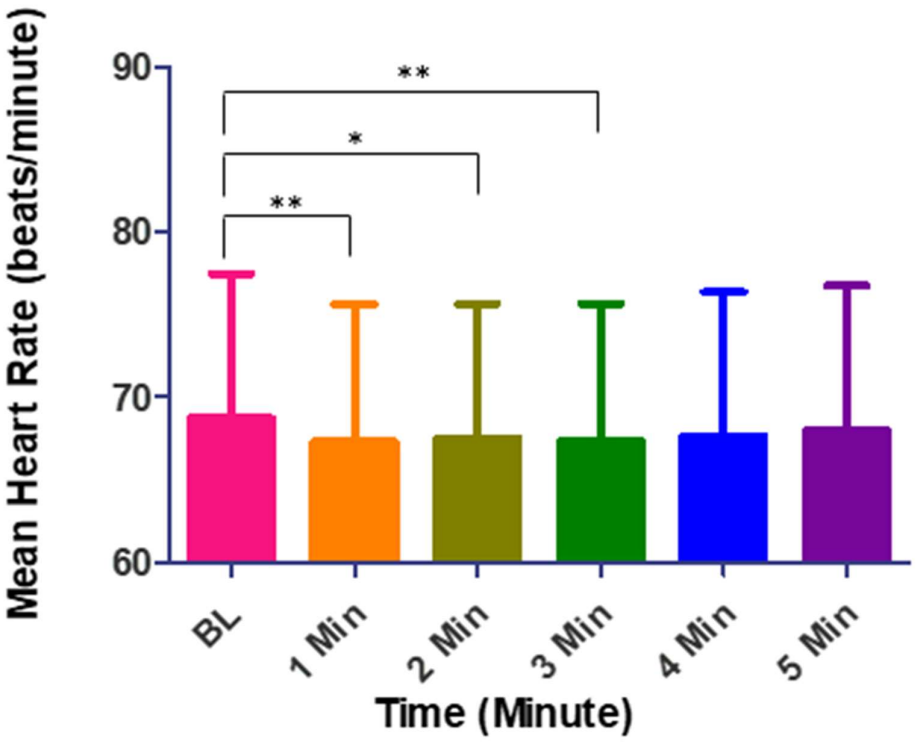


Figure 5.3: Response of mean heart rate after 6° HDT in healthy subjects. Data represented as bar plot. Error bar represents standard deviation (SD)

Pulse Transit Time response after 6° HDT

The Pulse Transit Time values at 1st minute (216.3 (198.4-228.7) ms), 2nd minute (213.4 (200.7-227.2) ms), 3rd minute (213.5 (200-226.5) ms), 4th minute (213.3 (202.1-226.8) ms) and 5th minute (213.3 (199.4-233.3) ms) did not shows any significant difference in comparison to baseline (213.7 (202-231) ms). (Friedman test, *p*- 0.62). The results are summarized in Figure 5.4.

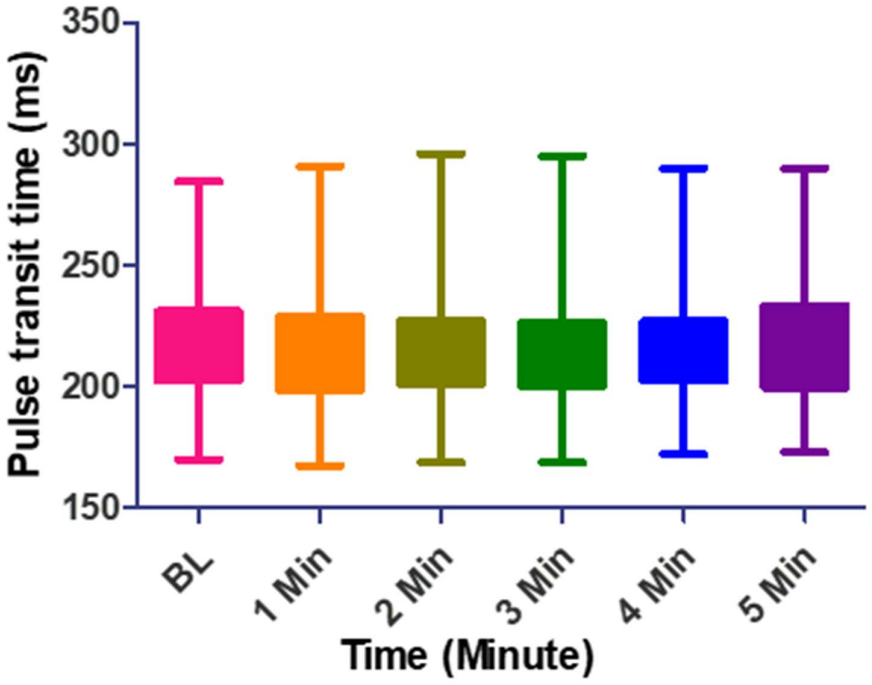


Figure 5.4: Response of pulse transit time after 6° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Systolic Blood Pressure response after 15° HDT

The Systolic blood pressure values at 1st minute (116 (106-124) mmHg), 2nd minute (115(102-126) mmHg), 3rd minute (115 (104-122.5) mmHg), 4th minute (115 (106-124) mmHg) and 5th minute (116 (106-124) mmHg) did not shows any significant change in comparison to baseline (116 (106-124) mmHg). (Friedman test, p=0.34). The results are summarized in Figure 5.5

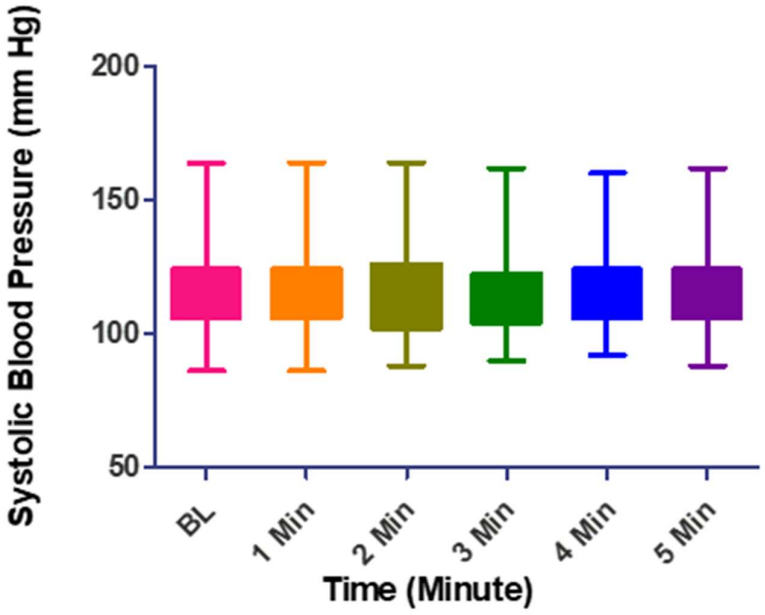


Figure 5.5: Response of systolic blood pressure after 15° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Diastolic Blood Pressure response after 15° HDT

The Diastolic blood pressure values at 2nd minute (70 (67.50-76.50) mmHg), 3rd minute (72 (68-76) mmHg), 4th minute (71 (68-78) mmHg) and 5th minute (70 (68-76) mmHg) were significantly lower then baseline value (74 (68-78.50) mmHg). (Friedman test, p=<0.0001). The results are summarized in Figure 5.6

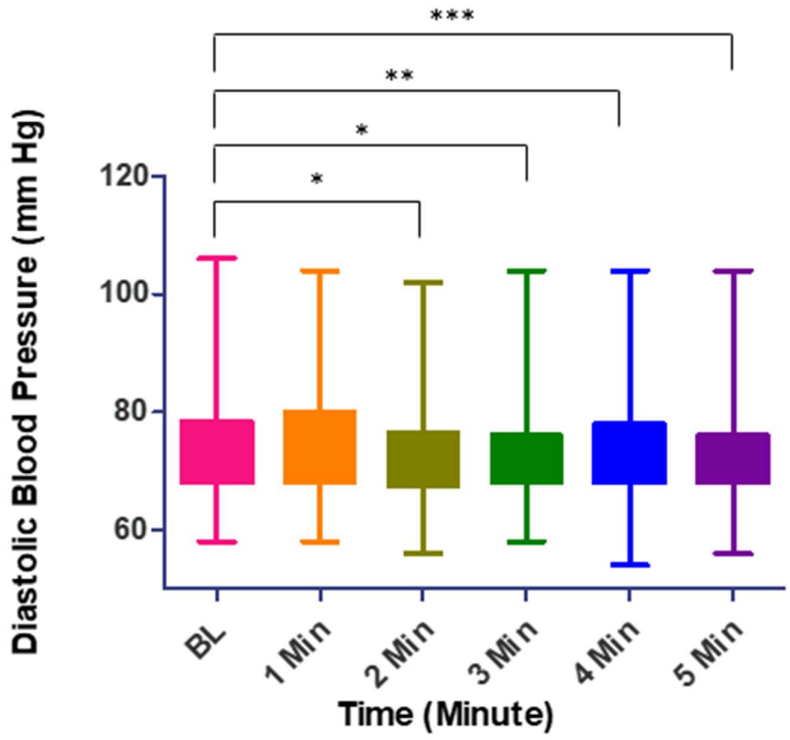


Figure 5.6: Response of diastolic blood pressure after 15° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Mean Heart Rate response after 15° HDT

The mean heart rate values at 1st minute (67.21±8.48), 2nd minute (67.33±8.61), 3rd minute (66.90±8.39), 4th minute (67.74±8.42) and 5th minute= 67.50±8.68) did not shows any significant difference in comparison to baseline (68.14±8.88).(Repeated Measures ANOVA, *p= 0.053).* The results are summarized in Figure 5.7

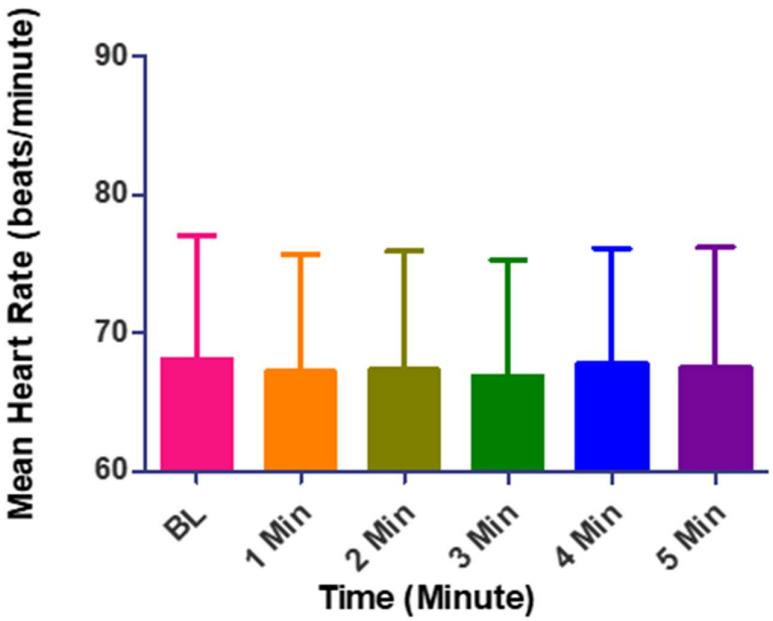


Figure 5.7: Response of mean heart rate after 15° HDT in healthy subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Pulse Transit Time response after 15° HDT

The Pulse transit time values at 1st minute (216.3 (203-239.2) ms), 2nd minute (217.6 (202.6-237) ms), 3rd minute (216.7 (202.4-232.7) ms), 4th minute (217.1 (201.4-235.4) ms)and 5th minute (216.6 (204.5-233.7) ms) did not shows any significant difference in comparison to baseline (216.6 (202.5-230)). (Friedman test, p=0.98). The results are summarized in Figure 5.8

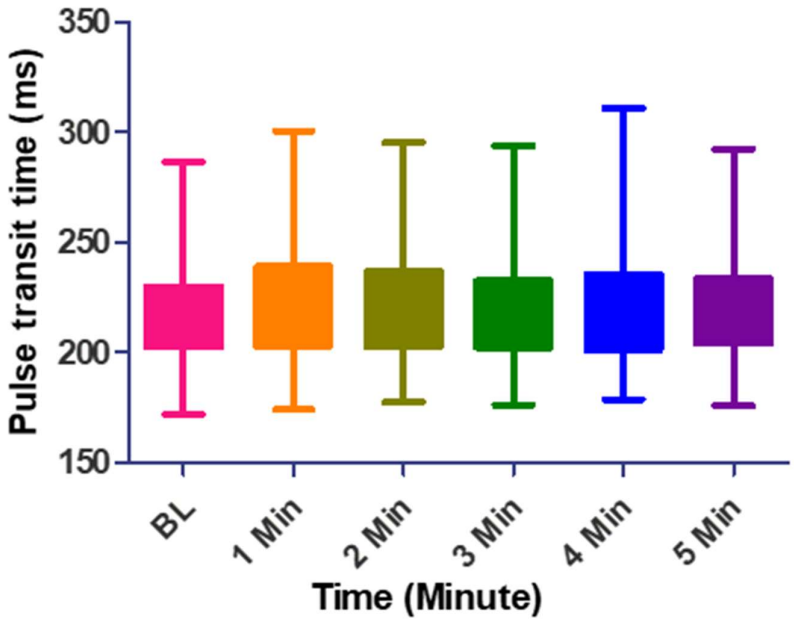


Figure 5.8: Response of Pulse transit time after 15° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Systolic Blood Pressure response after 30° HDT

The Systolic blood pressure values at 1st minute (116.6±14.46), 2nd minute (116.7±13.22), 3rd minute (117±13.45), 4th minute (117.4±14.07) and 5th minute (117.3±13) were significantly higher in comparison to baseline (114.4-13.13). (Repeated Measures ANOVA, p= <0.0001). The results are summarized in Figure 5.9

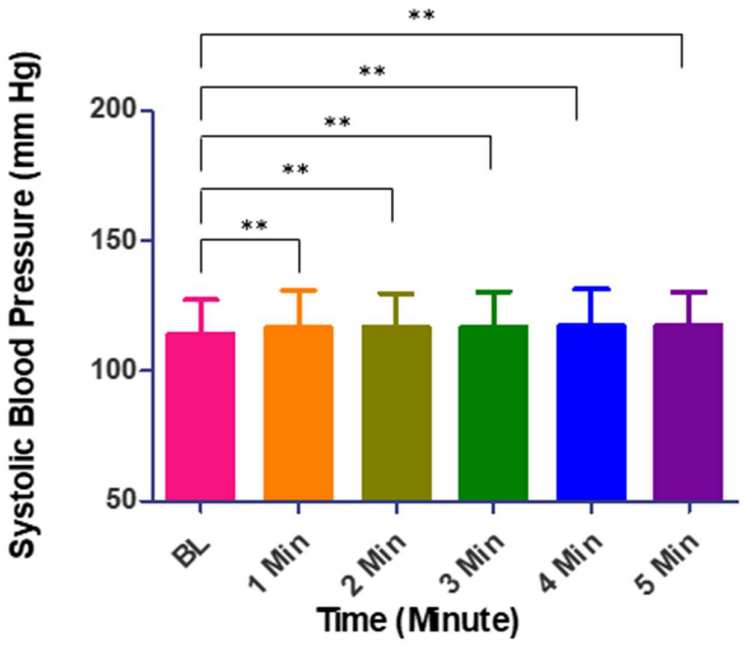


Figure 5.9: Response of systolic blood pressure after 30° HDT in healthy subjects. Data represented by bar plot. Error bar represented standard deviation (SD)

Diastolic Blood Pressure response after 30° HDT

The diastolic blood pressure values at 1st minute (72(69.50-78.50) mmHg), 2nd minute (71(68-76.50) mmHg), 3rd minute (72(66-78) mmHg), 4th minute (73(68-78) mmHg) and 5th minute (72(66-78) mmHg) did not shows any significant difference in comparison to baseline (73(68-80) mmHg).

The Diastolic blood pressure values at 2nd minute (71(68-76.50) mmHg), 3rd minute (72(66-78) mmHg) and 5th minute (72(66-78) mmHg) shows significant change in comparison to 1st minute (72(69.50-78.50) mmHg). (Friedman test, p= 0.0006). The results are summarized in Figure 5.10

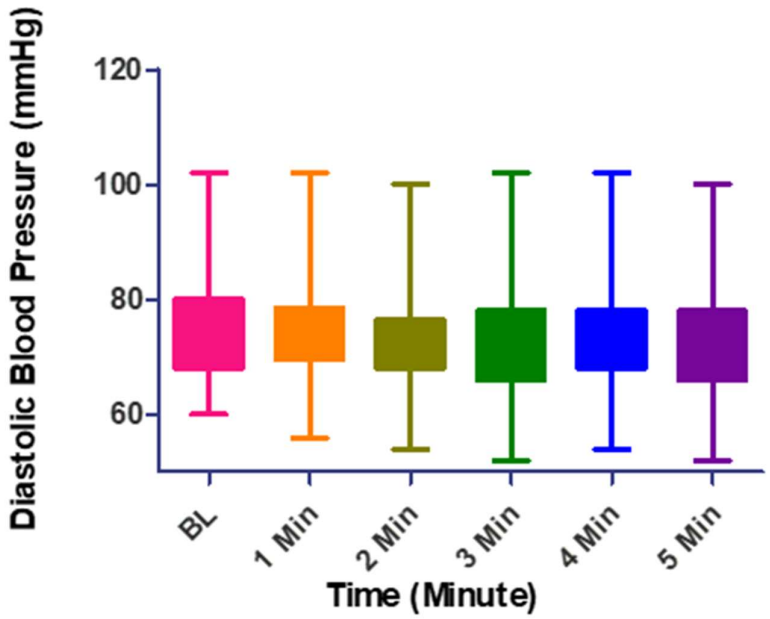


Figure 5.10: Response of diastolic blood pressure after 30° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Mean Heart Rate response after 30° HDT

The Mean heart rate values at 1st minute (69.15±8.78), 2nd minute (68.32±8.90), 3rd minute (68.31±8.70), 4th minute (67.89±8.70) and at 5th minute (68.08±8.98) did not shows any significant difference in comparison to baseline (68.95±8.76). (Repeated Measures ANOVA, p=0.16). The results are summarized in Figure 5.11

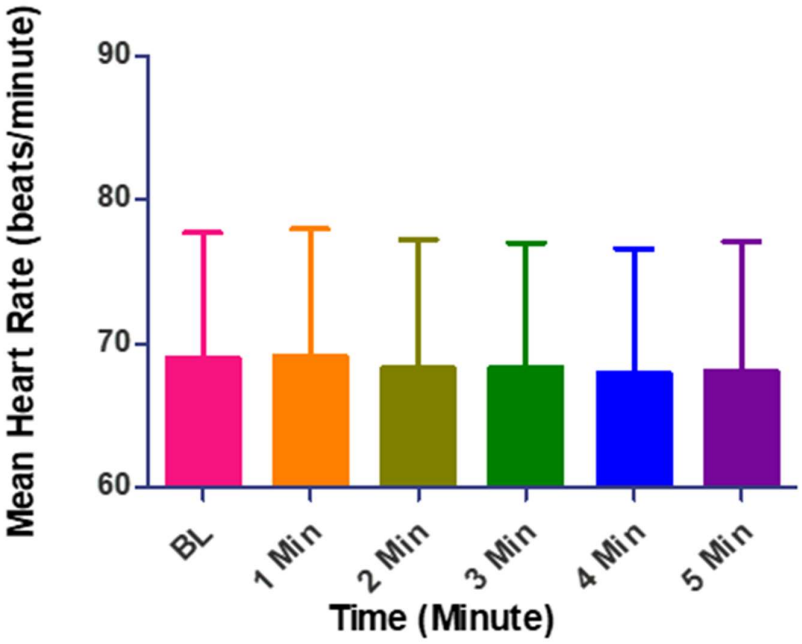


Figure 5.11: Response of mean heart rate after 30° HDT in healthy subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Pulse Transit Time response after 30° HDT

The Pulse transit time values at 1st minute (216.5(205.4-235.5) ms) and 2nd minute (217.2(205.8-235.8) ms) did not shows any significant difference in comparison to baseline (215.6(203.9-227.1).

The Pulse transit time values at 3rd minute (218.8(206.3-233.7) ms), 4th minute (219.1(207.3-234.3) ms) and 5th minute (218.6 (206.7-235.5) ms) shows significant increase in comparison to baseline (215.6(203.9-227.1).

The Pulse transit time values at 3rd minute (218.8(206.3-233.7) ms), 4th minute (219.1(207.3-234.3) ms) and 5th minute (218.6 (206.7-235.5) ms) shows significant increase in comparison to 1st minute (216.5(205.4-235.5) ms). (Friedman test, p= <0.0001). The results are summarized in Figure 5.12

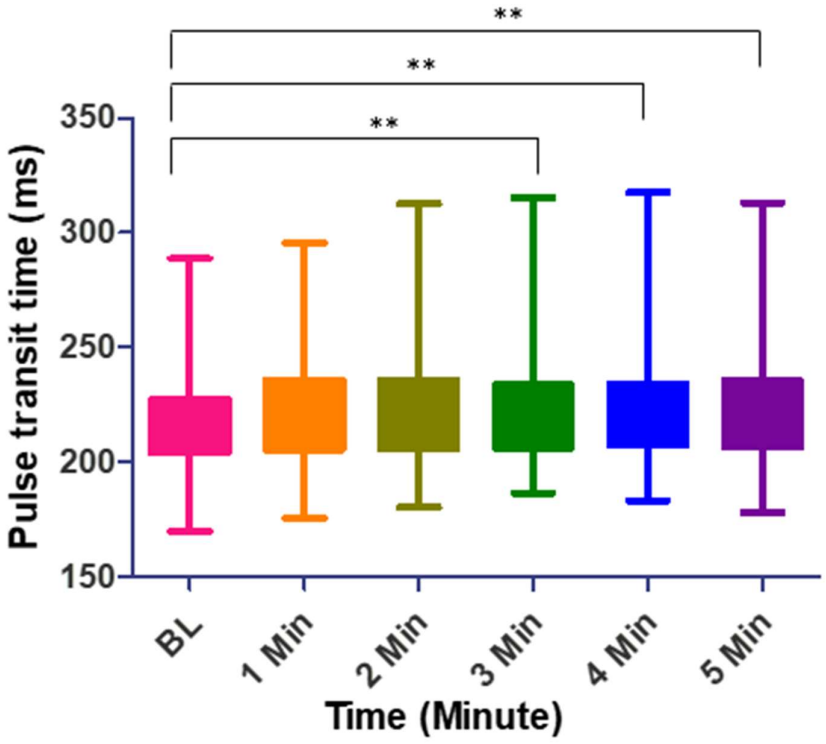


Figure 5.12: Response of pulse transit time after 30° HDT in healthy subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Cardiovascular parameters following HDT in patients with diabetes mellitus

Systolic Blood Pressure response in patients with diabetes mellitus after 6° HDT

The Systolic blood pressure values at 1st minute (124.8±14.80 mmHg) and 2nd minute (124.2±13.48 mmHg) were significantly higher in comparison to baseline (122.3±13.76 mmHg). (Repeated Measures ANOVA, p= <0.0001)

The Systolic blood pressure values at 3rd minute (123.3±13.97 mmHg), 4th minute (122.3±13.87 mmHg) and 5th minute (123±14.27 mmHg) did not shows any significant difference in comparison to baseline (122.3±13.76 mmHg). The results are summarized in Figure 5.13

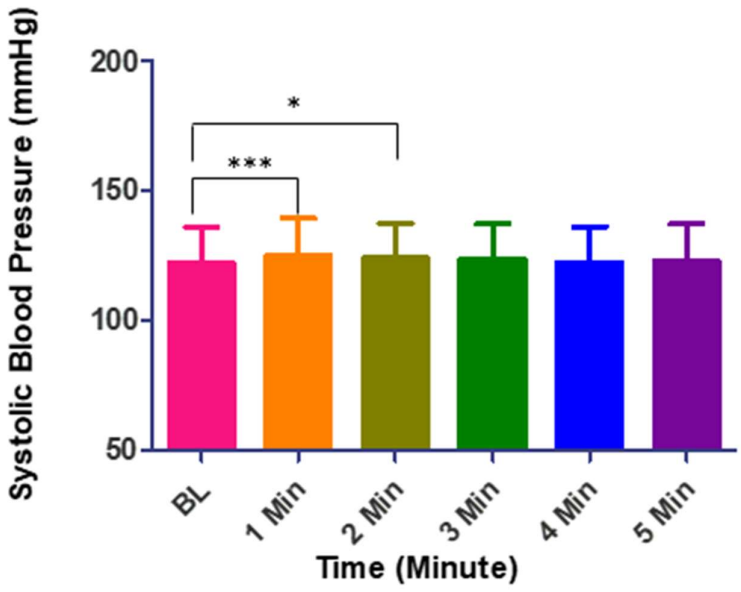


Figure 5.13: Response of systolic blood pressure after 6° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Diastolic Blood Pressure response in patients with diabetes mellitus after 6° HDT

The diastolic blood pressure values at 4th minute (78.42±10.26 mmHg) and at 5th minute (78.38±10.52 mmHg) were significantly lower in comparison to baseline (79.88±9.50 mmHg).

The diastolic blood pressure values at 1st minute (80.88±9.81 mmHg), 2nd minute (79.77±9.97 mmHg) and 3rd minute (79.35±10.01) did not show any significant difference in comparison to baseline (79.88±9.50 mmHg). (Repeated Measures ANOVA, p= <0.0001). The results are summarized in Figure 5.14

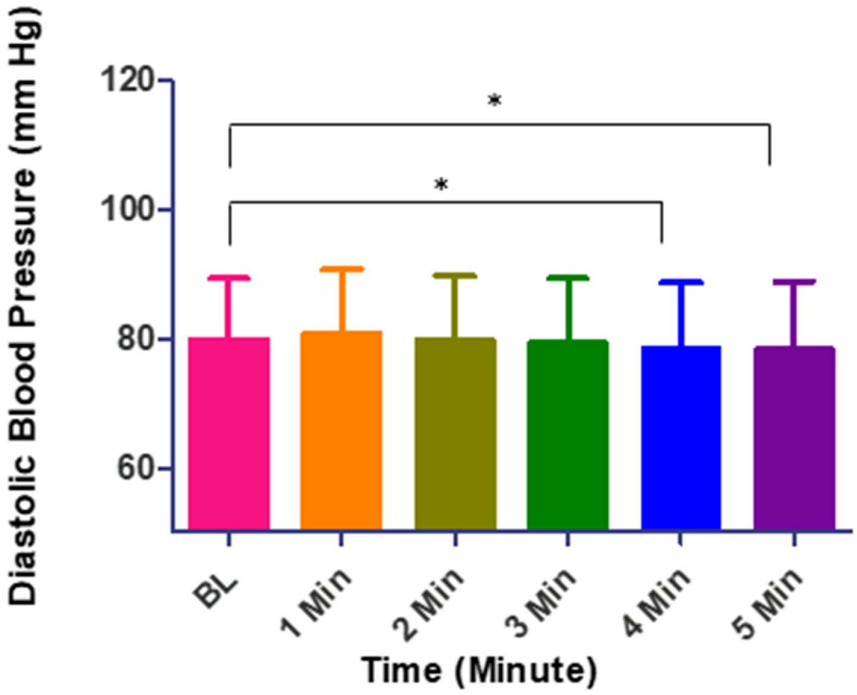


Figure 5.14: Response of diastolic blood pressure after 6° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Mean Heart Rate response in patients with diabetes mellitus after 6° HDT

The Mean heart rate values at 1st minute (72.52 (64.65-85.24) beats/ minute), 2nd minute (73.16(65.73-83.67) beats/ minute), 3rd minute (72.77(65.82-83.74) beats/minute), 4th minute (72.83(64.80-85.24) beats/minute) and 5th minute (72.95(65.85-84.30) beats/minute) did not shows any significant difference in comparison to baseline (73.60(65.02-85.29) beats/minute). (Friedman test, p= 0.05). The results are summarized in Figure 5.15

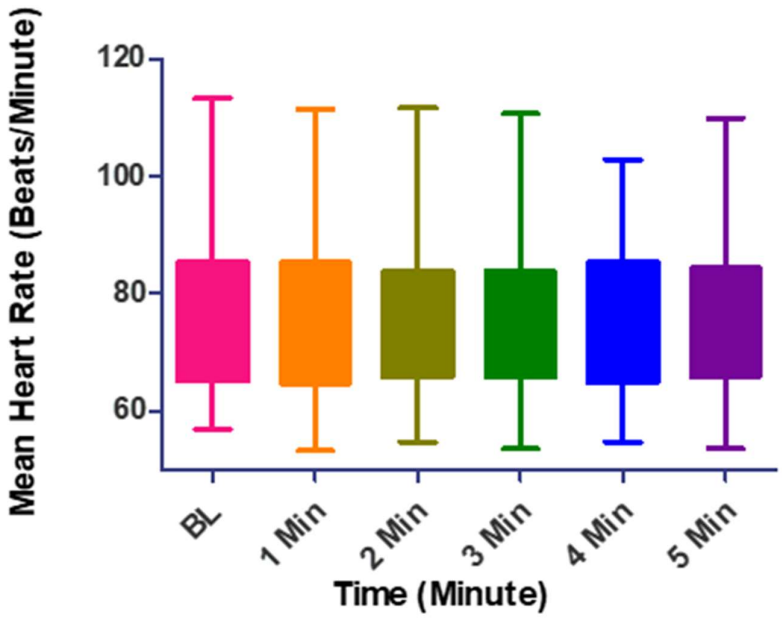


Figure 5.15: Response of mean heart rate after 6° HDT in DM subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Pulse Transit Time response in patients with diabetes mellitus after 6° HDT

The Pulse Transit time values at 1st minute (208.1(192.5-226.3) ms), 2nd minute (207.6 (194.8-225) ms), 3rd minute (207.9(195.1-222.6) ms), 4th minute (204.8(194.5-225.4) ms) and 5th minute (205.5(194.4-223.7) ms) did not show any significant difference in comparison to baseline (206.7(195.6-223.7) ms). (Friedman test, p= 0.17). The results are summarized in Figure 5.16

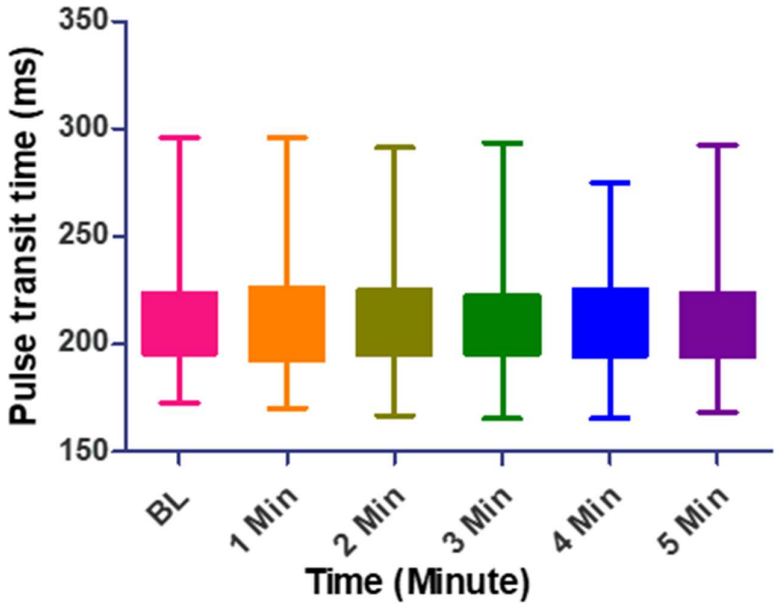


Figure 5.16: Response of pulse transit time after 6° HDT in DM subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Systolic Blood Pressure response in patients with diabetes mellitus after 15° HDT

The systolic blood pressure values at 1st minute (123.5±13.78) mmHg, 2nd minute (123±13.78) mmHg, 3rd minute (122.5±13.86) mmHg, 4th minute (123.1±14.01) mmHg and 5th minute (122.7±14.10) mmHg were significantly higher in comparison to baseline (120.7±12.08) mmHg. (Repeated Measures ANOVA, p= <0.0001). The results are summarized in Figure 5.17

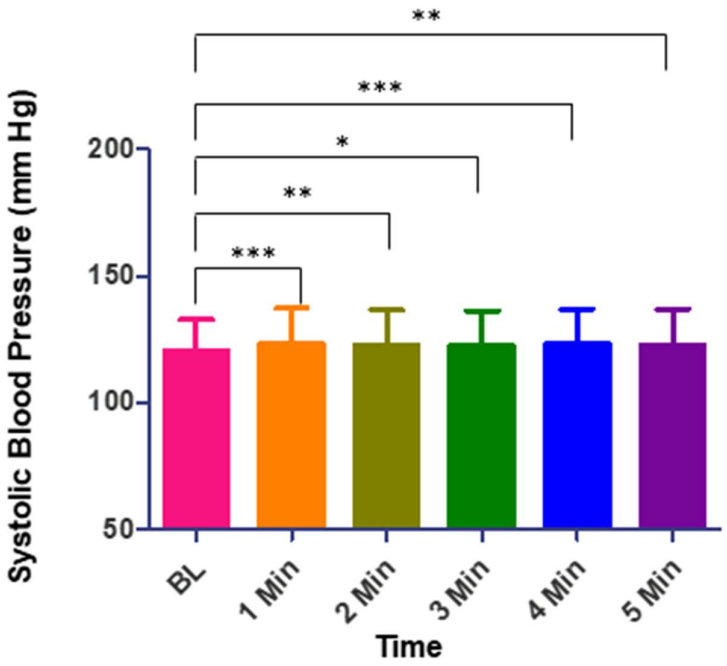


Figure 5.17: Response of systolic blood pressure after 15° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Diastolic Blood Pressure response in patients with diabetes mellitus after 15° HDT

The Diastolic blood pressure value at 3 minute (77.96±10.49) mmHg was significantly lower in comparison to value at 1st minute (79.46±10.75) mmHg. (Repeated Measures ANOVA, p= 0.0037). The diastolic blood pressure values at 1st minute, 2nd minute, 3rd minute, 4th minute, 5th minute did not show any significant difference in comparison to baseline. The results are summarized in Figure 5.18

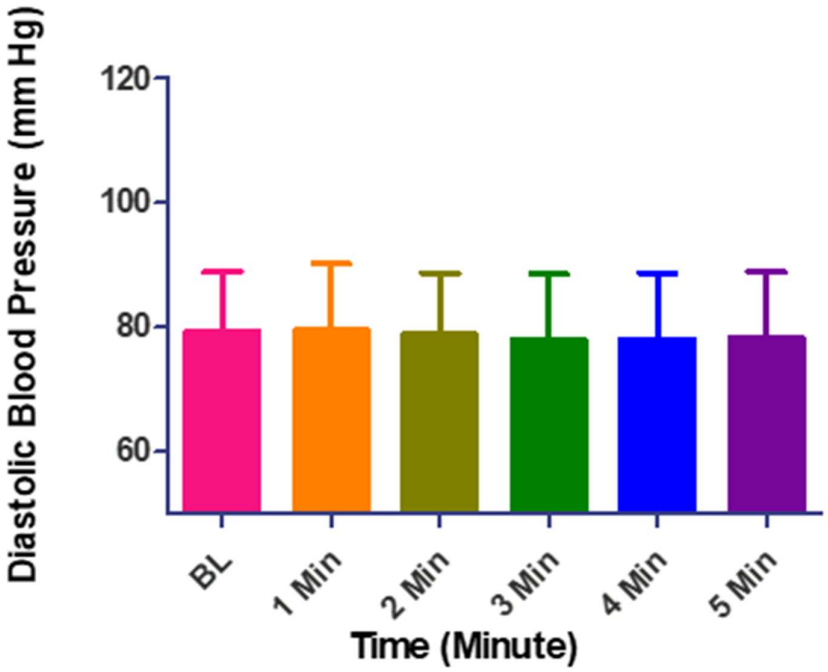


Figure 5.18: Response of diastolic blood pressure after 15° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Mean Heart Rate response in patients with diabetes mellitus after 15° HDT

The Mean heart rate values at 1st minute, 2nd minute, 4th minute and 5th minute did not show any significant change in comparison to baseline.

The Mean heart rate value at 3rd minute (72.34 (65.14-83.13) beats/minute) was significantly lower in comparison to baseline (73.92 (65.37-83.94) beats/minute). (Friedman test, p= 0.01). The results are summarized in Figure 5.19

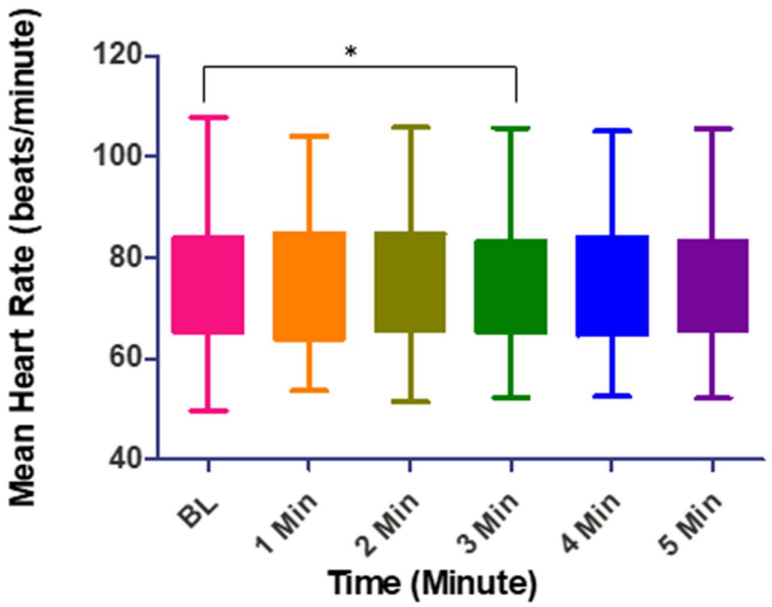


Figure 5.19: Response of mean heart rate after 15° HDT in DM subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Pulse Transit Time response in patients with diabetes mellitus after 15° HDT

The Pulse transit time values at 1st minute, 2nd minute, 3rd minute, 4th minute, 5th minute did not show any significant change in comparison to baseline. (Friedman test, p=0.76). The results are summarized in Figure 5.20

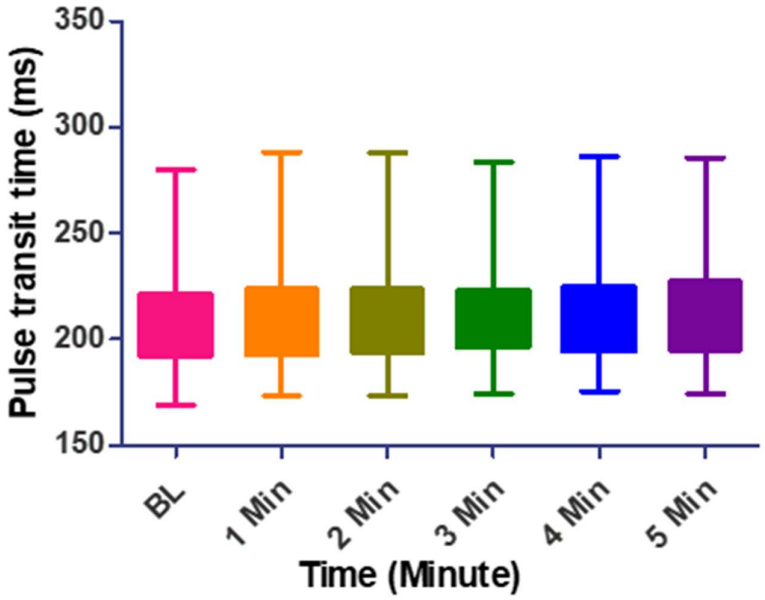


Figure 5.20: Response of Pulse transit time after 15° HDT in DM subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Systolic Blood Pressure response in patients with diabetes mellitus after 30° HDT

The Systolic blood pressure values at 1st minute (125.3±14.35) mmHg, 2nd minute (125.7±14.14) mmHg, 3rd minute (125.3±14.32) mmHg, 4th minute (126±13.59) mmHg and 5th minute (126.2±13.89) mmHg were significantly higher in comparison to baseline (121±12.79) mmHg. (Repeated Measures ANOVA, p= <0.0001). The results are summarized in Figure 5.21.

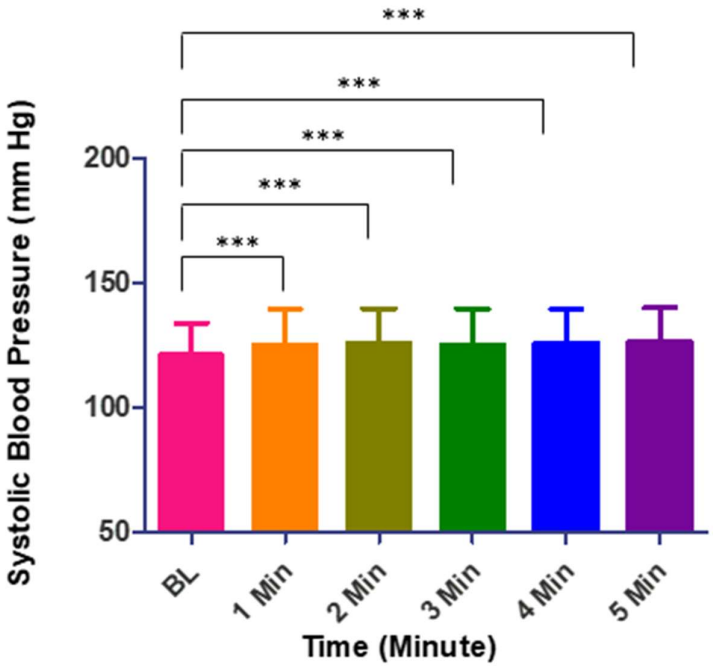


Figure 5.21: Response of systolic blood pressure after 30° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

Diastolic Blood Pressure response in patients with diabetes mellitus after 30° HDT

The Diastolic blood pressure value at 1st minute (81.12±10.41) mmHg was significantly higher in comparison to baseline (79.19±9.74) mmHg. (Repeated Measures ANOVA, p=0.01)

The Diastolic blood pressure values at 2nd minute, 3rd minute, 4th minute, 5th minute did not show any significant change in comparison to baseline. The results are summarized in Figure 5.22

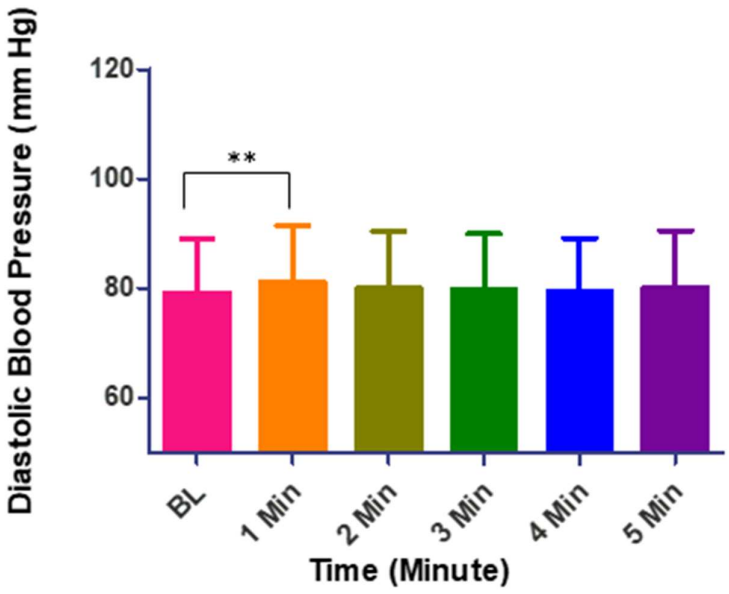


Figure 5.22: Response diastolic blood pressure after 30° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

.

Mean Heart Rate response in patients with diabetes mellitus after 30° HDT

The Mean heart rate values at 1st minute, 2nd minute, 3rd minute, 4th minute and 5th minute did not show any significant change in comparison to baseline. (Friedman test, p= 0.32). The results are summarized in Figure 5.23

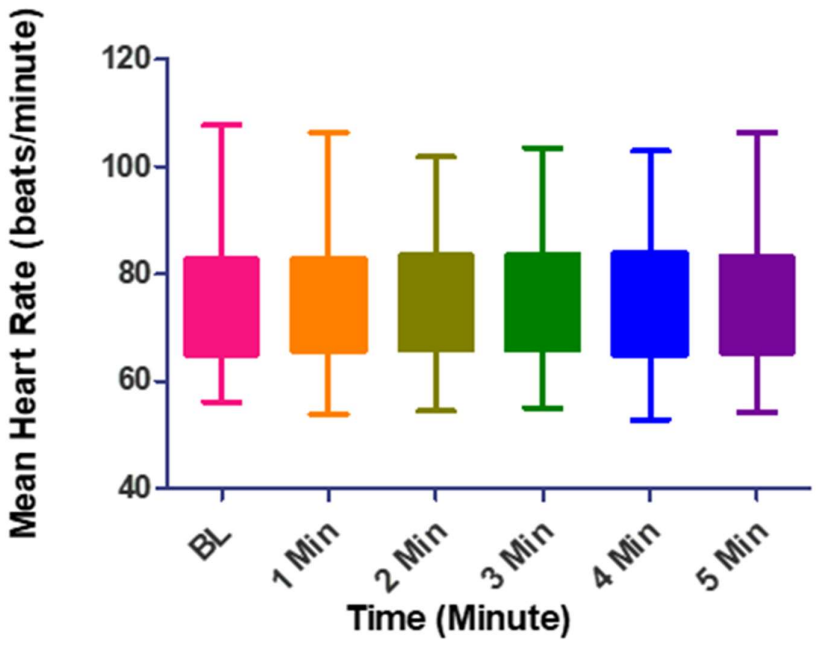


Figure 5.23: Response of mean heart rate after 30° HDT in DM subjects. Data represented as box and whisker plot. Whisker represents minimum to maximum values.

Pulse Transit Time response in patients with diabetes mellitus after 30° HDT

The Pulse transit time values at 1st minute (215.3±24.66) ms, 2nd minute (213.9±24.64) ms, 3rd minute (214.1±25.54) ms, 4th minute (214.7±25.68) ms were significantly higher in comparison to baseline (210.8±21.83) ms. (Repeated Measures ANOVA, p=0.0003). The results are summarized in Figure 5.24

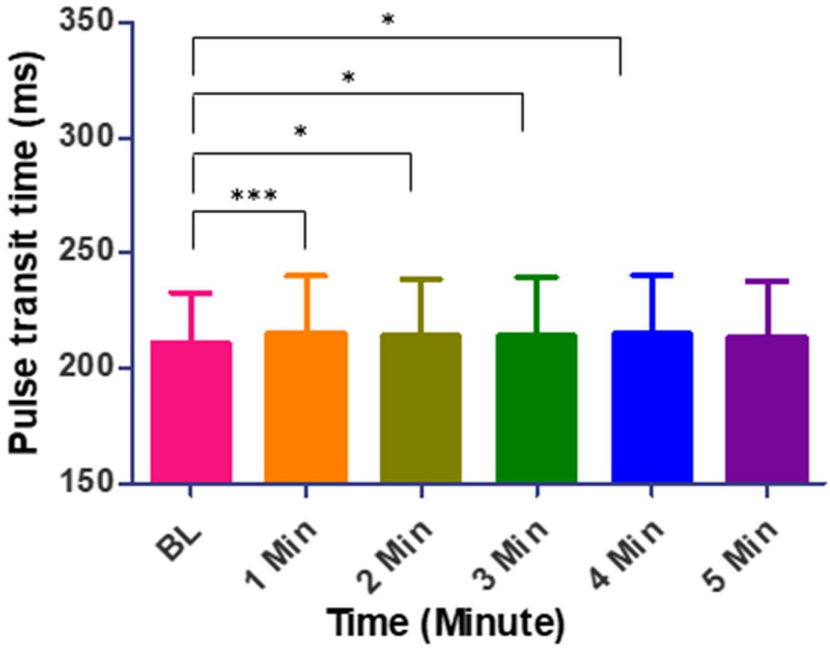


Figure 5.24: Response of pulse transit time after 30° HDT in DM subjects. Data represented by bar plot. Error bar represents standard deviation (SD)

6. Discussion

The present study was conducted at the Autonomic function laboratory, Department of Physiology AIIMS Jodhpur. We enrolled 50 healthy subjects (32 males, 18 females) and 50 patients with diabetes mellitus (32 males, 18 females).

Autonomic tone was assessed using Heart rate variability (HRV) indices and expressed in time and frequency domains. Autonomic reactivity was assessed by Ewing's battery of tests, as proposed by Ewing and colleagues and further modified as per AIIMS New Delhi criteria (37). Valsalva maneuver was excluded from the battery in view of the COVID-19 pandemic. Vascular stiffness was assessed using Pulse transit time (PTT), computed using photoplethysmography (PPG) signal and Lead II ECG.

Autonomic function testing is performed using assessment of autonomic tone and reactivity. HRV provides a window into the resting autonomic modulation of the cardiovascular system by quantifying the beat to beat fluctuations in the RR intervals recorded over a 5 minute time frame (74,75). Autonomic reactivity assesses response to standard stimuli such as posture change, cold temperature, deep breathing and isometric exercise to compare blood pressure and heart rate responses with baseline to find out the 'reactivity' of the physiological system to stimuli.

Diabetes mellitus is one of the commonest endocrinopathies seen in today's era. Cardiac autonomic neuropathy is the hallmark of this disease and manifests as a broad spectrum of symptoms. These may be as benign as resting tachycardia and as severe as myocardial arrhythmias culminating in sudden cardiac death. Therefore, a lot of emphasis has been placed on the screening of CAN in this patient population using tests of autonomic function described.

Our study population - both the healthy subjects and patients with DM - displayed normal results for the parameters of Autonomic reactivity. However, patients with DM exhibited reduced indices of HRV, which is suggestive of early setting in of autonomic neuropathy. This is in accordance with previous literature wherein changes in HRV precede deterioration in reactivity parameters by few years. (76,37,77). For the same reason, HRV is shown to be a sensitive and specific early predictor of cardiac autonomic neuropathy (78–80). Since the mean duration of diabetes in our subjects was 4.8 years, CAN was probably in its early stages manifesting as impaired tone with

normal reactivity parameters. In our study we found that E:I Ratio and ΔHR of DM subjects were significantly lower in comparison to healthy subjects. This is showing early vagal involvement in subjects with diabetes mellitus which is in accordance with findings of previous studies (77).

Head down tilt produces a fluid shift towards thoracic compartment leading to engagement of cardiopulmonary receptors. The currently available corpus of HDT is heterogenous with respect to the degree and duration of tilts administered. 6-degree HDT is the commonest tilt administered, followed by 10 and 30 degrees. However, the estimation of physiological parameters after HDT have been performed at different time points leading to difference in observations.

We observed no significant changes in Systolic blood pressure values in HDT at 6 degrees and 15 degrees in healthy subjects. This is consistent with previous reports of small magnitudes of HDT not leading to perturbation in blood pressure and heart rate (64,81,82). However, 30 degrees of HDT showed significant increases in SBP is both healthy subjects and patients with diabetes mellitus. We believe that a tilt of 30 degrees of inclination for 5 minutes causes sufficient loading of baroreceptors leading to an enhanced blood pressure response. Interestingly, a HDT of 15 degrees also causes statistically significant increases in SBP in patients with Diabetes mellitus. However, the magnitude of the changes reported at 15 degrees of HDT were found to be quite small (~2 mm Hg) and may not have much of physiological relevance.

A 6 degree HDT administered for 6 minutes has been shown to reduce MSNA suggestive of sympathetic withdrawal (4). However, we did not observe statistically significant increase in Pulse transit time (PTT) at 6 degree and 15-degree HDT either in healthy subjects or patients with diabetes mellitus. Only on administration of 30-degree HDT, did we observe a statistically significant increase in PTT, suggestive of reduction in vascular stiffness, as a result of administration of HDT. This may be due to the fact that during the short 5-minute window of HDT, significant pooling of blood occurred only at 30 degrees of HDT with subsequent vasodilatation in the forearm blood vessels. A tilt of 6 and 15 degrees may not have been sufficient to induce requisite pooling for headward fluid shift in the five-minute window.

In addition, the response is different between patients with diabetes mellitus and healthy subjects. In patients with diabetes mellitus, the increase in PTT manifests immediately

starting at 1st minute of HDT and continuing till 4th minute. However, in healthy subjects, the rise is PTT manifests with a lag of two minutes, continuing from 3rd to 4th minute of tilt. We had initially hypothesized that patients with diabetes mellitus were likely to show an increased latency of response due to underlying autonomic neuropathy. However, our present results of pulse transit time changes do not support our initial hypothesis. This may be due to the fact that all patients were in the early phase of CAN, as evident from results of autonomic testing. Therefore, this particular phenomenon needs further exploration taking into account the various factors that can affect vascular stiffness in patients with diabetes mellitus.

The present study highlights the important cardiovascular changes in the immediate post tilt period after 6, 15 and 30 degrees of head down tilt in healthy subjects and patients with diabetes mellitus. The patient population was in early stages of CAN, as assessed by reduced autonomic tone with preserved reactivity.

In our best knowledge there is no study available that compare PTT in healthy subjects and patients with diabetes mellitus in the immediate short-term window following 6, 15, 30 degrees of head down tilt. Therefore, this evaluation of vascular stiffness may open new avenues for exploration.

Further studies on larger sample sizes may provide a better understanding of the hemodynamic alterations resulting from head down tilt in healthy subjects and patients with diabetes mellitus. These observations will help understand the responses of the cardiovascular system during exposure to microgravity conditions.

7. Summary

We enrolled 50 healthy and 50 subjects with diabetes mellitus. Autonomic tone and reactivity parameters were evaluated. We administered 6, 15, 30 degrees of HDT for five minutes in random order to all subjects with 5-minute rest between each maneuver. We observed no significant changes in Systolic blood pressure values in HDT at 6 degrees and 15 degrees in healthy subjects, in accordance with previous literature. However, 30 degrees of HDT showed significant increases in SBP is both healthy subjects and patients with diabetes mellitus, suggestive of receptor engagement.

In addition, pulse transit time was evaluated post aforementioned HDT for 5 minutes. We observed increase in PTT post 30-degree HDT, with difference in latency of response seen in healthy subjects and patients with diabetes mellitus. This peculiar phenomenon may be further explored to understand underlying mechanisms.

The immediate 5-minute window post HDT is important to understand underlying cardiovascular regulatory mechanisms. Our study is an initial step in this direction, especially from a vascular stiffness perspective. Further studies with large sample sizes will help in evolution of better understanding of underlying physiological mechanisms.

8. Strengths and limitations

This study provides valuable information regarding the cardiovascular changes in the immediate period post HDT at 6, 15 and 30 degrees in healthy subjects and patients with DM. We observed significant increases in SBP after 30-degree HDT in both healthy subjects and patients with DM. In addition, reduction in vascular tone was observed after administration of 30-degree HDT suggestive of sympathetic withdrawal and resultant reduction in vascular stiffness.

Addition of beat-to-beat blood pressure may have provided valuable information regarding blood pressure transients as a result of HDT. Also, it may have given information about baroreflex sensitivity thus helping understand the underlying changes which we could completely explain in the present study. Since beat-to-beat blood pressure device was not available at the institute, we used Oscillometric measurement of BP which is an important limiting factor in our study.

Future directions

We were able to document the cardiovascular responses to HDT in healthy subjects and patients with diabetes mellitus. Future studies on large samples with addition of more parameters will help better understand the exact nature of the changes occurring in HDT and the underlying mechanisms.

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Annexure I: Institutional Ethics Certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3551 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3386**

Project title: **"Evaluation of cardiovascular responses to short term head down tilt in healthy subjects and diabetic patients"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Mamta Shobhawat**
Guide:	**Dr. Shival Srivastav**
Co-Guide:	**Dr. Om Lata Bhagat & Dr. Ravindra Kumar Shukla**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Pravesh Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
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Annexure II: Patient information sheet (English)

All India Institute of Medical Science Jodhpur, Rajasthan
Department of Physiology

PATIENT INFORMATION SHEET

Title of the Project- Evaluation of cardiovascular responses to short term head down tilt in healthy subjects and diabetic patients.

Candidate- Dr Mamta Shobhawat Mobile no- 9602782163

You are invited to take part in the above mentioned study. This study is being done to evaluate the cardiovascular responses in healthy subjects and diabetic patients when short term head down tilt is given. If you agree to participate in this study you will be required to perform 4 different tests of cardiac autonomic function for which we will record your blood pressure and breathing pattern with a belt and electrical activity of the heart with leads attached to your shoulder and neck. In the first test you will be required to stand from a lying down position and keep on standing for 5 minutes in which we will be recording your blood pressure intermittently. In the second test you will be asked to sustain a handgrip, following which your blood pressure changes will be recorded periodically. In the third test you have to immerse your hand in cold water for 1 minute and change in your blood pressure will be noted at 1 min, 2.5 min, and 5 min. In the 4^{th} test cold compresses (0-1°C) will be applied on your forehead for 30 sec while we will record your ECG and respiration continuously. For all test will have a gap of 5 min in between, rest will be provided during this time. Finger pulse will be recorded using a photoplethysmography probe attached to the index finger using a Velcro strap. After AFT you will be requested to lie supine on tilt table and rest for 5 minute will be given than a baseline BP will be measured at the end of 5 minute rest. Then you will be given 6° head down tilt for 5 minute followed by 5 minute of supine rest. HDT will be repeated for 15° and 30° giving a gap of 5 minutes between the manoeuvres. Lead II ECG and finger PPG will be recorded during the entire period. Blood pressure will be taken at baseline and 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} minute during every HDT maneuver.

The expected duration of your stay in the laboratory will be approximately one hour. The above mentioned tests will be conducted in the Autonomic function test laboratory, Department of Physiology, AIIMS Jodhpur. You are requested to attend all of the above mentioned tests in single sitting, depending upon your and your investigator's mutual time and comfort. Although there is no risk involved in performing the above stated tests even then these tests will be done under supervision of a physician. Test will be done 2 hour after light breakfast. You are also requested to refrain from tea, coffee, or any other beverage two hours prior to conduction of these tests. Your participation in this study is voluntary and you are free to leave this study anytime. Also, your identity shall be kept confidential.

For further Queries-

Dr Mamta Shobhawat-9602782163 **Dr Shival Srivastav-9560839841**

Dr Ravindra Kumar Shukla-8697190025 **Dr Om Lata Bhagat-9116076911**

Annexure III: Patient information sheet (Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान ,जोधपुर ,राजस्थान
शरीर क्रिया विज्ञान विभाग
रोगी सुचना पत्रक

परियोजना का शीर्षक - स्वस्थ्य व्यक्तियों एवं मधुमेह रोगियों में अल्प समय के लिए हेड डाउन टिल्ट देने पर होने वाले कार्डियोवैस्कुलर परिवर्तनों का परिक्षण

प्रधान शोधकर्ता - डॉ ममता शोभावत मोबाइल नंबर - 9602782163

आपको उपर्युक्त अध्ययन में भाग लेने के लिए आमंत्रित किया गया है। यह अध्ययन अल्प समय के लिए हेड डाउन टिल्ट देने पर स्वस्थ्य व्यक्तियों एवं मधुमेह रोगियों में होने वाले कार्डियोवैस्कुलर परिवर्तनों का परिक्षण करने के लिए किया जा रहा है , यदि आप इस अध्ययन में भाग लेने के लिए सहमत है तो आपको ऑटोनोमिक फंक्शन के ४ अलग अलग परिक्षण करने होंगे , जिसमे हम आपके रक्तचाप और साँस लेने के पैटर्न को एक बेल्ट और दिल की इलेक्ट्रिक गतिविधि के साथ के साथ आपके कंधे और गर्दन से जुड़े सुराग के साथ रिकॉर्ड करेंगे। पहले परिक्षण में आपको लेटने की स्थिति से खड़े होने की आवश्यकता होगी और ५ मिनट तक खड़े रहना होगा जिसमे हम आपके रक्तचाप को रुक रुक कर रिकॉर्ड कर रहे होंगे , दूसरे परिक्षण में आपको हैंडग्रिप बनाये रखने के लिए कहा जायेगा, जिसके बाद आपके रक्तचाप में परिवर्तन समय समय पर दर्ज किये जायेंगे। तीसरे परिक्षण में आपको १ मिनट के लिए ठन्डे पानी में अपने हाथ को डुबाना होगा और आपके रक्तचाप में परिवर्तन १ मिनट,२. ५ ,और ५ मिनट पर मापा जायेगा। चौथे परिक्षण में आपके माथे पे 0 -१ डिग्री सेंटीग्रेड के शीत संपीडन ३० सेकंड के लिये दिए जायेंगे और लगातार हृदय की इलेक्ट्रिक एक्टिविटी को रिकॉर्ड किया जायेगा। सभी परीक्षणों के बीच में ५ मिनट का अंतर रहेगा और इस दौरान आराम प्रदान किया जायेगा। अंगुली की पल्स को वेल्क्रो स्ट्रैप का उपयोग करते हुए तर्जनी से जुडी फोटोप्लेथीस्मोग्राफी जांच का उपयोग करते हुए रिकॉर्ड किया जायेगा। उसके पश्चात् आपको टिल्ट टेबल पर लेटना होगा और ५ मिनट आराम करना होगा, ५ मिनट के बाद आपके बेसलाइन रक्तचाप और हृदय की इलेक्ट्रिक एक्टिविटी को रिकॉर्ड किया जायेगा, फिर ६ डिग्री, १५ डिग्री और ३० डिग्री हेड डाउन टिल्ट ५-५ मिनट के लिए दिया जायेगा , हर टिल्ट के बाद ५ मिनट का आराम प्रदान किया जायेगा , टिल्ट के दौरान १,२ ,३ ,४ ,५ मिनट पर आपके रक्तचाप को रिकॉर्ड किया जायेगा और लगातार हृदय की इलेक्ट्रिक एक्टिविटी को रिकॉर्ड किया जायेगा। प्रयोगशाला में आपके रहने की अपेक्षित अवधि १ ऑवर होगी। उपर्युक्त परिक्षण ऑटोनोमिक फंक्शन लेबोरेटरी, फिजियोलॉजी डिपार्टमेंट ऐइम्स जोधपुर में किये जायेंगे। आपसे अनुरोध हे की आप अपनी और अपने अन्वेषक की सुविधा के हिसाब से एक ही बैठक में सभी परीक्षणों में भाग ले. यधपि उपरोक्त परीक्षणों में कोई भी जोखिम नहीं है फिर भी ये सभी परिक्षण चिकित्सक की देखरेख में किये जायेंगे। आपसे अनुरोध है की परिक्षण से २ ऑवर पहले चाय, कॉफ़ी या अन्य किसी पेय से परहेज करे. इस परिक्षण से २ ऑवर पहले हल्का नास्ता करने का आपसे अनुरोध किया जाता हे। इस परिक्षण में आपकी भागीदारी स्वेछिक हे, और आप बिना कारन बताये ये परिक्षण कभी भी छोड़ सकते हे , आपकी पूरी जानकारी गोपनीय रखी जाएगी।

आगे प्रश्न के लिए

डॉ ममता शोभावत -9602782163 डॉ शिवाल श्रीवास्तव -9560839841
डॉ रविंद्र कुमार शुक्ला - 8697190025 डॉ ओम लता भगत - 9116076911

Annexure IV: Patient informed consent form (English)

All India Institute of Medical Science

Jodhpur,Rajasthan

Informed Consent Form

Title of Thesis- Evaluation of cardiovascular responses to short term head down tilt in healthy subjects and diabetic patients.

Name of PG Student: Dr. Mamta Shobhawat Tel. No- 9602782163

Patient/Volunteer Identification No.:

I, _____ S/o or D/o _____

R/o_____

give my full, free, voluntary consent to be a part of the study "Evaluation of cardiovascular responses to short term head down tilt in healthy subjects and diabetic patients", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions. I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason. I understand that the information collected about me and any of my medical records may be looked at by responsible individual from AIIMS Jodhpur or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____

_____

Place: _____ Signature/Left thumb impression

This to certify that the above consent has been obtained in my presence.

Date: _____ Signature of PG student

Place: _____

Witness-1	Witness-2
Name-	Name-
Address-	Address-
Signature-	Signature-

Annexure V: Patient informed consent form (Hindi)

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर, राजस्थान

सूचित सहमति प्रपत्र

शोध का शीर्षक: " स्वस्थ व्यक्तियों एवं मधुमेह रोगियों में अल्प समय के लिए हेड डाउन टिल्ट देने पर होने वाले कार्डियोवैस्कुलर परिवर्तनों का परीक्षण"

प्रधान शोधकर्ता: डॉ ममता शोभावत मोबाइल नंबर: 9602782163

विषय क्रमांक संख्या -

में ---------- पुत्र /पुत्री श्री ,---------- --- निवासी ---------- "स्वस्थ्य व्यक्तियों एवं मधुमेह रोगियों में अल्प समय के लिए हेड डाउन टिल्ट देने पर होने वाले कार्डियोवैस्कुलर परिवर्तनों का परिक्षण" अध्ययन का हिस्सा बनने के लिए अपनी पूर्ण स्वतंत्र स्वेछिक सहमति देता /देती हु. यह अध्ययन मेरी खुद की भाषा में मुझे समझाया गया है। इस अध्ययन के बारे में मुझे सवाल पूछने का अवसर दिया गया है और में पूर्ण रूप से संतुष्ट हु। इस अध्ययन में मेरी भागीदारी स्वेछिक है और मुझे पता है की में बिना कोई कारन बताये किसी भी समय इस अध्ययन से बाहर हो सकता / सकती हु। मेरे मेडिकल रिकॉर्ड की जानकारी अखिल भारतीय आयुर्विज्ञान संस्थान जोधपुर के जिम्मेदार व्यक्तियों या नियामक अधिकारियो द्वारा देखी जा सकती है और में इसकी अनुमति देता/देती हु।

दिनांक : स्थान:
हस्ताक्षर:

गवाह -1 गवाह -2
हस्ताक्षर - हस्ताक्षर -
नाम- नाम-

Annexure VI: Case record form

DEPARTMENT OF PHYSIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

Autonomic Function Test Report

Name:..... Age: ..... Sex ..... Lab No. ..... Date ...

Address: ..... Ph. No. : .....

Email: ..... OPD / CR No: .....Unit :.....

Diagnosis:.....

Ward/OPD: ..... Consultant .....

Baseline Parameters: SBP ..... mmHg; DBP ..... mmHg; HR ..... bpm RR ..... per min

AUTONOMIC REACTIVITY TESTS

Test	Normal Range	Results
1. Cold face test: Percentage change in Heart Rate:	20%	
2. Isometric exercise (Hand-grip test): Rise in Diastolic BP:	≥ 10 mmHg	
3. Cold pressor test: Rise in Diastolic BP:	≥ 10 mmHg	
4. Postural Tests: Head-up tilt / Lying-to-standing		
a. Δ Systolic BP	Fall of ≤ 10 mmHg	
b. 30:15 Ratio:	≥ 1.04	

Comment:.....
.....

AUTONOMIC ACTIVITY TEST (HRV)

1. **Time domain:**
   a. **SDNN**
   b. **RMSSD**
2. **Freq. domain:**
   a. **Total power**
   b. **LF**
   c. **HF**
   d. **LF:HF**

Comment: .....
.....

OTHER TESTS:

Vascular Function **BRS (Baroreflex Sensitivity)** **Vagal Tone** **SSR (Skin Symp. Response)**

EGG **Cerebral Blood Flow Transcranial Doppler** **Cold Face Test:**

Impression:.....

Faculty Incharge
Autonomic Function Lab
Department of Physiology
AIIMS, Jodhpur

	DEPARTMENT OF PHYSIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

AUTONOMIC SYMPTOM FORM

Name ...../..... Age ..... Sex ..... Lab No. ..... Date .....

Ward/OPD ..... CR. No. ..... Diagnosis .....

Autonomic Symptoms **Yes / No**

1. Nasal **1.1 Dry nose** _____

1.2 Running nose _____

2. Sweating disturbances _____

3. Postural Fall/Dizziness _____

4. GIT **4.1 a. Alternate D/C** _____

b. Diarrhea _____

c. Constipation _____

4.2 Discomfort/Pain _____

5. Headache vascular: (Heaviness in head/throbbing headache, migraine) _____

6. Micturition disturbances _____

7. Occasional attack of bronchospasm: (after exercise laughter or emotion) _____

8. Do you often feel too hot/too cold: (Specify) _____

9. Do your extremities remain: Warm/Cold (Specify) _____

10. Impotence _____

Family History	**Father/ Mother**	**SELF Yes/No**

11. RISK FACTORS

a. – Alcoholism

b. – Diabetes mellitus

c. – Hypertension

d. – Heart attack (CHD)

e. – Obesity

f. – Smoking

12. Any stress related physical symptom: (Flushing, chocking, lump in throat, general weakness, tremor etc.)

13. Any other general symptoms

14. Allergy to any drugs

15. a) Occupation

b) Physical status, Athlete/Occasional Player/Regular exercise:

	DEPARTMENT OF PHYSIOLOGY, ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR

AUTONOMIC FUNCTION TEST FORM

Name _____ **Age/Sex** _____ **Lab. No.**_____

Address _____ **Patient ID**_____

Ward/OPD _____ **Date & Time** _____

Mob. No. _____ **Consultant**_____

Ht./ Wt. _____/_____ **Diagnosis** _____**Basal Parameters**_____

BP _____ _____ mm/Hg **HR** _____Per min. **RR** _____ Per Min.

I. Isometric Exercise Test (MVC = Kg.); Record blood pressure at:-

Basal			mmHg	**Diff.**
1st min			mmHg	
2nd min			mmHg	
4th min			mmHg	
6th min			mmHg	

HR	**Delta HR**	**PR**	**Delta PR**

Highest DBP – Baseline DBP =

II. Cold Pressor Test : Record Blood Pressure at:-

Basal			mmHg	**Diff.**
1st min			mmHg	
2.5th min			mmHg	
5thmin			mmHg	

HR	**Delta HR**	**PR**	**Delta PR**

Highest DBP – Baseline DBP =

III. Lying to Standing : Record Blood Pressure at:-

Basal			mmHg	**Diff.**
.5thmin			mmHg	
1st min			mmHg	
2nd min			mmHg	
2.5th min			mmHg	
5th min			mmHg	

Fall in SBP =

HEART RATE VARIABILITY REPORT

A) Time Domain

Mean	
SDNN	
SDSD	
RMSSD	
NN50	
PNN50	

Frequency Domain

LF (NU)	
HF (NU)	
LF/HF	
Total Power	

Poincare Analysis

SD1	
SD2	
SD1/SD2	

Remarks: ----------

HEAD DOWN TILT (6 degrees)

Basal BP		Difference
1 minute		
2 minute		
3 minute		
4 minute		
5 minute		

HEAD DOWN TILT (15 degrees)

Basal BP		Difference
1 minute		
2 minute		
3 minute		
4 minute		
5 minute		

HEAD DOWN TILT (30 degrees)

Basal BP		Difference
1 minute		
2 minute		
3 minute		
4 minute		
5 minute		

Annexure VI: Certificate of Poster presentation